BREAKING DOWN SCHIZOPHRENIA
into phenes, genes and environment

ISBN | 978-90-822-9280-0

design & layout | Jeroen Decoster
photography | Lieven Van Meulder
print | www.ridderprint.nl

BREAKING DOWN SCHIZOPHRENIA
into phenes, genes and environment

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit Maastricht,
op gezag van de Rector Magnificus, Prof. Dr. L.L.G. Soete,
volgens het besluit van het College van Decanen,
in het openbaar te verdedigen
op dinsdag 11 november 2014 om 16.00 uur

door

Jeroen Decoster
geboren op 16 maart 1982
te Ieper

Promotor

Prof. Dr. Inez Myin-Germeys

Copromotores

Prof. Dr. Marc De Hert (KU Leuven)
Dr. Ruud van Winkel

Beoordelingscommissie

Prof. Dr. Jim van Os (voorzitter)
Prof. Dr. Therese van Amelsvoort
Prof. Dr. Stephan Claes (KU Leuven)
Prof. Dr. Guido Pieters (KU Leuven)
Dr. Bart Rutten

The research presented in this thesis was conducted at the Department of Psychiatry and Psychology of the School for Mental Health and Neuroscience, Maastricht University and at the University Psychiatric Centre KU Leuven.

This research was funded by the European Community's Seventh Framework Program under grant agreement (HEALTH-F2-2009–241909, Project EU-GEI)

.

for my parents
for Nathalie
for Warre and Lowie

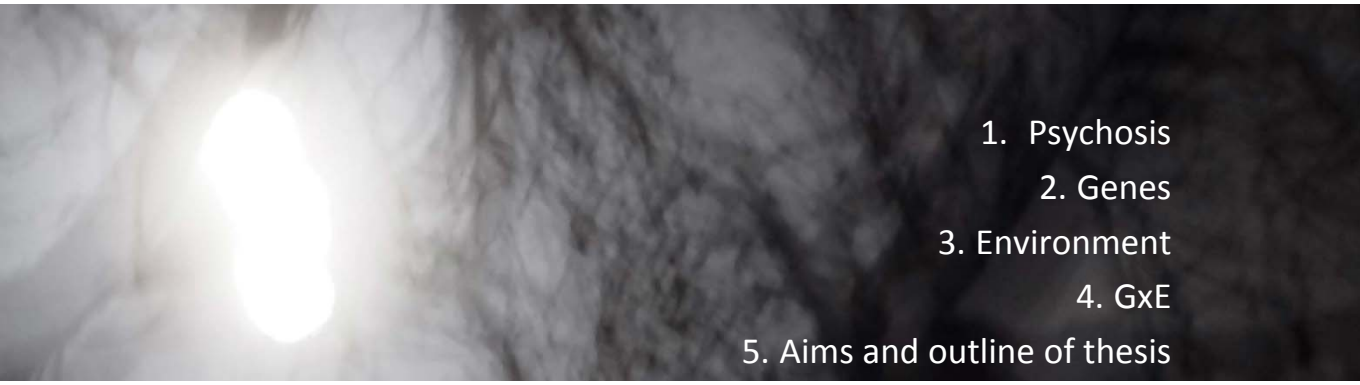
Paranimfen

Claudia Menne-Lothmann
Dieter Decoster

Contents

Chapter One

General introduction



1. Psychosis

From psychosis…

Psychosis, Ancient Greek for disturbance of the mind, refers to distortions of thinking (delusions and/or formal thought disorders) and/or perception (hallucinations) and is often described as problems in recognizing and interacting with reality. Psychotic symptoms occur in different nosological concepts; within the context of general medical conditions, during intoxication with or withdrawal from (illicit) drugs and in affective disorders, but are most common in non-affective psychotic disorders (American Psychiatric Association, 2000a; van Os and Kapur, 2009; World Health Organisation, 1992). Although the current classification of the non-affective psychotic disorders, and even their boundaries with affective disorders and developmental disorders, is subject of debate (van Os and Kapur, 2009), most epidemiological, etiological and clinical knowledge to date is based on research within those constructs, with schizophrenia as the most important (Tandon et al., 2008a; Tandon et al., 2009b, 2010).

…to schizophrenia…

Schizophrenia is a particularly heterogeneous disorder, characterized by the co-occurrence, in varying degrees, of several symptom domains which cause impairments in social and vocational functioning: (i) positive symptoms or psychosis, (ii) negative symptoms or diminished drive and volition (flattening of affect, lack of motivation, poverty of thoughts or speech, social withdrawal) and (iii) cognitive symptoms as difficulties in memory, attention and executive functioning. The latter symptom domain is not a formal diagnostic criterion.

Schizophrenia has a worldwide prevalence of 0.2 - 1% (Saha et al., 2005), with a greater lifetime risk (RR 1.15 - 1.40) in males (Aleman et al., 2003; van der Werf et al., 2012).

Schizophrenia, acute as well as residual state, is estimated to be among the health states with the highest disability (Salomon et al., 2013). Moreover, it is associated with a 2.5 times higher mortality rate than in the general population. This is only partly attributable to an elevated suicide risk and is suggested to reflect the increasing gap in health care accessibility, the less healthy life style and/or the possibly shared genetic and environmental risk factors between schizophrenia and comorbid somatic disorders, e.g. diabetes and cardiovascular diseases (Saha et al., 2007).

In addition to the impact on the patient, schizophrenia also impacts significantly the quality of life in caregivers (Awad and Voruganti, 2008; Caqueo-Urizar et al., 2009).

The course of schizophrenia is typically described in four phases; the premorbid, prodromal, psychotic and stable or plateau phase, with often insidious transition between two phases. Not all patients go through all phases; recovery, at variable degree, is possible at each stage (Tandon et al., 2009b). The first diagnosis is typically made in adolescence or early adulthood with a younger age at onset of the psychotic phase as an important predictor of more severe symptoms, worse treatment response and inferior overall prognosis (Crespo-Facorro et al., 2007; Luoma et al., 2008; Rabinowitz et al., 2006).
Since the 1950s, the cornerstone of an adequate treatment became antipsychotic medication, without ignoring the importance of psychological and social support and interventions (van Os and Kapur, 2009).

… and back to psychosis

The current concepts of schizophrenia and other non-affective psychotic disorders seem to reflect discrete nosological entities, but this can be firmly questioned (van Os, 2009). As mentioned above, schizophrenia is characterized by a marked heterogeneity which makes the existence of one specific etiological and pathophysiological pathway unlikely. Additionaly, several lines of research suggest shared (genetic) etiology between schizophrenia and several other psychiatric disorders, as indicated by (i) increased comorbidity rates with other psychiatric diseases (Buckley et al., 2009), (ii) molecular genetic overlap with other psychiatric diseases (Moreno-De-Luca et al., 2010; Ripke et al., 2011), (iii) familial history of schizophrenia associated with increased risk for most non-psychotic psychiatric disorders (DeVylder and Lukens, 2013) and (iv) broad psychiatric familial history being more predictive for schizophrenia than familial history of schizophrenia (Mortensen et al., 2010), …
In recent years, a more dimensional (instead of a purely categorical) classification model was proposed for psychiatric disorders in general (DeVylder and Lukens, 2013), and for psychotic disorders specifically (van Os et al., 2009). A dimensional classification allows for a more accurate reflection of the nature of psychosis, as psychosis can be seen as a continuum, ranging from subclinical psychotic experiences in the general population to clinical psychotic symptoms, requiring psychiatric treatment (van Os et al., 2009). The lifetime prevalence of clinically relevant psychotic symptoms can be estimated around 3% (Perala et al., 2007).
Convincing research is available to support an etiological continuity in the psychosis continuum, for genetic as well as environmental factors (van Os et al., 2009). Therefore, investigating the etiological and pathophysiological mechanisms along the

entire continuum may help to increase the knowledge about underlying causes of psychosis.

2. Genes (or nature)

Family, twin and adoption studies estimated the genetic portion of the risk to develop schizophrenia up to 80% (Kendler and Diehl, 1993; Sullivan et al., 2003), although more recent population-based studies lower the initial estimations to 67% and even less (Wray and Gottesman, 2012). Schizophrenia is considered to be a complex genetic disorder, which means that a single (Mendelian) genetic variant cannot explain its heritability, but rather the sum of a large number of genetic variants with individually small effects (Kendler and Diehl, 1993). During the last two decades, a lot of efforts were made to disentangle the complex, underlying molecular genetics of schizophrenia. A better understanding of these biological causes of psychotic disorders would provide the scientific world with new clues for the development of better biological treatments.

Family linkage studies were used to detect regions in the genome that could harbor disorder-relevant genetic variation (Cichon et al., 2009), without any conclusive, replicated results.

Association studies constitute another approach. A field synopsis of all gene association studies in schizophrenia can be found on www.szgene.org (Allen et al., 2008). Those association studies are mainly based on (a combination of) single nucleotide polymorphisms (SNPs). Of the 3 billion base pairs (two nucleotides) forming the human DNA, the vast majority is the same for all individuals. However, 1 in 1000 base pairs holds different information. If the same variation at a certain base pair position occurs in at least 1% of the overall population, it is called a SNP. In candidate SNP/gene association studies, SNPs/genes are considered candidate for association with schizophrenia mostly because of their position in the genome (follow-up of linkage studies) or their involvement in neurobiological pathways considered relevant in schizophrenia (e.g. dopamine metabolism, neurodevelopment processes, ...)(Cichon et al., 2009). So far, the candidate SNP/gene approach has not yielded widely accepted findings (Cichon et al., 2009).

A further step in genetic research was taken with the Genome Wide Association Studies (GWAS), based on the common-disease common-variant hypothesis (O'Donovan et al., 2008; Purcell et al., 2009; Ripke et al., 2011; Shi et al., 2009). According to this hypothesis, the genetic base of schizophrenia is formed by the combination of many different common genetic variants (with a prevalence of ≥ 5%),

each with small effect. GWAS could detect (some of) those variants by studying differential frequency of SNPs in large groups of patients and healthy controls. Huge international collaboration studies (e.g. International Schizophrenia Consortium, Psychiatric Genomics Consortium) were started to collect big enough samples to reach the needed statistical power. Although the power and/or the genome coverage could still be insufficient (Collins et al., 2011), some significant and replicated findings were made, including genetic variation in the major histocompatibility complex (MHC), TCF4 and ZNF804A (O'Donovan et al., 2008; Purcell et al., 2009a; Shi et al., 2009; Stefansson et al., 2009).

Parallel with the common-disease common-variant hypothesis, also the common-disease rare-variant hypothesis was put forward (McClellan et al., 2007). According to this, rare variants (rare SNPs or rare copy number variations (rare CNVs)) may have strong effects on the development of the disorder. Such a rare variant can even be present in only one individual or in one multiply affected family. CNVs are structural genomic variants; stretches of DNA (thousands to millions of base pairs) that are deleted, duplicated (once or more) or inversed compared to a reference genome. Some types of CNVs can be detected by the same techniques as used for the detection of SNPs, others are more difficult and expensive to investigate. Awaiting more affordable, technological possibilities, schizophrenia was already associated with a higher frequency of CNVs than in the general population (Bassett et al., 2010; Buizer-Voskamp et al., 2011) and the involvement of some chromosomal regions was replicated (Doherty et al., 2012).

Both the common-variant and the rare-variant approach could be able to explain the variance in genetic risk for schizophrenia, but so far, they only explain a fraction of the risk, leading to the so called 'missing heritability'. Further efforts and strategies in the search of the missing heritability are mainly announced as bigger and improved versions of the approaches described above. However, concerning these approaches, several caveats were raised (Cichon et al., 2009), two of them deserving special attention in the context of this thesis; 1. For some disorders there might be no detectable main effects of SNPs, only higher order gene-gene or gene-environment interactions (see further). 2. Current diagnostic categories might be inadequate and endophenotypic variables or intermediate phenotypes might better index the underlying gene effects.

A valid intermediate phenotype can be any measurable component along the pathway between genotype and disorder as long as it meets some criteria (Gottesman and Gould, 2003): (i) associated with the disorder in the general population, (ii) heritable, (iii) state-independent, (iv) cosegregation with the disorder within families and (v) non-

affected family members display the endophenotype in a higher degree than the general population. As elaborated in chapter two of this thesis, decreased P300 amplitude may be a valid endophenotype for psychosis, with a heritability of up to 80% (Bestelmeyer et al., 2009; Hall et al., 2009).

3. Environment (or nurture)

Although the heritability of psychosis/schizophrenia seems high, it certainly is not 100%, leaving still important causal influence for environmental factors (Tsuang et al., 2001). The causal role of the environment may even have been underestimated for a long time (Wray and Gottesman, 2012). Environmental components include psychosocial, biological and physical factors experienced by the individual from the moment of conception, through development, birth and maturation (Tsuang et al., 2001; van Os et al., 2005). Finding compelling, modifiable environmental risk factors of psychosis, would enable clinicians and policymakers to develop preventive and therapeutic psychosocial interventions.

Several biological, environmental factors are repeatedly associated with the risk to develop psychosis (not all of them with meta-analytic evidence); prenatal environmental factors like maternal infection (Brown and Derkits, 2010) and vitamin D deficiency (McGrath et al., 2010b), obstetrical complications (Mittal et al., 2008) and cannabis use (Henquet et al., 2005b; Moore et al., 2007). Also several psychosocial environmental factors were found to be associated with elevated risk for psychosis, possibly with social defeat as common underlying mechanism (Selten and Cantor-Graae, 2005); urban environment during development (March et al., 2008), childhood trauma (Varese et al., 2012), multiple traumatic life events (Shevlin et al., 2008; van Winkel et al., 2008b) and migration/minority status/discrimination (Bourque et al., 2011; Veling and Susser, 2011). It has been argued that stigmatization may be a precipitation factor too (van Zelst, 2009).

Collecting databases with phenotypes and adequate information about environmental exposure is an expensive and time-consuming process. However, reliable and precise measurement pays off by enhancing the power of an association study (Moffitt et al., 2005; Wong et al., 2003). Additionally, several strategies can improve the quality of data (Moffitt et al., 2005); prospective instead of retrospective, proximal instead of distal (e.g. individual cannabis use vs average use in community) and cumulative, repeated measures instead of snapshot measures. The latter because it provides more precise, sensitive, and reliable measurement of the environmental risk factor. The Experience Sampling Method (ESM), used in chapter six of this thesis, is an elegant

diary method that assesses thoughts, emotions and (social) context in the flow of daily life and meets the above mentioned criteria for optimal measurement of the environment (Myin-Germeys et al., 2009).

4. Gene-environment interactions (GxE)

Although not all strategies are optimized, pure molecular genetics and pure environmental epidemiology have not been very fruitful yet in detecting unequivocal causal factors of psychosis/schizophrenia. Ignoring nurture may have handicapped the field's ability to understand nature (Moffitt et al., 2005). And vice versa, since many environmental risk factors are ubiquitous, interactions with personal vulnerability must be invoked to explain the relatively low rates of psychosis (van Os et al., 2005).

In the last decade of psychosis research, a lot of scientific attention has been drawn to the concept of gene-environment interaction (GxE) (Duncan and Keller, 2011a; van Os et al., 2010; Van Winkel et al., 2010b). Genetic variation may alter the individual sensitivity to certain environmental factors, or conversely, certain environmental factors may disrupt some vulnerable genetic mechanisms. Such interactions have to be seen in a time-dependent model, with potentially differential effects according to the developmental stage of the individual (van Os et al., 2008b).

Analogously to the developments in molecular genetic research, large multi-centered and multidisciplinary, international research projects were initiated to investigate GxE in psychosis (EU-GEI, 2008). Herein, top notch genetic and environmental research strategies must be combined, along with the quest for solutions to the accompanying and challenging statistical problems. The psychosis research community eagerly awaits the first results of EU-GEI and other, large projects.

In the mean time, many interesting studies already suggested GxE in psychosis (Van Winkel et al., 2010b), although few with meta-analytic evidence.

Further research efforts to detect true GxE in psychosis will certainly contribute to enhancing the knowledge about the cause of psychosis. In a clinical perspective, GxE-findings may create possibilities for more individualized and differentiated interventions and for selective early intervention programs.

5. Aims and outline of thesis

The overall aim of this thesis was to further explore research strategies to unravel underlying genetics of psychosis, with particular focus on two strategies: (i) endophenotypes and (ii) gene-environment interactions.

Before zooming in on GxE, in **Chapter Two**, I study the electrophysiological endophenotypes, which may be a potentially useful leverage to crack the genetic code of psychosis. The results of a genetic association study with the P300-amplitude in patients with a psychotic disorder are presented.

Chapter Three, Chapter Four and **Chapter Five** discuss the differential effects of cannabis on the development of psychosis. In **Chapter Three**, a candidate-gene approach (BDNF Val66Met) was used to examine differences in age at onset of psychosis with or without preceding cannabis use. As described before, age at onset is an important predictor of the course of the illness. Additionally, it has been suggested that modifiers of the age at onset may offer an important clue to the etiology of psychosis (DeLisi, 1992). In **Chapter Four**, we report a replication study of an interesting GxE with rs2494732 in AKT1, suggested to contribute to the differential sensitivity to the psychotogenic effects of cannabis (van Winkel and GROUP Investigators, 2011). **Chapter Five** provides a critical review of the available evidence suggesting a GxE with cannabis and the associated molecular genetic findings (not considering Chapter Four). In this chapter, we also detect some pitfalls and formulate recommendations for future research.

Finally and in the spirit of the above-described etiological continuity in the psychosis continuum, we examined GxE in paranoia, a core symptom of psychotic disorders. In **Chapter Six**, the results of a large ESM-study, exploring underlying genetics of paranoid reactivity to social stress, are presented.

REFERENCES

Aleman, A., Kahn, R.S., Selten, J.P., 2003. Sex differences in the risk of schizophrenia: evidence from meta-analysis. Archives of General Psychiatry 60(6), 565-571.

Allen, N.C., Bagade, S., McQueen, M.B., Ioannidis, J.P., Kavvoura, F.K., Khoury, M.J., Tanzi, R.E., Bertram, L., 2008. Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: the SzGene database. Nat Genet 40(7), 827-834.

American Psychiatric Association, 2000. Diagnostic and statistical manual of mental disorders, Washington, DC: Author.

Awad, A.G., Voruganti, L.N., 2008. The burden of schizophrenia on caregivers: a review. Pharmacoeconomics 26(2), 149-162.

Bassett, A.S., Scherer, S.W., Brzustowicz, L.M., 2010. Copy number variations in schizophrenia: critical review and new perspectives on concepts of genetics and disease. The American journal of psychiatry 167(8), 899-914.

Bestelmeyer, P.E., Phillips, L.H., Crombie, C., Benson, P., St Clair, D., 2009. The P300 as a possible endophenotype for schizophrenia and bipolar disorder: Evidence from twin and patient studies. Psychiatry Res 169(3), 212-219.

Bourque, F., van der Ven, E., Malla, A., 2011. A meta-analysis of the risk for psychotic disorders among first- and second-generation immigrants. Psychological Medicine 41(5), 897-910.

Brown, A.S., Derkits, E.J., 2010. Prenatal infection and schizophrenia: a review of epidemiologic and translational studies. The American journal of psychiatry 167(3), 261-280.

Buckley, P.F., Miller, B.J., Lehrer, D.S., Castle, D.J., 2009. Psychiatric comorbidities and schizophrenia. Schizophr Bull 35(2), 383-402.

Buizer-Voskamp, J.E., Muntjewerff, J.W., Strengman, E., Sabatti, C., Stefansson, H., Vorstman, J.A., Ophoff, R.A., 2011. Genome-wide analysis shows increased frequency of copy number variation deletions in Dutch schizophrenia patients. Biological psychiatry 70(7), 655-662.

Caqueo-Urizar, A., Gutierrez-Maldonado, J., Miranda-Castillo, C., 2009. Quality of life in caregivers of patients with schizophrenia: a literature review. Health Qual Life Outcomes 7, 84.

Cichon, S., Craddock, N., Daly, M., Faraone, S.V., Gejman, P.V., Kelsoe, J., Lehner, T., Levinson, D.F., Moran, A., Sklar, P., Sullivan, P.F., 2009. Genomewide association studies: history, rationale, and prospects for psychiatric disorders. Am J Psychiatry 166(5), 540-556.

Collins, A.L., Kim, Y., Sklar, P., O'Donovan, M.C., Sullivan, P.F., 2011. Hypothesis-driven candidate genes for schizophrenia compared to genome-wide association results. Psychol Med, 1-10.

Crespo-Facorro, B., Pelayo-Teran, J.M., Perez-Iglesias, R., Ramirez-Bonilla, M., Martinez-Garcia, O., Pardo-Garcia, G., Vazquez-Barquero, J.L., 2007. Predictors of acute treatment response in patients with a first episode of non-affective psychosis: sociodemographics, premorbid and clinical variables. J Psychiatr Res 41(8), 659-666.

DeLisi, L.E., 1992. The significance of age of onset for schizophrenia. Schizophr Bull 18(2), 209-215.

DeVylder, J.E., Lukens, E.P., 2013. Family history of schizophrenia as a risk factor for axis I psychiatric conditions. Journal of psychiatric research 47(2), 181-187.

Doherty, J.L., O'Donovan, M.C., Owen, M.J., 2012. Recent genomic advances in schizophrenia. Clin Genet 81(2), 103-109.

Duncan, L.E., Keller, M.C., 2011. A Critical Review of the First 10 Years of Candidate Gene-by-Environment Interaction Research in Psychiatry. Am J Psychiatry 168(10), 1041-1049.

EU-GEI, 2008. Schizophrenia aetiology: do gene-environment interactions hold the key? Schizophr Res 102(1-3), 21-26.

Gottesman, II, Gould, T.D., 2003. The endophenotype concept in psychiatry: etymology and strategic intentions. Am J Psychiatry 160(4), 636-645.

Hall, M.H., Schulze, K., Rijsdijk, F., Kalidindi, S., McDonald, C., Bramon, E., Murray, R.M., Sham, P., 2009. Are auditory P300 and duration MMN heritable and putative endophenotypes of psychotic bipolar disorder? A Maudsley Bipolar Twin and Family Study. Psychol Med 39(8), 1277-1287.

Henquet, C., Murray, R., Linszen, D., van Os, J., 2005. The environment and schizophrenia: the role of cannabis use. Schizophr Bull 31(3), 608-612.

Kendler, K.S., Diehl, S.R., 1993. The genetics of schizophrenia: a current, genetic-epidemiologic perspective. Schizophr Bull 19(2), 261-285.

Luoma, S., Hakko, H., Ollinen, T., Jarvelin, M.R., Lindeman, S., 2008. Association between age at onset and clinical features of schizophrenia: the Northern Finland 1966 birth cohort study. European psychiatry : the journal of the Association of European Psychiatrists 23(5), 331-335.

March, D., Hatch, S.L., Morgan, C., Kirkbride, J.B., Bresnahan, M., Fearon, P., Susser, E., 2008. Psychosis and place. Epidemiol Rev 30, 84-100.

McClellan, J.M., Susser, E., King, M.C., 2007. Schizophrenia: a common disease caused by multiple rare alleles. The British journal of psychiatry : the journal of mental science 190, 194-199.

McGrath, J.J., Burne, T.H., Feron, F., Mackay-Sim, A., Eyles, D.W., 2010. Developmental vitamin D deficiency and risk of schizophrenia: a 10-year update. Schizophr Bull 36(6), 1073-1078.

Mittal, V.A., Ellman, L.M., Cannon, T.D., 2008. Gene-environment interaction and covariation in schizophrenia: the role of obstetric complications. Schizophr Bull 34(6), 1083-1094.

Moffitt, T.E., Caspi, A., Rutter, M., 2005. Strategy for investigating interactions between measured genes and measured environments. Archives of General Psychiatry 62(5), 473-481.

Moore, T.H., Zammit, S., Lingford-Hughes, A., Barnes, T.R., Jones, P.B., Burke, M., Lewis, G., 2007. Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. Lancet 370(9584), 319-328.

Moreno-De-Luca, D., Mulle, J.G., Kaminsky, E.B., Sanders, S.J., Myers, S.M., Adam, M.P., Pakula, A.T., Eisenhauer, N.J., Uhas, K., Weik, L., Guy, L., Care, M.E., Morel, C.F., Boni, C., Salbert, B.A., Chandrareddy, A., Demmer, L.A., Chow, E.W., Surti, U., Aradhya, S., Pickering, D.L., Golden, D.M., Sanger, W.G., Aston, E., Brothman, A.R., Gliem, T.J., Thorland, E.C., Ackley, T., Iyer, R., Huang, S., Barber, J.C., Crolla, J.A., Warren, S.T., Martin, C.L., Ledbetter, D.H., 2010. Deletion 17q12 is a recurrent copy number variant that confers high risk of autism and schizophrenia. American journal of human genetics 87(5), 618-630.

Mortensen, P.B., Pedersen, M.G., Pedersen, C.B., 2010. Psychiatric family history and schizophrenia risk in Denmark: which mental disorders are relevant? Psychological Medicine 40(2), 201-210.

Myin-Germeys, I., Oorschot, M., Collip, D., Lataster, J., Delespaul, P., van Os, J., 2009. Experience sampling research in psychopathology: opening the black box of daily life. Psychol Med 39(9), 1533-1547.

O'Donovan, M.C., Craddock, N., Norton, N., Williams, H., Peirce, T., Moskvina, V., Nikolov, I., Hamshere, M., Carroll, L., Georgieva, L., Dwyer, S., Holmans, P., Marchini, J.L., Spencer, C.C., Howie, B., Leung, H.T., Hartmann, A.M., Moller, H.J., Morris, D.W., Shi, Y., Feng, G., Hoffmann, P., Propping, P., Vasilescu, C., Maier, W., Rietschel, M., Zammit, S., Schumacher, J., Quinn, E.M., Schulze, T.G., Williams, N.M., Giegling, I., Iwata, N., Ikeda, M., Darvasi, A., Shifman, S., He, L., Duan, J., Sanders, A.R., Levinson, D.F., Gejman, P.V., Cichon, S., Nothen, M.M., Gill, M., Corvin, A., Rujescu, D., Kirov, G., Owen, M.J., Buccola, N.G., Mowry, B.J., Freedman, R., Amin, F., Black, D.W., Silverman, J.M., Byerley, W.F., Cloninger, C.R., 2008. Identification of loci associated with schizophrenia by genome-wide association and follow-up. Nat Genet 40(9), 1053-1055.

Perala, J., Suvisaari, J., Saarni, S.I., Kuoppasalmi, K., Isometsa, E., Pirkola, S., Partonen, T., Tuulio-Henriksson, A., Hintikka, J., Kieseppa, T., Harkanen, T., Koskinen, S., Lonnqvist, J., 2007. Lifetime prevalence of psychotic and bipolar I disorders in a general population. Archives of General Psychiatry 64(1), 19-28.

Purcell, S.M., Wray, N.R., Stone, J.L., Visscher, P.M., O'Donovan, M.C., Sullivan, P.F., Sklar, P., 2009. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature 460(7256), 748-752.

Rabinowitz, J., Levine, S.Z., Hafner, H., 2006. A population based elaboration of the role of age of onset on the course of schizophrenia. Schizophr Res 88(1-3), 96-101.

Ripke, S., Sanders, A.R., Kendler, K.S., Levinson, D.F., Sklar, P., Holmans, P., Lin, D., Duan, J., Ophoff, R.A., Andreassen, O.A., Scolnick, E.M., Cichon, S., St Clair, D., Corvin, A., Gurling, H., Werge, T., Rujescu, D., Blackwood, D., Pato, C.N., Malhotra, A.K., Purcell, S., Dudbridge, F., Neale, B., Rossin, L., Visscher, P.M., Posthuma, D., Ruderfer, D.M., Fanous, A., Stefansson, H., Steinberg, S., Mowry, B., Golimbet, V., De Hert, M., Jonsson, E.G., Bitter, I., Pietilainen, O.P., Collier, D., Tosato, S., Agartz, I., Albus, M., Alexander, M., Amdur, R., Amin, F., Bass, N., Bergen, S., Black, D.W., Borglum, A.D., Brown, M., Bruggeman, R., Buccola, N.G., Byerley, W., Cahn, W., Cantor, R.M., Carr, V., Catts, S., Choudhury, K., Cloninger, C.R., Cormican, P., Craddock, N., Danoy, P., Datta, S., De Haan, L., Demontis, D., Dikeos, D.G., Djurovic, S., Donnelly, P., Donohoe, G., Duong, L., Dwyer, S., Fink-Jensen, A., Freedman, R., Freimer, N.B., Friedl, M., Georgieva, L., Giegling, I., Gill, M., Glenthoj, B., Godard, S., Hamshere, M., Hansen, M., Hansen, T., Hartmann, A., Henskens, F., Hougaard, D., Hultman, C.M., Ingason, A., Jablensky, A., Jakobsen, K.D., Jay, M., Jurgens, G., Kahn, R.S., Keller, M.C., Kenis, G., Kenny, E., Kim, Y.S., Kirov, G., Konnerth, H., Konte, B., Krabbendam, L., Krasucki, R., Lasseter, V.K., Laurent, C., Lawrence, J., Lencz, T., Lerer, F., Liang, K.Y., Lichtenstein, P., Lieberman, J., Linszen, D., Lonnqvist, J., Loughland, C., Maclean, A.W., Maher, B.S.,

Maier, W., Mallet, J., Malloy, P., Mattheisen, M., Mattingsdal, M., McGhee, K.A., McGrath, J., McIntosh, A., McLean, D., McQuillin, A., Melle, I., Michie, P.T., Milanova, V., Morris, D.W., Mors, O., Mortensen, P.B., Moskvina, V., Muglia, P., Myin-Germeys, I., Nertney, D., Nestadt, G., Nielsen, J.B., Nikolov, I., Nordentoft, M., Norton, N., Nothen, M.M., O'Dushlaine, C.T., Olincy, A., Olsen, L., O'Neill, F.A., Orntoft, T., Owen, M., Pantelis, C., Papadimitriou, G.M., Pato, M.T., Peltonen, L., Petursson, H., Pickard, B., Pimm, J., Pulver, A.E., Puri, V., Quested, D., Quinn, E.M., Rasmussen, H.B., Rethelyi, J.M., Ribble, R., Rietschel, M., Riley, B., Ruggeri, M., Schall, U., Schulze, T.G., Schwab, S.G., Scott, R., Shi, J., Sigurdsson, E., Silverman, J.M., Spencer, C.C., Stefansson, H., Strange, A., Strengman, E., Stroup, T.S., Suvisaari, J., Terenius, L., Thirumalai, S., Thygesen, J., Timm, S., Toncheva, D., van den Oord, E., Van Os, J., van Winkel, R., Veldink, J., Walsh, D., Wang, A.G., Wiersma, D., Wildenauer, D.B., Williams, H., Williams, N., Wormley, B., Zammit, S., Sullivan, P., O'Donovan M, C., Daly, M.J., Gejman, P.V., Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium, 2011. Genome-wide association study identifies five new schizophrenia loci. Nat Genet 43(10), 969-976.

Saha, S., Chant, D., McGrath, J., 2007. A systematic review of mortality in schizophrenia: is the differential mortality gap worsening over time? Archives of General Psychiatry 64(10), 1123-1131.

Saha, S., Chant, D., Welham, J., McGrath, J., 2005. A systematic review of the prevalence of schizophrenia. PLoS Med 2(5), e141.

Salomon, J.A., Vos, T., Hogan, D.R., Gagnon, M., Naghavi, M., Mokdad, A., Begum, N., Shah, R., Karyana, M., Kosen, S., Farje, M.R., Moncada, G., Dutta, A., Sazawal, S., Dyer, A., Seiler, J., Aboyans, V., Baker, L., Baxter, A., Benjamin, E.J., Bhalla, K., Bin Abdulhak, A., Blyth, F., Bourne, R., Braithwaite, T., Brooks, P., Brugha, T.S., Bryan-Hancock, C., Buchbinder, R., Burney, P., Calabria, B., Chen, H., Chugh, S.S., Cooley, R., Criqui, M.H., Cross, M., Dabhadkar, K.C., Dahodwala, N., Davis, A., Degenhardt, L., Diaz-Torne, C., Dorsey, E.R., Driscoll, T., Edmond, K., Elbaz, A., Ezzati, M., Feigin, V., Ferri, C.P., Flaxman, A.D., Flood, L., Fransen, M., Fuse, K., Gabbe, B.J., Gillum, R.F., Haagsma, J., Harrison, J.E., Havmoeller, R., Hay, R.J., Hel-Baqui, A., Hoek, H.W., Hoffman, H., Hogeland, E., Hoy, D., Jarvis, D., Karthikeyan, G., Knowlton, L.M., Lathlean, T., Leasher, J.L., Lim, S.S., Lipshultz, S.E., Lopez, A.D., Lozano, R., Lyons, R., Malekzadeh, R., Marcenes, W., March, L., Margolis, D.J., McGill, N., McGrath, J., Mensah, G.A., Meyer, A.C., Michaud, C., Moran, A., Mori, R., Murdoch, M.E., Naldi, L., Newton, C.R., Norman, R., Omer, S.B., Osborne, R., Pearce, N., Perez-Ruiz, F., Perico, N., Pesudovs, K., Phillips, D., Pourmalek, F., Prince, M., Rehm, J.T., Remuzzi, G., Richardson, K., Room, R., Saha, S., Sampson, U., Sanchez-Riera, L., Segui-Gomez, M., Shahraz, S., Shibuya, K., Singh, D., Sliwa, K., Smith, E., Soerjomataram, I., Steiner, T., Stolk, W.A., Stovner, L.J., Sudfeld, C., Taylor, H.R., Tleyjeh, I.M., van der Werf, M.J., Watson, W.L., Weatherall, D.J., Weintraub, R., Weisskopf, M.G., Whiteford, H., Wilkinson, J.D., Woolf, A.D., Zheng, Z.J., Murray, C.J., 2013. Common values in assessing health outcomes from disease and injury: disability weights measurement study for the Global Burden of Disease Study 2010. Lancet 380(9859), 2129-2143.

Selten, J.P., Cantor-Graae, E., 2005. Social defeat: risk factor for schizophrenia? The British journal of psychiatry : the journal of mental science 187, 101-102.

Shevlin, M., Houston, J.E., Dorahy, M.J., Adamson, G., 2008. Cumulative traumas and psychosis: an analysis of the national comorbidity survey and the British Psychiatric Morbidity Survey. Schizophr Bull 34(1), 193-199.

Shi, J., Levinson, D.F., Duan, J., Sanders, A.R., Zheng, Y., Pe'er, I., Dudbridge, F., Holmans, P.A., Whittemore, A.S., Mowry, B.J., Olincy, A., Amin, F., Cloninger, C.R., Silverman, J.M., Buccola, N.G., Byerley, W.F., Black, D.W., Crowe, R.R., Oksenberg, J.R., Mirel, D.B., Kendler, K.S., Freedman, R., Gejman, P.V., 2009. Common variants on chromosome 6p22.1 are associated with schizophrenia. Nature 460(7256), 753-757.

Stefansson, H., Ophoff, R.A., Steinberg, S., Andreassen, O.A., Cichon, S., Rujescu, D., Werge, T., Pietilainen, O.P., Mors, O., Mortensen, P.B., Sigurdsson, E., Gustafsson, O., Nyegaard, M., Tuulio-Henriksson, A., Ingason, A., Hansen, T., Suvisaari, J., Lonnqvist, J., Paunio, T., Borglum, A.D., Hartmann, A., Fink-Jensen, A., Nordentoft, M., Hougaard, D., Norgaard-Pedersen, B., Bottcher, Y., Olesen, J., Breuer, R., Moller, H.J., Giegling, I., Rasmussen, H.B., Timm, S., Mattheisen, M., Bitter, I., Rethelyi, J.M., Magnusdottir, B.B., Sigmundsson, T., Olason, P., Masson, G., Gulcher, J.R., Haraldsson, M., Fossdal, R., Thorgeirsson, T.E., Thorsteinsdottir, U., Ruggeri, M., Tosato, S., Franke, B., Strengman, E., Kiemeney, L.A., Melle, I., Djurovic, S., Abramova, L., Kaleda, V., Sanjuan, J., de Frutos, R., Bramon, E., Vassos, E., Fraser, G., Ettinger, U., Picchioni, M., Walker, N., Toulopoulou, T., Need, A.C., Ge, D., Yoon, J.L., Shianna, K.V., Freimer, N.B., Cantor, R.M., Murray, R., Kong, A., Golimbet, V., Carracedo, A., Arango, C., Costas, J., Jonsson, E.G., Terenius, L., Agartz, I., Petursson, H., Nothen, M.M., Rietschel, M., Matthews, P.M., Muglia, P., Peltonen,

L., St Clair, D., Goldstein, D.B., Stefansson, K., Collier, D.A., 2009. Common variants conferring risk of schizophrenia. Nature 460(7256), 744-747.
Sullivan, P.F., Kendler, K.S., Neale, M.C., 2003. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Archives of General Psychiatry 60(12), 1187-1192.
Tandon, R., Keshavan, M.S., Nasrallah, H.A., 2008. Schizophrenia, "just the facts" what we know in 2008. 2. Epidemiology and etiology. Schizophr Res 102(1-3), 1-18.
Tandon, R., Nasrallah, H.A., Keshavan, M.S., 2009. Schizophrenia, "just the facts" 4. Clinical features and conceptualization. Schizophr Res 110(1-3), 1-23.
Tandon, R., Nasrallah, H.A., Keshavan, M.S., 2010. Schizophrenia, "just the facts" 5. Treatment and prevention. Past, present, and future. Schizophr Res 122(1-3), 1-23.
Tsuang, M.T., Stone, W.S., Faraone, S.V., 2001. Genes, environment and schizophrenia. The British journal of psychiatry. Supplement 40, s18-24.
van der Werf, M., Hanssen, M., Kohler, S., Verkaaik, M., Verhey, F.R., van Winkel, R., van Os, J., Allardyce, J., 2012. Systematic review and collaborative recalculation of 133 693 incident cases of schizophrenia. Psychological Medicine, 1-8.
van Os, J., 2009. A salience dysregulation syndrome. The British journal of psychiatry : the journal of mental science 194(2), 101-103.
van Os, J., Kapur, S., 2009. Schizophrenia. Lancet 374(9690), 635-645.
van Os, J., Kenis, G., Rutten, B.P., 2010. The environment and schizophrenia. Nature 468(7321), 203-212.
van Os, J., Krabbendam, L., Myin-Germeys, I., Delespaul, P., 2005. The schizophrenia envirome. Curr Opin Psychiatry 18(2), 141-145.
van Os, J., Linscott, R.J., Myin-Germeys, I., Delespaul, P., Krabbendam, L., 2009. A systematic review and meta-analysis of the psychosis continuum: evidence for a psychosis proneness-persistence-impairment model of psychotic disorder. Psychol Med 39(2), 179-195.
van Os, J., Rutten, B.P., Poulton, R., 2008. Gene-environment interactions in schizophrenia: review of epidemiological findings and future directions. Schizophrenia bulletin 34(6), 1066-1082.
Van Winkel, R., Esquivel, G., Kenis, G., Wichers, M., Collip, D., Peerbooms, O., Rutten, B., Myin-Germeys, I., Van Os, J., 2010. REVIEW: Genome-wide findings in schizophrenia and the role of gene-environment interplay. CNS Neurosci Ther 16(5), e185-192.
van Winkel, R., GROUP Investigators, 2011. Family-based analysis of genetic variation underlying psychosis-inducing effects of cannabis: sibling analysis and proband follow-up. Arch Gen Psychiatry 68(2), 148-157.
van Winkel, R., Stefanis, N.C., Myin-Germeys, I., 2008. Psychosocial stress and psychosis. A review of the neurobiological mechanisms and the evidence for gene-stress interaction. Schizophr Bull 34(6), 1095-1105.
van Zelst, C., 2009. Stigmatization as an environmental risk in schizophrenia: a user perspective. Schizophr Bull 35(2), 293-296.
Varese, F., Smeets, F., Drukker, M., Lieverse, R., Lataster, T., Viechtbauer, W., Read, J., van Os, J., Bentall, R.P., 2012. Childhood adversities increase the risk of psychosis: a meta-analysis of patient-control, prospective- and cross-sectional cohort studies. Schizophr Bull 38(4), 661-671.
Veling, W., Susser, E., 2011. Migration and psychotic disorders. Expert Rev Neurother 11(1), 65-76.
Wong, M.Y., Day, N.E., Luan, J.A., Chan, K.P., Wareham, N.J., 2003. The detection of gene-environment interaction for continuous traits: should we deal with measurement error by bigger studies or better measurement? International journal of epidemiology 32(1), 51-57.
World Health Organisation, 1992. ICD-10 Classifications of Mental and Behavioural Disorder: Clinical Descriptions and Diagnostic Guidelines. World Health Organisation, Geneva.
Wray, N.R., Gottesman, II, 2012. Using summary data from the danish national registers to estimate heritabilities for schizophrenia, bipolar disorder, and major depressive disorder. Front Genet 3, 118.

Chapter Two

Genetic association study of the P300 endophenotype in schizophrenia

Jeroen Decoster[1,2], Marc De Hert [1], Wolfgang Viechtbauer[1], Guy Nagels[1,4], Inez Myin-Germeys[2], Joseph Peuskens[1], Jim van Os[2,3], Ruud van Winkel[1,2]

Schizophrenia Research, Volume 141, Issue 1, October 2012, Pages 54-59

[1] Dept. of Psychiatry and Psychology, School for Mental Health and Neuroscience, EURON, Maastricht University Medical Centre, PO BOX 616, 6200 MD Maastricht, The Netherlands

[2] University Psychiatric Centre Catholic University Louvain, campus Kortenberg, Leuvensesteenweg 517, 3070 Kortenberg, Belgium

[3] Division of Psychological Medicine, Institute of Psychiatry, De Crespigny Park, London, United Kingdom SE5 8AF

[4] Service d'orthopédagogie clinique, faculté de psychologie et des sciences de l'éducation, Université de Mons, Place du Parc 18, 7000 Mons, Belgium.

ABSTRACT

Objective

Although reduced amplitude of the P300 event-related potential is a well-documented intermediate phenotype of schizophrenia, little is known about its genetic underpinnings in patients with schizophrenia. This study aims to examine associations between P300 and a range of candidate genetic variants, selected from either candidate gene studies or genome-wide association studies, in a large sample of patients with schizophrenia.

Methods

P300 amplitude at the midline parietal electrode and 193 single nucleotide polymorphisms (SNPs) in 67 genes were assessed in 336 patients with schizophrenia. The association between each SNP and P300 amplitude, controlled for illness duration and gender, was evaluated. Associations at $p < .01$ were considered of potential relevance, while Bonferroni correction was applied to determine formal statistical significance (Bonferroni-corrected threshold of significance $p = .0003$).

Results

Of the 193 selected SNPs, 4 SNPs showed potentially relevant association with P300 amplitude at a significance level of $p < .01$. One of these SNPs, rs1045642 in ABCB1, was most convincingly associated with P300 amplitude, reaching formal (Bonferroni-corrected) significance, while there was evidence for possible association with rs1572899 in DISC-1, rs6265 in BDNF and rs1625579 in MIR137.

Conclusion

Genetic variation in ABCB1 may be associated with P300 amplitude in patients with schizophrenia. This result may encourage further efforts to elucidate the genetic underpinnings of P300 generation.

1. INTRODUCTION

The auditory P300 is an event-related potential (ERP) that is typically elicited by an auditory target stimulus serving as the signal for the participant to execute a predefined task, such as pushing a button or counting. It is named after its typical peak 300ms after the target stimulus. The P300 is believed to reflect a summation of simultaneous brain processes, including directed attention and contextual updating of working memory (Turetsky et al., 2007; van der Stelt and Belger, 2007). It is described by its amplitude and latency. Two subcomponents can be distinguished: the P3a subcomponent with a predominantly frontal distribution, which reflects the unexpectedness of the stimulus and the P3b subcomponent with a predominantly parietal distribution, reflecting cognitive processing of task-relevant or contextually salient stimuli (Turetsky et al., 2007).

Reduced amplitude of the auditory P300, especially the P3b subcomponent, has been consistently reported in patients with schizophrenia (Turetsky et al., 2007; van der Stelt and Belger, 2007). Although reduced P300 amplitude is not specific to schizophrenia, the observed deficits are distinguishable in several aspects from the P300-deficits in Alzheimer disease (marked latency prolongation), alcoholism (more visual than auditory abnormalities) and depression (state-dependent abnormalities), suggesting different underlying neural mechanisms (Salisbury et al., 1999; Souza et al., 1995; Turetsky et al., 2007). However, since P300 amplitudes at centro-parietal sites in patients with bipolar disorder manifesting psychotic symptoms were not distinguishable from those of patients with schizophrenia, it was suggested that decreased P300 amplitude at these sites may mark functional psychosis in general (Bestelmeyer et al., 2009).

The heritability of the P300 was established by several twin studies (Bestelmeyer et al., 2009; Hall et al., 2009; O'Connor et al., 1994). Moreover, part of the genetic contribution to the P300 waveform is shared with the genetic contribution to schizophrenia (Hall et al., 2007) and family members of patients with schizophrenia also show significantly reduced P300 amplitudes compared to the general population, although to a lesser degree than their ill relatives (Bramon et al., 2005).

Reduced P300 amplitude was found in first-episode patients, recent-onset, chronic patients and even people at ultra-high risk for psychosis (Turetsky et al., 2007; Umbricht et al., 2006; van Beijsterveldt et al., 2001; van der Stelt and Belger, 2007; van Tricht et al., 2011). Given these findings, a decrease in the amplitude of the P300 is commonly accepted as an intermediate phenotype for schizophrenia (Turetsky et al., 2007; van der Stelt and Belger, 2007).

Although a body of evidence supports reduced P300 amplitude as an intermediate phenotype for schizophrenia, there is limited knowledge of the actual genetic underpinnings of P300 generation in general, and P300 disruption in schizophrenia specifically (Turetsky et al., 2007; van der Stelt and Belger, 2007). Blackwood and colleagues (Blackwood et al., 2001) reported a reduction of P300 amplitude in a large Scottish family multiply affected with schizophrenia. In this family, a balanced translocation of chromosome 1 and 11, disrupting the DISC1 gene, was strongly associated with both the diagnosis of schizophrenia and reduced P300 amplitude (Blackwood et al., 2001). In addition, reduced P300 amplitude was also observed in unaffected carriers of the translocation (Blackwood et al., 2001). Further studies used a candidate gene approach to examine the association with P300 amplitude in healthy controls (Gallinat et al., 2007; Mulert et al., 2006; Schofield et al., 2009; Shaikh et al., 2011; Tsai et al., 2003a; Tsai et al., 2003b; Vogel et al., 2006), patients with a diagnosis of depression (Chen et al., 2002) or addiction (Antolin et al., 2009; Berman et al., 2006; Hill et al., 1998; Johnson et al., 1997), as well as patients with schizophrenia (Bramon et al., 2006; Bramon et al., 2008; Gallinat et al., 2003; Golimbet et al., 2006; Shaikh et al., 2011; Wang et al., 2009). These studies commonly focused on a single gene, often even limited to a single nucleotide polymorphism (SNP). This is potentially problematic since there are increasing concerns about the candidate gene approach, as the high prevalence of false-positive findings in genetic research may not always be adequately taken into account, especially in the context of undisclosed multiple statistical comparisons (Sullivan, 2007). On the other hand, sample sizes are often too small to allow for the use of genome-wide chips, suggesting that a candidate gene approach with a larger number of SNPs and adequate control for multiple testing may be the most viable option for the research of candidate endophenotypes (Greenwood et al., 2011). Therefore, the current study examined a range of candidate SNPs, selected from either candidate gene studies or genome-wide association studies, in a sample of 336 patients with a schizophrenia spectrum disorder.

2. METHODS AND MATERIALS

2.1. Subjects

The sample of this study was recruited between October 1999 and November 2006. Psychiatric diagnoses according to DSM-IV criteria were established by experienced psychiatrists affiliated with the University Centre at Louvain, Belgium, and responsible for the patient's treatment. In the University Centre, P300 analysis forms part of a comprehensive neurological and neurophysiological assessment conducted in

inpatients, after clinical stabilization. Conform to international guidelines (De Hert et al., 2009), patients receive an elaborate physical health screening including assessment of fasting glucose, lipids and other parameters as described previously (De Hert et al., 2010; van Winkel et al., 2006). On this occasion, they were asked for permission to store a blood sample for genetic analyses and for the anonymous analysis of clinical data recorded during their treatment. The study was approved by the standing ethics committee.

2.2. P300-recording

P300 data were recorded using a Neurofax Portable Electroencephalograph EEG-7414 (Nihon Kohden Corporation, Tokyo). During the recording patients were seated in a slightly reclined chair and were asked to fix their gaze at a mark approximately 1 meter in front of them. Evoked responses were recorded with 3 mid-line electrodes (Fz, Cz and Pz), positioned according to the international 10/20 system and online referenced to left and right ear-electrodes (A1 and A2). Electro-oculogram (EOG) was recorded in order to reject P300-epochs distorted by eye-movement artefacts. All electrodes were attached with a skin-electrode impedance of less than 5 kOhm.

120 sinus tones of 800Hz (standard) and 30 sinusoidal tones of 1470Hz (deviant), both with a duration of 40ms and an intensity of 70dB sound pressure level, were presented binaurally through earphones. Each inter-stimulus interval (ISI) was 1s. Standard and deviant tones were mixed randomly. Patients were asked to push a button as quickly as possible when hearing a deviant tone. Data were collected with a sampling rate of 1024 Hz and with a high cut-off at 70Hz. The event-related potentials (ERP) elicited by correctly processed standard (without push on button) and deviant tones (push on button) were averaged separately for each subject, using the EEG epochs from 100 ms pre-stimulus to 600 ms post-stimulus. The obtained curves (Fz, Cz, Pz and EOG) were displayed on a LCD-screen and for each electrode the N100 and P300 peaks after the deviant tone were manually indicated. The most negative deflection between 50ms and 150ms post-stimulus was considered as the N100, the most positive deflection between 250ms and 400ms as the P300. The obtained curves and the indicated peak values were printed and the paper report was stored in the patient's file. For 336 patients both DNA and P300 data were available. Because a reduction of P300 amplitude over the midline parietal electrode Pz was described as a very robust finding in patients with schizophrenia (Turetsky et al., 2007; van der Stelt and Belger, 2007), P300 amplitude at Pz was *a priori* used for all analyses. During the retrieval of the P300-data, the printed curves were visually inspected. The amplitude of the averaged EOG-curves exceeded the amplitude of the P300-waves in 145 patients. Although EOG-

artefacts are not expected to affect measurements at Pz, analysis of the entire sample of 336 patients was complemented with a sensitivity analysis in the sample of 191 patients for whom the averaged EOG curves did not exceed the P300 amplitude.

2.3. Genetic variation

A previous study of our group, which examined molecular-genetic interactions with cannabis, selected a total of 179 SNPs, 152 of which passed quality control and were subsequently analyzed (van Winkel and GROUP Investigators, 2011). Gene selection in this study was based on previous evidence of association with schizophrenia, involvement in dopamine or endocannabinoid signaling or an involvement in the regulation of environmental influences including epigenetic mechanisms. Since this selection included the most studied candidate genes for schizophrenia prior to the genome-wide studies, this set was used as the starting point for our SNP selection. This set was updated with 24 SNPs either showing association with schizophrenia at grade 'A' or 'B' level in the SzGene database (Allen et al., 2008) (update 26 February 2010) or identified by genome-wide association studies (situated in PGBD1, NRGN, NOTCH4, PDE4B, TCF4, TPH1, HTR2A, RELN, MDGA1, CCKAR, DRD4, APOE, GWAS 11p14.1, PLXNA2, GABRB2, SRR, ANK3, CACNA1C, ZNF804A, MHC, MIR137). Finally, a set of 10 SNPs was selected for intended pharmacogenetic studies (in ABCB1, ADH1C, AS3MT, CYP17A1, CYP1A2, CYP2D6, FOXA2, GSTP1, SOD2). It was decided to also analyze these SNPs in the context of this study, since for one of these SNPs, rs1045642 in ABCB1, an association with P300-amplitude was found in a sample of healthy controls using a data-driven analysis with 384 SNPs in 222 genes, which survived stringent correction for multiple testing (Liu et al., 2009).

The sample used in this article is completely independent of the GROUP sample that was used to examine the molecular-genetic interactions with cannabis (van Winkel and GROUP Investigators, 2011). The sample analyzed here is part of a larger sample (UPC-CUL sample) previously described in the context of metabolic syndrome (van Winkel et al., 2010c), which now has been genotyped for the same markers as the GROUP sample with the intention of replication of the molecular-genetic cannabis findings. From the UPC-CUL sample, only the patients for whom a P300 measurement was available (n=336) were included in this current study.

The selected SNPs were determined by Sequenom (Hamburg, Germany) using the MassARRAY iPLEX platform at the facilities of the manufacturer; SNPs, therefore, were not selected from a larger set of genomewide markers.

Of the 205 SNPs originally included, (see supplementary table S1), 8 SNPs were excluded because they had more than 10% genotyping failure (rs165599 in COMT,

rs1800955 in DRD4, rs2032582 in ABCB1, rs265981 in DRD1, rs3892097 in CYP2D6, rs403636 in SLC6A3, rs6465084 in GRM3 and rs9296158 in FKBP5). 3 SNPs were excluded because of Hardy-Weinberg disequilibrium ($p < .001$) (rs2023239 in CNR1, rs28362317 in SLC6A3 and rs743572 in CYP17A1) and no variation was found for 1 SNP (rs1799961 in DRD1). Thus, a final set of 193 SNPs in 67 genes was suitable for further analysis.

2.4. Statistical analyses

All analyses were conducted using STATA/SE 10.1 for Windows (StataCorp., 2007), regressing continuous P300 amplitude, as dependent variable, on each SNP. Genotypes were coded '0', '1' or '2' according to the number of minor alleles and modeled as a linear effect, since this method can deal with different genotype distributions, including distributions with a low minor allele frequency, as it avoids stratification into small subgroups (Cordell and Clayton, 2005). Given the amount of multiple testing involved, associations at $p < .01$ were arbitrarily considered of potential relevance, while Bonferroni correction was applied to determine formal statistical significance (Bonferroni-corrected threshold of significance $p = .0003$). Thus, SNPs that reached $p < .01$ significance in the main analysis were also analyzed in the sample of 191 patients with a P300 measurement surpassing the most stringent quality control as a sensitivity analysis to maximize the signal to noise ratio, however at the cost of reduced statistical power. All P300 values differing more than 3 standard deviations from the mean were considered as outliers and excluded from the regression analyses (n=4 in the main analysis, n=1 in the sensitivity analysis), as recommended by Osborne and Overbay (Osborne and Overbay, 2004). Analyses were controlled for the *a priori* defined confounders illness duration and gender.

A statistical power calculation was performed for the total sample as well as for the sensitivity analysis. In the total sample, this study has a power of 100% to detect a SNP that explains 10% of the variation in P300 amplitude according to the used regression model (with $\alpha = .01$), 95% to detect a SNP accounting for 5% and 53% to detect a SNP accounting for 2% of the P300 variation. In the sensitivity analyses, the power decreases to 98%, 74% and 29%, respectively.

3. RESULTS

3.1. Sample

The sample consisted of 336 individuals with a psychotic disorder who were on average 32.6 years old (SD 11.0, range 14.4 – 64.2) and of whom 68.5% were male. The

average illness duration was 8.5 year (SD 9.6, range 0 – 42). Patients had clinical diagnoses of schizophrenia (64.3%), schizophreniform disorder (12.2%) or schizoaffective disorder (23.5%).

For the sensitivity analysis, 191 individuals were included with an average age of 31.3 years (SD 10.2, range 14.4 – 57.1), 74.4% were male. The average illness duration was 7.6 year (SD 8.8, range 0 – 34), also with clinical diagnoses of schizophrenia (67.0%), schizophreniform disorder (8.4%) or schizoaffective disorder (24.6%) (See Table 1 for more details). Because of the significant differences in diagnosis, Clinical Global Impression (CGI) scale and Global Assessment of Functioning (GAF) score, those three variables were added as possible confounders in the regression model of the sensitivity analysis.

Table 1

Epidemiological and clinical information of the total sample and the sensitivity analysis subsample.

	Total sample (n=332)	**Sensitivity analysis (n=190)**	**p [a]**
Male (%)	230 (68.5%)	142 (74.4%)	**.010**
Mean age (SD)	32.4 (11.0)	31.3 (10.2)	**.030**
Age range	14.4 – 64.2	14.4 – 57.1	
DSM-IV Diagnosis			**.039**
schizophrenia	216 (64.3%)	128 (67.0%)	
schizophreniform disorder	41 (12.2%)	16 (8.4%)	
schizo-affective disorder	79 (23.5%)	47 (24.6%)	
Illness duration in years (SD)	8.5 (9.6)	7.6 (8.8)	.092
Antipsychotic medication [b]			
fenothiazines	22 (8.8%)	11 (8.1%)	.776
thioxanthenes	46 (18.47%)	24 (17.7%)	.633
butyrophenones	28 (11.2%)	21 (15.4%)	**.026**
diphenylbutylpiperidine	2 (0.8%)	1 (0.7%)	.879
amisulpride	22 (8.8%)	14 (10.3%)	.411
aripiprazole	5 (2.0 %)	1 (0.7%)	.219
clozapine	25 (10.0%)	13 (9.6%)	.724
olanzapine	76 (30.5%)	43 (31.6%)	.888
quetiapine	27 (10.8%)	13 (9.6%)	.724
risperidone	88 (36.1%)	49 (36.0%)	.933
Cannabis use			.489
non-user	196 (59.2%)	110 (57.9%)	
user, outside heaviest period	115 (34.7%)	66 (34.7%)	
user, during heaviest period	20 (6.0%)	14 (7.3%)	
CGI (SD)	4.3 (0.8)	4.1 (0.7)	**<.001**
GAF (SD)	57.6 (11.1)	60.0 (9.0)	**<.001**

Chi-squared test for categorical variables, double-sided paired t-test for continuous variables.
SD = standard deviation / CGI = clinical global impression (scale form 1-7) / GAF = global assessment of functioning

[a] P-values of statistical comparison between patients in sensitivity analyses and those who were dropped on the basis of EOG artefacts.

[b] Data available for 249 patients (74.1%) of the total sample, of whom 136 (71.2%) in the sensitivity analysis.

3.2. P300

The overall mean P300 amplitude at Pz was 13.2 µV (SD 6.4; range -.4 – 48.8). Four outliers were excluded from the analyses, resulting in a corrected average of 12.9 µV (SD 5.6; range -.4 – 32.4). P300 amplitude was significantly associated with illness duration (Coef = -.107; 95%-CI: -.170 – -.044; p = .001), but not with gender (Coef = -.478; 95%-CI: -1.794 – .837; p = .475). Four SNPs showed an association with P300 amplitude at p < .01; situated in ABCB1, MIR137, BDNF and DISC-1 (Table 2). The sensitivity analysis supported an association with P300 amplitude for rs1045642 in ABCB1 (reaching Bonferroni-corrected statistical significance), and to a lesser degree for rs1572899 in DISC-1 and for rs6265 in BDNF, but not for rs1625579 in MIR137 (Table 2). Patients homozygous for the C-allele of rs1045642 (n=76) had an average P300 amplitude of 11.4 µV (SD 5.1 µV) versus 12.8 µV (SD 5.8 µV) in heterozygous patients (n=167) and 14.4 µV (SD 5.6 µV) in patients with the T/T genotype (n=89)(Figure 1). A QQ-plot of the residuals of the regression model indicated no departure from normality.

Table 2

Regression model of P300 amplitude, controlled for illness duration and sex in the main analysis, with 3 additional confounders in the sensitivity analysis; diagnosis, CGI and GAF-score. A positive coefficient (coeff) reflects an association of higher P300 peak amplitude with the minor allele, a negative coefficient reflects an association with the major allele. Results of SNPs with p-value <.01 in the main analysis are displayed, as well as the coefficient and p-value in the sensitivity analysis. The percentage of P300 variation explained (EV) by adding the SNP in the significant models, is also reported.

SNP (gene)	coeff main analysis	95%-confidence interval	p-value main analysis	EV	coeff sensitivity analysis	p-value sensitivity analysis	EV
rs1045642 (ABCB1)	-1.37	-2.15 – 0.59	0.0021	2.9%	-2.01	0.00017	7.0%
rs1625579 (MIR137)	1.70	.52 – 2.88	0.0048	2.0%	.91	0.2215	
rs6265 (BDNF)	-1.47	-2.53 – 0.41	0.0068	2.6%	-1.61	0.0265	2.5%
rs1572899 (DISC-1)	-1.26	-2.19 –-0.34	0.0076	2.2%	-1.56	0.0242	2.7%

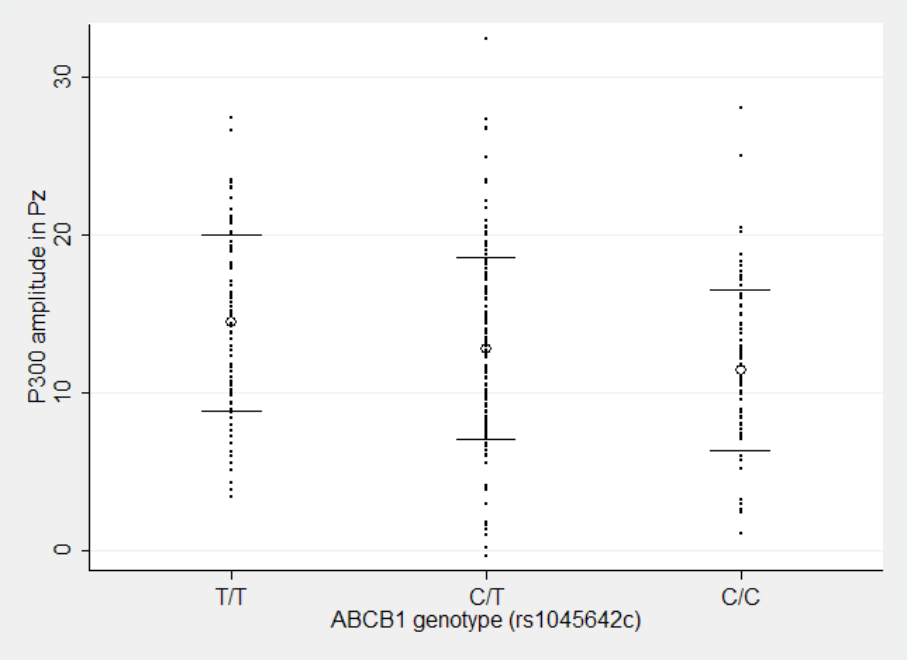
3.3. Follow-up analyses of the ABCB1 finding

The ABCB1-gene, formerly called Multi Drug Resistance 1 (MDR1) gene, encodes for the P-glycoprotein that has a known function in ATP-driven cellular excretion of a wide range of exogenous and endogenous substrates, like drugs, hormones (Moons et al., 2011) and tetrahydrocannabinol (THC) (Bonhomme-Faivre et al., 2008). Therefore, *post-hoc* analyses examined possible confounding of the association between P300 and ABCB1 by antipsychotic medication and cannabis use.

None of the different classes of antipsychotics was significantly associated with P300 amplitude (Table 3). Moreover, when covarying for type of antipsychotic, the

association between rs1045642 in ABCB1 and P300 amplitude remained significant (Coef = -1.822; 95%-CI: -2.826 – -.818; p = .00042).

Figure 1
Scatterplot of P300 amplitude over ABCB1-genotype.



° indicates average. Whiskers indicate interval of 1 standard deviation under and above mean.

Lifetime cannabis use was assessed using the Composite International Diagnostic Interview (CIDI)-lifetime section on substance abuse. Patients were thus identified as non-users (n=196), users with P300-measurement outside the period of heaviest use (n=116) and users with P300-measurement during the period of heaviest use (n=20). There was no significant association between cannabis use and P300 (Coef = .712; 95%-CI: -.290 – 1.714; p=.163) and controlling for lifetime cannabis use did not reduce the association between rs1045642 in ABCB1 and P300 amplitude (Coef = -1.469; 95%-CI: -2.322 – -.616; p = .00079).

Table 3

Outcome parameters (regression coefficient (coef), standard-error (SE), p-value (p) and 95%-confidence interval (95%-CI)) of a multiple regression model with P300 amplitude as dependent variable and dummy variables for each class of anti-psychotic medication as independent variables. Number of patients taking each class of anti-psychotics is indicated in the second column (n).

Class of anti-psychotic	n	coef	SE	p	95%-CI
fenothiazines	21	-2.111	1.310	0.108	-4.691 – 0.470
thioxanthenes	46	-1.577	0.969	0.105	-3.488 – 0.333
butyrophenones	28	0.833	1.223	0.496	-1.576 – 3.242
diphenylbutylpiperidine	2	/	/	/	/
amisulpride	22	1.995	1.507	0.187	-0.975 – 4.964
aripiprazole	4	/	/	/	/
clozapine	25	0.246	1.433	0.864	-2.576 – 3.069
olanzapine	76	-1.214	1.249	0.332	-3.675 – 1.247
quetiapine	25	-1.139	1.540	0.460	-4.173 – 1.895
risperidone	88	-.639	1.196	0.593	-2.996 – 1.717

4. DISCUSSION

Previous research, as reviewed by Turetsky (Turetsky et al., 2007) and van der Stelt (van der Stelt and Belger, 2007), supports the reliability of reduced P300 amplitude as intermediate phenotype for schizophrenia. Nevertheless, the knowledge of the genetic underpinnings of P300 generation in schizophrenia is limited. This study examined a range of 193 candidate SNPs for their association with P300 amplitude in 336 patients with schizophrenia.

Of the 193 selected SNPs, 4 SNPs showed potentially relevant association with P300 amplitude at a significance level of $p < .01$, situated in ABCB1, DISC-1, BDNF and MIR137. One of these SNPs, rs1045642 in ABCB1, was most convincingly associated with P300 amplitude, while there was modest evidence for rs1572899 in DISC-1 and rs6265 in BDNF. The association between P300 amplitude and the novel genome-wide supported risk variant rs1625579 in MIR137 was not supported in the sensitivity analysis.

The ABCB1 finding is in line with the results of Liu and colleagues (Liu et al., 2009), who used a parallel independent component analysis of electrophysiological and genetic data (384 SNPs) in order to investigate the genetic underpinnings of auditory ERP components in a sample of healthy individuals. They found the exact same SNP in ABCB1 (rs1045642), which encodes for P-glycoprotein, to be associated with P300 amplitude. The fact that two studies in different samples, one in healthy volunteers and one in patients with schizophrenia, identify the same SNP from a set of hundreds

of markers at the Bonferroni-corrected threshold of significance, makes it unlikely that this is a chance finding.

P-glycoprotein has a known function in ATP-driven cellular excretion of, among others, drugs (Moons et al., 2011) and THC (Bonhomme-Faivre et al., 2008). Although rs1045642 is a synonymous polymorphism, it changes the substrate specificity of the translated protein (Kimchi-Sarfaty et al., 2007) and can thus be considered functional. Little is known about the specific influence of the polymorphism on the cellular excretion of different substrates, like antipsychotic drugs and THC. However, *post-hoc* analyses in the present study, controlling for type of used antipsychotic drug, could not explain the association between rs1045642 and P300 amplitude. This is in line with expectations, since Liu et al. (Liu et al., 2009) found the same genotype-phenotype association in healthy individuals, free of antipsychotic drugs. Similarly, controlling for cannabis did not reduce the association between rs1045642 in ABCB1 and P300 amplitude, indicating that the association is unlikely to be mediated by genetically determined differences in P-glycoprotein excretion of THC. The underlying biology of the strong genotype-phenotype association between rs1045642 and P300 amplitude, as well as the specific significance for schizophrenia, remains to be elucidated.

Previous research on a balanced translocation implicated DISC1 in P300 generation in schizophrenia (Blackwood et al., 2001), supported by a recent study of common genetic variation in this gene (Shaikh et al., 2011). The present study may add further weight to this hypothesis, although it should be noted that the associated SNP in this study (rs1572899) did not survive Bonferroni correction for multiple testing, and is not in linkage disequilibrium with rs821597, which was found to be associated with P300 amplitude in the study of Shaikh and colleagues (Shaikh et al., 2011).

Earlier research has also examined the role of rs6265, better known as BDNF Val66Met, in P300 generation in the general population; Schofield reported a slowed P300 response in Met-homozygotes (Schofield et al., 2009), whereas Hansell failed to find an association between rs6265 and P300 amplitude (Hansell et al., 2007). In this study in schizophrenia patients, the Met-allele was associated with a lower P300 amplitude, although not at the Bonferroni corrected threshold of significance.

Rs1625579 is situated in an intron of a putative primary transcript for microRNA 137 (MIR137), a regulator of neuronal maturation and function. The recent GWAS of Ripke et al. suggested that MIR137 forms part of a newly detected etiological mechanism in schizophrenia (Ripke et al., 2011). Unlike the three other SNPs with a p-value <.01 in the main analysis, the association between P300 amplitude and rs1625579 was not confirmed in the sensitivity analysis.

Some limitations of this study are worth noting. Although considerable in the light of previous studies, the sample size of 336 patients with a psychotic disorder may have been underpowered to detect more subtle genetic determinants of P300 generation. With the additional sensitivity analysis in 191 patients, lower statistical power was partially compensated by a gain of signal-to-noise ratio. Secondly, the lack of a healthy control sample makes it impossible to draw inferences about the specificity of the findings. However, since P300 is an important intermediate phenotype of schizophrenia, every component explaining some of the individual differences in P300 amplitude may be of significance, as for example, P300 amplitude may help to predict transition in subjects at high risk for developing psychosis (van Tricht et al., 2011). Lastly, since the molecular pathway underlying P300 generation is not fully known, the *a priori* SNP selection can be questioned. Therefore, future work could use a genome-wide or candidate gene tag-SNP approach to unravel the genetic underpinnings of the P300 generation, but the sample of 336 patients was insufficiently powered to allow for this strategy. Nevertheless, we would argue that the selection in this study harbours most of the relevant SNPs based on the available information and includes SNPs from the classical candidate genes as well as those from genome-wide studies.

Since P300 is one of the most established intermediate phenotypes of schizophrenia, improving the efforts to unravel its genetic underpinnings could be an interesting complementary strategy to increase our understanding of the genetic and biological mechanisms of this disorder.

REFERENCES

Allen, N.C., Bagade, S., McQueen, M.B., Ioannidis, J.P., Kavvoura, F.K., Khoury, M.J., Tanzi, R.E., Bertram, L., 2008. Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: the SzGene database. Nat Genet 40(7), 827-834.

Antolin, T., Berman, S.M., Conner, B.T., Ozkaragoz, T.Z., Sheen, C.L., Ritchie, T.L., Noble, E.P., 2009. D2 dopamine receptor (DRD2) gene, P300, and personality in children of alcoholics. Psychiatry Res 166(2-3), 91-101.

Berman, S.M., Noble, E.P., Antolin, T., Sheen, C., Conner, B.T., Ritchie, T., 2006. P300 development during adolescence: effects of DRD2 genotype. Clin Neurophysiol 117(3), 649-659.

Bestelmeyer, P.E., Phillips, L.H., Crombie, C., Benson, P., St Clair, D., 2009. The P300 as a possible endophenotype for schizophrenia and bipolar disorder: Evidence from twin and patient studies. Psychiatry Res 169(3), 212-219.

Blackwood, D.H., Fordyce, A., Walker, M.T., St Clair, D.M., Porteous, D.J., Muir, W.J., 2001. Schizophrenia and affective disorders--cosegregation with a translocation at chromosome 1q42 that directly disrupts brain-expressed genes: clinical and P300 findings in a family. Am J Hum Genet 69(2), 428-433.

Bonhomme-Faivre, L., Benyamina, A., Reynaud, M., Farinotti, R., Abbara, C., 2008. Disposition of Delta tetrahydrocannabinol in CF1 mice deficient in mdr1a P-glycoprotein. Addict Biol 13(3-4), 295-300.

Bramon, E., Dempster, E., Frangou, S., McDonald, C., Schoenberg, P., MacCabe, J.H., Walshe, M., Sham, P., Collier, D., Murray, R.M., 2006. Is there an association between the COMT gene and P300 endophenotypes? Eur Psychiatry 21(1), 70-73.

Bramon, E., Dempster, E., Frangou, S., Shaikh, M., Walshe, M., Filbey, F.M., McDonald, C., Sham, P., Collier, D.A., Murray, R., 2008. Neuregulin-1 and the P300 waveform--a preliminary association study using a psychosis endophenotype. Schizophr Res 103(1-3), 178-185.

Bramon, E., McDonald, C., Croft, R.J., Landau, S., Filbey, F., Gruzelier, J.H., Sham, P.C., Frangou, S., Murray, R.M., 2005. Is the P300 wave an endophenotype for schizophrenia? A meta-analysis and a family study. Neuroimage 27(4), 960-968.

Chen, T.J., Yu, Y.W., Chen, J.Y., Wang, Y.C., Chen, M.C., Hong, C.J., Tsai, S.J., 2002. Association analysis of two dopamine D2 receptor gene polymorphisms and p300 event-related potential in depressive patients. Neuropsychobiology 46(3), 141-144.

Cordell, H.J., Clayton, D.G., 2005. Genetic association studies. Lancet 366(9491), 1121-1131.

De Hert, M., Dekker, J.M., Wood, D., Kahl, K.G., Holt, R.I., Moller, H.J., 2009. Cardiovascular disease and diabetes in people with severe mental illness position statement from the European Psychiatric Association (EPA), supported by the European Association for the Study of Diabetes (EASD) and the European Society of Cardiology (ESC). Eur Psychiatry 24(6), 412-424.

De Hert, M., van Winkel, R., Silic, A., Van Eyck, D., Peuskens, J., 2010. Physical health management in psychiatric settings. Eur Psychiatry 25 Suppl 2, S22-28.

Gallinat, J., Bajbouj, M., Sander, T., Schlattmann, P., Xu, K., Ferro, E.F., Goldman, D., Winterer, G., 2003. Association of the G1947A COMT (Val(108/158)Met) gene polymorphism with prefrontal P300 during information processing. Biol Psychiatry 54(1), 40-48.

Gallinat, J., Gotz, T., Kalus, P., Bajbouj, M., Sander, T., Winterer, G., 2007. Genetic variations of the NR3A subunit of the NMDA receptor modulate prefrontal cerebral activity in humans. J Cogn Neurosci 19(1), 59-68.

Golimbet, V., Gritsenko, I., Alfimova, M., Lebedeva, I., Lezheiko, T., Abramova, L., Kaleda, V., Ebstein, R., 2006. Association study of COMT gene Val158Met polymorphism with auditory P300 and performance on neurocognitive tests in patients with schizophrenia and their relatives. World J Biol Psychiatry 7(4), 238-245.

Greenwood, T.A., Lazzeroni, L.C., Murray, S.S., Cadenhead, K.S., Calkins, M.E., Dobie, D.J., Green, M.F., Gur, R.E., Gur, R.C., Hardiman, G., Kelsoe, J.R., Leonard, S., Light, G.A., Nuechterlein, K.H., Olincy, A., Radant, A.D., Schork, N.J., Seidman, L.J., Siever, L.J., Silverman, J.M., Stone, W.S., Swerdlow, N.R., Tsuang, D.W., Tsuang, M.T., Turetsky, B.I., Freedman, R., Braff, D.L., 2011. Analysis of 94 candidate genes and 12 endophenotypes for schizophrenia from the Consortium on the Genetics of Schizophrenia. Am J Psychiatry 168(9), 930-946.

Hall, M.H., Rijsdijk, F., Picchioni, M., Schulze, K., Ettinger, U., Toulopoulou, T., Bramon, E., Murray, R.M., Sham, P., 2007. Substantial shared genetic influences on schizophrenia and event-related potentials. Am J Psychiatry 164(5), 804-812.

Hall, M.H., Schulze, K., Rijsdijk, F., Kalidindi, S., McDonald, C., Bramon, E., Murray, R.M., Sham, P., 2009. Are auditory P300 and duration MMN heritable and putative endophenotypes of psychotic bipolar disorder? A Maudsley Bipolar Twin and Family Study. Psychol Med 39(8), 1277-1287.

Hansell, N.K., James, M.R., Duffy, D.L., Birley, A.J., Luciano, M., Geffen, G.M., Wright, M.J., Montgomery, G.W., Martin, N.G., 2007. Effect of the BDNF V166M polymorphism on working memory in healthy adolescents. Genes Brain Behav 6(3), 260-268.

Hill, S.Y., Locke, J., Zezza, N., Kaplan, B., Neiswanger, K., Steinhauer, S.R., Wipprecht, G., Xu, J., 1998. Genetic association between reduced P300 amplitude and the DRD2 dopamine receptor A1 allele in children at high risk for alcoholism. Biol Psychiatry 43(1), 40-51.

Johnson, J.P., Muhleman, D., MacMurray, J., Gade, R., Verde, R., Ask, M., Kelley, J., Comings, D.E., 1997. Association between the cannabinoid receptor gene (CNR1) and the P300 event-related potential. Mol Psychiatry 2(2), 169-171.

Kimchi-Sarfaty, C., Oh, J.M., Kim, I.W., Sauna, Z.E., Calcagno, A.M., Ambudkar, S.V., Gottesman, M.M., 2007. A "silent" polymorphism in the MDR1 gene changes substrate specificity. Science 315(5811), 525-528.

Liu, J., Kiehl, K.A., Pearlson, G., Perrone-Bizzozero, N.I., Eichele, T., Calhoun, V.D., 2009. Genetic determinants of target and novelty-related event-related potentials in the auditory oddball response. Neuroimage 46(3), 809-816.

Moons, T., de Roo, M., Claes, S., Dom, G., 2011. Relationship between P-glycoprotein and second-generation antipsychotics. Pharmacogenomics 12(8), 1193-1211.

Mulert, C., Juckel, G., Giegling, I., Pogarell, O., Leicht, G., Karch, S., Mavrogiorgou, P., Moller, H.J., Hegerl, U., Rujescu, D., 2006. A Ser9Gly polymorphism in the dopamine D3 receptor gene (DRD3) and event-related P300 potentials. Neuropsychopharmacology 31(6), 1335-1344.

O'Connor, S., Morzorati, S., Christian, J.C., Li, T.K., 1994. Heritable features of the auditory oddball event-related potential: peaks, latencies, morphology and topography. Electroencephalogr Clin Neurophysiol 92(2), 115-125.

Osborne, J.W., Overbay, A., 2004. The power of outliers (and why researchers should always check for them). Practical Assessment, Research & Evaluation 9(6).

Ripke, S., Sanders, A.R., Kendler, K.S., Levinson, D.F., Sklar, P., Holmans, P.A., Lin, D.Y., Duan, J., Ophoff, R.A., Andreassen, O.A., Scolnick, E., Cichon, S., St Clair, D., Corvin, A., Gurling, H., Werge, T., Rujescu, D., Blackwood, D.H., Pato, C.N., Malhotra, A.K., Purcell, S., Dudbridge, F., Neale, B.M., Rossin, L., Visscher, P.M., Posthuma, D., Ruderfer, D.M., Fanous, A., Stefansson, H., Steinberg, S., Mowry, B.J., Golimbet, V., De Hert, M., Jonsson, E.G., Bitter, I., Pietilainen, O.P., Collier, D.A., Tosato, S., Agartz, I., Albus, M., Alexander, M., Amdur, R.L., Amin, F., Bass, N., Bergen, S.E., Black, D.W., Borglum, A.D., Brown, M.A., Bruggeman, R., Buccola, N.G., Byerley, W.F., Cahn, W., Cantor, R.M., Carr, V.J., Catts, S.V., Choudhury, K., Cloninger, C.R., Cormican, P., Craddock, N., Danoy, P.A., Datta, S., de Haan, L., Demontis, D., Dikeos, D., Djurovic, S., Donnelly, P., Donohoe, G., Duong, L., Dwyer, S., Fink-Jensen, A., Freedman, R., Freimer, N.B., Friedl, M., Georgieva, L., Giegling, I., Gill, M., Glenthoj, B., Godard, S., Hamshere, M., Hansen, M., Hansen, T., Hartmann, A.M., Henskens, F.A., Hougaard, D.M., Hultman, C.M., Ingason, A., Jablensky, A.V., Jakobsen, K.D., Jay, M., Jurgens, G., Kahn, R.S., Keller, M.C., Kenis, G., Kenny, E., Kim, Y., Kirov, G.K., Konnerth, H., Konte, B., Krabbendam, L., Krasucki, R., Lasseter, V.K., Laurent, C., Lawrence, J., Lencz, T., Lerer, F.B., Liang, K.Y., Lichtenstein, P., Lieberman, J.A., Linszen, D.H., Lonnqvist, J., Loughland, C.M., Maclean, A.W., Maher, B.S., Maier, W., Mallet, J., Malloy, P., Mattheisen, M., Mattingsdal, M., McGhee, K.A., McGrath, J.J., McIntosh, A., McLean, D.E., McQuillin, A., Melle, I., Michie, P.T., Milanova, V., Morris, D.W., Mors, O., Mortensen, P.B., Moskvina, V., Muglia, P., Myin-Germeys, I., Nertney, D.A., Nestadt, G., Nielsen, J., Nikolov, I., Nordentoft, M., Norton, N., Nothen, M.M., O'Dushlaine, C.T., Olincy, A., Olsen, L., O'Neill, F.A., Orntoft, T.F., Owen, M.J., Pantelis, C., Papadimitriou, G., Pato, M.T., Peltonen, L., Petursson, H., Pickard, B., Pimm, J., Pulver, A.E., Puri, V., Quested, D., Quinn, E.M., Rasmussen, H.B., Rethelyi, J.M., Ribble, R., Rietschel, M., Riley, B.P., Ruggeri, M., Schall, U., Schulze, T.G., Schwab, S.G., Scott, R.J., Shi, J., Sigurdsson, E., Silverman, J.M., Spencer, C.C., Stefansson, K., Strange, A., Strengman, E., Stroup, T.S., Suvisaari, J., Terenius, L., Thirumalai, S., Thygesen, J.H., Timm, S., Toncheva, D., van den Oord, E., van Os, J., van Winkel, R., Veldink, J., Walsh, D., Wang, A.G., Wiersma, D., Wildenauer, D.B., Williams, H.J., Williams, N.M., Wormley, B., Zammit, S., Sullivan, P.F., O'Donovan, M.C., Daly, M.J., Gejman, P.V., 2011. Genome-wide association study identifies five new schizophrenia loci. Nat Genet 43(10), 969-976.

Salisbury, D.F., Shenton, M.E., McCarley, R.W., 1999. P300 topography differs in schizophrenia and manic psychosis. Biol Psychiatry 45(1), 98-106.

Schofield, P.R., Williams, L.M., Paul, R.H., Gatt, J.M., Brown, K., Luty, A., Cooper, N., Grieve, S., Dobson-Stone, C., Morris, C., Kuan, S.A., Gordon, E., 2009. Disturbances in selective information processing associated with the BDNF Val66Met polymorphism: evidence from cognition, the P300 and fronto-hippocampal systems. Biol Psychol 80(2), 176-188.

Shaikh, M., Hall, M.H., Schulze, K., Dutt, A., Li, K., Williams, I., Walshe, M., Constante, M., Broome, M., Picchioni, M., Toulopoulou, T., Collier, D., Stahl, D., Rijsdijk, F., Powell, J., Murray, R.M., Arranz, M., Bramon, E., 2011. Effect of DISC1 on the P300 Waveform in Psychosis. Schizophr Bull.

Souza, V.B., Muir, W.J., Walker, M.T., Glabus, M.F., Roxborough, H.M., Sharp, C.W., Dunan, J.R., Blackwood, D.H., 1995. Auditory P300 event-related potentials and neuropsychological performance in schizophrenia and bipolar affective disorder. Biol Psychiatry 37(5), 300-310.

StataCorp., 2007. Stata/SE statistical software, release 10. StataCorp LP, College Station.

Sullivan, P.F., 2007. Spurious genetic associations. Biol Psychiatry 61(10), 1121-1126.

Tsai, S.J., Yu, Y.W., Chen, T.J., Chen, J.Y., Liou, Y.J., Chen, M.C., Hong, C.J., 2003a. Association study of a functional catechol-O-methyltransferase-gene polymorphism and cognitive function in healthy females. Neurosci Lett 338(2), 123-126.

Tsai, S.J., Yu, Y.W., Chen, T.J., Chen, M.C., Hong, C.J., 2003b. Association analysis for dopamine D3 receptor, dopamine D4 receptor and dopamine transporter genetic polymorphisms and P300 event-related potentials for normal young females. Psychiatr Genet 13(1), 51-53.

Turetsky, B.I., Calkins, M.E., Light, G.A., Olincy, A., Radant, A.D., Swerdlow, N.R., 2007. Neurophysiological endophenotypes of schizophrenia: the viability of selected candidate measures. Schizophr Bull 33(1), 69-94.

Umbricht, D.S., Bates, J.A., Lieberman, J.A., Kane, J.M., Javitt, D.C., 2006. Electrophysiological indices of automatic and controlled auditory information processing in first-episode, recent-onset and chronic schizophrenia. Biol Psychiatry 59(8), 762-772.

van Beijsterveldt, C.E., van Baal, G.C., Molenaar, P.C., Boomsma, D.I., de Geus, E.J., 2001. Stability of genetic and environmental influences on P300 amplitude: a longitudinal study in adolescent twins. Behav Genet 31(6), 533-543.

van der Stelt, O., Belger, A., 2007. Application of electroencephalography to the study of cognitive and brain functions in schizophrenia. Schizophr Bull 33(4), 955-970.

van Tricht, M.J., Nieman, D.H., Koelman, J.H., van der Meer, J.N., Bour, L.J., de Haan, L., Linszen, D.H., 2011. Reduced parietal P300 amplitude is associated with an increased risk for a first psychotic episode. Biol Psychiatry 68(7), 642-648.

van Winkel, R., De Hert, M., Van Eyck, D., Hanssens, L., Wampers, M., Scheen, A., Peuskens, J., 2006. Screening for diabetes and other metabolic abnormalities in patients with schizophrenia and schizoaffective disorder: evaluation of incidence and screening methods. J Clin Psychiatry 67(10), 1493-1500.

van Winkel, R., GROUP Investigators, 2011. Family-based analysis of genetic variation underlying psychosis-inducing effects of cannabis: sibling analysis and proband follow-up. Arch Gen Psychiatry 68(2), 148-157.

van Winkel, R., Rutten, B.P., Peerbooms, O., Peuskens, J., van Os, J., De Hert, M., 2010. MTHFR and risk of metabolic syndrome in patients with schizophrenia. Schizophr Res 121(1-3), 193-198.

Vogel, C.I., Laucht, M., Furtado, E.F., Becker, K., Schmidt, M.H., 2006. Association of DRD4 exon III polymorphism with auditory P300 amplitude in 8-year-old children. J Neural Transm 113(12), 1935-1941.

Wang, Y., Hu, Y., Fang, Y., Zhang, K., Yang, H., Ma, J., Xu, Q., Shen, Y., 2009. Evidence of epistasis between the catechol-O-methyltransferase and aldehyde dehydrogenase 3B1 genes in paranoid schizophrenia. Biol Psychiatry 65(12), 1048-1054.

Supplementary table S1

All 203 selected Single Nucleotide Polymorphisms (SNPs) and the genes (abbreviation and full name) in which they are situated. Chi-squared values of Hardy-Weinberg (HW χ^2) equilibrium are indicated. SNPs with a callrate beneath 0.90 (marked with *) and a chi-squared value above 10.83 (marked with #, corresponding with a p-value < .001) were excluded for analyses.

SNP	Gene	Full gene name	callrate	HW χ^2
hCV219779	DISC-1	disrupted in schizophrenia 1	1.00	0.34
rs1006737	CACNA1C	calcium channel, voltage-dependent, L type, alpha 1C subunit	1.00	0.98
rs1011313	DTNBP1	dystrobrevin binding protein 1 (Dysbindin)	1.00	0.60
rs10138227	AKT1	v-akt murine thymoma viral oncogene homolog 1	1.00	0.73
rs10149785	AKT1	v-akt murine thymoma viral oncogene homolog 1	1.00	1.45
rs1018381	DTNBP1	dystrobrevin binding protein 1 (Dysbindin)	1.00	0.93
rs10452197	FGF2	fibroblast growth factor 2	1.00	0.08
rs1045642	ABCB1 (MDR1)	ATP-binding cassette, sub-family B (MDR/TAP), member 1	1.00	0.02
rs1049353	CNR1	cannabinoid receptor 1	1.00	0.12
rs10520303	GPM6A	glycoprotein M6A	0.99	0.05
rs10748835	AS3MT	arsenic methyltransferase	1.00	0.01
rs1079597	DRD2	dopamine receptor D2	1.00	0.03
rs10828317	PIP5K2A	phosphatidylinositol-5-phosphate 4-kinase, type II, alpha	0.99	0.21
rs10917670	RGS4	regulator of G-protein signaling 4	1.00	2.28
rs10934254	DRD3	dopamine receptor D3	1.00	0.18
rs10994336	ANK3	ankyrin 3 (ankyrin G)	1.00	0.16
rs1130214	AKT1	v-akt murine thymoma viral oncogene homolog 1	1.00	1.04
rs11564752	SLC6A3	solute carrier family 6 (neurotransmitter transporter, dopamine), member 3	0.96	0.79
rs11651497	PPP1R1B	protein phosphatase 1, regulatory (inhibitor) subunit 1B	1.00	0.13
rs11759115	MDGA1	MAM domain containing glycosylphosphatidylinositol anchor 1	1.00	3.44
rs1212275	FOXA2	forkhead box A1	1.00	0.46
rs12185692	GAD1	glutamate decarboxylase 1	1.00	0.53
rs12506776	FGF2	fibroblast growth factor 2	1.00	0.08
rs12516948	SLC6A3	solute carrier family 6 (neurotransmitter transporter, dopamine), member 3	1.00	0.04

SNP	Gene	Full gene name	callrate	HW χ^2
rs12807809	NRGN	neurogranin (protein kinase C substrate, RC3)	1.00	2.22
rs1316830	SLC6A3	solute carrier family 6 (neurotransmitter transporter, dopamine), member 3	1.00	0.22
rs13211507	PGBD1	piggyBac transposable element derived 1	1.00	0.06
rs13219181	GWAS	6p21.3	1.00	0.69
rs133894	FKBP5	fibroblast growth factor receptor 1	0.97	2.44
rs1344706	ZNF804A	zinc finger protein 804A	1.00	0.02
rs1360780	FKBP5	FK506 binding protein 5	1.00	0.03
rs1421292	DAOA (G72)	D-amino acid oxidase activator (G72 transcript)	1.00	2.18
rs1455832	ROBO1	roundabout, axon guidance receptor, homolog 1	1.00	0.10
rs1468412	GRM3	glutamate receptor, metabotropic 3	1.00	0.09
rs1507754	RGS4	regulator of G-protein signaling 4	1.00	1.10
rs1535255	CNR1	cannabinoid receptor 1	1.00	3.74
rs1572899	DISC-1	disrupted in schizophrenia 1	0.99	0.33
rs1602565	GWAS	11p14.1	1.00	0.14
rs16139	NPY	neuropeptide Y	1.00	0.39
rs1625579	GWAS	within intron of a putative primary transcript for MIR137 (microRNA 137)	1.00	3.69
rs165599	COMT	catechol-O-methyltransferase	0.00*	
rs165815	COMT	catechol-O-methyltransferase	0.99	0.05
rs1695	GSTP1	glutathione S-transferase pi 1	1.00	0.19
rs17149106	NPY	neuropeptide Y	1.00	0.50
rs174696	COMT	catechol-O-methyltransferase	1.00	0.07
rs1799732	DRD2	dopamine receptor D2	1.00	1.68
rs1799961	DRD1	dopamine receptor D1	1.00	no variation
rs1799978	DRD2	dopamine receptor D2	0.99	0.38
rs1800497	ANKK1	ankyrin repeat and kinase domain containing 1	1.00	0.17
rs1800498	DRD2	dopamine receptor D2	1.00	0.50
rs1800532	TPH1	tryptophan hydroxylase 1	0.99	1.86
rs1800566	NQO1	NAD(P)H dehydrogenase, quinone 1	1.00	0.14
rs1800828	DRD3	dopamine receptor D3	1.00	0.28

SNP	Gene	Full gene name	callrate	HW χ^2
rs1800955	DRD4	dopamine receptor D4	0.89*	4.65
rs1801131	MTHFR	methylenetetrahydrofolate reductase (NAD(P)H)	1.00	1.20
rs1801394	MTRR	5-methyltetrahydrofolate-homocysteine methyltransferase reductase	1.00	0.08
rs1805087	MTR	5-methyltetrahydrofolate-homocysteine methyltransferase	1.00	0.00
rs187993	GRM3	glutamate receptor, metabotropic 3	1.00	1.64
rs1978340	GAD1	glutamate decarboxylase 1	1.00	0.01
rs2008720	PRODH	proline dehydrogenase (oxidase) 1	0.99	2.28
rs2023239	CNR1	cannabinoid receptor 1	1.00	12.04#
rs2032582	ABCB1 (MDR1)	ATP-binding cassette, sub-family B (MDR/TAP), member 1	0.00*	
rs2058725	GAD1	glutamate decarboxylase 1	1.00	0.05
rs2134655	DRD3	dopamine receptor D3	1.00	2.44
rs221132	NRG1	neuregulin 1	1.00	0.16
rs2228595	GRM3	glutamate receptor, metabotropic 3	0.99	0.05
rs2230912	P2RX7	purinergic receptor P2X, ligand-gated ion channel, 7	1.00	0.46
rs2235515	PRDM2	PR domain containing 2, with ZNF domain	1.00	0.19
rs2241165	GAD1	glutamate decarboxylase 1	1.00	0.51
rs2269726	TBX1	guanine nucleotide binding protein (G protein), beta polypeptide 1-like	1.00	3.39
rs2288696	FGFR1	fibroblast growth factor receptor 1	1.00	0.02
rs2297235	GSTO2 (GST-2)	glutathione S-transferase omega 2	1.00	0.48
rs2391191	DAOA (G72)	D-amino acid oxidase activator (G72 transcript)	1.00	0.06
rs241930	NRG1	neuregulin 1	1.00	0.01
rs2494750	AKT1	v-akt murine thymoma viral oncogene homolog 1	0.99	4.85
rs2498784	AKT1	v-akt murine thymoma viral oncogene homolog 1	1.00	4.18
rs2498799	AKT1	v-akt murine thymoma viral oncogene homolog 1	1.00	0.02
rs2550956	SLC6A3	solute carrier family 6 (neurotransmitter transporter, dopamine), member 3	1.00	0.37
rs2619522	DTNBP1	dystrobrevin binding protein 1 (Dysbindin)	1.00	0.20
rs2619528	DTNBP1	dystrobrevin binding protein 1 (Dysbindin)	0.98	0.24
rs2619538	DTNBP1	dystrobrevin binding protein 1 (Dysbindin)	1.00	0.30
rs2619539	DTNBP1	dystrobrevin binding protein 1 (Dysbindin)	1.00	0.00

SNP	Gene	Full gene name	callrate	HW χ^2
rs2652510	SLC6A3	solute carrier family 6 (neurotransmitter transporter, dopamine), member 3	1.00	0.52
rs265981	DRD1	dopamine receptor D1	0.09*	281.50
rs2661319	RGS4	regulator of G-protein signaling 4	0.99	1.02
rs2682826	NOS1	nitric oxide synthase 1 (neuronal)	0.99	0.07
rs27027	SLC6A3	solute carrier family 6 (neurotransmitter transporter, dopamine), member 3	1.00	1.27
rs274622	GRM3	glutamate receptor, metabotropic 3	1.00	0.11
rs28362317	SLC6A3	solute carrier family 6 (neurotransmitter transporter, dopamine), member 3	0.99	30.65#
rs28757217	AKT1	v-akt murine thymoma viral oncogene homolog 1	1.00	0.90
rs2978073	FGFR1	fibroblast growth factor receptor 1	1.00	0.06
rs3037354	NPY	neuropeptide Y	0.99	0.21
rs308379	FGF2	fibroblast growth factor 2	1.00	0.27
rs308420	FGF2	fibroblast growth factor 2	1.00	1.56
rs308428	FGF2	fibroblast growth factor 2	1.00	0.55
rs308439	FGF2	fibroblast growth factor 2	1.00	0.47
rs3131296	NOTCH4	notch 4	1.00	0.01
rs3213207	DTNBP1	dystrobrevin binding protein 1 (Dysbindin)	1.00	0.89
rs3219151	GABRA6	gamma-aminobutyric acid (GABA) A receptor, alpha 6	1.00	0.09
rs324029	DRD3	dopamine receptor D3	1.00	2.44
rs324030	DRD3	dopamine receptor D3	0.99	2.29
rs363227	SLC18A2	solute carrier family 18 (vesicular monoamine), member 2	1.00	1.15
rs363338	SLC18A2	solute carrier family 18 (vesicular monoamine), member 2	1.00	0.04
rs363393	SLC18A2	solute carrier family 18 (vesicular monoamine), member 2	1.00	0.20
rs372055	PRODH	proline dehydrogenase (oxidase) 1	0.99	1.39
rs3737597	DISC-1	disrupted in schizophrenia 1	1.00	0.82
rs3738435	CHRM3	cholinergic receptor, muscarinic 3	0.99	0.55
rs3756450	SLC6A3	solute carrier family 6 (neurotransmitter transporter, dopamine), member 3	1.00	4.77
rs3764352	PPP1R1B	protein phosphatase 1, regulatory (inhibitor) subunit 1B	0.99	0.03
rs3764353	PPP1R1B	protein phosphatase 1, regulatory (inhibitor) subunit 1B	1.00	0.02
rs3791851	GAD1	glutamate decarboxylase 1	1.00	0.43

SNP	Gene	Full gene name	callrate	HW χ^2
rs379850	GAD1	glutamate decarboxylase 1	0.99	4.05
rs3800373	FKBP5	FK506 binding protein 5	0.99	0.27
rs3803300	AKT1	v-akt murine thymoma viral oncogene homolog 1	0.99	2.35
rs3892097	CYP2D6	cytochrome P450, family 2, subfamily D, polypeptide 6	0.72*	28.24
rs3916967	DAOA (G72)	D-amino acid oxidase activator (G72 transcript)	1.00	0.07
rs3918342	DAOA (G72)	D-amino acid oxidase activator (G72 transcript)	1.00	4.29
rs3925	FGFR1	fibroblast growth factor receptor 1	1.00	0.29
rs403636	SLC6A3	solute carrier family 6 (neurotransmitter transporter, dopamine), member 3	0.23*	199.22
rs406193	DNMT3B	DNA (cytosine-5-)-methyltransferase 3 beta	1.00	0.64
rs408067	SRR	Smg-6 homolog, nonsense mediated mRNA decay factor (C. elegans)	0.90	3.83
rs4298458	NRG1	neuregulin 1	1.00	1.52
rs4309482	GWAS		0.99	1.34
rs4331006	NRG1	neuregulin 1	0.99	0.99
rs4452759	NRG1	neuregulin 1	1.00	0.72
rs4476964	NRG1	neuregulin 1	1.00	1.81
rs450046	PRODH	proline dehydrogenase (oxidase) 1	1.00	1.24
rs4532	DRD1	dopamine receptor D1	1.00	0.76
rs456082	SLC6A3	solute carrier family 6 (neurotransmitter transporter, dopamine), member 3	1.00	2.27
rs4606	RGS2	regulator of G-protein signaling 2	1.00	2.08
rs463379	SLC6A3	solute carrier family 6 (neurotransmitter transporter, dopamine), member 3	0.99	2.19
rs4633	COMT	catechol-O-methyltransferase	1.00	0.75
rs4634737	EHMT1	euchromatic histone-lysine N-methyltransferase 1	0.99	1.01
rs464049	SLC6A3	solute carrier family 6 (neurotransmitter transporter, dopamine), member 3	1.00	2.17
rs4680	COMT	catechol-O-methyltransferase	1.00	0.96
rs4713916	FKBP5	FK506 binding protein 5	0.92	2.95
rs4733263	NRG1	neuregulin 1	1.00	8.22
rs4733946	FGFR1	fibroblast growth factor receptor 1	0.99	0.09
rs4795390	PPP1R1B	protein phosphatase 1, regulatory (inhibitor) subunit 1B	1.00	0.07
rs4818	COMT	catechol-O-methyltransferase	0.99	0.13

SNP	Gene	Full gene name	callrate	HW χ^2
rs4880	SOD2	superoxide dismutase 2, mitochondrial	1.00	1.76
rs4925	GSTO1 (GST-1)	glutathione S-transferase omega 1	1.00	0.65
rs535586	EHMT2	euchromatic histone-lysine N-methyltransferase 2	1.00	0.68
rs5573	NPY	neuropeptide Y	1.00	2.21
rs5574	NPY	neuropeptide Y	1.00	2.35
rs5746832	TBX1	guanine nucleotide binding protein (G protein), beta polypeptide 1-like	1.00	0.58
rs61888800	BDNF	brain derived neurotrophic factor	1.00	0.13
rs6265	BDNF	brain derived neurotrophic factor	1.00	0.84
rs6269	COMT	catechol-O-methyltransferase	1.00	0.57
rs6277	DRD2	dopamine receptor D2	1.00	1.23
rs6280	DRD3	dopamine receptor D3	1.00	5.30
rs6311	HTR2A	5-hydroxytryptamine (serotonin) receptor 2A	1.00	1.77
rs6313	HTR2A	5-hydroxytryptamine (serotonin) receptor 2A	1.00	1.93
rs6314	HTR2A	5-hydroxytryptamine (serotonin) receptor 2A	1.00	0.75
rs6347	SLC6A3	solute carrier family 6 (neurotransmitter transporter, dopamine), member 3	1.00	1.62
rs6454674	CNR1	cannabinoid receptor 1	1.00	1.65
rs6465084	GRM3	glutamate receptor, metabotropic 3	0.00*	
rs6556547	GABRB2	gamma-aminobutyric acid (GABA) A receptor, beta 2	0.99	0.09
rs673871	LRRTM1	leucine rich repeat transmembrane neuronal 1	1.00	0.53
rs6904071	GWAS	6p21.3	1.00	0.15
rs6913660	GWAS	6p21.3	1.00	0.16
rs6928499	CNR1	cannabinoid receptor 1	0.99	3.94
rs6987534	FGFR1	fibroblast growth factor receptor 1	1.00	1.79
rs698	ADH1C (ADH3)	alcohol dehydrogenase 1C (class I), gamma polypeptide	1.00	2.93
rs6996321	FGFR1	fibroblast growth factor receptor 1	1.00	2.99
rs7004633	GWAS		1.00	1.57
rs7012413	FGFR1	fibroblast growth factor receptor 1	1.00	0.10
rs7014762	NRG1	neuregulin 1	1.00	0.33
rs7341475	RELN	reelin	1.00	0.42

SNP	Gene	Full gene name	callrate	HW χ^2
rs737054	FKBP5	FK506 binding protein 5	1.00	0.17
rs737865	COMT	catechol-O-methyltransferase	1.00	0.28
rs743572	CYP17A1	cytochrome P450, family 17, subfamily A, polypeptide 1	1.00	32.37#
rs746187	DAOA (G72)	D-amino acid oxidase activator (G72 transcript)	1.00	0.31
rs7557793	GAD1	glutamate decarboxylase 1	0.97	1.50
rs760761	DTNBP1	dystrobrevin binding protein 1 (Dysbindin)	1.00	0.22
rs762551	CYP1A2	cytochrome P450, family 1, subfamily A, polypeptide 2	0.99	1.89
rs769407	GAD1	glutamate decarboxylase 1	1.00	0.56
rs7700205	FGF2	fibroblast growth factor 2	1.00	0.08
rs778293	DAOA (G72)	D-amino acid oxidase activator (G72 transcript)	0.99	3.49
rs7825208	FGFR1	fibroblast growth factor receptor 1	0.99	1.02
rs806308	CNR1	cannabinoid receptor 1	1.00	0.34
rs806377	CNR1	cannabinoid receptor 1	1.00	1.71
rs806379	CNR1	cannabinoid receptor 1	1.00	1.35
rs821597	DISC-1	disrupted in schizophrenia 1	1.00	0.46
rs821616	DISC-1	disrupted in schizophrenia 1	1.00	0.42
rs841865	PLXNA2	plexin A2	1.00	1.62
rs879606	PPP1R1B	protein phosphatase 1, regulatory (inhibitor) subunit 1B	0.99	0.15
rs907094	PPP1R1B	protein phosphatase 1, regulatory (inhibitor) subunit 1B	1.00	0.02
rs909706	DTNBP1	dystrobrevin binding protein 1 (Dysbindin)	1.00	0.09
rs910694	PDE4B	phosphodiesterase 4B, cAMP-specific	1.00	0.01
rs917071	GRM3	glutamate receptor, metabotropic 3	1.00	0.01
rs926300	GWAS	6p21.3	0.99	0.18
rs929493	SLC18A2	solute carrier family 18 (vesicular monoamine), member 2	1.00	2.16
rs9296158	FKBP5	FK506 binding protein 5	0.87*	6.43
rs9470080	FKBP5	FK506 binding protein 5	1.00	0.01
rs951436	RGS4	regulator of G-protein signaling 4	1.00	0.44
rs951439	RGS4	regulator of G-protein signaling 4	0.95	2.88
rs9804190	ANK3	ankyrin 3, node of Ranvier (ankyrin G)	1.00	0.02

SNP	Gene	Full gene name	callrate	
rs980989	DISC-1	disrupted in schizophrenia 1	0.99	3.98
rs988748	BDNF	brain derived neurotrophic factor	1.00	0.56
rs992105	FKBP5	FK506 binding protein 5	1.00	1.66
rs9960767	TCF4	transcription factor 4	0.97	0.79
rs999710	DISC-1	disrupted in schizophrenia 1	0.98	1.68

Chapter Three

Age at onset of psychotic disorder: cannabis, BDNF Val66Met and sex-specific models of gene-environment interaction.

Jeroen Decoster[1,2], Jim van Os[1,3], Gunter Kenis[1], Cécile Henquet[1], Joseph Peuskens[2], Marc De Hert[2], Ruud van Winkel[1,2]

American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, Volume 156, Issue 3, April 2011, pages 363–369

[1] Dept. of Psychiatry and Psychology, School for Mental Health and Neuroscience, EURON, Maastricht University Medical Centre, PO BOX 616, 6200 MD Maastricht, The Netherlands
[2] University Psychiatric Centre Catholic University Louvain, campus Kortenberg, Leuvensesteenweg 517, 3070 Kortenberg, Belgium
[3] Division of Psychological Medicine, Institute of Psychiatry, De Crespigny Park, London, United Kingdom SE5 8AF

ABSTRACT

Background

Discovering modifiable predictors for age at onset may help to identify predictors of transition to psychotic disorder in the 'at-risk mental state'. Inconsistent effects of sex, BDNF Val66Met (rs6265) and cannabis use on age of onset were previously reported.

Methods

BDNF Val66Met and cannabis use before illness onset were retrospectively assessed in a sample of 585 patients with schizophrenia and their association with age at onset was evaluated.

Results

Cannabis use was significantly associated with earlier age at onset of psychotic disorder (AOP; average difference 2.7 years, p<.001), showing dose-response effects with higher frequency and earlier age at first use. There was a weak association between BDNF Val66Met genotype and AOP (difference 1.2 years; p=.050). No evidence was found for BDNF X cannabis interaction (interaction $\chi 2(1)=0.65$, p=.420). However, a significant BDNF X cannabis X sex interaction was found (interaction $\chi 2(1)=4.99$, p=.026). In female patients, cannabis use was associated with earlier AOP in BDNF Met-carriers (difference 7 years), but not in Val/Val-genotypes. In male patients, cannabis use was associated with earlier AOP irrespective of BDNF Val66Met genotype (difference 1.3 years). BDNF Val66Met genotype in the absence of cannabis use did not influence AOP, neither in female or male patients with psychotic disorder.

Conclusion

Complex interactions between cannabis and BDNF may shape age at onset in female individuals at risk of psychotic disorder. No compelling evidence was found that BDNF genotype is associated with age at onset of psychotic disorder in the absence of cannabis use.

Keywords

Schizophrenia – Tetrahydrocannabinol (THC) – Neurotrophins – Gender

1. INTRODUCTION

In light of increasing efforts to identify predictors of transition to psychotic disorder in persons presenting with an 'at-risk mental state', discovering modifiable predictors for age at onset of psychotic disorder (AOP) is relevant (Compton et al., 2009). A growing body of literature suggests that cannabis use may decrease age AOP (Compton et al., 2009; De Hert et al., 2011b; Gonzalez-Pinto et al., 2008; Sugranyes et al., 2009; Veen et al., 2004). Veen (Veen et al., 2004) reported a difference of 7,5 years on AOP between cannabis using and non-using patients with a first psychotic episode (n=133). Sugranyes (Sugranyes et al., 2009) found the same association, but with a smaller difference of 1,93 years (n=116) and Gonzalez-Pinto (Gonzalez-Pinto et al., 2008) reported a difference of 7 to 12 years, which was dependent on the frequency of cannabis use, in a sample of 131 patients with a first psychotic episode. Compton (Compton et al., 2009) did not find a significant effect of frequency of cannabis use on AOP, but did find a significant association with progression to daily use.

In addition, some studies have examined possible associations between genetic variation and AOP (Peerbooms et al., 2010; Vares et al., 2010), given evidence for a genetic contribution to AOP (Hare et al., 2010). Genes regulating neurotrophic factors may be of special interest in this regard, since neurotrophins are key factors for maintenance of neuroplasticity and neuronal survival in response to environmental exposures. Most notably, a Val to Met substitution at codon 66 (rs6265) of the Brain Derived Neurotrophic Factor (BDNF) gene, resulting in less efficient intracellular trafficking and decreased activity-dependent BDNF secretion (Egan et al., 2003), was reported to be associated with AOP (Chao et al., 2008; Numata et al., 2006). Met-carriers were significantly younger at the onset of psychotic disorder in a cohort of 159 Japanese patients with a diagnosis of schizophrenia (Numata et al., 2006) and in a cohort of 42 African-American patients (Chao et al., 2008), although other work failed to replicate this association in a sample of Japanese patients with a diagnosis of schizophrenia (Naoe et al., 2007) and in 2 white patient samples (Gourion et al., 2005; Renou et al., 2007).

All studies had fairly small sample sizes (less than 240 patients) and did not take possible interactions with cannabis use into account, which may be important since animal and human studies suggest that cannabis use may directly influence BDNF-regulated physiological mechanisms (D'Souza et al., 2009; Derkinderen et al., 2003; Jockers-Scherubl et al., 2004). For example, a study in mice reported significant up-regulation of BDNF mRNA in the hippocampus within one hour of injection with Δ9-THC (Derkinderen et al., 2003), which may be in agreement with a study in first-episode patients with psychotic disorder suggesting that cannabis-using patients had

significantly higher BDNF serum levels than both non-using patients and matched controls (Jockers-Scherubl et al., 2004). These findings may suggest that BDNF release in response to THC represents a neuronal adaptive response to the psychotogenic effects of THC. Since BDNF upregulation in response to cannabis use may be less efficient in BDNF Met-carriers, any effect of cannabis use on AOP would be greater in Met-carriers. In agreement with this hypothesis, the possibility of gene-environment interaction (GxE) between cannabis and BDNF Val66Met genotype was recently put forward (D'Souza et al., 2009). In addition, a recent family-based study of patients and their unaffected siblings found that genetic variation in AKT1 may be involved in cannabis-induced psychosis (van Winkel and GROUP Investigators, 2011), which may be in support of a BDNF X cannabis interaction since BDNF and cannabis signaling both involve the AKT1-GSK3 pathway downstream of their receptors, TrkB and CB1 respectively.

In examining the possible synergistic effects of BDNF and cannabis use on AOP, it may further be important to consider the role of sex. Female sex is associated with later AOP (Angermeyer and Kuhn, 1988; Larsen et al., 1996), although not all studies confirmed this (Kohler et al., 2007; Ongur et al., 2009), and some authors also suggested that this association may be attenuated when controlling for cannabis use (De Hert et al., 2011b). In addition, several studies have suggested sex-specific BDNF effects in phenotypes of potential relevance to psychosis. For example, gender-dependent effects of the Val66Met BDNF polymorphism were reported for cognitive abilities (Raz et al., 2009), HPA axis reactivity to stress (Shalev et al., 2009) and for the genetic vulnerability to major depressive disorder (Verhagen et al., 2008). The reason for sex-dependent BDNF Val66Met effects are unclear, but may result from complex interactions with sex hormones such as estrogen (Scharfman and MacLusky, 2006). There is replicated evidence showing similar effects of estrogen and BDNF on measures of neuroplasticity and neurogenesis in the hippocampus (Scharfman and MacLusky, 2006). These shared actions of estrogen and BDNF in the hippocampus may have relevance for individuals at imminent risk for psychotic disorder, as several studies have shown hippocampal alterations occurring in individuals with at-risk mental states (Hurlemann et al., 2008), with more severe reductions in volume in more severely ill patients (Hurlemann et al., 2008).

Therefore, the current study aimed to examine the association between cannabis use and BDNF Val66Met genotype on the one hand, and AOP on the other, in a cohort of 587 patients with a diagnosis of schizophrenia. In addition, models of gene-environment interaction between BDNF Val66Met genotype and cannabis use were examined, taking into account the possibility of sex-specific models of GxE.

2. SUBJECTS AND METHODS

2.1 Sample

In a catchment area of about 300.000 Dutch-speaking Belgians, patients with psychotic disorder are usually treated at the University Psychiatric Centre of the Catholic University in Louvain and affiliate services (UPC), in both outpatient and inpatient settings. The sample of this study was collected between July 2003 and February 2008. Conform to international guidelines (De Hert et al., 2009), these patients are routinely screened for the presence of metabolic abnormalities as described previously (van Winkel et al., 2006). On this occasion, they were asked for permission to store a blood sample for genetic analyses and additionally provided information on lifetime substance use, assessed using the Composite International Diagnostic Interview (CIDI) - lifetime section on substance abuse or, in case the patients could not be contacted for interview, by case-note review (Smeets, 1993). The CIDI was assessed by a trained research assistant who was not involved in the treatment of the patients. Patients were included in the study sample if their AOP was below 65 years. Psychiatric diagnoses according to DSM-IV criteria were established by experienced psychiatrists affiliated with the University Centre and responsible for the patient's treatment. Patients provided written informed consent and the study was approved by the standing ethics committee.

AOP was defined as the age at first admission for psychotic disorder. This was assessed by patient interview and cross-checked by case-note review. Age at first admission was chosen because it can be assessed reliably and because it is a good indicator of clinical relevance of psychotic symptoms, especially given the fact that there were no community teams for the treatment of psychotic disorder in the catchment area during the study period.

2.2 Cannabis measures

Cannabis use was considered present when subjects reported cannabis use at least five times in their lives, and when first use of cannabis preceded the first admission for psychotic disorder. This dichotomous variable, describing the presence of pre-onset cannabis use, was used in all analyses concerning main and interaction effects. Patients who only started using after their first admission for psychotic disorder, were included in the analyses as non-users, since in their case cannabis use was unrelated to the onset of the psychotic disorder. Other drug measures (cocaine, stimulants, phencyclidine, psychedelics and opiates) were defined similarly. Furthermore, to examine dose-response effects, suggested by previous literature (Gonzalez-Pinto et al., 2008), two ordinal cannabis variables were constructed, each reflecting a separate

aspect of cannabis use: intensity of use and age at first use. By definition, the effect of these variables on AOP can only be examined in the subgroup with pre-onset cannabis use, as described above. Intensity of use was defined as the frequency of use in the most intense period of cannabis use (1=less than once per week, 2=at least weekly but less than daily, 3=daily use) and was only constructed in patients for whom the most intense period of cannabis use started before their first admission for psychotic disorder. Age at first use was defined as the age at which the first use of cannabis took place (1=18 years or above, 2=from 16 to 18 years, 3= under 16), given the evidence that the risk-increasing effects of cannabis are particularly present when first used in early adolescence, but moderate to negligible when first used above the age of 18 years old (Arseneault et al., 2002; Konings et al., 2008; Stefanis et al., 2004).

2.3 Genotyping

The BDNF Val66Met polymorphism was genotyped using the rs6265 TaqMan® SNP Genotyping assay (assay ID C__11592758_10, Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). The assay was run on a 7900HT Fast Real-Time PCR System (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands).

2.4 Statistical analysis

All analyses were conducted using STATA/SE 10.1 for Windows (StataCorp., 2007). Survival analysis of univariable main effects was conducted using the Log-rank STS TEST procedure, thus taking advantage of the continuous nature of AOP as outcome variable. For the ordinal cannabis measures, the additional TREND function within the STS TEST procedure was used, in order to evaluate whether there was a linear decrease in AOP associated with increasing intensity of use and earlier age at first use, respectively. Subsequently, multivariable Cox regression models were fitted to examine 1) main effects of sex, BDNF genotype and cannabis use, controlled for each other, 2) two-way interaction between BDNF genotype and cannabis use, controlled for sex and 3) BDNF X cannabis X sex interaction. Logistic regression and linear regression were used respectively to test for a possible association between BDNF genotype and (i) cannabis use (dichotomous variable) and (ii) age of first use (continuous variable). Since the frequency of BDNF Met homozygosity is low in people of European Caucasian origin (<5%) (Egan et al., 2003), BDNF genotype was divided into Val/Val individuals (‘0’) versus Met-carriers (‘1’).

3. RESULTS

3.1 Sample

The sample consisted of 587 individuals with a psychotic disorder who were on average 36.1 years old (SD 12.1) and of whom 65.4% were male. They had clinical diagnoses of schizophrenia (68.7%), schizophreniform disorder (11.2%) or schizoaffective disorder (20.1%). Two patients were excluded because their age at first admission was above 65 years, leaving a final sample of 585 patients. Of this sample, 97% was white, 2% was black African and 1% was Asian.

CIDI information on substance abuse was obtained for 559 patients (95.6%); for 23 patients, case-note information on cannabis use was available (3.9%) and for 3 patients no reliable information on substance use could be obtained (0.5%). Use of cannabis on at least 5 occasions before the onset of the psychotic disorder was reported by 35.0% of the sample. Overall lifetime use was reported by 36.4% of the sample. Cannabis users were significantly younger and more likely to be male (Table 1). In their most intensive period of use, 62.4% were daily cannabis users, 19.3% were weekly users and 18.3% of the cannabis users was using at a less than weekly frequency. The most intensive period lasted on average 3.6 years (SD 3.7, range <1 year – 20 years).

Table 1

Demographics of cannabis users versus non-users (before onset of psychosis).

	Cannabis (n=205)	No cannabis (n=377)	P
Sex (% male)	82.9	55.2	<0.001
Age (years)	28.6	39.9	<0.001
Illness duration (years)	5.7	14.4	<0.001
BDNF Met (%)	38.5	39.6	0.793

Use of other drugs (cocaine, stimulants, phencyclidine, psychedelics and opiates) before first admission for psychotic disorder was reported by 17.2% (n=96) of the CIDI-documented patients. Only 6.3% (n=6) of this group did not use cannabis before the AOP.

Of the 587 genotyped patients, 60,6% had the Val/Val, 34.7% the Val/Met and 4,7% the Met-Met genotype. The BDNF Val66Met polymorphism did not show departure from Hardy-Weinberg equilibrium ($\chi 2$= 0.05, p=.83) and its distribution was not dissimilar for male and female patients (Table 2). Genotyping failed in eleven patients.

Table 2
Demographics of BDNF Met-carriers and Val/Val genotypes.

	BDNF Met (n=226)[a]	BDNF Val/Val (n=348)[a]	P
Sex (% male)	61.9	67.2	0.193
Age (years)	34.9	36.7	0.073
Illness duration (years)	11.0	11.6	0.503
Pre-onset cannabis use (%)	34.4	35.4	0.793
Overall lifetime cannabis use (%)	35.3	36.9	0.694
Intensity of cannabis use (n=158)[b]	(n=60)	(n=98)	0.328
Daily (%)	70.0	58.2	
Weekly (%)	16.7	23.5	
less than weekly (%)	13.3	18.4	
Age first cannabis use (years) (n=186)[c]	16.5	16.9	0.341

[a] 11 patients (5 pre-onset cannabis users) could not be genotyped due to technical obstacles.
[b] only tabulated if the most intense period of cannabis use started before age at onset of psychosis
[c] only tabulated if cannabis use before age at onset of psychosis (age at first use not available for 14 patients)

3.2 Main effects of sex, cannabis use and BDNF Val66Met genotype

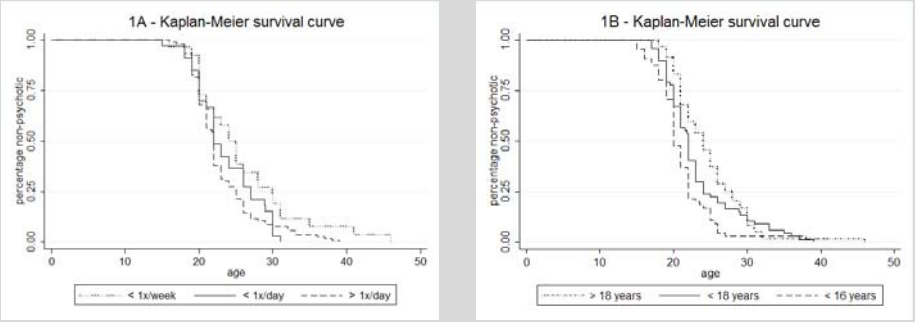
The mean AOP was 24.6 years (SD 7.2, range 14.0-62.9), with a median AOP of 22 and an inter-quartile range of 20-27 years. Male patients had a significantly earlier AOP than female patients: 23.3 years versus 27.0 years (Log rank $\chi2(1)=40.1$, $p<.001$).

Cannabis use was also significantly associated with AOP in a univariable log-rank survival analysis (Log-rank $\chi2(1)= 22.2$, $p<.001$), decreasing the age at first admission with 2.7 years. As depicted in figure 1, univariate log-rank analysis showed that AOP was significantly predicted by the frequency of cannabis use in the most intense period (Log-rank $\chi2(1)=5.8$, $p=.016$) and a younger age at first use of cannabis (Log-rank $\chi2(1)=14.9$, $p<.001$). Intensity of use (Hazard Ratio (HR) 1.29, 95% CI 1.03 – 1.60, $p=.023$) and age at first use (HR 1.50, 95% CI 1.24 – 1.82, $p<.001$) remained both significantly associated with AOP in multivariate models controlling for the effects of other drug use and sex. When both were entered in the Cox regression model, age at first use rather than intensity of use was most strongly associated with an earlier AOP (HR age first use 1.56, 95% CI 1.26 – 1.93, $p<.001$, HR intensity of use 1.22, 95% CI 0.98 – 1.51, $p=.072$; controlled for sex and other drug use).

There was a weak association between BDNF Val66Met genotype and AOP (log-rank $\chi2(1)= 3.8$, $p=.050$), Met-carriers on average displaying onset 1.2 years earlier than Val/Val genotypes. BDNF Met was not associated with cannabis use or age at first use of cannabis (Table 2).

Multivariable Cox regression confirmed an association between AOP and sex (HR 1.64, 95% CI 1.36 – 1.99, $p<.001$), cannabis use (HR 1.43, 95% CI 1.14 – 1.79, $p=.002$) and

Figure 1
Kaplan-Meier survival curves indicating the percentage of patients not yet converted to psychotic disorder as a function of frequency of cannabis use during the most intensive period (1A) and age of first use (1B).



BDNF genotype (HR 1.23, 95% CI 1.03 – 1.46, p=.021) controlled for each other and the effects of the use of other drugs. Results were similar when only individuals of European Caucasian origin were considered. None of the differences in any of the cannabis measures between BDNF Met individuals and Val/Val individuals were large or statistically significant (Table 2).

3.3 Models of GxE

No evidence was found for BDNF X cannabis interaction (interaction $\chi 2(1) = 0.65$, p= .420). However, a statistically significant BDNF X cannabis X sex interaction was found (interaction $\chi 2(1) = 4.99$, p= .026), showing a significant BDNF genotype X cannabis interaction in female (interaction $\chi 2(1) = 5.15$, p= .023) but not in male patients (interaction $\chi 2(1) = 0.04$, p= .833).

In female patients, cannabis use was associated with decreased AOP only in BDNF Met carriers. When both cannabis use and BDNF Met genotype were present, AOP decreased more than 7 years (Table 3 & Figure 2). No association was found between BDNF genotype and AOP in the absence of cannabis use or between cannabis use and AOP in Val/Val genotypes (Table 3).

In contrast, cannabis use was associated with a decreased AOP in male patients irrespective of their BDNF Val66Met genotype (HR 1.27, 95% CI 1.03 – 1.57, p= .024), while no association between AOP and BDNF Met genotype was found (HR 1.20, 95% CI 0.97 – 1.48, p= .095). AOP was 1.3 years earlier for cannabis using male patients. Again, results were similar when only individuals of European Caucasian origin were considered.

Table 3

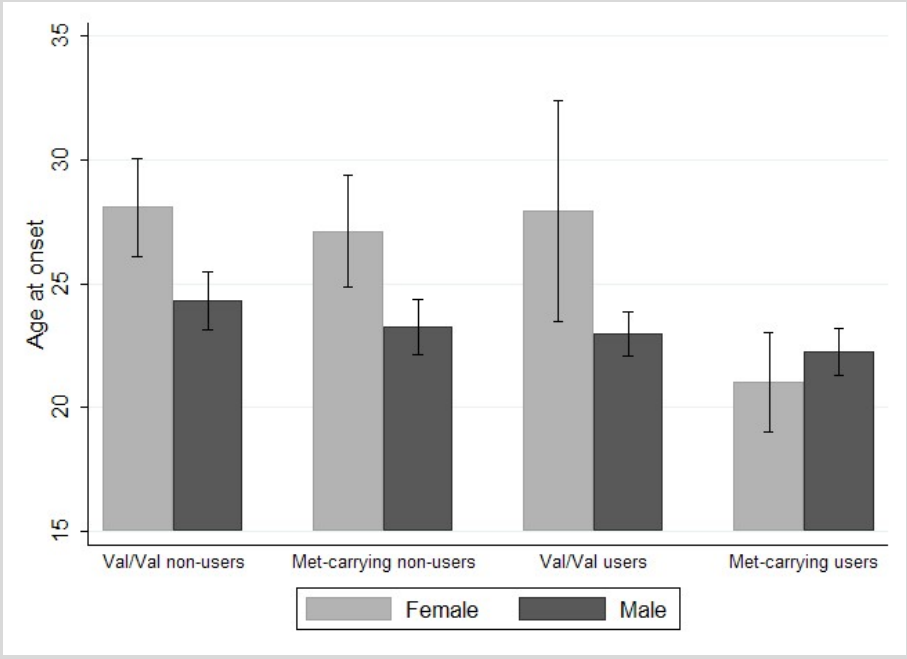
Mean age at onset (in years), difference with reference (in years), Hazard Ratio and p-value for the female schizophrenia patients stratified for cannabis use and BDNF Val66Met genotype.

	Female non-users		Female users	
	BDNF Val/Val (n=97)	**BDNF Met (n=69)**	**BDNF Val/Val (n=17)**	**BDNF Met (n=17)**
Age at onset	28.1	27.1	27.9	21.0
Difference with reference (95%-CI)	0.0[#]	1.0 (-2.0 – 4.0)	0.2 (-4.7 – 4.9)	7.1 (4.3 – 9.8)
Hazard ratio (95%-CI)	--[#]	1.11 (0.82 – 1.52)	1.01 (0.60 – 1.70)	2.39 (1.13 – 5.07)
p-value	--[#]	0.494	0.966	**0.023**

[#] reference category

Figure 2

Diagram with mean age at onset for the different patient groups, stratified by sex, cannabis use and BDNF Val66Met genotype. 95%-confidence intervals are indicated.



4. DISCUSSION

In this large sample of patients with schizophrenia, earlier findings about the unfavorable influence of cannabis use on the AOP were replicated. Cannabis use before illness onset was associated with a 2.7 years earlier AOP. Similar to the findings of Gonzalez-Pinto and colleagues (Gonzalez-Pinto et al., 2008), a dose-dependent association between cannabis use and AOP, with an earlier onset of psychotic disorder in heavier users, was found. Furthermore, a significant association between a younger age of first cannabis use and an earlier onset of psychotic disorder was found; this association remained significant after controlling for possible differences in intensity of use in early users of cannabis. This is in agreement with existent literature suggesting that the brain may be more vulnerable to the psychotogenic effects of cannabis during adolescence (Arseneault et al., 2002; Konings et al., 2008; Stefanis et al., 2004).

A main effect of BDNF Met-allele has been reported before, although not always replicated (Chao et al., 2008; Gourion et al., 2005; Naoe et al., 2007; Numata et al., 2006; Renou et al., 2007). These earlier studies, however, all had fairly small sample sizes compared to the current study and did not take cannabis use into account. In the current sample of 585 patients, a weak association between AOP and BDNF genotype was found, with an earlier AOP (difference 1.2 years) in Met-carriers. Importantly, however, the present results may also suggest that the above-reported main effects cannot be interpreted without taking a sex-specific gene-environment interaction between the BDNF Val66Met polymorphism and cannabis use into account. Thus, cannabis use independently predicted AOP in male patients, whereas in female patients, cannabis use was only associated with AOP in BDNF Met-carriers. This group had a 7 years earlier AOP than their non-using counterparts with the BDNF Val/Val genotype. We were unable to find a significant effect of BDNF genotype in the absence of cannabis use, in either female or male patients. These findings may be in agreement with the hypothesis, derived from earlier findings (D'Souza et al., 2009; Derkinderen et al., 2003; Jockers-Scherubl et al., 2004), that BDNF excretion could be a neuronal adaptive response to the psychotogenic effects of THC, although this could only be demonstrated in female and not in male participants.

The hippocampus may play a central role in an attempt to explain this observation, since hippocampal alterations have been shown in individuals with at-risk mental states (Hurlemann et al., 2008; Witthaus et al., 2009). Especially in the hippocampus, shared cellular mechanisms and a complex interaction of BDNF and estrogen may play an important role in neuroplasticity and neuroprotection, as reviewed by Scharfmann and MacLusky (Scharfman and MacLusky, 2006). Smaller hippocampal volumes have been shown in users of cannabis (Matochik et al., 2005; Yucel et al., 2008) and a recent

study reported a sex-specific influence of cannabis on brain morphometry of the prefrontal cortex with a decrease in prefrontal cortex volume in male users and an increase in female users, compared to non-users of the same sex (Medina et al., 2009). The present results may thus suggest that complex interactions between cannabinoids, BDNF and estrogen shape AOP in individuals already at risk. Further research focused on elucidating the role of cannabis use, estrogen and BDNF in key structures such as the hippocampus in patients at high risk for psychotic disorder is warranted.

In the current sample a low frequency of cannabis use was found in women (17%). Green and collegeas (Green et al., 2005) reported in their concise review that both age and gender may contribute to observed differences in prevalence of cannabis use. It is important to note that the rate of male pre-onset cannabis-users (44%) and the overall rate of pre-onset cannabis users (35%) is also relatively low compared to other samples, e.g. Foti (Foti et al., 2010). This may reflect differences in age, since the present sample is not a first-episode sample but a more chronic sample (mean age 36.1 years, SD 12.1). In addition, the female subgroup is significantly older than the male subgroup in the present sample (average of 40.2 vs 33.6 years, $p<0.0001$), which together may explain the relatively low percentage of female users.

The current findings should also be interpreted in the light of some limitations. First, AOP was defined as the age at first admission. Since there were no community teams in the catchment area to treat psychotic episodes in the community, age at first admission may be a good indicator of the clinical relevance of positive symptoms. Moreover, the reliability of this variable is high, perhaps in contrast to other retrospective measures of symptoms that rely heavily on the mnemonic capacity of patients and thus are prone to recall bias.

Since age at first admission was used as outcome variable, no information on the length of the prodromal period was available. It is possible that the current findings are a reflection of a shorter prodromal period resulting in an earlier AOP, but even if this were the case, this would not make the findings of the current study less relevant. A second limitation is that psychiatric diagnoses were not formally examined by means of a diagnostic interview, but were made by experienced psychiatrists. They may therefore be considered reliable. Cannabis use, on the other hand, was documented formally using the Composite International Diagnostic Interview, which can be considered a strength of the current study, as is the large sample size. In spite of the large sample size, however, it must be acknowledged that the group of cannabis-using female patients was relatively small. Therefore, given the well-known risk of spurious association in candidate gene studies, especially in stratified subgroups (Sullivan, 2007), replication of the current findings is required.

5. CONCLUSION

The current study found sex-specific effects of cannabis use and BDNF Val66Met genotype on age at onset of psychotic disorder in patients with schizophrenia. These findings may help to gain new insights into important genetic and environmental factors operating in the crucial phase of transition from vulnerability to onset of psychotic disorder.

REFERENCES

Angermeyer, M.C., Kuhn, L., 1988. Gender differences in age at onset of schizophrenia. An overview. European archives of psychiatry and neurological sciences 237(6), 351-364.

Arseneault, L., Cannon, M., Poulton, R., Murray, R., Caspi, A., Moffitt, T.E., 2002. Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study. BMJ 325(7374), 1212-1213.

Chao, H.M., Kao, H.T., Porton, B., 2008. BDNF Val66Met variant and age of onset in schizophrenia. Am J Med Genet B Neuropsychiatr Genet 147B(4), 505-506.

Compton, M.T., Kelley, M.E., Ramsay, C.E., Pringle, M., Goulding, S.M., Esterberg, M.L., Stewart, T., Walker, E.F., 2009. Association of pre-onset cannabis, alcohol, and tobacco use with age at onset of prodrome and age at onset of psychosis in first-episode patients. The American journal of psychiatry 166(11), 1251-1257.

D'Souza, D.C., Pittman, B., Perry, E., Simen, A., 2009. Preliminary evidence of cannabinoid effects on brain-derived neurotrophic factor (BDNF) levels in humans. Psychopharmacology (Berl) 202(4), 569-578.

De Hert, M., Dekker, J.M., Wood, D., Kahl, K.G., Holt, R.I., Moller, H.J., 2009. Cardiovascular disease and diabetes in people with severe mental illness position statement from the European Psychiatric Association (EPA), supported by the European Association for the Study of Diabetes (EASD) and the European Society of Cardiology (ESC). Eur Psychiatry 24(6), 412-424.

De Hert, M., Wampers, M., Jendricko, T., Franic, T., Vidovic, D., De Vriendt, N., Sweers, K., Peuskens, J., van Winkel, R., 2011. Effects of cannabis use on age at onset in schizophrenia and bipolar disorder. Schizophrenia research 126(1-3), 270-276.

Derkinderen, P., Valjent, E., Toutant, M., Corvol, J.C., Enslen, H., Ledent, C., Trzaskos, J., Caboche, J., Girault, J.A., 2003. Regulation of extracellular signal-regulated kinase by cannabinoids in hippocampus. J Neurosci 23(6), 2371-2382.

Egan, M.F., Kojima, M., Callicott, J.H., Goldberg, T.E., Kolachana, B.S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B., Weinberger, D.R., 2003. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112(2), 257-269.

Foti, D.J., Kotov, R., Guey, L.T., Bromet, E.J., 2010. Cannabis use and the course of schizophrenia: 10-year follow-up after first hospitalization. The American journal of psychiatry 167(8), 987-993.

Gonzalez-Pinto, A., Vega, P., Ibanez, B., Mosquera, F., Barbeito, S., Gutierrez, M., Ruiz de Azua, S., Ruiz, I., Vieta, E., 2008. Impact of cannabis and other drugs on age at onset of psychosis. The Journal of clinical psychiatry 69(8), 1210-1216.

Gourion, D., Goldberger, C., Leroy, S., Bourdel, M.C., Olie, J.P., Krebs, M.O., 2005. Age at onset of schizophrenia: interaction between brain-derived neurotrophic factor and dopamine D3 receptor gene variants. Neuroreport 16(12), 1407-1410.

Green, B., Young, R., Kavanagh, D., 2005. Cannabis use and misuse prevalence among people with psychosis. Br J Psychiatry 187, 306-313.

Hare, E., Glahn, D.C., Dassori, A., Raventos, H., Nicolini, H., Ontiveros, A., Medina, R., Mendoza, R., Jerez, A., Munoz, R., Almasy, L., Escamilla, M.A., 2010. Heritability of age of onset of psychosis in schizophrenia. Am J Med Genet B Neuropsychiatr Genet 153B(1), 298-302.

Hurlemann, R., Jessen, F., Wagner, M., Frommann, I., Ruhrmann, S., Brockhaus, A., Picker, H., Scheef, L., Block, W., Schild, H.H., Moller-Hartmann, W., Krug, B., Falkai, P., Klosterkotter, J., Maier, W., 2008. Interrelated neuropsychological and anatomical evidence of hippocampal pathology in the at-risk mental state. Psychol Med 38(6), 843-851.

Jockers-Scherubl, M.C., Danker-Hopfe, H., Mahlberg, R., Selig, F., Rentzsch, J., Schurer, F., Lang, U.E., Hellweg, R., 2004. Brain-derived neurotrophic factor serum concentrations are increased in drug-naive schizophrenic patients with chronic cannabis abuse and multiple substance abuse. Neuroscience letters 371(1), 79-83.

Kohler, S., van Os, J., de Graaf, R., Vollebergh, W., Verhey, F., Krabbendam, L., 2007. Psychosis risk as a function of age at onset: a comparison between early- and late-onset psychosis in a general population sample. Social psychiatry and psychiatric epidemiology 42(4), 288-294.

Konings, M., Henquet, C., Maharajh, H.D., Hutchinson, G., Van Os, J., 2008. Early exposure to cannabis and risk for psychosis in young adolescents in Trinidad. Acta psychiatrica Scandinavica 118(3), 209-213.

Larsen, T.K., McGlashan, T.H., Moe, L.C., 1996. First-episode schizophrenia: I. Early course parameters. Schizophr Bull 22(2), 241-256.

Matochik, J.A., Eldreth, D.A., Cadet, J.L., Bolla, K.I., 2005. Altered brain tissue composition in heavy marijuana users. Drug Alcohol Depend 77(1), 23-30.

Medina, K.L., McQueeny, T., Nagel, B.J., Hanson, K.L., Yang, T.T., Tapert, S.F., 2009. Prefrontal cortex morphometry in abstinent adolescent marijuana users: subtle gender effects. Addict Biol 14(4), 457-468.

Naoe, Y., Shinkai, T., Hori, H., Fukunaka, Y., Utsunomiya, K., Sakata, S., Matsumoto, C., Shimizu, K., Hwang, R., Ohmori, O., Nakamura, J., 2007. No association between the brain-derived neurotrophic factor (BDNF) Val66Met polymorphism and schizophrenia in Asian populations: Evidence from a case-control study and meta-analysis. Neuroscience letters 415(2), 108-112.

Numata, S., Ueno, S., Iga, J., Yamauchi, K., Hongwei, S., Ohta, K., Kinouchi, S., Shibuya-Tayoshi, S., Tayoshi, S., Aono, M., Kameoka, N., Sumitani, S., Tomotake, M., Kaneda, Y., Taniguchi, T., Ishimoto, Y., Ohmori, T., 2006. Brain-derived neurotrophic factor (BDNF) Val66Met polymorphism in schizophrenia is associated with age at onset and symptoms. Neuroscience letters 401(1-2), 1-5.

Ongur, D., Lin, L., Cohen, B.M., 2009. Clinical characteristics influencing age at onset in psychotic disorders. Compr Psychiatry 50(1), 13-19.

Peerbooms, O., Rutten, B.P., Decoster, J., van Os, J., Kenis, G., De Hert, M., van Winkel, R., 2010. No association between MTHFR C677T or A1298C and age at onset of schizophrenia. Am J Med Genet B Neuropsychiatr Genet 153B(7), 1362-1363; author reply 1361.

Raz, N., Rodrigue, K.M., Kennedy, K.M., Land, S., 2009. Genetic and vascular modifiers of age-sensitive cognitive skills: effects of COMT, BDNF, ApoE, and hypertension. Neuropsychology 23(1), 105-116.

Renou, J., De Luca, V., Zai, C.C., Bulgin, N., Remington, G., Meltzer, H.Y., Lieberman, J.A., Le Foll, B., Kennedy, J.L., 2007. Multiple variants of the DRD3, but not BDNF gene, influence age-at-onset of schizophrenia. Molecular psychiatry 12(12), 1058-1060.

Scharfman, H.E., MacLusky, N.J., 2006. Estrogen and brain-derived neurotrophic factor (BDNF) in hippocampus: complexity of steroid hormone-growth factor interactions in the adult CNS. Front Neuroendocrinol 27(4), 415-435.

Shalev, I., Lerer, E., Israel, S., Uzefovsky, F., Gritsenko, I., Mankuta, D., Ebstein, R.P., Kaitz, M., 2009. BDNF Val66Met polymorphism is associated with HPA axis reactivity to psychological stress characterized by genotype and gender interactions. Psychoneuroendocrinology 34(3), 382-388.

Smeets, R., Dingemans, P., 1993. Composite International Diagnostic Interview (CIDI) version1.1. World Health Organization, Amsterdam/Genava.

StataCorp., 2007. Stata/SE statistical software, release 10. StataCorp LP, College Station.

Stefanis, N.C., Delespaul, P., Henquet, C., Bakoula, C., Stefanis, C.N., Van Os, J., 2004. Early adolescent cannabis exposure and positive and negative dimensions of psychosis. Addiction 99(10), 1333-1341.

Sugranyes, G., Flamarique, I., Parellada, E., Baeza, I., Goti, J., Fernandez-Egea, E., Bernardo, M., 2009. Cannabis use and age of diagnosis of schizophrenia. Eur Psychiatry 24(5), 282-286.

Sullivan, P.F., 2007. Spurious genetic associations. Biol Psychiatry 61(10), 1121-1126.

van Winkel, R., De Hert, M., Van Eyck, D., Hanssens, L., Wampers, M., Scheen, A., Peuskens, J., 2006. Screening for diabetes and other metabolic abnormalities in patients with schizophrenia and schizoaffective disorder: evaluation of incidence and screening methods. The Journal of clinical psychiatry 67(10), 1493-1500.

van Winkel, R., GROUP Investigators, 2011. Family-based analysis of genetic variation underlying psychosis-inducing effects of cannabis: sibling analysis and proband follow-up. Archives of general psychiatry 68(2), 148-157.

Vares, M., Saetre, P., Deng, H., Cai, G., Liu, X., Hansen, T., Rasmussen, H.B., Werge, T., Melle, I., Djurovic, S., Andreassen, O.A., Agartz, I., Hall, H., Terenius, L., Jonsson, E.G., 2010. Association between methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and age of onset in schizophrenia. Am J Med Genet B Neuropsychiatr Genet 153B(2), 610-618.

Veen, N.D., Selten, J.P., van der Tweel, I., Feller, W.G., Hoek, H.W., Kahn, R.S., 2004. Cannabis use and age at onset of schizophrenia. The American journal of psychiatry 161(3), 501-506.

Verhagen, M., van der Meij, A., van Deurzen, P.A., Janzing, J.G., Arias-Vasquez, A., Buitelaar, J.K., Franke, B., 2008. Meta-analysis of the BDNF Val66Met polymorphism in major depressive disorder: effects of gender and ethnicity. Molecular psychiatry.

Witthaus, H., Kaufmann, C., Bohner, G., Ozgurdal, S., Gudlowski, Y., Gallinat, J., Ruhrmann, S., Brune, M., Heinz, A., Klingebiel, R., Juckel, G., 2009. Gray matter abnormalities in subjects at ultra-high risk for schizophrenia and first-episode schizophrenic patients compared to healthy controls. Psychiatry Res 173(3), 163-169.

Yucel, M., Solowij, N., Respondek, C., Whittle, S., Fornito, A., Pantelis, C., Lubman, D.I., 2008. Regional brain abnormalities associated with long-term heavy cannabis use. Arch Gen Psychiatry 65(6), 694-701.

Chapter Four

AKT1 rs2494732 and risk of psychosis in cannabis users

A prospective study of unaffected siblings and case-only analysis of a novel patient sample.

Ruud van Winkel [1,2], Jeroen Decoster*[1,2], Marc De Hert*[2], Gunter Kenis[1], Genetic Risk and Outcome in Psychosis (GROUP) Investigators[#].

* contributed equally.
[#] GROUP investigators: Richard Bruggeman [3], Wiepke Cahn [4], Lieuwe de Haan [5], René S. Kahn [4], Carin Meijer[5], Inez Myin-Germeys [1], Jim van Os[1,6], Durk Wiersma[3].

Submitted

[1] *Dept. of Psychiatry and Psychology, School for Mental Health and Neuroscience, EURON, Maastricht University Medical Centre, The Netherlands*
[2] *University Psychiatric Centre Catholic University Louvain, campus Kortenberg, Belgium*
[3] *University Medical Center Groningen, Department of Psychiatry, University of Groningen, The Netherlands.*
[4] *University Medical Center Utrecht, Department of Psychiatry, Rudolf Magnus Institute of Neuroscience, The Netherlands.*
[5] *Academic Medical Centre University of Amsterdam, Department of Psychiatry, Amsterdam, The Netherlands.*
[6] *King's College, King's Health Partners, Department of Psychosis Studies Institute of Psychiatry, De Crespigny Park, Denmark Hill, London, UK.*

ABSTRACT

Background

Studies have suggested that AKT1 rs2494732 influences the risk for psychosis in cannabis users. However, longitudinal data are lacking and sample sizes were relatively modest.

Methods

Data from two studies are reported: (1) A 3-year follow-up study examining the course of schizotypy as a function of AKT1 genotype and cannabis use (by urinalysis) in 598 unaffected siblings of patients with psychosis, and (2) Case-only replication study in a novel sample of 533 patients and individual participant meta-analysis of the combined discovery and replication patient-samples (n=1,222).

Results

In the unaffected siblings, there was significant interaction between AKT1 genotype and cannabis on the course of schizotypy. In persistent cannabis users (n=21), C-carriers had significantly higher schizotypy at follow-up than T/T genotypes ((Wald χ2(df 1)=9.9, p=.002), whereas no differences were found in non-users. Moreover, while separate analysis of the patient-replication sample was non-significant (odds ratio (OR) linear trend for history of cannabis use 1.26, 95% CI 0.93 – 1.69), the direction and magnitude of effect was similar to the discovery patient-sample, and meta-analysis of both patient samples confirmed the association between cannabis use and C-allele loading at AKT1 rs2494732 (OR linear trend 1.22, 95% CI 1.02 – 1.46). The association was somewhat stronger in white European patients (n=1010, OR linear trend 1.32, 95% CI 1.08 – 1.60).

Conclusion

These data provide additional evidence that the AKT1 rs2494732 polymorphism may influence the risk for cannabis-induced psychosis, in a manner consistent with gene-environment interaction rather than correlation.

1. INTRODUCTION

Cannabis use is consistently associated with psychosis in epidemiological studies (Henquet et al., 2005b; Moore et al., 2007) and the available evidence is in support of a causal contribution (Kuepper et al., 2011). The associated risk is still low, however, and genetic make-up is likely to play a roll in determining sensitivity to the psychosis-inducing effects of cannabis (Decoster et al., 2012).

A recent study in a large sample of unaffected siblings of patients with psychotic disorder, collected as part of the Genetic Risk and Outcome of Psychosis (GROUP) study (Genetic Risk and Outcome of Psychosis (GROUP) Investigators, 2011; van Winkel and Genetic Risk and Outcome of Psychosis (GROUP) Investigators, 2011), examined possible interactions between cannabis and 152 single nucleotide polymorphisms (SNPs) selected from 42 candidate genes and found 3 SNPs that significantly influenced the effects of recent cannabis use on the expression of schizotypy, in AKT1 (2 SNPs) and LRRTM1 (1 SNP). Follow-up of these SNPs in the patients confirmed the presence of interaction between rs2494732 in AKT1 and cannabis in a case-only, case-sibling and case-control design. Compared to those with the T/T genotype, individuals with a C/C genotype displayed a 2-fold increased risk of being diagnosed with a psychotic disorder when having used cannabis (van Winkel and Genetic Risk and Outcome of Psychosis (GROUP) Investigators, 2011).

AKT1 is a protein kinase that is involved in multiple cellular processes (Freyberg et al., 2010). It has a role in regulating neuronal cell size and survival (Franke, 2008) and is also a key signaling molecule downstream of the dopamine D2 (DRD2) receptor; decreased AKT1 functionality may result in exacerbated responses to DRD2 receptor stimulation in the striatum (Arguello and Gogos, 2008). The biological plausibility of its interaction with cannabis is strengthened by the fact that cannabinoids are able to activate AKT1/GSK3 signalling through CB1 and CB2 endocannabinoid receptors *in vitro* (Sanchez et al., 2003), as well as *in vivo*, in several brain areas including the striatum, independent of dopamine D1 and D2 receptor blockade (Ozaita et al., 2007).

A recent case-control study of an independent sample of 489 patients and 278 controls from the UK was in support of an interaction between rs2494732 in AKT1 and cannabis (Di Forti et al., 2012a), while indirect evidence supporting this interaction was also found at the level of AKT1-dependent prefrontal functioning under the influence of cannabis, as measured by the Continuous Performance Test (van Winkel et al., 2011a).

Although these studies provide initial support that rs2494732 in AKT1 may indeed influence the risk for psychosis in cannabis users, important questions remain. First, all of the reported findings were based on cross-sectional data, making it difficult to distinguish true gene-environment interaction (GxE) from gene-environment

correlation. Although cross-sibling, cross-trait analyses indicated no clear evidence for such gene-environment correlation in previous studies (Genetic Risk and Outcome of Psychosis (GROUP) Investigators, 2011; van Winkel and Genetic Risk and Outcome of Psychosis (GROUP) Investigators, 2011), longitudinal follow-up data would provide validity to the notion of a temporal relationship between cannabis use, AKT1 genotype and schizotypy. Second, a recent study demonstrated that the risk for false-positive findings in candidate GxE studies is significantly reduced with increasing sample size and greater 'a priori probability', indicating the need to collect additional samples with the aim to specifically follow up on a promising candidate interaction in order to increase the likelihood that the reported finding is actually true (Duncan and Keller, 2011b).

The present paper combines the two strategies described above. First, we report longitudinal data of the sample of unaffected siblings who participated in the GROUP study, in which we specifically investigate whether the combination of cannabis use and AKT1 rs2494732 genotype predicts levels of schizotypy at the 3-year follow-up assessment. Second, we report case-only data from a novel sample of 533 patients from Belgium (University Psychiatric Centre – Catholic University of Leuven [UPC-CUL sample])(Decoster et al., 2011) as well as a combined analysis of the UPC-CUL and GROUP samples using individual participant genotype and phenotype data (1,222 patients in total). The rational of combining these strategies is that the issue of temporality in the relationship between cannabis use and expression of psychosis (i.e. what comes first) is addressed by the analysis in the unaffected siblings, while the risk of spurious findings from relatively small sample sizes is reduced by the joint analysis of the two patient samples.

2. METHODS AND MATERIALS

2.1. Samples

2.2.1. Patients and their unaffected siblings – the GROUP study

A detailed description of this study can be found elsewhere (Genetic Risk and Outcome of Psychosis (GROUP) Investigators, 2011). In short, the full GROUP sample consists of 1119 patients with non-affective psychotic disorder, 1057 siblings of these 1119 patients, 919 of their parents and 590 unrelated controls. Inclusion criteria for patients were: (i) age range 16 to 50 years, (ii) diagnosis of non-affective psychotic disorder and (iii) good command of Dutch language. Diagnosis was based on the Diagnostic and Statistical Manual of Mental Disorder-IV (DSM-IV) criteria (American Psychiatric Association, 2000b), assessed with the Comprehensive Assessment of Symptoms and History (CASH) interview (Andreasen et al., 1992) or Schedules for Clinical Assessment for Neuropsychiatry (SCAN 2.1)(Wing et al., 1990).

Of 1119 patients included, genetic data were available in 801 (76.8% male, mean age 27.9 [SD 8.2]). As this study aimed to examine the etiological relevance of cannabis use on the development of psychotic disorder, and no information on the age of first use of cannabis was available, patients whose period of most heavy cannabis use occurred after the onset of their psychotic disorder (22.7% of the cannabis using patients) were excluded from the analyses, resulting in a final sample size of 689 patients with psychotic disorder (van Winkel and Genetic Risk and Outcome of Psychosis (GROUP) Investigators, 2011).

Of the 1057 unaffected siblings included in the GROUP sample, 598 individuals provided DNA and a urine sample at the baseline assessment and 3-year follow-up and also participated in the Structured Interview for Schizotypy-Revised (SIS-R) at both time-points. The SIS-R is a semi-structured interview containing 20 schizotypal symptoms and 11 schizotypal signs, rated on a four-point scale. Symptoms are defined as verbal responses to standardized questions concerning, for example, magical ideation, illusions, and referential thinking. Signs refer to behaviours that are rated by the interviewer such as, for example, goal directedness of thinking and flatness of affect. Questions and rating procedures are standardized (Kendler et al., 1989; Vollema and Ormel, 2000). In addition to the 598 siblings who remained well, 8 siblings experienced a transition to a diagnosable psychotic disorder over the course of the follow-up period, as assessed by CASH interview (Andreasen et al., 1992). For these participants no SIS-R was done at follow-up, as this interview is not valid for individuals with a diagnosed psychotic disorder (Kendler et al., 1989; Vollema and Ormel, 2000).

2.2.2. The Belgian University Psychiatric Centre - Catholic University Leuven (UPC-CUL) sample

This sample was collected between July 2003 and February 2008 at the University Psychiatric Centre (UPC) of the Catholic University in Leuven, Belgium. Patients presenting at the UPC are routinely screened for metabolic abnormalities as described previously (De Hert et al., 2011a). On this occasion, they were asked for permission to store a blood sample for genetic analyses and additionally provided information on lifetime substance use, assessed using the Composite International Diagnostic Interview (CIDI) -lifetime section on substance abuse (De Hert et al., 2011b; Decoster et al., 2011). Psychiatric diagnoses according to DSM-IV criteria were established by experienced psychiatrists affiliated with the University Centre and responsible for the patient's treatment. Patients provided written informed consent and the study was approved by the standing ethics committee. The UPC-CUL sample consists of 585 patients with a non-affective psychotic disorder. For 574 patients genetic data were available; for 548 of these detailed CIDI cannabis information was also available. In agreement with the original report (van Winkel and Genetic Risk and Outcome of Psychosis (GROUP) Investigators, 2011), patients whose period of most heavy cannabis use occurred after the onset of their psychotic disorder (8.1% of the cannabis using patients) were excluded from the analyses, resulting in a final sample size of 533. Patients had clinical diagnoses of schizophrenia (68.5%), schizophreniform disorder (10.9%) or schizoaffective disorder (20.6%).

2.2. Cannabis measures

In the prospective study of unaffected siblings, the measure applied was cannabis use as established by urinalysis. Since cannabis in urine was measured both at baseline and at 3-year follow-up, this variable ('follow-up urinalysis') had 4 levels: (0) negative urinalysis at both time-points ('no use'), (1) negative at baseline, positive at follow-up ('follow-up only'), (2) positive at baseline, negative at follow-up ('baseline-only') and (3) positive at both baseline and follow-up ('persistent cannabis use').

In the case-only analysis, cannabis measures were a history of cannabis use (of which the lifetime period of heaviest use preceded the onset of psychosis; yes/no) and frequency of use during the lifetime period of heaviest use (none [0], less than weekly [1] weekly [2] and daily [3]), as applied in the original publication (van Winkel and Genetic Risk and Outcome of Psychosis (GROUP) Investigators, 2011) and in the study by Di Forti and colleagues (Di Forti et al., 2012a).

2.3. AKT1 genotyping

AKT1 rs2494732 genotype was determined by Sequenom (Hamburg, Germany) in both samples using the Sequenom MassARRAY iPLEX platform at the facilities of the manufacturer, using the SNP array described in previous work (van Winkel and Genetic Risk and Outcome of Psychosis (GROUP) Investigators, 2011). Genotyping of AKT1 rs2494732 was successful in 98.5% of the GROUP sample and 97.7% of the UPC-CUL sample. Hardy-Weinberg Equilibrium was examined and confirmed ($p>.05$) in all samples, using the GENHW command in STATA.

2.4. Statistical analysis

2.4.1. Longitudinal course of schizotypy as a function of AKT1 rs2494732 and cannabis use

SIS-R schizotypy at follow-up was regressed on cannabis urinalysis, rs2494732 (genotypes coded 0, 1 or 2 and modeled as a linear effect), and their interaction while co-varying for baseline schizotypy. This was done in two separate analyses using the XTREG routine in STATA, which takes into account the multilevel structure of the data (multiple assessments per person, as well as clustering of participants within families). First, in order to investigate whether the combination of *baseline cannabis use* and AKT1 genotype predicts SIS-R schizotypy at follow-up, this analysis was conducted using cannabis information from the baseline measurement only (i.e. positive urinalysis versus negative urinalysis at baseline). Second, in order to further differentiate *subgroups of cannabis use over the follow-up period*, the variable 'follow-up urinalysis' as described above (no use, baseline only, follow-up only, persistent cannabis use) was used as a dummy variable in the regression model.

Analyses were *a priori* adjusted for age, sex and ethnicity. Post-hoc comparisons of genotype (AKT1 rs2494732 T/T versus C/T + C/C) and follow-up urinalysis groups (no use, baseline only, follow-up only, persistent cannabis use) were conducted using Wald tests (Clayton and Hills, 1993). In addition, a sensitivity analysis re-examined the interaction excluding the siblings scoring in the highest tertile of schizotypy at baseline, thus removing the individuals driving the significant baseline interaction. A second sensitivity analysis examined the interaction in individuals from white European descent only, since the minor allele frequency (MAF) of AKT1 rs2494732 was significantly different in the white versus non-white siblings (MAF white 40.6%, MAF non-white 52.5%, $\chi 2(2)=9.2$, $p=.010$).

2.4.2. Case-only analysis

A case-only design determines presence of GxE on the basis of an association between SNP and environmental exposure in patients, while assuming independence between the two in the general population (Khoury and Flanders, 1996). This assumption cannot hold when using a mass-marker approach (Murcray et al., 2009) but is acceptable in the case of selective follow-up of previously established interactions with high prior probability. A previous analysis of the healthy controls and the unaffected siblings of the GROUP sample indeed demonstrated no association between AKT1 genotype and cannabis (van Winkel and Genetic Risk and Outcome of Psychosis (GROUP) Investigators, 2011), and the same was true in the report by Di Forti and colleagues (Di Forti et al., 2012a), in support of the independence assumption.

AKT1 rs2494732 was coded (0, 1, 2) and modeled as a linear effect. Analyses were *a priori* adjusted for age, sex and ethnicity. Power analysis using Quanto version 1.2.4 (http://hydra.usc.edu/gxe/) revealed a power of .57 in the UPC-CUL sample for an interaction effect of 2.0 (T/T versus C/C, based on (van Winkel and Genetic Risk and Outcome of Psychosis (GROUP) Investigators, 2011)) at an alpha of .05, while the individual participant meta-analysis of both samples had a power of .90. Again, sensitivity analyses were conducted in patients from white European descent only, since differences in genotype distribution of AKT1 rs2494732 between white and non-white participants were trend-significant in the GROUP sample ($\chi 2(2)=5.8$, p=.056) and in the combined sample ($\chi 2(2)=4.7$, p=.093), but not in the UPC-CUL sample, which contained very few non-white individuals (4.7%; $\chi 2(2)=1.1$, p=.585).

3. RESULTS

3.1. Study 1: prospective analysis of unaffected siblings

Of the 598 unaffected siblings, slightly more than half were female (54.4%) and the mean age at follow-up was 30.3 years (SD 7.7). Eighty-six percent was of white European origin, 9% of mixed origin while the remaining siblings were of non-white or unclear ethnicity. A proportion of 32.7% had the T/T genotype of AKT1 rs2494732, 50.1% the C/T and 17.3% the C/C genotype. In whites, percentages were 34.1% (T/T), 50.4% (C/T) and 15.5% (C/C), whereas in non-whites these were 23.5%, 48.2% and 28.4%, respectively.

The vast majority of the unaffected siblings did not screen positive for cannabis use on either occasion (n=551, 92.1%), 2.0% (n= 12) screened positive only at baseline, 2.3% (n= 14) only at follow-up and 3.5% (n= 21) screened positive on both occasions. Overall, there was a significant decrease in schizotypy over the follow-up period (mean

t0 = .31, mean t1 = .28, t=2.6, p = .009). This decrease was absent in those with persistent cannabis use (mean t0 = .42, mean t1 = .44, t= -.3, p= .80). Persistent cannabis use was associated with higher levels of schizotypy at follow-up compared to non-use (b=.10, SE .04, p=.024), whereas AKT1 rs2494732 genotype was not (b=.01, SE .01, p=.466).

There was a significant interaction between *baseline urinalysis* and rs2494732 in the model of schizotypy at 3-year follow-up (interaction $\chi 2$(df 1)=6.9, b=.17, 95% CI .04 - .30, p=.009). Sensitivity analyses showed that the interaction remained significant when restricting the analysis to individuals from white European ancestry (interaction $\chi 2$(df 1)=6.4, b=.18, 95% CI .04 - .32, p=.012) and when excluding the siblings scoring in the highest tertile of schizotypy scores at baseline (interaction $\chi 2$(df 1)=8.0, b=.18, 95% CI .06 - .31, p=.005). Similarly, there was a significant interaction between *follow-up urinalysis* and rs2494732 in the model of schizotypy at 3-year follow-up (interaction $\chi 2$(df 1)=9.3, b=.06, 95% CI .02 - .10, p=.002). Again, this interaction remained significant when restricting the analysis to individuals from white European ancestry (interaction $\chi 2$(df 1)=5.9, b=.05, 95% CI .01 - .10, p=.016) and when excluding the siblings scoring in the highest tertile of schizotypy scores at baseline (interaction $\chi 2$(df 1)=8.4, b=.06, 95% CI .02 - .10, p=.004).

Table 1

Course of schizotypy in unaffected siblings as a function of cannabis use and AKT1 rs2494732 genotype in the overall sample and in the sample without the siblings scoring in the highest tertile of schizotypy at baseline.

Overall sample			
	Baseline schizotypy (SD)	**Follow-up schizotypy (SD)**	**p[#]**
No cannabis use			.50
AKT1 rs2494732 T/T (n=177)	.30 (.27)	.29 (.25)	
AKT1 rs2494732 C-carrier (n=374)	.31 (.28)	.28 (.22)	
Persistent cannabis use			**.002**
AKT1 rs2494732 T/T (n=10)	.25 (.20)	.22 (.21)	
AKT1 rs2494732 C-carrier (n=11)	.58 (.46)	.64 (.41)	
Without siblings scoring in highest tertile at baseline			
	Baseline schizotypy (SD)	**Follow-up schizotypy (SD)**	**p[#]**
No cannabis use			.60
AKT1 rs2494732 T/T (n=130)	.17 (.13)	.22 (.21)	
AKT1 rs2494732 C-carrier (n=283)	.18 (.13)	.22 (.17)	
Persistent cannabis use			**.015**
AKT1 rs2494732 T/T (n=8)	.18 (.13)	.17 (.11)	
AKT1 rs2494732 C-carrier (n=7)	.30 (.13)	.45 (.23)	

[#] p-value refers to the comparison between AKT1 rs2494732 T/T and C-carrier groups using Wald tests.

Further stratification showed that, in non-cannabis users, there were no significant differences in schizotypy at the 3-year follow-up according to AKT1 rs2494732 genotype (Wald χ2(df 1) =0.5, p=.50). The same was true for individuals that used cannabis only at baseline (Wald χ2(df 1) =0.6, p=.43) or follow-up (Wald χ2(df 1) =0.6, p=.45). In persistent (baseline + follow-up) cannabis users, however, C-carriers had significantly higher schizotypy scores at follow-up than individuals with the T/T genotype (Wald χ2(df 1)=9.8, p=.002). The group of C-carriers with persistent cannabis use was also the only group for which mean schizotypy scores had increased over the follow-up period (table 1). When excluding the siblings scoring in the highest tertile at baseline, the largest increases were similarly observed in C-carriers with persistent cannabis use (table 1).

3.2. Study 2: case-only replication and individual participant meta-analysis

Compared to the GROUP sample, the UPC-CUL sample was older and consisted of more individuals of white European ancestry (table 2). AKT1 rs2494732 allele frequencies were similar, but the GROUP sample had a higher prevalence of lifetime cannabis use and an earlier age at onset of psychosis (table 2).

Table 2
Sample characteristics.

	GROUP (n=689)	UPC-CUL (n=533)
Age (SD)	28.2 (8.4)	36.2 (11.9)
Sex (% male)	75.3	64.4
Lifetime cannabis use (%)	55.9	31.9
Less than weekly (%)	7.4	5.3
Weekly (%)	11.0	6.2
Daily (%)	37.5	20.5
Age at onset of psychosis	22.9 (7.1)	24.8 (7.3)
AKT1 rs2494732 genotype		
T/T	34.9	33.4
C/T	46.1	47.4
C/C	19.0	19.2
Ethnicity		
White European (%)	75.5	95.3
Black African (%)	N/A[#]	1.5
Asian (%)	0.2	0.6
Mahreb (%)	2.2	1.3
Iran/Pakistan/India (%)	0.0	0.6
Turkey (%)	2.3	0.8
Antillian/Surinam (%)	3.5	0.0
Mixed (%)	10.6	N/A[*]
Unknown/other (%)	5.8	0.0

[*]Participants were considered 'non-white' if one of the parents was of non-white ethnicity.
[#]In category 'other'.

Analysis of the UPC-CUL sample showed no significant association between AKT1 rs2494732 and a history of cannabis use (OR linear trend 1.26, 95% CI 0.93 – 1.69, p= .13) or frequency of use (b = .07, SE .07, p= .28). Results were similar when analyzing white European patients only (history of use: OR linear trend 1.30, 95% CI 0.96 – 1.76, p= .095; frequency of use: b = .07, SE .07, p= .29). Nevertheless, the pattern of association was in the expected direction and the effect sizes were comparable to those in the original report (table 3).

Individual participant meta-analysis of the combined GROUP and UPC-CUL samples did show significant evidence for association between the AKT1 rs2494732 polymorphism and history of cannabis use (OR linear trend 1.22, SE = .11, p = .027) as well as lifetime frequency of use (b = .12, SE .05, p= .014), C/C genotypes displaying ORs of 1.49 (95% CI 1.04 – 2.14) for a history of cannabis use and 1.58 for daily lifetime use (95% CI 1.06 – 2.37). Associations were also significant when analyzing only white European patients (history of use: OR linear trend 1.32, 95% CI 1.08 – 1.60, p= .007; frequency of use: b = .13, SE .05, p= .016), but effect sizes were somewhat higher (table 4).

Table 3

Relative risk and 95% confidence interval (n, CI) for cannabis use before the onset of psychosis as a function of AKT1 rs2494732 genotype in 1200* patients with a psychotic disorder.

	T/T	C/T	C/C	Linear trend
GROUP (n=679*)	1.0[a] (n=237)	1.14 (n=313, 0.80 – 1.63)	1.49 (n=129, 0.94 – 2.38)	1.21 (0.96 – 1.52)
UPC-CUL (n=521*)	1.0[a] (n=174)	1.42 (n=247, 0.87 – 2.31)	1.53 (n=100, 0.84 – 2.80)	1.26 (0.93 – 1.69)
Combined (n=1200*)	1.0[a] (n=411)	1.22 (n=560, 0.92 – 1.63)	1.49 (n=229, 1.04 – 2.14)	1.22 (1.02 – 1.46)

*Genotyping was unsuccessful in 22 patients

[a] reference category of genotype effect

Table 4

Relative risk and 95% confidence interval (n, CI) for cannabis use before the onset of psychosis as a function of AKT1 rs2494732 genotype in 1010 white European patients with a psychotic disorder.

	T/T	C/T	C/C	Linear trend
GROUP (n=513)	1.0[a] (n=185)	1.17 (n=241, 0.78 – 1.76)	1.89 (n=87, 1.08 – 3.31)	1.33 (1.02 – 1.74)
UPC-CUL (n=497)	1.0[a] (n=164)	1.55 (n=238, 0.93 – 2.56)	1.62 (n=95, 0.87 – 3.02)	1.30 (0.96 – 1.76)
Combined (n=1010)	1.0[a] (n=349)	1.30 (n=479, 0.95 – 1.78)	1.74 (n=182, 1.16 – 2.61)	1.32 (1.08 – 1.60)

[a] reference category of genotype effect

4. DISCUSSION

This study examined the interaction between AKT1 rs2494732 and cannabis, specifically following up on a previous report (van Winkel and Genetic Risk and Outcome of Psychosis (GROUP) Investigators, 2011). In a prospective follow-up of 598 unaffected siblings, a significant interaction on the course of schizotypy over the

follow-up period was found, C-carriers with persistent cannabis use showing significantly higher schizotypy at follow-up than persistent cannabis users with the T/T genotype, whereas no such difference was observed in non-users. In addition, although an attempt at direct replication in the independent UPC-CUL sample produced a non-significant result, the direction and magnitude of the effect was similar as in the original GROUP sample, and an individual participant meta-analysis of both samples combined confirmed the association between cannabis use and C-allele loading at AKT1 rs2494732, with an odds ratio of 1.6 for daily cannabis use in patients with the C/C genotype.

These data provide important new information about the implicated SNP in AKT1 and its interaction with cannabis use. First, the extension of the patient sample to 1,222 participants, together with an independent replication by researchers from the UK (Di Forti et al., 2012a), considerably decreases the likelihood that the reported interaction is due to chance, as it was clearly shown that the risk for false positives in GxE research depends on (i) a priori probability of the polymorphism involved, (ii) sample size and statistical power, as well as (iii) the degree of 'direct' (as opposed to indirect or approximate) replication (Duncan and Keller, 2011b). This paper examined a SNP x cannabis interaction that stood out in a screening of a large list of candidate interactions in a previous study (van Winkel and Genetic Risk and Outcome of Psychosis (GROUP) Investigators, 2011), thus increasing prior probability, in the largest sample to date in this area of investigation. That sample size is important is illustrated by the results reported here, as separate analysis of the Belgian sample did not result in a positive finding despite reasonable sample size (n=533). Indeed, the fact that the reported direction and effect sizes were similar in both samples and that analysis of the combined sample did produce a significant association suggests that lack of power is the most likely reason for the 'non-replication' in the separate analysis of the Belgian sample, in agreement with our estimations showing a power of only .57 in this sample. It may also be important to consider the effect size: while larger than the effect sizes typically reported in genome-wide studies of schizophrenia, which are usually under 1.10 (Ripke et al., 2011; Shi et al., 2009), it is much smaller than the effect size reported in a previous study involving COMT Val158Met – interaction with cannabis (OR of 10.9 for individuals with adolescent cannabis use and the Val/Val genotype) (Caspi et al., 2005). Given the evidence for a polygenic contribution to schizophrenia, with possibly thousands of SNPs involved (Purcell et al., 2009b), we would argue that effect sizes for interactions will also most likely be under 2.0 (Decoster et al., 2012).

The effect size reported in the present study is also somewhat smaller than the OR of 2 for a history of cannabis use and the OR of 7 for daily use in AKT1 rs2494732 C/C

genotypes in the study of Di Forti and colleagues (Di Forti et al., 2012a). A possible reason for this difference may be situated in the THC-content of the cannabis that was used, which was reported to be very high in a previous study by the same researchers (Di Forti et al., 2009). It should also be noted that the confidence interval for daily use in Di Forti's study was large (OR 7.32, 95% CI 1.37 – 38.12), indicating the need for further follow-up in larger samples.

The second important finding reported in the present paper is the prospective validation of AKT1 rs2494732 – cannabis interaction in the sample of unaffected siblings. Although this analysis was conducted in the same sample of siblings that was used in the paper which first described this interaction (van Winkel and Genetic Risk and Outcome of Psychosis (GROUP) Investigators, 2011), it provides important new information about the nature of the interaction, as it suggests that the reported interaction is not the result of gene-environment correlation (i.e. high schizotypy leading to cannabis use in individuals with AKT1 rs2494732 C-alleles), but that the synergistic effects of genotype and exposure result in prospective increases in schizotypy. Nevertheless, given the lack of independent replication, further efforts to examine this finding in other cohorts at risk for psychosis remains necessary.

It should also be noted that AKT1 rs2494732 is not among the few top hits in the latest genome-wide association study of schizophrenia (Ripke et al., 2011). However, it is becoming increasingly clear that the risk for schizophrenia is polygenic, and that several thousands of SNPs are likely to be involved, only a handful of which have been identified (Sullivan et al., 2012). Nevertheless, 5 SNPs with genome-wide association to schizophrenia (in MIR-137, PCGEM1, CSMD1, MMP16 and CNNM2) were shown to be associated with the ratio of phosphorylated (active) versus total AKT1 in 115 healthy volunteers, individuals carrying the schizophrenia risk alleles displaying lower ratios (Balog et al., 2012). Similar results were found for rs1006737 in CACNA1C, which was also found to be associated with schizophrenia and bipolar disorder in genome-wide association studies (Green et al., 2010; Hamshere et al., 2012), leading the authors to speculate that "the complex array of proteins encoded by schizophrenia genes may converge on common intracellular molecular pathways that convert information from the environment to the biological system" (Balog et al., 2012). Our results may be in agreement with this interpretation, suggesting that genetic variation in AKT1 may be an important determinant in modifying the psychosis-inducing effects of environmental factors such as cannabis use (Di Forti et al., 2012a; van Winkel and Genetic Risk and Outcome of Psychosis (GROUP) Investigators, 2011) and obstetric complications (Nicodemus et al., 2008), possibly in concert with other dopamine-related SNPs (Blasi et al., 2011; Tan et al., 2011; Tan et al., 2008).

A number of limitations need to be considered when interpreting the data of the current paper. First and most importantly, only 21 of the 597 unaffected siblings were persistent cannabis users, limiting statistical power. On the other hand, we used a biological measure, thus increasing reliability, and the overall sample size may be considered large given the well-recognized difficulty of recruiting subjects at risk. Second, cannabis use was assessed twice, three years apart, meaning that subjects testing negative could also have used cannabis over the follow-up period without this being detected. Third, the results of this paper are not independent from those reported earlier (van Winkel and Genetic Risk and Outcome of Psychosis (GROUP) Investigators, 2011). Nevertheless, as argued above, they do provide evidence that the combination of cannabis use and AKT1 genotype predict prospective increases in schizotypy, in agreement with a causal explanation (i.e. gene-environment interaction rather than gene-environment correlation), as well as providing a greater degree of confidence that the reported interaction is actually true. Fourth, we used a case-only design, which relies on the independence assumption. However, the available data from previous reports concerning AKT1 rs2494732 and cannabis were in support of this assumption (Di Forti et al., 2012a; van Winkel and Genetic Risk and Outcome of Psychosis (GROUP) Investigators, 2011). It must be noted, however, that the sample sizes of the unaffected siblings (n=740) and healthy controls (GROUP, n=419, Di Forti, n=278) used in these papers may have resulted in moderate statistical power to detect an association. Assuming that lack of statistical power is the reason that no association is found in the unaffected individuals, rather than a genuine absence of association, this would point to gene-environment correlation as the most likely explanation for the association between AKT1 genotype and cannabis use. However, this interpretation is not compatible with the results of the prospective analysis of the unaffected siblings, which clearly argue against gene-environment correlation. In conclusion, while further efforts at replication and validation remain necessary, there is now reasonable evidence that AKT1 rs2494732 may influence the risk for psychosis in cannabis users.

REFERENCES

American Psychiatric Association, 2000. Diagnostic and Statistical Manual of Mental Disorders, 4th edition. Text revision. American Psychiatric Association, Washington, D.C.

Andreasen, N.C., Flaum, M., Arndt, S., 1992. The Comprehensive Assessment of Symptoms and History (CASH). An instrument for assessing diagnosis and psychopathology. Archives of general psychiatry 49(8), 615-623.

Arguello, P.A., Gogos, J.A., 2008. A signaling pathway AKTing up in schizophrenia. J Clin Invest 118(6), 2018-2021.

Balog, Z., Kiss, I., Keri, S., 2012. Five new schizophrenia loci may converge on the same cellular mechanism: the AKT pathway. The American journal of psychiatry 169(3), 335.

Blasi, G., Napolitano, F., Ursini, G., Taurisano, P., Romano, R., Caforio, G., Fazio, L., Gelao, B., Di Giorgio, A., Iacovelli, L., Sinibaldi, L., Popolizio, T., Usiello, A., Bertolino, A., 2011. DRD2/AKT1 interaction on D2 cAMP independent signaling, attentional processing, and response to olanzapine treatment in schizophrenia. Proc Natl Acad Sci U S A 108(3), 1158-1163.

Caspi, A., Moffitt, T.E., Cannon, M., McClay, J., Murray, R., Harrington, H., Taylor, A., Arseneault, L., Williams, B., Braithwaite, A., Poulton, R., Craig, I.W., 2005. Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. Biological psychiatry 57(10), 1117-1127.

Clayton, D., Hills, M., 1993. Wald tests. Oxford University Press., Oxford.

De Hert, M., van Winkel, R., Silic, A., Van Eyck, D., Peuskens, J., 2011a. Physical health management in psychiatric settings. Eur Psychiatry 25 Suppl 2, S22-28.

De Hert, M., Wampers, M., Jendricko, T., Franic, T., Vidovic, D., De Vriendt, N., Sweers, K., Peuskens, J., van Winkel, R., 2011b. Effects of cannabis use on age at onset in schizophrenia and bipolar disorder. Schizophr Res 126(1-3), 270-276.

Decoster, J., Van Os, J., Kenis, G., Henquet, C., Peuskens, J., De Hert, M., van Winkel, R., 2011. Age at onset of psychotic disorder: cannabis, BDNF Val66Met, and sex-specific models of gene-environment interaction. Am J Med Genet B Neuropsychiatr Genet 156B(3), 363-369.

Decoster, J., Van Os, J., Myin-Germeys, I., De Hert, M., van Winkel, R., 2012. Genetic Variation Underlying Psychosis-inducing Effects of Cannabis: Critical Review and Future Directions. Curr Pharm Des 18(32), 5015-5023.

Di Forti, M., Iyegbe, C., Sallis, H., Kolliakou, A., Falcone, A., Papparelli, A., Siriani, M., La Cascia, C., Stilo, S.A., Reis Marques, T., Handley, R., Mondelli, V., Dazzan, P., Pariante, C.M., David, A.S., Morgan, C., Powell, J., Murray, R.M., 2012. Confirmation that the AKT1 (rs2494732) genotype influences the risk of psychosis in cannabis users. Biol Psychiatry 72(10), 811-816.

Di Forti, M., Morgan, C., Dazzan, P., Pariante, C.M., Mondelli, V., Marques, T.R., Handley, R., Luzi, S., Russo, M., Paparelli, A., Butt, A., Stilo, S.A., Wiffen, B., Powell, J., Murray, R.M., 2009. High-potency cannabis and the risk of psychosis. Br J Psychiatry 195(6), 488-491.

Duncan, L.E., Keller, M.C., 2011. A critical review of the first 10 years of candidate gene-by-environment interaction research in psychiatry. The American journal of psychiatry 168(10), 1041-1049.

Franke, T.F., 2008. PI3K/Akt: getting it right matters. Oncogene 27, 6473-6488.

Freyberg, Z., Ferrando, S.J., Javitch, J.A., 2010. Roles of the Akt/GSK-3 and Wnt signaling pathways in schizophrenia and antipsychotic drug action. Am J Psychiatry 167(4), 388-396.

Genetic Risk and Outcome of Psychosis (GROUP) Investigators, 2011. Evidence that Familial Liability for Psychosis is Expressed as Differential Sensitivity to Cannabis: an Analysis of Patient-Sibling and Sibling-Control Pairs. Arch Gen Psychiatry 68(2), 138-147.

Green, E.K., Grozeva, D., Jones, I., Jones, L., Kirov, G., Caesar, S., Gordon-Smith, K., Fraser, C., Forty, L., Russell, E., Hamshere, M.L., Moskvina, V., Nikolov, I., Farmer, A., McGuffin, P., Holmans, P.A., Owen, M.J., O'Donovan, M.C., Craddock, N., 2010. The bipolar disorder risk allele at CACNA1C also confers risk of recurrent major depression and of schizophrenia. Mol Psychiatry 15(10), 1016-1022.

Hamshere, M., Walters, J., Smith, R., Richards, A.L., Green, E., Grozeva, D., Jones, I., Forty, L., Jones, L., Gordon-Smith, K., Riley, B., O'Neill, T., Kendler, K.S., Sklar, P., Purcell, S., Kranz, J., Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium, Welcome Trust Case Control Consortium+ (WTCCC+), Welcome Trust Case Control Consortium 2 (WTCCC2), Morris, D.W., Gill, M., Holmans, P., Craddock, N., Corvin, A., Owen, M., O'Donovan M, C., 2012. Genome-wide significant

associations in schizophrenia to ITIH3/4, CACNA1C and SDCCAG8, and extensive replication of associations reported by the Schizophrenia PGC. Mol Psychiatry, doi: 10.1038/mp.2012.1067.

Henquet, C., Murray, R., Linszen, D., van Os, J., 2005. The environment and schizophrenia: the role of cannabis use. Schizophrenia bulletin 31(3), 608-612.

Kendler, K.S., Lieberman, J.A., Walsh, D., 1989. The Structured Interview for Schizotypy (SIS): a preliminary report. Schizophrenia bulletin 15(4), 559-571.

Khoury, M.J., Flanders, W.D., 1996. Nontraditional epidemiologic approaches in the analysis of gene-environment interaction: case-control studies with no controls! Am J Epidemiol 144(3), 207-213.

Kuepper, R., Van Os, J., Lieb, R., Wittchen, H.U., Hofler, M., Henquet, C., 2011. Continued cannabis use and risk of incidence and persistence of psychotic symptoms: 10 year follow-up cohort study. Bmj 342, d738.

Moore, T.H., Zammit, S., Lingford-Hughes, A., Barnes, T.R., Jones, P.B., Burke, M., Lewis, G., 2007. Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. Lancet 370(9584), 319-328.

Murcray, C.E., Lewinger, J.P., Gauderman, W.J., 2009. Gene-environment interaction in genome-wide association studies. American journal of epidemiology 169(2), 219-226.

Nicodemus, K.K., Marenco, S., Batten, A.J., Vakkalanka, R., Egan, M.F., Straub, R.E., Weinberger, D.R., 2008. Serious obstetric complications interact with hypoxia-regulated/vascular-expression genes to influence schizophrenia risk. Mol Psychiatry 13(9), 873-877.

Ozaita, A., Puighermanal, E., Maldonado, R., 2007. Regulation of PI3K/Akt/GSK-3 pathway by cannabinoids in the brain. J Neurochem 102(4), 1105-1114.

Purcell, S.M., Wray, N.R., Stone, J.L., Visscher, P.M., O'Donovan, M.C., Sullivan, P.F., Sklar, P., Purcell Leader, S.M., Stone, J.L., Sullivan, P.F., Ruderfer, D.M., McQuillin, A., Morris, D.W., O'Dushlaine, C.T., Corvin, A., Holmans, P.A., O'Donovan, M.C., Sklar, P., Wray, N.R., Macgregor, S., Sklar, P., Sullivan, P.F., O'Donovan, M.C., Visscher, P.M., Gurling, H., Blackwood, D.H., Corvin, A., Craddock, N.J., Gill, M., Hultman, C.M., Kirov, G.K., Lichtenstein, P., McQuillin, A., Muir, W.J., O'Donovan, M.C., Owen, M.J., Pato, C.N., Purcell, S.M., Scolnick, E.M., St Clair, D., Stone, J.L., Sullivan, P.F., Sklar Leader, P., O'Donovan, M.C., Kirov, G.K., Craddock, N.J., Holmans, P.A., Williams, N.M., Georgieva, L., Nikolov, I., Norton, N., Williams, H., Toncheva, D., Milanova, V., Owen, M.J., Hultman, C.M., Lichtenstein, P., Thelander, E.F., Sullivan, P., Morris, D.W., O'Dushlaine, C.T., Kenny, E., Quinn, E.M., Gill, M., Corvin, A., McQuillin, A., Choudhury, K., Datta, S., Pimm, J., Thirumalai, S., Puri, V., Krasucki, R., Lawrence, J., Quested, D., Bass, N., Gurling, H., Crombie, C., Fraser, G., Leh Kuan, S., Walker, N., St Clair, D., Blackwood, D.H., Muir, W.J., McGhee, K.A., Pickard, B., Malloy, P., Maclean, A.W., Van Beck, M., Wray, N.R., Macgregor, S., Visscher, P.M., Pato, M.T., Medeiros, H., Middleton, F., Carvalho, C., Morley, C., Fanous, A., Conti, D., Knowles, J.A., Paz Ferreira, C., Macedo, A., Helena Azevedo, M., Pato, C.N., Stone, J.L., Ruderfer, D.M., Kirby, A.N., Ferreira, M.A., Daly, M.J., Purcell, S.M., Sklar, P., Purcell, S.M., Stone, J.L., Chambert, K., Ruderfer, D.M., Kuruvilla, F., Gabriel, S.B., Ardlie, K., Moran, J.L., Daly, M.J., Scolnick, E.M., Sklar, P., 2009. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature 460(7256), 748-752.

Ripke, S., Sanders, A.R., Kendler, K.S., Levinson, D.F., Sklar, P., Holmans, P., Lin, D., Duan, J., Ophoff, R.A., Andreassen, O.A., Scolnick, E.M., Cichon, S., St Clair, D., Corvin, A., Gurling, H., Werge, T., Rujescu, D., Blackwood, D., Pato, C.N., Malhotra, A.K., Purcell, S., Dudbridge, F., Neale, B., Rossin, L., Visscher, P.M., Posthuma, D., Ruderfer, D.M., Fanous, A., Stefansson, H., Steinberg, S., Mowry, B., Golimbet, V., De Hert, M., Jonsson, E.G., Bitter, I., Pietilainen, O.P., Collier, D., Tosato, S., Agartz, I., Albus, M., Alexander, M., Amdur, R., Amin, F., Bass, N., Bergen, S., Black, D.W., Borglum, A.D., Brown, M., Bruggeman, R., Buccola, N.G., Byerley, W., Cahn, W., Cantor, R.M., Carr, V., Catts, S., Choudhury, K., Cloninger, C.R., Cormican, P., Craddock, N., Danoy, P., Datta, S., De Haan, L., Demontis, D., Dikeos, D.G., Djurovic, S., Donnelly, P., Donohoe, G., Duong, L., Dwyer, S., Fink-Jensen, A., Freedman, R., Freimer, N.B., Friedl, M., Georgieva, L., Giegling, I., Gill, M., Glenthoj, B., Godard, S., Hamshere, M., Hansen, M., Hansen, T., Hartmann, A., Henskens, F., Hougaard, D., Hultman, C.M., Ingason, A., Jablensky, A., Jakobsen, K.D., Jay, M., Jurgens, G., Kahn, R.S., Keller, M.C., Kenis, G., Kenny, E., Kim, Y.S., Kirov, G., Konnerth, H., Konte, B., Krabbendam, L., Krasucki, R., Lasseter, V.K., Laurent, C., Lawrence, J., Lencz, T., Lerer, F., Liang, K.Y., Lichtenstein, P., Lieberman, J., Linszen, D., Lonnqvist, J., Loughland, C., Maclean, A.W., Maher, B.S., Maier, W., Mallet, J., Malloy, P., Mattheisen, M., Mattingsdal, M., McGhee, K.A., McGrath, J., McIntosh, A., McLean, D., McQuillin, A., Melle, I., Michie, P.T., Milanova, V., Morris, D.W., Mors, O., Mortensen, P.B., Moskvina, V., Muglia, P., Myin-Germeys, I., Nertney, D., Nestadt, G., Nielsen, J.B., Nikolov, I., Nordentoft, M., Norton, N., Nothen, M.M., O'Dushlaine, C.T., Olincy, A., Olsen, L., O'Neill, F.A., Orntoft, T., Owen, M., Pantelis, C., Papadimitriou, G.M., Pato, M.T., Peltonen, L., Petursson, H., Pickard, B., Pimm,

J., Pulver, A.E., Puri, V., Quested, D., Quinn, E.M., Rasmussen, H.B., Rethelyi, J.M., Ribble, R., Rietschel, M., Riley, B., Ruggeri, M., Schall, U., Schulze, T.G., Schwab, S.G., Scott, R., Shi, J., Sigurdsson, E., Silverman, J.M., Spencer, C.C., Stefansson, H., Strange, A., Strengman, E., Stroup, T.S., Suvisaari, J., Terenius, L., Thirumalai, S., Thygesen, J., Timm, S., Toncheva, D., van den Oord, E., Van Os, J., van Winkel, R., Veldink, J., Walsh, D., Wang, A.G., Wiersma, D., Wildenauer, D.B., Williams, H., Williams, N., Wormley, B., Zammit, S., Sullivan, P., O'Donovan M, C., Daly, M.J., Gejman, P.V., Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium, 2011. Genome-wide association study identifies five new schizophrenia loci. Nat Genet 43(10), 969-976.

Sanchez, M.G., Ruiz-Llorente, L., Sanchez, A.M., Diaz-Laviada, I., 2003. Activation of phosphoinositide 3-kinase/PKB pathway by CB(1) and CB(2) cannabinoid receptors expressed in prostate PC-3 cells. Involvement in Raf-1 stimulation and NGF induction. Cell Signal 15(9), 851-859.

Shi, J., Levinson, D.F., Duan, J., Sanders, A.R., Zheng, Y., Pe'er, I., Dudbridge, F., Holmans, P.A., Whittemore, A.S., Mowry, B.J., Olincy, A., Amin, F., Cloninger, C.R., Silverman, J.M., Buccola, N.G., Byerley, W.F., Black, D.W., Crowe, R.R., Oksenberg, J.R., Mirel, D.B., Kendler, K.S., Freedman, R., Gejman, P.V., 2009. Common variants on chromosome 6p22.1 are associated with schizophrenia. Nature 460(7256), 753-757.

Sullivan, P., Daly, M.J., O'Donovan M, C., 2012. Genetic architectures of psychiatric disorders: the emerging picture and its implications. Nat Rev Genet 13, 537-551.

Tan, H., Chen, A., Chen, Q., Browne, L.B., Verchinski, B., Kolachana, B., Zhang, F., Apud, J., Callicott, J.H., Mattay, V., Weinberger, D.R., 2011. Epistatic interactions of AKT1 on human medial temporal lobe biology and pharmacogenetic implications. Mol Psychiatry, doi: 10.1038/mp.2011.1091.

Tan, H.Y., Nicodemus, K.K., Chen, Q., Li, Z., Brooke, J.K., Honea, R., Kolachana, B.S., Straub, R.E., Meyer-Lindenberg, A., Sei, Y., Mattay, V.S., Callicott, J.H., Weinberger, D.R., 2008. Genetic variation in AKT1 is linked to dopamine-associated prefrontal cortical structure and function in humans. J Clin Invest 118(6), 2200-2208.

van Winkel, R., Genetic Risk and Outcome of Psychosis (GROUP) Investigators, 2011. Family-based Analysis of Genetic Variation underlying Psychosis-Inducing Effects of Cannabis: Sibling Analysis and Proband Follow-Up. Archives of general psychiatry 68(2), 148-157.

van Winkel, R., van Beveren, J.M., Genetic Risk and Outcome of Psychosis (GROUP) Investigators, 2011. AKT1 moderation of cannabis-induced cognitive alterations in psychotic disorder. Neuropsychopharmacology 36(12), 2529-2537.

Vollema, M.G., Ormel, J., 2000. The reliability of the structured interview for schizotypy-revised. Schizophr Bull 26(3), 619-629.

Wing, J.K., Babor, T., Brugha, T., Burke, J., Cooper, J.E., Giel, R., Jablenski, A., Regier, D., Sartorius, N., 1990. SCAN. Schedules for Clinical Assessment in Neuropsychiatry. Archives of general psychiatry 47(6), 589-593.

Chapter Five

Genetic Variation Underlying Psychosis-inducing Effects of Cannabis: Critical Review and Future Directions.

Jeroen Decoster[1,2], Jim van Os[1, 3], Inez Myin-Germeys[1], Marc De Hert[2], Ruud van Winkel[1,2]

Current Pharmaceutical Design, Volume 18, Issue 32, 2012, Pages 5015-5023

[1] Dept. of Psychiatry and Psychology, School for Mental Health and Neuroscience, EURON, Maastricht University Medical Centre, The Netherlands

[2] University Psychiatric Centre Catholic University Louvain, campus Kortenberg, Belgium

[3] King's College London, King's Health Partners, Department of Psychosis Studies, Institute of Psychiatry, London SE5 8AF, UK

ABSTRACT

Cannabis use is associated with an increased risk for psychotic disorder, yet most cannabis users do not develop psychosis, suggesting that other factors are also involved.

This paper reviews the available evidence suggesting that differential sensitivity to the psychosis-inducing effects of cannabis may be related to underlying genetic liability.

There is robust evidence that persons at psychometric risk for psychosis are most vulnerable to display psychotic symptoms subsequent to the use of cannabis. Multiple studies have also found that persons at familial risk for psychosis have an increased sensitivity to the effects of cannabis. Together, these findings support the concept of a biological interaction between cannabis use and one's underlying genetic vulnerability.

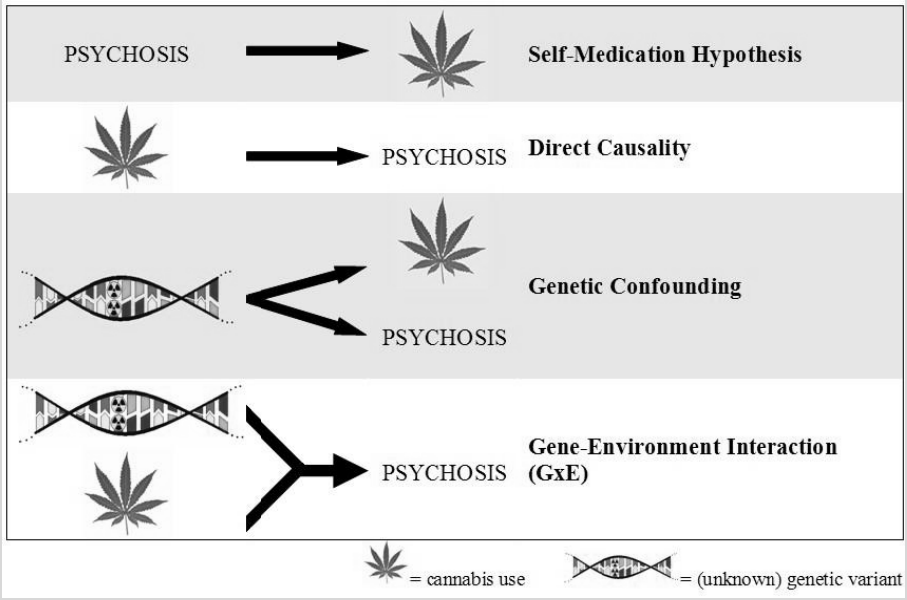
At the molecular-genetic level, however, few (if any) interactions have been consistently replicated, although a reported interaction with variation in AKT1 is promising and deserves further follow-up. The apparent lack of consistent replication can be ascribed to problems of initial gene selection, statistical power, a bias towards positive results and insufficient attempts at true replication, leading to the conclusion that increased sample sizes, greater density of genetic markers and a stronger focus on true replication are necessary.

The major challenge for molecular-genetic gene-environment interaction research will be to combine the agnostic detection of disorder-associated genetic variants from genome-wide studies with the hypothesis-based approach from epidemiological and neurobiological studies. Possible strategies for future cannabis interaction studies are discussed.

1. INTRODUCTION

Epidemiological studies and meta-analytic work have consistently shown an association between psychotic disorder and cannabis use, with a two-fold increased risk for psychotic disorder associated with the use of relevant amounts of cannabis (Arseneault et al., 2004; Henquet et al., 2005b; Moore et al., 2007). Some authors have argued that emerging psychotic symptoms may lead to cannabis use as a way of dealing with these symptoms, also referred to as the 'self-medication hypothesis' (Goswami et al., 2004; Hambrecht and Hafner, 1996), while others have argued in favour of a causal contribution of cannabis use (Hambrecht and Hafner, 1996; Kuepper et al., 2011b). It has also been proposed that the observed association may be explained by residual, non-measured (genetic) confounding (Macleod et al., 2004) (for schematic overview, see Figure 1). A number of studies have addressed the directionality of the association between cannabis use and psychosis in longitudinal designs. Henquet and colleagues (Henquet et al., 2005a) found no significant evidence for self-medication but did find a significant association between cannabis use at baseline and psychotic symptoms at 4-year follow-up in 2,437 individuals from the general population aged between 14 and 24 years. Fergusson and co-workers drew the same conclusions from a 7-year follow-up (from age 18 to 25) of a New-Zealand birth-cohort (n=1,055)(Fergusson et al., 2005). These findings were recently supported by

Figure 1
Possible mechanisms explaining association between cannabis use and psychosis



Kuepper and colleagues in a 10-year follow-up of 1,923 individuals from the general population (aged 14-24 at baseline) (Kuepper et al., 2011b). In this study, the authors found that in individuals who had no psychotic symptoms and no cannabis use at baseline, incident cannabis use at the 4-year follow-up measurement increased the risk of later incident psychotic symptoms at 9-year follow-up, while there was no evidence for self-medication effects (Kuepper et al., 2011b). However, Ferdinand and co-workers found a more complex bi-directional temporal association between cannabis use and psychotic symptoms in a 14-year follow-up of a general population sample of 1,580 individuals interviewed about lifetime cannabis use (aged 4 – 16 at baseline) (Ferdinand et al., 2005) as did McGrath and colleagues in a birth-cohort study of 3,801 individuals assessed at age 14 and 21 years (McGrath et al., 2010a). Importantly, the latter study also included a sibling-pair analysis (228 pairs) confirming the association between duration since first cannabis use (as proxy for age at first cannabis exposure) and incident delusional-like experiences in non-affected sibling-pairs. The sibling analysis reduces the likelihood that the reported association may be explained by unmeasured residual confounding, since many of the unmeasured potential confounders can be assumed to be similar for both siblings.

In addition, Veling and colleagues addressed the issue of possible genetic confounding in a study examining cannabis use in 100 patients, their unaffected siblings (n=63) and 100 healthy controls (matched for age, gender and ethnicity) (Veling et al., 2008). In this sample the healthy siblings displayed similar rates of cannabis use as did the healthy controls, arguing against the possibility that a genetic predisposition for schizophrenia also leads to a genetic predisposition for cannabis use. Similarly, the Genetic Risk and Outcome in Psychosis (GROUP) investigators did not find evidence for genetic confounding, using a cross-sibling, cross-trait design investigating sensitivity to the psychotomimetic effect of cannabis in a sample of 1,120 patients with psychotic disorder, 1,057 siblings of these patients and 590 community controls (Genetic Risk and Outcome in Psychosis (GROUP) Investigators, 2011). By contrast, Smith and colleagues did find higher rates of cannabis use in 53 non-psychotic sibling of patients with schizophrenia, as compared to 75 siblings of community controls (Smith et al., 2008).

Taken together, most of the available evidence points to a direct risk-increasing effect associated with the use of cannabis. Although some studies have found an indication of self-medication or genetic confounding, these, even if confirmed, cannot entirely explain the association between cannabis and psychosis.

Any causal effect of cannabis may be rather modest, however, since only a minority of cannabis users develops subclinical psychotic symptoms (Freeman et al., 2010a; van Os

et al., 2009) and an even smaller subsample will develop a clinical psychotic disorder (Tandon et al., 2008b) despite high rates of cannabis use in Western countries, with up to 50% of all adolescents having used cannabis at least once (Perkonigg et al., 2008). This apparently modest effect is likely explained by the fact that cannabis may only increase risk in interaction with other environmental and genetic risk factors (Van Winkel et al., 2010a) (Figure 1). For instance, urban environment (Kuepper et al., 2011a) and childhood trauma (Harley et al., 2010; Houston et al., 2008) were recently put forward as environmental factors that interact with cannabis use to increase the likelihood of psychotic symptoms. In addition, as will be argued in this paper, genetic factors may determine one's individual sensitivity to the psychosis-inducing effects of cannabis. These so-called 'gene-environment interactions' (GxE) may help to explain the observed differential sensitivity to cannabis (Henquet et al., 2008; van Os et al., 2010). Molecular gene-environment interactions (at the level of individual genes or single nucleotide polymorphisms (SNPs)) may only be valid and relevant, however, given evidence that one's underlying genetic vulnerability for psychosis is indeed associated with sensitivity to the effects of cannabis.

2. OVERVIEW OF THE LITERATURE

2.1. Vulnerable individuals (G) and cannabis use (E): differential sensitivity (GxE)?

Underlying genetic vulnerability to psychotic disorder is currently not directly measurable and remains latent in many individuals. Two approaches that attempt to model liability to psychosis, at the group level, use (i) familial risk, or (ii) psychometric risk as a proxy of the underlying genetic risk. The use of familial risk as a proxy of psychosis liability is based on the observation that the risk to develop a psychotic disorder increases as the degree of genetic affinity with an affected family member increases (Kendler et al., 1993). Adoption studies have established that familial liability for psychosis has a clear genetic component (Tandon et al., 2008b), and heritability rates of schizophrenia are estimated at around 60% (Lichtenstein et al., 2009).

In addition to familial risk, many researchers have used psychometric psychosis liability as a proxy for the underlying genetic vulnerability. Psychometric psychosis liability refers to the level of subtle psychotic experiences, which are seen as the behavioural expression of genetic vulnerability in non-patients. It can be assessed by means of self-report questionnaires (the Community Assessment of Psychic Experiences scale (CAPE) (Konings et al., 2006), the Symptom Checklist-90-R (SCL-90-R) (Derogatis, 1983), and the Schizotypal Personality Questionnaire (SPQ) (Raine, 1991)) or structured interview (Structured Interview for Schizotypy-Revised (SIS-R) (Vollema and Ormel, 2000)).

Earlier research provides arguments to assume that psychometric psychosis liability in the general population reflects a genetic liability that is coherent with the genetic vulnerability in patients with a clinical psychosis phenotype (Henquet et al., 2008; van Os et al., 2009).

These findings imply that the use of familial or psychometric risk to examine GxE on psychosis-related outcomes may be an informative and valid approach (van Os et al., 2008a). Psychosis-related outcomes already examined in GxE designs include the development of psychotic symptoms, but also other phenotypes such as cognition and neurobiological alterations.

2.1.1. Psychotic symptoms

In the previously cited study by Henquet and colleagues the association between cannabis use and incident psychotic symptoms was significantly stronger in those at psychometric risk for psychosis (Henquet et al., 2005a). In a sample of 532 university students, Barkus and Lewis found a positive correlation between psychometric psychosis liability and psychotic symptoms experienced during and after cannabis use (Barkus and Lewis, 2008). This finding was replicated in a pooled sample of 431 young cannabis users by Stirling and colleagues (Stirling et al., 2008). Mason and colleagues compared the level of psychotic symptoms in 142 controls without cannabis use with the symptoms in 140 cannabis-users immediately after cannabis use in their familiar environment (Mason et al., 2009). A second measurement of psychotic symptoms was performed 3 days later and after at least 24 hours of abstinence in the cannabis users. Higher levels of psychotic symptoms were reported for cannabis users at increased psychometric psychosis liability in reaction to acute cannabis use, but also when not acutely intoxicated (Mason et al., 2009). Furthermore, in a sample of 79 cannabis-using undergraduate university students, Verdoux and colleagues used the Experience Sampling Method (ESM) to investigate cannabis use and psychotic symptoms in daily life (Verdoux et al., 2003) and similarly found that the acute effects of cannabis were greater with increasing psychometric psychosis liability.

Using familial risk as the proxy of underlying genetic vulnerability, Hollis compared mental health functioning in relation to cannabis use in three groups of young people: non-psychotic siblings of adolescents with schizophrenia (n=36), 25 adolescents with Attention Deficit Hyperactivity Disorder (ADHD) (n=25) and 72 healthy controls (Hollis et al., 2008). In this study an association between cannabis use and mental health disturbance was specific for the group at high familial liability for psychosis. The authors concluded that this subgroup was particularly vulnerable to the detrimental effects of cannabis use (Hollis et al., 2008). In addition, the Genetic Risk and Outcome

in Psychosis (GROUP) Investigators recently demonstrated an increased sensitivity to cannabis associated with familial risk for psychosis, using a sibling-control and cross-sibling design (1,120 patients with psychotic disorder, 1,057 siblings of these patients and 590 community controls) (Genetic Risk and Outcome in Psychosis (GROUP) Investigators, 2011).

2.1.2. Cognition

As described above, there is a wealth of evidence showing that psychosis liability contributes to differential sensitivity at the level of (subclinical) psychotic symptoms. Research on possible differential effects of cannabis use on cognition remains scarce and is complicated by the fact that the relationship between cannabis use, cognition and psychosis is unclear. Most studies agree that acute cannabis use is associated with cognitive impairments in the short-term (Morrison et al., 2009; Ramaekers et al., 2009). With regard to the possible long-term consequences, however, recent meta-analyses reported better cognition in cannabis-using patients (Rabin et al., 2011; Yücel et al., 2010). Several explanations for superior cognitive abilities in cannabis-using patients have been put forward, including possible neuroprotective effects of cannabis in individuals developing psychotic symptoms (Jockers-Scherubl et al., 2007) and a selective mechanism of causal contribution of cannabis, such that persons with less neurocognitive impairment make a transition to psychotic disorder that they would not have made in the absence of cannabis use (Yücel et al., 2010).

In an experimental design with intravenous delta-9-tetrahydrocannabinol (THC) administration in 13 patients with schizophrenia and 22 healthy controls (D'Souza et al., 2005; D'Souza et al., 2004), patients were found to be more vulnerable to the acute effects of THC on learning and recall. However, another experimental study with controlled THC-exposure in 30 patients with a psychotic disorder, 12 relatives and 32 healthy controls was unable to demonstrate differential effects of THC on cognitive measures as a function of psychometric psychosis liability in these individuals (Henquet et al., 2006).

Concerning the possible long-term consequences of cannabis use, Jockers-Scherübl et al suggested a different effect on cognitive function in schizophrenia patients and healthy controls (Jockers-Scherubl et al., 2007). However, this conclusion was based on the results of only a few of the administered neuropsychological tests, in a study sample of only 39 patients (19 users) and 39 controls (18 users), the results of which did not survive control for multiple testing. Scholes and Martin-Iverson only found a subtle difference in cognitive performance between cannabis users and non-users in both healthy individuals (n=71) and patients with schizophrenia (n=71) (Scholes and

Martin-Iverson, 2010). They reported no differential effects between both groups. Similarly, Meijer and colleagues (Meijer et al., 2011) were unable to find a significant interaction between cannabis use and group status on cognitive functioning in a large cross-sectional sample of 956 patients with non-affective psychosis, 953 healthy siblings and 554 controls. Given these findings, it can be concluded that, at the level of cognition, the evidence for differential sensitivity to cannabis associated with pre-existing liability is relatively inconsistent, for both the acute as well as the longer-term effects.

2.1.3. Neurobiological changes

Several imaging studies investigated the influence of cannabis use on brain morphology in schizophrenia patients and reported greater reduction of grey matter density in cerebral regions known to have a high density of cannabinoid receptors (CB1R) in cannabis-using patients (Bangalore et al., 2008; James et al., 2011; Rais et al., 2010; Szeszko et al., 2007), although this was not always found (Cahn et al., 2004). Ambiguous results were reported with regard to the effect of cannabis use on white matter integrity (Dekker et al., 2010; James et al., 2011). Since no cannabis-using healthy controls were included in these studies, they do not allow for the examination of possible differential sensitivity to cannabis at the level of brain morphology. However, in a recent study, Habets and colleagues used T1-weighted MRI scans to measure cortical thickness in 88 patients with schizophrenia, 98 non-psychotic siblings of a schizophrenia patient and 87 healthy controls, and examined the association between cortical thickness and cannabis use (Habets et al., 2011). A group X cannabis interaction was found, showing a dose dependent decrease in cortical thickness associated with the use of cannabis in patients and siblings, but not in control subjects, suggesting differential sensitivity (Habets et al., 2011).

Earlier research found that the concentration of the endocannabinoid anandamide in cerebrospinal fluid (CSF) may be elevated in antipsychotic-naïve psychotic patients (Giuffrida et al., 2004) and a possible protective role of this increased concentration in early psychosis was hypothesized (Koethe et al., 2009). In addition, Leweke and co-workers measured CSF anandamide levels in 44 first-episode, antipsychotic-naïve patients with schizophrenia and 81 healthy volunteers, among them 19 and 26 cannabis users, respectively (Leweke et al., 2007). The findings supported possible differential sensitivity to cannabis exposure, with down-regulation of CSF anandamide associated with cannabis use in patients, but not in healthy controls, suggesting that alterations of endocannabinoid signalling may underlie the mechanism through which cannabis impacts mental health.

2.2. Molecular genetic findings

Despite the evidence for interaction between pre-existing vulnerability and cannabis use, research efforts to discover the underlying molecular genetic variation are relatively scarce and mainly limited to a number of specific candidate genes.

2.2.1. Psychotic symptoms

Caspi and colleagues were the first to report a molecular genetic GxE with cannabis in psychosis (Caspi et al., 2005). Their study focused on a functional polymorphism (Val158Met or rs4680) in the catechol-O-methyltransferase (COMT) gene. COMT is an enzymatic inactivator of dopamine and other mono-amines and is essential for dopamine signalling in the prefrontal cortex. The Val allele is associated with a 40% higher enzyme activity (Lachman et al., 1996; Lotta et al., 1995). Although COMT Val158Met is probably not associated with schizophrenia (Okochi et al., 2009), Caspi and colleagues found that the COMT polymorphism moderated the risk of developing schizophreniform disorder at age 26 following adolescent-onset cannabis use in a birth-cohort of 1,037 individuals, Val carriers displaying a higher risk (Caspi et al., 2005).

Following Caspi's initial paper, several studies focused on this particular COMT x cannabis interaction. Using an experimental design in which patients with a psychotic disorder, their relatives and healthy controls were exposed to THC, Henquet and co-workers tried to further validate this finding (Henquet et al., 2006). In this study, examining acute effects of cannabis, the same interaction between COMT polymorphism and cannabis was found, but only in individuals with a pre-existing psychometric psychosis liability. Henquet and colleagues also demonstrated this conditional (COMT x cannabis x psychosis liability) interaction in an ESM-study with 31 patients and 25 controls. In this study, carriers of the Val allele with prior evidence of psychometric psychosis liability showed an increase in hallucinations after acute cannabis exposure in daily life (Henquet et al., 2009a).

Zammit and colleagues used a case-only design in a sample of 493 schizophrenia patients in order to examine possible COMT x cannabis interaction (Zammit et al., 2007). A case-only design determines presence of GxE on the basis of an association between SNP and exposure, while assuming independence between SNP and exposure in the general population (Khoury and Flanders, 1996). However, this study was unable to find an association between COMT Val158Met and cannabis use. In addition, the same study could not find an association between cannabis use and (i) 2 other SNPs in COMT (rs737865 and rs165599) and (ii) rs1049353 in the cannabinoid receptor CB1R (Zammit et al., 2007). Kantrowitz and colleagues also failed to replicate the

COMT rs4680 finding using a case-only design in a (markedly underpowered) sample of 92 patients with psychotic disorder (Kantrowitz et al., 2009). In contrast with the report of Caspi et al. (Caspi et al., 2005), Costas and co-workers (Costas et al., 2011) found in their case-only study with 748 patients with schizophrenia an association between lifetime cannabis use and (i) low activity haplotype defined by 5 SNPs in COMT and (ii) homozygosity for the Met (rather than Val) allele at rs4680. Finally, Zammit and colleagues (Zammit et al., 2011) studied the possible interaction between cannabis use and COMT in a longitudinal design with 2,630 individuals of a birth cohort, assessed at age 14 for cannabis use and at age 16 for incident psychotic symptoms. No interaction between COMT rs4680 and cannabis use was found. Also analyses with the rs6269-rs4818-rs4680 haplotype and additional SNPs (rs737865, rs2097603 and rs165599) revealed no interaction between COMT and cannabis use. The authors of this study also conducted 26 sensitivity analyses, using different measures of exposure, different samples and different designs. In all these analyses the main effect of cannabis (significantly associated with psychotic symptoms, in a dose-dependent way) remained relatively stable. By contrast, interactions between cannabis and COMT showed greater variation, even with opposite directions in different analyses (Zammit et al., 2011).

Given increasing concerns in the psychiatric genetics field about the risk of spurious findings associated with the use of the candidate gene approach, especially in the context of studies (i) examining the effects of single SNPs (ii) without a valid replication strategy and (iii) using liberal significance thresholds (Van Winkel et al., 2010a), van Winkel and the GROUP investigators tried to use a more systematic, yet hypothesis-driven approach towards gene-selection (van Winkel and GROUP Investigators, 2011). The authors examined possible interactions between 152 SNPs selected from 42 candidate genes and recent cannabis use in 740 unaffected siblings, and found 3 SNPs to survive correction for multiple testing, in AKT1 (2 SNPs) and LRRTM1 (1 SNP). Follow-up of these SNPs in the patients confirmed the presence of GxE between rs2494732 in AKT1 and cannabis using a case-only (801 patients with psychotic disorder), case-sibling and case-control (419 unrelated controls) design. Compared to those with the T/T genotype, individuals with a C/C genotype displayed a 2-fold increased risk of being diagnosed with a psychotic disorder when having used cannabis (van Winkel and GROUP Investigators, 2011). In a recent study, Bhattacharyya and co-workers, found a trend for a main effect of the AKT1 rs1130233 polymorphism (G-homozygotes vs A-carriers; in linkage disequilibrium with rs2494732) on the increase of psychotic symptoms induced by experimental cannabis administration in 35 healthy volunteers (placebo-controlled, within-subject design) (Bhattacharyya et al., 2012).

Also a main effect of the polymorphic 40-basepair variable number of tandem repeats (VNTR) in the 3' untranslated region (UTR) in the DAT1 gene (9-repeat vs 10-repeat allele) and an interaction between the AKT1 and the DAT1 polymorphisms was reported. The volunteers who were G homozygotes of AKT1 rs1130233 and also carriers of the 9-repeat allele of the DAT1 3'UTR VNTR (the GG/9-repeat carriers), had a greater increase in psychotic symptoms after cannabis than the others (Bhattacharyya et al., 2012).

2.2.2. Cognition

Although the evidence for differential sensitivity to cannabis with regard to cognition is inconsistent, as outlined above, a few reports attempted to identify molecular-genetic interactions with cannabis use, specifically following-up on previously reported interactions in COMT and AKT1. For example, COMT Val carriers were found to be more sensitive to the acute effects of cannabis in some domains of verbal memory and sustained attention, compared to Met allele carriers (Henquet et al., 2006). Only the interactions with regard to sustained attention (as measured by the Continuous Performance Test [CPT]) survived correction for multiple testing (Henquet et al., 2006). Building on the recent finding that AKT1 may be associated with a differential sensitivity to the psychosis-inducing effects of cannabis use (van Winkel and GROUP Investigators, 2011), van Winkel and colleagues studied the effects of this interaction on verbal memory and sustained attention in patients with psychotic disorder (van Winkel et al., 2011b). No interaction was found between AKT1 rs2494732 and cannabis use in 654 patients with a psychotic disorder in terms of verbal memory impairment. By contrast, in the CPT, patients with the C/C genotype and lifetime cannabis use (especially those with daily use) were slower and less accurate, whereas cannabis-using patients with the T/T genotype performed similar or even slightly better than non-using patients with the same genotype. This interaction was also apparent in patients with psychotic disorder who had not used cannabis in the 12 months preceding assessment, but was absent in the unaffected siblings of these patients and in healthy controls. In their recent study, Bhattacharyya and colleagues reported the same DAT1-AKT1 interaction, that was associated with the acute psychotogenic effects of cannabis, to be associated with cannabis-dependent changes in striatal and midbrain function during a verbal learning task inside a MRI scanner (Bhattacharyya et al., 2012). The effects of cannabis administration on activation in the striatum during encoding and in the midbrain during recall, as measured by the blood oxygen level-dependent (BOLD) haemodynamic response, were also greater in the GG/9-repeat carriers (Bhattacharyya et al., 2012).

2.2.3. Neurobiological changes

Recently, Ho and co-workers studied the impact of cannabis abuse/dependency and 12 SNPs in the cannabinoid receptor 1 gene (CB1R) on MRI measured white matter volume and performance in a comprehensive neurocognitive test battery in 235 patients with schizophrenia (Ho et al., 2011). They reported GxE between CB1R rs12720071 and cannabis abuse on white matter (WM) volume and neurocognition. Compared to A/A homozygotes, G-allele carrying patients had significantly smaller frontal and temporal WM volumes and for the parietal WM volumes this was only the case for cannabis-using G-allele carriers. The same interaction was found in problem solving skills, but not in other neurocognitive domains. This study based its conclusions on a relatively small proportion of patients with the heaviest cannabis use (52 out of 235) and did not include a control group to evaluate whether the reported effects were specific for patients with psychotic disorder.

2.2.4. Age at onset

In addition to the phenotypes discussed above, some studies have focused on age at onset of psychosis, following (i) robust evidence that cannabis use is associated with a 2.7 years earlier onset of psychosis (De Hert et al., 2011; Large et al., 2011) and (ii) evidence that age at onset of psychosis may be under considerable genetic control (Hare et al., 2010). This evidence furthers the possibility that gene-by-cannabis interactions may also play a role in determining age at onset of psychosis, which is potentially important given the known clinical and prognostic relevance of age at onset of psychosis (Crespo-Facorro et al., 2007; Rabinowitz et al., 2006).

In a sample of 80 patients with a psychotic disorder and 77 patients with a non-psychotic disorder (conduct, affective and personality disorders), the interaction between COMT Val158Met genotype and cannabis use was significantly associated with age at onset, but only for the patients with a psychotic disorder (Estrada et al., 2011). Val/Val-genotype cannabis users showed an earlier age at onset than Met carriers. The data also suggested that the interaction was particularly present in those who started cannabis use at a young age.

Another GxE study using age at onset of psychosis as primary outcome is the study of Decoster and colleagues (Decoster et al., 2011). Building on previous findings that a functional polymorphism in the brain-derived neurotrophic factor (BDNF) gene (Val66Met in rs6265) may be associated with age at onset in psychosis, with earlier onset in Met-carriers (Chao et al., 2008; Numata et al., 2006), and that cannabis use may directly influence BDNF-regulated physiological mechanisms (D'Souza et al., 2009; Derkinderen et al., 2003; Jockers-Scherubl et al., 2004), an interaction between

cannabis use and BDNF genotype was investigated in a cohort of 587 patients with a psychotic disorder. Since age at onset is known to be associated with gender (Larsen et al., 1996) and, furthermore, gender-dependent effects of BDNF (Raz et al., 2009; Shalev et al., 2009; Verhagen et al., 2010) and shared effects of BDNF and estrogen (Scharfman and MacLusky, 2006) were reported, a three-way BDNF x cannabis x gender interaction was hypothesized. A significant interaction between cannabis use and BDNF-genotype in females was demonstrated, although findings were based on a relatively small group of cannabis-using female patients (n=34).

3. CRITICAL REVIEW OF THE AVAILABLE EVIDENCE

Considering the available evidence regarding the gene-cannabis interaction, several things can be noted. First, there is robust evidence that persons at psychometric risk for psychosis are most vulnerable to display psychotic symptoms following cannabis use. In addition, multiple studies have confirmed that persons at familial risk for psychosis have an increased sensitivity to the effects of cannabis, in terms of psychotic symptoms. Together, these findings support the concept of a biological interaction between cannabis and a person‘s underlying genetic vulnerability. Less evidence is available regarding possible underlying neurobiological changes, although the available studies consistently support differential sensitivity to cannabis use in terms of neurobiology as well. For cognition, the reported findings are inconsistent.

At the molecular-genetic level, few (if any) interactions have been consistently replicated (see Table 1), although the reported interaction with AKT1 is promising and deserves further follow-up. Several reasons for this apparent lack of consistent replication can be identified, such as problems with gene selection, underpowered samples, lack of adequate attempts for replication and ‘pseudo-replications’, considerable heterogeneity in research designs and outcome measures.

3.1 Gene selection

It is increasingly clear that appropriate gene selection is a very challenging task. For example, a recent study showed that there was no evidence for enrichment of genetic variation implicated in the etiology of schizophrenia in the most studied candidate genes (e.g. COMT, NRG1, DTNBP1, etc)(Collins et al., 2011). Although gene selection may be somewhat easier for GxE research, as it is partly known how cannabis exerts its

Table 1
Overview of studies examining molecular gene x cannabis interactions, reported in clinical psychotic phenotype.

Genetic Variant (*Risk Genotype*)	**First Report**	**Replication attempts**	**Replication outcome**	**Indirect validation with studied phenotype[a]**
COMT rs4680 *(Val/Val)*	Caspi (Caspi et al., 2005); birth cohort study	Zammit (Zammit et al., 2007); case-only study	Negative	Henquet (Henquet et al., 2006); Acute effects of experimental cannabis exposure on psychotic symptoms and cognition (sustained attention, measured by CPT)
		Kantrowitz (Kantrowitz et al., 2009); case-only study	Negative	Henquet (Henquet et al., 2009a); Psychotic symptoms after cannabis use in daily life
		Costas (Costas et al., 2011); case-only study	Negative [b]	Estrada (Estrada et al., 2011); Age at onset of psychosis
		Zammit (Zammit et al., 2011); birth cohort study	Negative	
AKT1 rs2494732 *(C/C)*	van Winkel (van Winkel and GROUP Investigators, 2011); case-only, case-control and case-sibling study	/		van Winkel(van Winkel and GROUP Investigators, 2011); Schizotypy in unaffected siblings van Winkel (van Winkel et al., 2011b); Cognition (performance on CPT) Bhattacharyya (Bhattacharyya et al., 2012) [c]; Acute psychotogenic effects of cannabis, correlated with effects on midbrain and striatal function

[a] No attempts of indirect validation with negative finding were reported
[b] Costas and colleagues (Costas et al., 2011) reported an association between homozygosity for the Met allele and cannabis use
[c] Bhattacharyya and colleagues (Bhattacharyya et al., 2012) reported an association with rs1130233 which is in high linkage disequilibrium with rs2494732

effects in the brain, the lack of consistent interactions supports the view that gene selection, even in the context of GxE, is very difficult. A recent paper proposed to use a broad range of SNPs with either a reliable association to the disorder or a plausible relation to the underlying biology by which the environmental factor may exert its effects (Van Winkel et al., 2010a). The first attempt that used this strategy (van Winkel and GROUP Investigators, 2011), although limited by the fact that no genome-wide supported variants were included and the selected SNPs did not cover the entire genetic variation within the selected genes, resulted in the identification of the AKT1 x cannabis interaction. This finding, which was robust across different samples and study designs, may support the claim that such a strategy may be fruitful.

3.2 Statistical power and 'replication'

It is estimated that the detection of a novel interaction requires an up to four times larger sample than the detection of a main effect of similar magnitude (Thomas, 2010). Although one hopes for larger effect sizes associated with GxE than the Odds Ratios of 1.10 or lower reported in GWA (Genome Wide Association) studies, it is now evident that GxE studies have generally been underpowered (Burton et al., 2009; Duncan and Keller, 2011a; Luan et al., 2001). In addition, fuelled by reports about chance findings in random (i.e. simulated) datasets as long as enough statistical tests were conducted (Eaves, 2006; Sullivan, 2007), there are increasing concerns about undisclosed multiple statistical comparisons (post-hoc analyses or statistical 'fishing expeditions')(Munafo and Flint, 2009), resulting in a high prevalence of false positive chance findings (type I errors). Furthermore, as argued by Sullivan, once a false positive finding is reported and acclaimed, there is a high risk of propagating this false positive finding by approximate, indirect or pseudo-replication (Sullivan, 2007). This view was recently supported by Duncan and Keller, who showed clear evidence for publication bias in GxE research, favouring positive 'new' findings and positive replication attempts (Duncan and Keller, 2011a).

Finally, there is the issue of the many designs that can be used to demonstrate gene-environment interaction, both at the level of the clinical disorder as well as at the subclinical level, and the associated uncertainty about what actually constitutes 'true replication'. The often cited COMT x cannabis interaction (Caspi et al., 2005) may serve as a good example, since the initial report has never been replicated with the same outcome-measure, the same genotype (SNP or haplotype), the same direction of association, the same type of interaction (2-way or 3-way) or the same definition of environmental exposure (Zammit et al., 2011). Importantly, also studies that failed to

replicate the finding did not fulfil these criteria (Costas et al., 2011; Zammit et al., 2011; Zammit et al., 2007).

4. FUTURE DIRECTIONS FOR GxE RESEARCH

In order to understand the differential sensitivity to the psychosis-inducing effects of cannabis at the molecular-genetic level, the challenge is to deal with the shortcomings of previous research (See Box 1 for summary).

4.1 Adequate replication

It is disappointing that even the most studied gene-cannabis interaction (COMT Val158Met), has never been the subject of a true attempt at replication. It should be noted that the (negative) studies by Zammit and colleagues came closest to the original study design, in terms of phenotype under study (Zammit et al., 2007) or in terms of the prospective design (Zammit et al., 2011) (although it should be noted that the follow-up was short, from age 14 to age 16 and that the measure of psychosis outcome was uncertain as it was not associated with family history of psychotic disorder (Zammit et al., 2008)). Therefore, as also argued by Duncan and Keller (Duncan and Keller, 2011a), greater efforts at replication are necessary. We would argue that, in terms of replication, efforts to show relevance of any reported interaction at the level of the clinical phenotype are most important, whereas the subclinical phenotype may be an additional tool in the discovery of novel interactions, as large general population samples with detailed environmental info are available and these studies may have increased statistical power. In addition, studies of the subclinical phenotype may serve to further validate existing findings, although the focus should be on the clinical phenotype since only these studies are able to truly establish the clinical relevance of the reported interaction.

4.2 Larger samples and improved gene selection

The observations that GxE studies have generally been underpowered (Burton et al., 2009; Duncan and Keller, 2011a; Luan et al., 2001) and biased towards positive results (Duncan and Keller, 2011a) lead to the obvious conclusion that increased sample sizes and density of genetic markers are necessary, as has been the case in genetics in general (Cichon et al., 2009). While collaborative efforts are underway (EU-GEI, 2008), appropriate gene selection for GxE studies remains a considerable challenge. The major challenge in GxE research is to combine the agnostic detection of disorder-associated genetic variance from GWAS with the hypothesis-based approach from

Box 1
Future challenges in GxE research

Large samples

- Collaborative efforts

Appropriate gene selection

- Caution with (single) candidate gene approaches
- Possibility for broad SNP range approaches: SNPs associated with disorder or underlying biology by which the environmental factor may exert its effects
- Genes implicated in underlying pathways detected by gene ontology analyses
- GEWIS and subsequent pathway detection

Adequate replication

- Importance of replication in clinical phenotype
- Efforts for true replication of reported findings

epidemiological and neurobiological studies (van Os and Rutten, 2009). The strategy of using a broad range of SNPs with a reliable association to the disorder or SNPs that can be plausibly related to the underlying biology by which the environmental factor may exert its effects, tries to find a midway between hypothesis-based testing on the one hand and systematic reporting of all investigated markers and controlling for the associated number of statistical tests on the other, thus trying to avoid a bias towards positive results (van Winkel and GROUP Investigators, 2011; Van Winkel et al., 2010a). Application of this strategy using the first SNPs reliably associated with schizophrenia by genome-wide studies (Ripke et al., 2011) would be an interesting and uncomplicated first step, with the important advantage that the required sample sizes need not be very large. In addition, elegant tools and strategies were recently developed to detect disease-implicated biological pathways based on GWAS findings; the so-called gene ontology analyses, of which the applicability was also demonstrated for psychiatric disorders (Holmans et al., 2009; Poelmans et al., 2011; Weng et al., 2011). The detection of underlying pathways may enable a more directed and hypothesis-based selection of genes for further investigation of their role in the disease and possible interactions with cannabis.

A final strategy, although in need of statistical and conceptual refinement, is to enrich the agnostic GWAS strategy with an environmental risk factor (e.g. cannabis use) to agnostically investigate interaction effects: the so-called Gene-Environment Wide Interaction Studies (GEWIS)(Khoury and Wacholder, 2009). Several approaches have been proposed to deal with the large number of statistical tests and associated low

power for detecting genome-wide significant signals (Chatterjee and Wacholder, 2009; Kooperberg and Leblanc, 2008; Murcray et al., 2011; Murcray et al., 2009; Thomas, 2010). In addition to pathway-based approaches based on GWAS, GEWIS studies may also serve as the first step in the search for pathways associated with schizophrenia following, for example, cannabis use.

Further refinement of the proposed strategies and continued joint efforts, like the EU-GEI project (EU-GEI, 2008), will hopefully result in a better understanding of the molecular-genetic variation underlying sensitivity to the psychosis-inducing effects of cannabis within the foreseeable future.

REFERENCES

Arseneault, L., Cannon, M., Witton, J., Murray, R.M., 2004. Causal association between cannabis and psychosis: examination of the evidence. Br J Psychiatry 184, 110-117.

Bangalore, S.S., Prasad, K.M., Montrose, D.M., Goradia, D.D., Diwadkar, V.A., Keshavan, M.S., 2008. Cannabis use and brain structural alterations in first episode schizophrenia--a region of interest, voxel based morphometric study. Schizophr Res 99(1-3), 1-6.

Barkus, E., Lewis, S., 2008. Schizotypy and psychosis-like experiences from recreational cannabis in a non-clinical sample. Psychol Med 38(9), 1267-1276.

Bhattacharyya, S., Atakan, Z., Martin-Santos, R., Crippa, J.A., Kambeitz, J., Prata, D., Williams, S., Brammer, M., Collier, D.A., McGuire, P.K., 2012. Preliminary report of biological basis of sensitivity to the effects of cannabis on psychosis: AKT1 and DAT1 genotype modulates the effects of delta-9-tetrahydrocannabinol on midbrain and striatal function. Mol Psychiatry doi: 10.1038/mp.2011.187.

Burton, P.R., Hansell, A.L., Fortier, I., Manolio, T.A., Khoury, M.J., Little, J., Elliott, P., 2009. Size matters: just how big is BIG?: Quantifying realistic sample size requirements for human genome epidemiology. Int J Epidemiol 38(1), 263-273.

Cahn, W., Hulshoff Pol, H.E., Caspers, E., van Haren, N.E., Schnack, H.G., Kahn, R.S., 2004. Cannabis and brain morphology in recent-onset schizophrenia. Schizophr Res 67(2-3), 305-307.

Caspi, A., Moffitt, T.E., Cannon, M., McClay, J., Murray, R., Harrington, H., Taylor, A., Arseneault, L., Williams, B., Braithwaite, A., Poulton, R., Craig, I.W., 2005. Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. Biol Psychiatry 57(10), 1117-1127.

Chao, H.M., Kao, H.T., Porton, B., 2008. BDNF Val66Met variant and age of onset in schizophrenia. Am J Med Genet B Neuropsychiatr Genet 147B(4), 505-506.

Chatterjee, N., Wacholder, S., 2009. Invited commentary: efficient testing of gene-environment interaction. Am J Epidemiol 169(2), 231-233; discussion 234-235.

Cichon, S., Craddock, N., Daly, M., Faraone, S.V., Gejman, P.V., Kelsoe, J., Lehner, T., Levinson, D.F., Moran, A., Sklar, P., Sullivan, P.F., 2009. Genomewide association studies: history, rationale, and prospects for psychiatric disorders. Am J Psychiatry 166(5), 540-556.

Collins, A.L., Kim, Y., Sklar, P., O'Donovan, M.C., Sullivan, P.F., 2011. Hypothesis-driven candidate genes for schizophrenia compared to genome-wide association results. Psychol Med, 1-10.

Costas, J., Sanjuan, J., Ramos-Rios, R., Paz, E., Agra, S., Tolosa, A., Paramo, M., Brenlla, J., Arrojo, M., 2011. Interaction between COMT haplotypes and cannabis in schizophrenia: a case-only study in two samples from Spain. Schizophr Res 127(1-3), 22-27.

Crespo-Facorro, B., Pelayo-Teran, J.M., Perez-Iglesias, R., Ramirez-Bonilla, M., Martinez-Garcia, O., Pardo-Garcia, G., Vazquez-Barquero, J.L., 2007. Predictors of acute treatment response in patients with a first episode of non-affective psychosis: sociodemographics, premorbid and clinical variables. J Psychiatr Res 41(8), 659-666.

D'Souza, D.C., Abi-Saab, W.M., Madonick, S., Forselius-Bielen, K., Doersch, A., Braley, G., Gueorguieva, R., Cooper, T.B., Krystal, J.H., 2005. Delta-9-tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. Biol Psychiatry 57(6), 594-608.

D'Souza, D.C., Perry, E., MacDougall, L., Ammerman, Y., Cooper, T., Wu, Y.T., Braley, G., Gueorguieva, R., Krystal, J.H., 2004. The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. Neuropsychopharmacology 29(8), 1558-1572.

D'Souza, D.C., Pittman, B., Perry, E., Simen, A., 2009. Preliminary evidence of cannabinoid effects on brain-derived neurotrophic factor (BDNF) levels in humans. Psychopharmacology (Berl) 202(4), 569-578.

De Hert, M., Wampers, M., Jendricko, T., Franic, T., Vidovic, D., De Vriendt, N., Sweers, K., Peuskens, J., van Winkel, R., 2011. Effects of cannabis use on age at onset in schizophrenia and bipolar disorder. Schizophr Res 126(1-3), 270-276.

Decoster, J., van Os, J., Kenis, G., Henquet, C., Peuskens, J., De Hert, M., van Winkel, R., 2011. Age at onset of psychotic disorder: cannabis, BDNF Val66Met, and sex-specific models of gene-environment interaction. Am J Med Genet B Neuropsychiatr Genet 156B(3), 363-369.

Dekker, N., Schmitz, N., Peters, B.D., van Amelsvoort, T.A., Linszen, D.H., de Haan, L., 2010. Cannabis use and callosal white matter structure and integrity in recent-onset schizophrenia. Psychiatry Res 181(1), 51-56.

Derkinderen, P., Valjent, E., Toutant, M., Corvol, J.C., Enslen, H., Ledent, C., Trzaskos, J., Caboche, J., Girault, J.A., 2003. Regulation of extracellular signal-regulated kinase by cannabinoids in hippocampus. J Neurosci 23(6), 2371-2382.

Derogatis, J., 1983. SCL-90-R: Administration, Scoring, and Procedures Manual - II. Md: Clinical Psychometric Research, Towson.

Duncan, L.E., Keller, M.C., 2011. A Critical Review of the First 10 Years of Candidate Gene-by-Environment Interaction Research in Psychiatry. Am J Psychiatry 168(10), 1041-1049.

Eaves, L.J., 2006. Genotype x Environment interaction in psychopathology: fact or artifact? Twin Res Hum Genet 9(1), 1-8.

Estrada, G., Fatjo-Vilas, M., Munoz, M.J., Pulido, G., Minano, M.J., Toledo, E., Illa, J.M., Martin, M., Miralles, M.L., Miret, S., Campanera, S., Bernabeu, C., Navarro, M.E., Fananas, L., 2011. Cannabis use and age at onset of psychosis: further evidence of interaction with COMT Val158Met polymorphism. Acta Psychiatr Scand 123(6), 485-492.

EU-GEI, 2008. Schizophrenia aetiology: do gene-environment interactions hold the key? Schizophr Res 102(1-3), 21-26.

Ferdinand, R.F., Sondeijker, F., van der Ende, J., Selten, J.P., Huizink, A., Verhulst, F.C., 2005. Cannabis use predicts future psychotic symptoms, and vice versa. Addiction 100(5), 612-618.

Fergusson, D.M., Horwood, L.J., Ridder, E.M., 2005. Tests of causal linkages between cannabis use and psychotic symptoms. Addiction 100(3), 354-366.

Freeman, D., McManus, S., Brugha, T., Meltzer, H., Jenkins, R., Bebbington, P., 2010. Concomitants of paranoia in the general population. Psychol Med 41(5), 923-936.

Genetic Risk and Outcome in Psychosis (GROUP) Investigators, 2011. Evidence that familial liability for psychosis is expressed as differential sensitivity to cannabis: an analysis of patient-sibling and sibling-control pairs. Arch Gen Psychiatry 68(2), 138-147.

Giuffrida, A., Leweke, F.M., Gerth, C.W., Schreiber, D., Koethe, D., Faulhaber, J., Klosterkotter, J., Piomelli, D., 2004. Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. Neuropsychopharmacology 29(11), 2108-2114.

Goswami, S., Mattoo, S.K., Basu, D., Singh, G., 2004. Substance-abusing schizophrenics: do they self-medicate? Am J Addict 13(2), 139-150.

Habets, P., Marcelis, M., Gronenschild, E., Drukker, M., van Os, J., 2011. Reduced cortical thickness as an outcome of differential sensitivity to environmental risks in schizophrenia. Biol Psychiatry 69(5), 487-494.

Hambrecht, M., Hafner, H., 1996. Substance abuse and the onset of schizophrenia. Biol Psychiatry 40(11), 1155-1163.

Hare, E., Glahn, D.C., Dassori, A., Raventos, H., Nicolini, H., Ontiveros, A., Medina, R., Mendoza, R., Jerez, A., Munoz, R., Almasy, L., Escamilla, M.A., 2010. Heritability of age of onset of psychosis in schizophrenia. Am J Med Genet B Neuropsychiatr Genet 153B(1), 298-302.

Harley, M., Kelleher, I., Clarke, M., Lynch, F., Arseneault, L., Connor, D., Fitzpatrick, C., Cannon, M., 2010. Cannabis use and childhood trauma interact additively to increase the risk of psychotic symptoms in adolescence. Psychol Med 40(10), 1627-1634.

Henquet, C., Di Forti, M., Morrison, P., Kuepper, R., Murray, R.M., 2008. Gene-environment interplay between cannabis and psychosis. Schizophr Bull 34(6), 1111-1121.

Henquet, C., Krabbendam, L., Spauwen, J., Kaplan, C., Lieb, R., Wittchen, H.U., van Os, J., 2005a. Prospective cohort study of cannabis use, predisposition for psychosis, and psychotic symptoms in young people. BMJ 330(7481), 11.

Henquet, C., Murray, R., Linszen, D., van Os, J., 2005b. The environment and schizophrenia: the role of cannabis use. Schizophr Bull 31(3), 608-612.

Henquet, C., Rosa, A., Delespaul, P., Papiol, S., Fananas, L., van Os, J., Myin-Germeys, I., 2009. COMT ValMet moderation of cannabis-induced psychosis: a momentary assessment study of 'switching on' hallucinations in the flow of daily life. Acta Psychiatr Scand 119(2), 156-160.

Henquet, C., Rosa, A., Krabbendam, L., Papiol, S., Fananas, L., Drukker, M., Ramaekers, J.G., van Os, J., 2006. An experimental study of catechol-o-methyltransferase Val158Met moderation of delta-9-tetrahydrocannabinol-induced effects on psychosis and cognition. Neuropsychopharmacology 31(12), 2748-2757.

Ho, B.C., Wassink, T.H., Ziebell, S., Andreasen, N.C., 2011. Cannabinoid receptor 1 gene polymorphisms and marijuana misuse interactions on white matter and cognitive deficits in schizophrenia. Schizophr Res 128(1-3), 66-75.

Hollis, C., Groom, M.J., Das, D., Calton, T., Bates, A.T., Andrews, H.K., Jackson, G.M., Liddle, P.F., 2008. Different psychological effects of cannabis use in adolescents at genetic high risk for schizophrenia and with attention deficit/hyperactivity disorder (ADHD). Schizophr Res 105(1-3), 216-223.

Holmans, P., Green, E.K., Pahwa, J.S., Ferreira, M.A., Purcell, S.M., Sklar, P., Owen, M.J., O'Donovan, M.C., Craddock, N., 2009. Gene ontology analysis of GWA study data sets provides insights into the biology of bipolar disorder. Am J Hum Genet 85(1), 13-24.

Houston, J.E., Murphy, J., Adamson, G., Stringer, M., Shevlin, M., 2008. Childhood sexual abuse, early cannabis use, and psychosis: testing an interaction model based on the National Comorbidity Survey. Schizophr Bull 34(3), 580-585.

James, A., Hough, M., James, S., Winmill, L., Burge, L., Nijhawan, S., Matthews, P.M., Zarei, M., 2011. Greater white and grey matter changes associated with early cannabis use in adolescent-onset schizophrenia (AOS). Schizophr Res 128(1-3), 91-97.

Jockers-Scherubl, M.C., Danker-Hopfe, H., Mahlberg, R., Selig, F., Rentzsch, J., Schurer, F., Lang, U.E., Hellweg, R., 2004. Brain-derived neurotrophic factor serum concentrations are increased in drug-naive schizophrenic patients with chronic cannabis abuse and multiple substance abuse. Neurosci Lett 371(1), 79-83.

Jockers-Scherubl, M.C., Wolf, T., Radzei, N., Schlattmann, P., Rentzsch, J., Gomez-Carrillo de Castro, A., Kuhl, K.P., 2007. Cannabis induces different cognitive changes in schizophrenic patients and in healthy controls. Prog Neuropsychopharmacol Biol Psychiatry 31(5), 1054-1063.

Kantrowitz, J.T., Nolan, K.A., Sen, S., Simen, A.A., Lachman, H.M., Bowers, M.B., Jr., 2009. Adolescent cannabis use, psychosis and catechol-O-methyltransferase genotype in African Americans and Caucasians. Psychiatr Q 80(4), 213-218.

Kendler, K.S., McGuire, M., Gruenberg, A.M., O'Hare, A., Spellman, M., Walsh, D., 1993. The Roscommon Family Study. III. Schizophrenia-related personality disorders in relatives. Arch Gen Psychiatry 50(10), 781-788.

Khoury, M.J., Flanders, W.D., 1996. Nontraditional epidemiologic approaches in the analysis of gene-environment interaction: case-control studies with no controls! Am J Epidemiol 144(3), 207-213.

Khoury, M.J., Wacholder, S., 2009. Invited commentary: from genome-wide association studies to gene-environment-wide interaction studies--challenges and opportunities. Am J Epidemiol 169(2), 227-230; discussion 234-225.

Koethe, D., Giuffrida, A., Schreiber, D., Hellmich, M., Schultze-Lutter, F., Ruhrmann, S., Klosterkotter, J., Piomelli, D., Leweke, F.M., 2009. Anandamide elevation in cerebrospinal fluid in initial prodromal states of psychosis. Br J Psychiatry 194(4), 371-372.

Konings, M., Bak, M., Hanssen, M., van Os, J., Krabbendam, L., 2006. Validity and reliability of the CAPE: a self-report instrument for the measurement of psychotic experiences in the general population. Acta Psychiatr Scand 114(1), 55-61.

Kooperberg, C., Leblanc, M., 2008. Increasing the power of identifying gene x gene interactions in genome-wide association studies. Genet Epidemiol 32(3), 255-263.

Kuepper, R., van Os, J., Lieb, R., Wittchen, H.U., Henquet, C., 2011a. Do cannabis and urbanicity co-participate in causing psychosis? Evidence from a 10-year follow-up cohort study. Psychol Med 41(10), 2121-2129.

Kuepper, R., van Os, J., Lieb, R., Wittchen, H.U., Hofler, M., Henquet, C., 2011b. Continued cannabis use and risk of incidence and persistence of psychotic symptoms: 10 year follow-up cohort study. BMJ 342, d738.

Lachman, H.M., Papolos, D.F., Saito, T., Yu, Y.M., Szumlanski, C.L., Weinshilboum, R.M., 1996. Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenetics 6(3), 243-250.

Large, M., Sharma, S., Compton, M.T., Slade, T., Nielssen, O., 2011. Cannabis use and earlier onset of psychosis: a systematic meta-analysis. Arch Gen Psychiatry 68(6), 555-561.

Larsen, T.K., McGlashan, T.H., Moe, L.C., 1996. First-episode schizophrenia: I. Early course parameters. Schizophr Bull 22(2), 241-256.

Leweke, F.M., Giuffrida, A., Koethe, D., Schreiber, D., Nolden, B.M., Kranaster, L., Neatby, M.A., Schneider, M., Gerth, C.W., Hellmich, M., Klosterkotter, J., Piomelli, D., 2007. Anandamide levels in cerebrospinal fluid of first-episode schizophrenic patients: impact of cannabis use. Schizophr Res 94(1-3), 29-36.

Lichtenstein, P., Yip, B.H., Bjork, C., Pawitan, Y., Cannon, T.D., Sullivan, P.F., Hultman, C.M., 2009. Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. Lancet 373(9659), 234-239.

Lotta, T., Vidgren, J., Tilgmann, C., Ulmanen, I., Melen, K., Julkunen, I., Taskinen, J., 1995. Kinetics of human soluble and membrane-bound catechol O-methyltransferase: a revised mechanism and description of the thermolabile variant of the enzyme. Biochemistry 34(13), 4202-4210.

Luan, J.A., Wong, M.Y., Day, N.E., Wareham, N.J., 2001. Sample size determination for studies of gene-environment interaction. Int J Epidemiol 30(5), 1035-1040.

Macleod, J., Oakes, R., Copello, A., Crome, I., Egger, M., Hickman, M., Oppenkowski, T., Stokes-Lampard, H., Davey Smith, G., 2004. Psychological and social sequelae of cannabis and other illicit drug use by young people: a systematic review of longitudinal, general population studies. Lancet 363(9421), 1579-1588.

Mason, O., Morgan, C.J., Dhiman, S.K., Patel, A., Parti, N., Curran, H.V., 2009. Acute cannabis use causes increased psychotomimetic experiences in individuals prone to psychosis. Psychol Med 39(6), 951-956.

McGrath, J., Welham, J., Scott, J., Varghese, D., Degenhardt, L., Hayatbakhsh, M.R., Alati, R., Williams, G.M., Bor, W., Najman, J.M., 2010. Association between cannabis use and psychosis-related outcomes using sibling pair analysis in a cohort of young adults. Arch Gen Psychiatry 67(5), 440-447.

Meijer, J.H., Dekker, N., Koeter, M.W., Quee, P.J., van Beveren, N.J., Meijer, C.J., 2011. Cannabis and cognitive performance in psychosis: a cross-sectional study in patients with non-affective psychotic illness and their unaffected siblings. Psychol Med, 1-12.

Moore, T.H., Zammit, S., Lingford-Hughes, A., Barnes, T.R., Jones, P.B., Burke, M., Lewis, G., 2007. Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. Lancet 370(9584), 319-328.

Morrison, P.D., Zois, V., McKeown, D.A., Lee, T.D., Holt, D.W., Powell, J.F., Kapur, S., Murray, R.M., 2009. The acute effects of synthetic intravenous Delta9-tetrahydrocannabinol on psychosis, mood and cognitive functioning. Psychol Med 39(10), 1607-1616.

Munafo, M.R., Flint, J., 2009. Replication and heterogeneity in gene x environment interaction studies. Int J Neuropsychopharmacol 12(6), 727-729.

Murcray, C.E., Lewinger, J.P., Conti, D.V., Thomas, D.C., Gauderman, W.J., 2011. Sample size requirements to detect gene-environment interactions in genome-wide association studies. Genet Epidemiol 35(3), 201-210.

Murcray, C.E., Lewinger, J.P., Gauderman, W.J., 2009. Gene-environment interaction in genome-wide association studies. Am J Epidemiol 169(2), 219-226.

Numata, S., Ueno, S., Iga, J., Yamauchi, K., Hongwei, S., Ohta, K., Kinouchi, S., Shibuya-Tayoshi, S., Tayoshi, S., Aono, M., Kameoka, N., Sumitani, S., Tomotake, M., Kaneda, Y., Taniguchi, T., Ishimoto, Y., Ohmori, T., 2006. Brain-derived neurotrophic factor (BDNF) Val66Met polymorphism in schizophrenia is associated with age at onset and symptoms. Neurosci Lett 401(1-2), 1-5.

Okochi, T., Ikeda, M., Kishi, T., Kawashima, K., Kinoshita, Y., Kitajima, T., Yamanouchi, Y., Tomita, M., Inada, T., Ozaki, N., Iwata, N., 2009. Meta-analysis of association between genetic variants in COMT and schizophrenia: an update. Schizophr Res 110(1-3), 140-148.

Perkonigg, A., Goodwin, R.D., Fiedler, A., Behrendt, S., Beesdo, K., Lieb, R., Wittchen, H.U., 2008. The natural course of cannabis use, abuse and dependence during the first decades of life. Addiction 103(3), 439-449; discussion 450-431.

Poelmans, G., Pauls, D.L., Buitelaar, J.K., Franke, B., 2011. Integrated genome-wide association study findings: identification of a neurodevelopmental network for attention deficit hyperactivity disorder. Am J Psychiatry 168(4), 365-377.

Rabin, R.A., Zakzanis, K.K., George, T.P., 2011. The effects of cannabis use on neurocognition in schizophrenia: a meta-analysis. Schizophrenia research 128(1-3), 111-116.

Rabinowitz, J., Levine, S.Z., Hafner, H., 2006. A population based elaboration of the role of age of onset on the course of schizophrenia. Schizophr Res 88(1-3), 96-101.

Raine, A., 1991. The SPQ: a scale for the assessment of schizotypal personality based on DSM-III-R criteria. Schizophr Bull 17(4), 555-564.

Rais, M., van Haren, N.E., Cahn, W., Schnack, H.G., Lepage, C., Collins, L., Evans, A.C., Hulshoff Pol, H.E., Kahn, R.S., 2010. Cannabis use and progressive cortical thickness loss in areas rich in CB1 receptors during the first five years of schizophrenia. Eur Neuropsychopharmacol 20(12), 855-865.

Ramaekers, J.G., Kauert, G., Theunissen, E.L., Toennes, S.W., Moeller, M.R., 2009. Neurocognitive performance during acute THC intoxication in heavy and occasional cannabis users. J Psychopharmacol 23(3), 266-277.

Raz, N., Rodrigue, K.M., Kennedy, K.M., Land, S., 2009. Genetic and vascular modifiers of age-sensitive cognitive skills: effects of COMT, BDNF, ApoE, and hypertension. Neuropsychology 23(1), 105-116.

Ripke, S., Sanders, A.R., Kendler, K.S., Levinson, D.F., Sklar, P., Holmans, P.A., Lin, D.Y., Duan, J., Ophoff, R.A., Andreassen, O.A., Scolnick, E., Cichon, S., St Clair, D., Corvin, A., Gurling, H., Werge, T., Rujescu, D., Blackwood, D.H., Pato, C.N., Malhotra, A.K., Purcell, S., Dudbridge, F., Neale, B.M., Rossin, L., Visscher, P.M., Posthuma, D., Ruderfer, D.M., Fanous, A., Stefansson, H., Steinberg, S., Mowry, B.J., Golimbet, V., De Hert, M., Jonsson, E.G., Bitter, I., Pietilainen, O.P., Collier, D.A., Tosato, S., Agartz, I., Albus, M., Alexander, M., Amdur, R.L., Amin, F., Bass, N., Bergen, S.E., Black, D.W., Borglum, A.D., Brown, M.A., Bruggeman, R., Buccola, N.G., Byerley, W.F., Cahn, W., Cantor, R.M., Carr, V.J., Catts, S.V., Choudhury, K., Cloninger, C.R., Cormican, P., Craddock, N., Danoy, P.A., Datta, S., de Haan, L., Demontis, D., Dikeos, D., Djurovic, S., Donnelly, P., Donohoe, G., Duong, L., Dwyer, S., Fink-Jensen, A., Freedman, R., Freimer, N.B., Friedl, M., Georgieva, L., Giegling, I., Gill, M., Glenthoj, B., Godard, S., Hamshere, M., Hansen, M., Hansen, T., Hartmann, A.M., Henskens, F.A., Hougaard, D.M., Hultman, C.M., Ingason, A., Jablensky, A.V., Jakobsen, K.D., Jay, M., Jurgens, G., Kahn, R.S., Keller, M.C., Kenis, G., Kenny, E., Kim, Y., Kirov, G.K., Konnerth, H., Konte, B., Krabbendam, L., Krasucki, R., Lasseter, V.K., Laurent, C., Lawrence, J., Lencz, T., Lerer, F.B., Liang, K.Y., Lichtenstein, P., Lieberman, J.A., Linszen, D.H., Lonnqvist, J., Loughland, C.M., Maclean, A.W., Maher, B.S., Maier, W., Mallet, J., Malloy, P., Mattheisen, M., Mattingsdal, M., McGhee, K.A., McGrath, J.J., McIntosh, A., McLean, D.E., McQuillin, A., Melle, I., Michie, P.T., Milanova, V., Morris, D.W., Mors, O., Mortensen, P.B., Moskvina, V., Muglia, P., Myin-Germeys, I., Nertney, D.A., Nestadt, G., Nielsen, J., Nikolov, I., Nordentoft, M., Norton, N., Nothen, M.M., O'Dushlaine, C.T., Olincy, A., Olsen, L., O'Neill, F.A., Orntoft, T.F., Owen, M.J., Pantelis, C., Papadimitriou, G., Pato, M.T., Peltonen, L., Petursson, H., Pickard, B., Pimm, J., Pulver, A.E., Puri, V., Quested, D., Quinn, E.M., Rasmussen, H.B., Rethelyi, J.M., Ribble, R., Rietschel, M., Riley, B.P., Ruggeri, M., Schall, U., Schulze, T.G., Schwab, S.G., Scott, R.J., Shi, J., Sigurdsson, E., Silverman, J.M., Spencer, C.C., Stefansson, K., Strange, A., Strengman, E., Stroup, T.S., Suvisaari, J., Terenius, L., Thirumalai, S., Thygesen, J.H., Timm, S., Toncheva, D., van den Oord, E., van Os, J., van Winkel, R., Veldink, J., Walsh, D., Wang, A.G., Wiersma, D., Wildenauer, D.B., Williams, H.J., Williams, N.M., Wormley, B., Zammit, S., Sullivan, P.F., O'Donovan, M.C., Daly, M.J., Gejman, P.V., 2011. Genome-wide association study identifies five new schizophrenia loci. Nat Genet 43(10), 969-976.

Scharfman, H.E., MacLusky, N.J., 2006. Estrogen and brain-derived neurotrophic factor (BDNF) in hippocampus: complexity of steroid hormone-growth factor interactions in the adult CNS. Front Neuroendocrinol 27(4), 415-435.

Scholes, K.E., Martin-Iverson, M.T., 2010. Cannabis use and neuropsychological performance in healthy individuals and patients with schizophrenia. Psychol Med 40(10), 1635-1646.

Shalev, I., Lerer, E., Israel, S., Uzefovsky, F., Gritsenko, I., Mankuta, D., Ebstein, R.P., Kaitz, M., 2009. BDNF Val66Met polymorphism is associated with HPA axis reactivity to psychological stress characterized by genotype and gender interactions. Psychoneuroendocrinology 34(3), 382-388.

Smith, M.J., Barch, D.M., Wolf, T.J., Mamah, D., Csernansky, J.G., 2008. Elevated rates of substance use disorders in non-psychotic siblings of individuals with schizophrenia. Schizophr Res 106(2-3), 294-299.

Stirling, J., Barkus, E.J., Nabosi, L., Irshad, S., Roemer, G., Schreudergoidheijt, B., Lewis, S., 2008. Cannabis-induced psychotic-like experiences are predicted by high schizotypy. Confirmation of preliminary results in a large cohort. Psychopathology 41(6), 371-378.

Sullivan, P.F., 2007. Spurious genetic associations. Biol Psychiatry 61(10), 1121-1126.

Szeszko, P.R., Robinson, D.G., Sevy, S., Kumra, S., Rupp, C.I., Betensky, J.D., Lencz, T., Ashtari, M., Kane, J.M., Malhotra, A.K., Gunduz-Bruce, H., Napolitano, B., Bilder, R.M., 2007. Anterior cingulate grey-matter deficits and cannabis use in first-episode schizophrenia. Br J Psychiatry 190, 230-236.

Tandon, R., Keshavan, M.S., Nasrallah, H.A., 2008. Schizophrenia, "just the facts" what we know in 2008. 2. Epidemiology and etiology. Schizophr Res 102(1-3), 1-18.

Thomas, D., 2010. Gene--environment-wide association studies: emerging approaches. Nat Rev Genet 11(4), 259-272.

van Os, J., Kenis, G., Rutten, B.P., 2010. The environment and schizophrenia. Nature 468(7321), 203-212.

van Os, J., Linscott, R.J., Myin-Germeys, I., Delespaul, P., Krabbendam, L., 2009. A systematic review and meta-analysis of the psychosis continuum: evidence for a psychosis proneness-persistence-impairment model of psychotic disorder. Psychol Med 39(2), 179-195.

van Os, J., Rutten, B.P., 2009. Gene-environment-wide interaction studies in psychiatry. Am J Psychiatry 166(9), 964-966.

van Os, J., Rutten, B.P., Poulton, R., 2008. Gene-environment interactions in schizophrenia: review of epidemiological findings and future directions. Schizophr Bull 34(6), 1066-1082.

Van Winkel, R., Esquivel, G., Kenis, G., Wichers, M., Collip, D., Peerbooms, O., Rutten, B., Myin-Germeys, I., Van Os, J., 2010. REVIEW: Genome-wide findings in schizophrenia and the role of gene-environment interplay. CNS Neurosci Ther 16(5), e185-192.

van Winkel, R., van Beveren, N.J., Simons, C., RS, S.K., Linszen, D.H., van Os, J., Wiersma, D., Bruggeman, R., Cahn, W., de Haan, L., Krabbendam, L., Myin-Germeys, I., 2011. AKT1 Moderation of Cannabis-Induced Cognitive Alterations in Psychotic Disorder. Neuropsychopharmacology 36(12), 2529-2537.

Veling, W., Mackenbach, J.P., van Os, J., Hoek, H.W., 2008. Cannabis use and genetic predisposition for schizophrenia: a case-control study. Psychol Med 38(9), 1251-1256.

Verdoux, H., Gindre, C., Sorbara, F., Tournier, M., Swendsen, J.D., 2003. Effects of cannabis and psychosis vulnerability in daily life: an experience sampling test study. Psychol Med 33(1), 23-32.

Verhagen, M., van der Meij, A., van Deurzen, P.A., Janzing, J.G., Arias-Vasquez, A., Buitelaar, J.K., Franke, B., 2010. Meta-analysis of the BDNF Val66Met polymorphism in major depressive disorder: effects of gender and ethnicity. Mol Psychiatry 15(3), 260-271.

Vollema, M.G., Ormel, J., 2000. The reliability of the structured interview for schizotypy-revised. Schizophr Bull 26(3), 619-629.

Weng, L., Macciardi, F., Subramanian, A., Guffanti, G., Potkin, S.G., Yu, Z., Xie, X., 2011. SNP-based pathway enrichment analysis for genome-wide association studies. BMC Bioinformatics 12, 99.

Yücel, M., Bora, E., Lubman, D.I., Solowij, N., Brewer, W.J., Cotton, S.M., Conus, P., Takagi, M.J., Fornito, A., Wood, S.J., McGorry, P.D., Pantelis, C., 2010. The impact of cannabis use on cognitive functioning in patients with schizophrenia: a meta-analysis of existing findings and new data in a first-episode sample. Schizophrenia bulletin, doi: 10.1093/schbul/sbq1079.

Zammit, S., Horwood, J., Thompson, A., Thomas, K., Menezes, P., Gunnell, D., Hollis, C., Wolke, D., Lewis, G., Harrison, G., 2008. Investigating if psychosis-like symptoms (PLIKS) are associated with family history of schizophrenia or paternal age in the ALSPAC birth cohort. Schizophr Res 104(1-3), 279-286.

Zammit, S., Owen, M.J., Evans, J., Heron, J., Lewis, G., 2011. Cannabis, COMT and psychotic experiences. Br J Psychiatry 199, 380-385.

Zammit, S., Spurlock, G., Williams, H., Norton, N., Williams, N., O'Donovan, M.C., Owen, M.J., 2007. Genotype effects of CHRNA7, CNR1 and COMT in schizophrenia: interactions with tobacco and cannabis use. Br J Psychiatry 191, 402-407.

CHAPTER SIX

Paranoia evoked by social stress: evidence for moderation by genetic variation in AKT1.

Decoster Jeroen[1,2], van Nierop Martine[1], Pishva Ehsan[1], Gunter Kenis[1], Viechtenbauer Wolfgang[1], Nele Jacobs[1], Viviane Thewissen[1], Marielle Lardinois[1], Claudia Simons[1], Tineke Lataster[1], Johan Lataster[1], Dina Collip[1], Margreet Oorschot[1], Mayke Janssens[1], Claudia Menne-Lothmann[1], Jessica Hartmann[1], Philippe Delespaul[1], Marjan Drukker[1], Bart Rutten[1], Marieke Wichers[1], van Os Jim[1,3], Myin-Germeys Inez[1], van Winkel Ruud[1,2]

Submitted

[1] Dept. of Psychiatry and Psychology, School for Mental Health and Neuroscience, EURON, Maastricht University Medical Centre, PO BOX 616, 6200 MD Maastricht, The Netherlands

[2] Department of Neurosciences KU Leuven, University Psychiatric Centre Catholic University Louvain, Herestraat 49, 3000 Leuven, Belgium

[3] Division of Psychological Medicine, Institute of Psychiatry, De Crespigny Park, London, UK SE5 8AF

ABSTRACT

Background

Paranoia is a core symptom of schizophrenia and is also highly prevalent in affective and anxiety disorders. Previous evidence shows that both genetic vulnerability and social stressors are important in the formation of paranoia. This study aimed to investigate possible interactions between social stress and relevant single nucleotide polymorphisms (SNPs).

Method

Paranoid reactivity to social stress was measured using the Experience Sampling Method in five different samples across the paranoia continuum (total n=867). 33 SNPs emerging from either agnostic genome-wide association studies of relevant phenotypes or from previous candidate gene-environment interaction studies were selected. Replicated interactions at $p<.05$ in two healthy subsamples (1. healthy control group [n=112] and 2. female general population twin sample [n=434]) were followed-up in an at-risk (unaffected siblings of psychotic patients, n=86) and two patient samples (1. schizophrenia [n=109] and 2. depression [n=126]) for further assessment of etiological and clinical relevance.

Results

6 SNPs moderated the effects of social stress on paranoia in both healthy samples ($p<.05$); rs3803300 in AKT1 and 5 SNPs in the Major Histocompatibility Complex (MHC). Rs3803300 in AKT1 also moderated paranoia in the unaffected siblings and the psychotic patients, whereas the five MHC-SNPs moderated paranoia in the patients with remitted depression.

Conclusions

Genetic variation in AKT1, and possibly also in the MHC region, is important in explaining individual differences in paranoid reactivity to social stress, a fundamental psychological mechanism of relevance across traditional diagnostic boundaries.

1. INTRODUCTION

Paranoia is one of the core symptoms of schizophrenia (Tandon et al., 2009a) and is also highly prevalent in affective and anxiety disorders, in which case it is associated with worse outcome, higher genetic risk for psychiatric disorders, greater exposure to social stress in early life as well as recent life events (Wigman et al., 2012). Subclinical paranoid ideation is detectable in the general population with a prevalence of up to 18 % (Freeman et al., 2010b). Similar cognitive, social and clinical characteristics were found in persons with subclinical paranoia and in individuals with clinical persecutory delusions (Combs et al., 2007), suggesting that paranoid beliefs in healthy persons and persecutory delusions in patients with schizophrenia form part of a 'paranoia continuum' (American Psychiatric Association, 2000a).

Recent observational (Simons et al., 2009) and experimental work in the general population (Lincoln et al., 2009) and in patients with schizophrenia (Moritz et al., 2010) has found that paranoid ideation increases with higher levels of perceived stress. Since misinterpretation in social interactions seems to be a key feature of paranoid ideation (Freeman, 2008), perceived social stress is considered one of the crucial elements in the developmentof paranoid beliefs (Bentall and Fernyhough, 2008).

The experience sampling method (ESM), a diary method that assesses thoughts, emotions and social context, is an elegant approach to capture subtle paranoid reactions to minor (social) stressors as they occur in the flow of daily life (Henquet et al.; Myin-Germeys et al., 2005a; Myin-Germeys et al., 2005b; Simons et al., 2009; van Winkel et al., 2008a). Previous ESM work has shown significant increases in paranoia under stressful circumstances in patients with a psychotic disorder and their first-degree relatives (Myin-Germeys et al., 2005a), as well as in healthy volunteers (Simons et al., 2009). There is also evidence of familial covariation of stress-reactivity and psychotic symptoms (Lataster et al., 2010). Furthermore, when combined with genetic information, ESM offers a unique way to test gene-environment interactions (GxE) (Moffitt et al., 2005; Myin-Germeys et al., 2009). The large number of within-person measurements results in improved statistical power to detect GxE interactions, yielding highly significant findings using moderate sample sizes (Collip et al., 2011c; Peerbooms et al., 2012). Preliminary studies found evidence of (i) genetic moderation of stress-induced psychotic symptoms by functional polymorphisms in COMT (Val158Met)(Collip et al., 2011c; van Winkel et al., 2008a) and BDNF (Val66Met)(Simons et al., 2009).

Given the importance of paranoia across a range of mental disorders, and abundant evidence for the role of interaction between environmental factors and underlying vulnerability in producing mental disorders (Lesch, 2004; van Os et al., 2010), the

present study aimed to examine possible interaction between social stress and carefully selected single nucleotide polymorphisms (SNPs) in different samples across the paranoia continuum, totalling a combined number of 867 individuals.

2. METHODS

2.1. Subjects

For this study, subjects were pooled from five separate samples; inclusion criteria were described elsewhere for four of these (Collip et al., 2011a; Geschwind et al., 2011; Thewissen et al., 2008; Wichers et al., 2009) and one unpublished sample. In the latter, 22 patients with non-affective psychotic disorder, 25 first-degree relatives of patients with psychotic disorder and 38 controls were included. In total, the combination of ESM and genetic data was collected in 905 participants. Participants were classified on the basis of their psychosis liability and need for care: healthy controls (n=568), non-affected relatives of psychotic patients (n=97), formerly depressed patients with residual symptoms (n=126) and psychotic patients (n=114). The healthy control group consisted of two different samples: a group of healthy controls (n=115, detection sample) and a group of female general population twins and their non-twin sisters (n=453 (in 226 families), replication sample)(see Table 1). In the group of non-affected relatives, 41 individuals were a first degree relative of 35 patients in the psychosis subsample.

2.2. Experience Sampling Method

ESM is a structured diary technique for assessing subjects in their daily living environment (Myin-Germeys et al., 2009), and has been validated for studying paranoid reactions to stress (Oorschot et al., 2009; Simons et al., 2009; Thewissen et al., 2008). Subjects received a digital wristwatch and a set of ESM self-assessment forms collated in a booklet for each day. Ten times a day on 5 consecutive days for the twins and 6 for all other participants, the watch emitted a signal (beep) at unpredictable moments between 7:30 AM and 10:30 PM. After every “beep,” reports of thoughts, current context (activity, persons present, location), appraisals of the current situation, current symptomatology and mood were collected. All self-assessments were rated on 7-point Likert scales. The ESM procedure was explained to the subjects during an initial briefing session, and a practice form was completed to confirm that subjects were able to understand the 7-point Likert scale format. Subjects were instructed to complete their reports immediately after the beep, thus minimizing memory distortions, and to record the time at which they completed the form. During the actual sampling period, research staff repeatedly called the subjects to assess

Table 1
Description of participants included in analyses.

Group	N (all[$])	male (%)	mean age (SD)	mean ESM moments in company (SD)	% of ESM moments in company (SD)	mean perceived social stress (SD)	mean ESM paranoia in company (SD)	original study (reference)
healthy controls	112 (115)	34 (30.4%)	33,3 (11.6)	26.9 (7.3)	61.4 (16.0)	1.84 (.56)	1.08 (.19)	- GROUP (Collip et al., 2011b; Thewissen et al., 2008): 78 -STRIP : 38
general population twins	434 (453)	0 (0%)	27.7 (7.9)	27.7 (7.3)	72.3 (16.3)	2.33 (.70)	1.15 (.30)	(Wichers et al., 2009)
non-affected first degree relatives	86 (97)	29 (33.7%)	35.9 (14.2)	27.4 (9.6)	61.7 (15.7	1.78 (.55)	1.07 (.17)	-GROUP (Collip et al., 2011b; Thewissen et al., 2008): 71 -STRIP: 25
psychotic patients	109 (114)	75 (68.8%)	34.2 (11.4)	22.5 (10.2)	53.7 (22.2)	2.25 (.86)	1.54 (.88)	-GROUP (Lataster et al., 2012; Thewissen et al., 2008): 90 -STRIP: 22
residual depressive symptoms	126 (126)	29 (23.0%)	43.7 (9.7)	26.6 (10.5)	55.1 (19.1)	2.12 (.70)	1.67 (.78)	MindMaastricht (Geschwind et al., 2011)
total sample	867 (905)	167 (19.3%)	32.4 (11.4)	26.4 (8.9)	65.0 (19.1)	2.17 (.72)	1.26 (.54)	

STRIP = STRess In Psychosis, the current paper is the first paper reporting data of this study. [$]Including participants who were dropped because of ESM quality criteria.

whether they were complying with the instructions. Based on the times subjects indicated that they completed the report, all reports completed more than 15 min after the actual signal were excluded from the analysis. Previous work has shown that reports completed after this interval are less reliable and consequently less valid (Delespaul, 1995). Subjects with less than 17 or 20 valid reports, during 5 or 6 consecutive days respectively, were excluded from the analysis (n=38, see Table 1), in agreement with previous ESM work in these samples (Collip et al., 2011a; Geschwind et al., 2011; Thewissen et al., 2008; Wichers et al., 2009).

2.2.1. Social stress

Social stress was conceptualized as the subject's appraisal of the current social situation, as previously applied (Collip et al., 2011a; Simons et al., 2009). First, subjects were asked to indicate whether they were alone or with other people. If they were not alone, they were asked to state (1) how comfortable they felt in the present company and (2) whether they would rather be alone. Both items were rated on a 7-point Likert scale (1 not at all – 7 very much). The first item was inversed and combined with the second item, by calculating the mean of the two scores to generate one social stress score, varying from 1 to 7. In the general population twin sample, the second item was not available; therefore only the first item was used in this sample.

2.2.2. Paranoia

Paranoid stress response was assessed by asking subjects on each ESM moment to state whether they felt suspicious, on a 7-point Likert scale (1 not at all – 7 very much) (Myin-Germeys et al., 2005b; Simons et al., 2009; Thewissen et al., 2008).

2.3. Gene selection

As previously described (Decoster et al., 2012a; Van Winkel et al., 2010b), the major challenge for gene-environment interaction research is to combine the agnostic detection of disorder-associated genetic variants from genome-wide studies with the hypothesis-based approach from epidemiological and neurobiological studies. For the SNP selection of this study, we therefore combined SNPs emerging from agnostic genome-wide association studies (GWAS) of relevant phenotypes on the one hand, and candidate SNPs emerging from previous GxE studies on the other hand. Thus, 20 SNPs were included based on GWAS results in schizophrenia (situated in the 6p21.3 Major Histocompatibility Complex (MHC) region, NOTCH4, NRGN, RELN, TCF4, ZNF804A), bipolar disorder (ANK3, CACNA1C) and neuroticism (MDGA2, PDE4D, SNAP25). 17 SNPs were added based on previous GxE studies in which the selected

SNPs interacted with stress-related environmental factors (AKT1, BDNF, COMT, CRHR1, DRD2, FKBP5, HTR2A, NR3C1, TPH1). See table 2 for further details.
DNA was extracted from blood, saliva, or buccal epithelium for different samples. The selected SNPs were determined by Sequenom MassARRAY iPLEX platform at the facilities of the manufacturer (Hamburg, Germany) ; SNPs, therefore, were not selected from a larger set of genomewide markers.
For every monozygotic twin pair in the sample, only one individual was genotyped and the same genotypic data was included for the co-twin, assuming that both twins had identical genotypes.
Of the 37 SNPs originally included, 3 SNPs were excluded because they had more than 10% genotyping failure (rs3800316 of the 6p21.3 MHC region, rs3131296 in NOTCH4, rs2494735 in AKT1) and 1 SNP was excluded because of Hardy-Weinberg disequilibrium ($p < .001$) (rs4713916 in FKBP5). A final set of 33 SNPs in 19 genes was therefore used for further analysis.

2.4. Plan of analysis

2.4.1. Statistical analysis

ESM data have a hierarchical structure; multiple observations (level 1) are clustered within subjects (level 2), and some of themwere related (level 3). Multilevel random regression analysis was applied since it takes the variability associated with each level of nesting into account (Snijders and Bosker, 1999). Thus, multilevel random regression analysis models both fixed and random effects. The fixed effects are interpreted similarly as standard regression coefficients and are estimated directly; the random effects portion of the model is specified by considering the nesting of the data. The XTMIXED command in STATA 11.0 (StataCorp LP, 2012) was thus used for the 3 level analyses.
First, the association between perceived social stress and ESM paranoia was assessed for each sample. Multilevel regression models, as described above, with ESM paranoia as dependent variable, were used. Second, using the same strategy, possible associations between the selected SNPs and ESM paranoia were examined. Finally, ESM paranoia, as dependent variable, was modeled by the two-way interaction of perceived social stress and each selected SNP separately. Genotypes were coded ‘0’, ‘1’ or ‘2’ according to the number of minor alleles and modeled as a linear effect, since this method can deal with different genotype distributions, including distributions with a low minor allele frequency, as it avoids stratification into small subgroups (Cordell and Clayton, 2005). All analyses were controlled for age and sex as *a priori* confounders.

Table 2
Overview of selected SNPs. Callrates (rate of succesfully genotyped individuals) and chi-squared values of Hardy-Weinberg (HW χ^2) equilibrium are indicated. SNPs with a callrate beneath 0.90 (marked with *) or a chi-squared value above 10.83 (marked with #, corresponding with a p-value < .001) were excluded for analyses.

SNP	gene	location	function	alleles	alternative name(s)	inclusion criteria (references)	callrate	HW χ^2
rs10994336	ANK3	10q21		C/T		GWAS in bipolar disorder (Ferreira et al., 2008; Schulze et al., 2009)	0.997	0.023
rs9804190	ANK3	10q21		C/T		GWAS in bipolar disorder (Schulze et al., 2009)	0.998	0.007
rs1006737	CACNA1C	12p13.3	intronic	G/A		GWAS in bipolar disorder (Ferreira et al., 2008)	0.999	0.21
rs13219181	MHC[$]	6p21.3		A/G		GWAS in schizophrenia (Shi et al., 2009)	0.998	0.378
rs3800307	MHC[$]	6p21.3		T/A		GWAS in schizophrenia (Shi et al., 2009)	0.999	0.006
*rs3800316	MHC[$]	6p21.3		A/C		GWAS in schizophrenia (Shi et al., 2009)	0	/
rs6904071	MHC[$]	6p21.3		A/G		GWAS in schizophrenia (Shi et al., 2009)	1	0.117
rs6913660	MHC[$]	6p21.3		A/C		GWAS in schizophrenia (Shi et al., 2009)	1	0.146
rs926300	MHC[$]	6p21.3		A/T		GWAS in schizophrenia (Shi et al., 2009)	0.998	0.192
rs1288334	MDGA2	14q21.3		A/G		GWAS in neuroticism (van den Oord et al., 2008)	0.997	0.084
rs1959813	MDGA2	14q21.3		A/G		GWAS in neuroticism (van den Oord et al., 2008)	0.983	0.56
rs3007105	MDGA2	14q21.3	intronic	C/T		GWAS in neuroticism (van den Oord et al., 2008)	1	0.596
rs7151262	MDGA2	14q21.3	intronic	C/G		GWAS in neuroticism (van den Oord et al., 2008)	0.999	0.572
*rs3131296	NOTCH4	6p21.3	intronic	G/A		GWAS in schizophrenia (Li et al., 2010; Stefansson et al., 2009)	0	/
rs12807809	NRGN	11q24		T/C		GWAS in schizophrenia (Stefansson et al., 2009)	1	0.346
rs702543	PDE4D	5q12	intronic	A/G		GWAS neuroticism (Calboli et al., 2010; Shifman et al., 2008a)	1	2.433
rs7341475	RELN	7q22	intronic	G/A		GWAS in schizophrenia, only in women (Shifman et al., 2008b)	1	1.264
rs362584	SNAP25	20p12-p11.2	intronic	G/A		GWAS in neuroticism	1	1.953
rs9960767	TCF4	18q21.1	intronic	A/C		GWAS in schizophrenia (Li et al., 2010; Stefansson et al., 2009)	0.998	4.14

[$]MHC = Major Histocompatibility Complex region (specific gene not known).

SNP	gene	location	function	alleles	alternative name(s)	inclusion criteria (references)	callrate	HW χ^2
rs1344706	ZNF804A	2q32.1	intronic	T/G		GWAS in schizophrenia (O'Donovan et al., 2008)	1	4.506
rs1130233	AKT1	14q32.32	synonymous	G/A	rs2498799 SNP4	GxE; interaction with obstetric complicationson risk for schizophrenia (Nicodemus et al., 2008), crucial role for AKT in cellular and behavioral response on chronic stress/social defeat in mice (Krishnan et al., 2008)	1	0.004
*rs2494735	AKT1	14q32.32		C/T		GxE; interaction with obstetric complications on risk for schizophrenia(Nicodemus et al., 2008) , crucial role for AKT in cellular and behavioral response on chronic stress/social defeat in mice (Krishnan et al., 2008)	0	/
rs3803300	AKT1	14q32.33		G/A	SNP1	GxE; interaction with obstetric complicationson risk for schizophrenia (Nicodemus et al., 2008) , crucial role for AKT in cellular and behavioral response on chronic stress/social defeat in mice (Krishnan et al., 2008)	1	0.209
rs6265	BDNF	11p13	missense (Val -> Met)	G/A	Val66Met	GxE; interaction with childhood adversity on risk for depression (Grabe et al., 2012; Juhasz et al., 2011; Yang et al., 2010) and bipolar disorder (Hosang et al., 2010)	0.998	2.077
rs4680	COMT	22q11.21	missense	G/A	Val158Met	GxE; interaction with stress on momentary psychotic symptoms (Collip et al., 2011c; van Winkel et al., 2008a)	0.999	0.222
rs110402	CRHR1	17q12-q22	intronic	C/T		GxE; interaction with childhood adversity on depression (Bradley et al., 2008; Heim et al., 2009), on HPA-axis reactivity (Tyrka et al., 2009), and on neuroticism (DeYoung et al., 2011)	1	0.928
rs242924	CRHR1	17q12-q22	intronic	C/A		GxE; interaction with childhood adversity on HPA-axis reactivity (Tyrka et al., 2009) and on neuroticism (DeYoung et al., 2011)	0.997	0.99

SNP	gene	location	function	alleles	alternative name(s)	inclusion criteria (references)	callrate	HW χ^2
rs7209436	CRHR1	17q12-q22	intronic	C/T		GxE; interaction with childhood adversity on depression (Bradley et al., 2008) and on neuroticism (DeYoung et al., 2011)	0.992	2.296
rs6277	DRD2	11q23	synonymous; exon 7	C/T	C957T	GxE; interaction with psychosocial stressor on reward-related behavioral impulsivity (White et al., 2009)	1	0.134
rs1360780	FKBP5	6p21.31	intron 2	C/T		GxE; interaction with childhood adversity on PTSD symptom severity (Binder et al., 2008), on depression (Appel et al., 2011), on suicide attempt (Roy et al., 2010), and on agressive behavior (Bevilacqua et al., 2012), with social stress on cortisol secretion (Ising et al., 2008), with attachment on cortisol reactivity in 14m old infants (Luijk et al., 2010)	1	2.632
#rs4713916	FKBP5	6p21.31	intronic 5' upstream	G/A		GxE; interaction with social stress on cortisol secretion (Ising et al., 2008)	0.999	14.193
rs9296158	FKBP5	6p21.31	intron 5	G/A		GxE; interaction with childhood adversity on PTSD symptom severity (Binder et al., 2008) and on suicide attempt (Roy et al., 2010)	0.994	0.632
rs6311	HTR2A	13q14-q21	promotor region	C/T	A-1438 G	GxE; interaction with maternal overprotection on harm avoidance (personality trait) (Nakamura et al., 2010)	0.999	7.122
rs6313	HTR2A	13q14-q21	synonymous	C/T	T102C	GxE; interaction with maternal overprotection on harm avoidance (personality trait) (Nakamura et al., 2010), with urban/rural residency on depressive symptoms (Jokela et al., 2007), with childhood maternal nurturance on adulthood social attachment (Salo et al., 2011)	0.995	6.866
rs6190	NR3C1	5q31.3	missense (Arg -> Lys)	G/A	ER22/23EK	GxE; interaction with psychosocial stress on cortisol response (van West et al., 2010)	1	0.02

SNP	gene	location	function	alleles	alternative name(s)	inclusion criteria (references)	callrate	HW χ^2
rs1800532	TPH1	11p15.3-p14	intron 7	C/A	A218C	GxE; interaction with childhood abuse on borderline personality disorder (Wilson et al., 2012)	0.999	0.141
rs4537731	TPH1	11p15.3-p14	promotor region	C/T	G-6526A	GxE; interaction with childhood abuse on borderline personality disorder (Wilson et al., 2012)	1	2.06

Table 2

Overview of selected SNPs. Callrates (rate of succesfully genotyped individuals) and chi-squared values of Hardy-Weinberg (HW χ^2) equilibrium are indicated. SNPs with a callrate beneath 0.90 (marked with *) or a chi-squared value above 10.83 (marked with #, corresponding with a p-value < .001) were excluded for analyses.

2.4.2. Analytic strategy

The large number of within-person measurements in the ESM-method results in improved statistical power to detect GxE interactions, yielding highly significant findings using moderate sample sizes (Collip et al., 2011c; Peerbooms et al., 2012). Some of these findings may reflect true interactions with the genotype under study, while others, in spite of a convincing p-value, may be specific to a particular sample. Therefore, a two-step strategy focusing on replication rather than absolute statistical significance was applied, as this strategy best takes the ESM data structure into account in separating true from false positives. In the first 'detection' step, the selected SNPs were thus tested for their main effects and for possible interactions with social stress in the healthy control sample (n=112) and the general population twin sample (n=434). Secondly, replicated, directionally similar main effects and SNP-social stress interaction models (at alpha .05) were then followed up in the genetic at-risk sample as well as in the patient samples. Main effects and interactions were considered potentially relevant when, in addition to replication in the control samples, (i) at least one at-risk/patient sample also yielded significant, directionally similar, association and (ii) the p-value in the combined sample reached Bonferroni-corrected statistical significance. For the latter a threshold of p=.002 was applied, since 33 genotype x stress interactions were tested.

3. RESULTS

3.1. Detection

The healthy control sample consisted of 112 participants, of whom DNA material and valid ESM data were available. 30.4% of them was male. The general population twin sample consisted of 434 female participants. Mean age of the included controls was 33.3 years (SD 11.6) and 27.7 years (SD 7.9) for the twins. See table 1 for more details.

ESM paranoia was associated with perceived social stress in the healthy control sample (b= .082, 95%-CI .064-.099, p<.001) and in the general population twin sample (b= .045, 95%-CI .036-.054, p<.001). None of the SNPs had a replicated association with ESM paranoia.

For six SNPs of the selection, replicated, directionally similar interactions with social stress were found in both the control and the twin sample (see Table 3): rs3803300 in AKT1 and 5 SNPs in the Major Histocompability Complex (MHC; rs13219181, rs3800307, rs6904071, rs6913660 and rs926300).

Table 3
Multilevel regression models of ESM paranoia with perceived social stress, SNP and their interaction term as independent variables, controlled for age and sex. Coefficients (b) and p-values (p) of the significant models are displayed.

			controls		twins		relatives of psychotic patients		psychotic patients		residual depressive symptoms		total sample	
SNP	gene	location	b	p	b	p	b	p	b	p	b	p	b	p
rs3803300	AKT1	14q32.32	-0.04	0.043	-0.028	0.013	-0.046	0.015	-0.116	0.002	-	n.s.	-0.046	$8.14*10^{-06}$
rs13219181	MHC	6p21.3	0.1	<.001	0.058	<.001	-	n.s.	-	n.s.	0.082	0.008	0.058	$1.25*10^{-11}$
rs3800307	MHC	6p21.3	0.088	<.001	0.04	<.001	-	n.s.	-	n.s.	0.096	<.001	0.048	$6.14*10^{-10}$
rs6904071	MHC	6p21.3	0.076	<.001	0.058	<.001	-	n.s.	-	n.s.	0.082	0.008	0.056	$3.56*10^{-11}$
rs6913660	MHC	6p21.3	0.076	<.001	0.058	<.001	-	n.s.	-	n.s.	0.082	0.008	0.056	$3.67*10^{-11}$
rs926300	MHC	6p21.3	0.075	<.001	0.06	<.001	-	n.s.	-	n.s.	0.082	0.008	0.056	$2.51*10^{-11}$

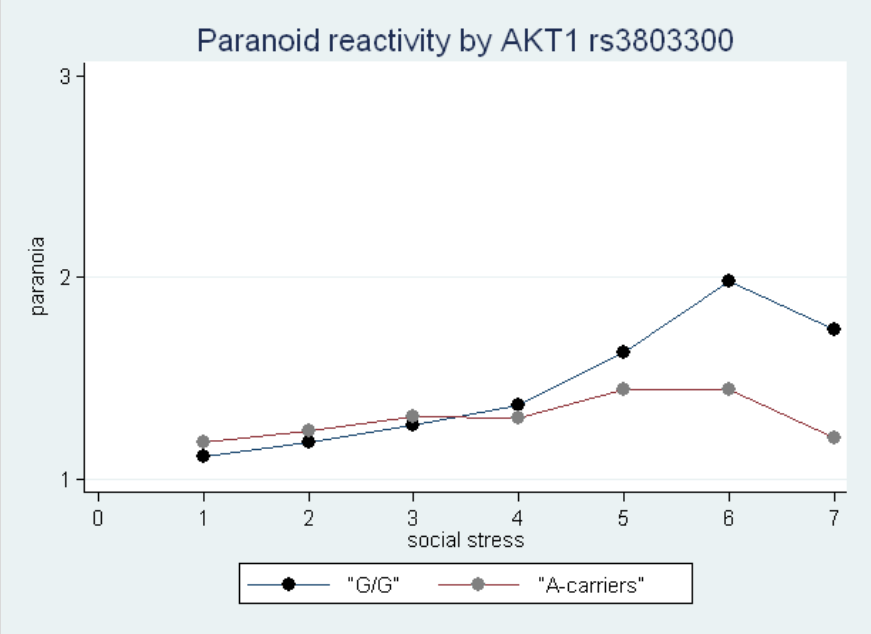
3.2. Follow-up

86 non-affected relatives of psychotic patients, 109 psychotic patients and 126 depressed patients formed three separate follow-up samples (see table 1). ESM paranoia was associated with perceived social stress in the relatives (b= .036, 95%-CI .020-.051, p<.001), the psychotic patients (b= .106, 95%-CI .076-.137, p<.001), and the depressed patients (b= .215, 95%-CI .183-.247, p<.001).

Follow-up of rs3803300 in AKT1 confirmed (directionally similar) interaction between rs3803300 and social stress in psychotic patients, as well as in the genetic at-risk sample (Table 3). In the minor allele carriers (A-allele), paranoia is significantly less reactive to the social environment. The interactions between the MHC SNPs and social stress were replicated in the subgroup of depression patients, but not in the at-risk or psychotic patient sample (Table 3). For the MHC SNPs, carriers of the minor allele consistently displayed the greatest reactivity to social stress.

In the overall sample, the AKT1 and MHC SNPs remained significant predictors of stress-evoked paranoia, at the Bonferroni corrected treshold of significance (Table 3). For rs3803300 in AKT1, the minor A-allele was associated with a decreased paranoid reactivity to social stress (see Figure 1). For all 5 MHC SNPs, the minor alleles displayed an increased reactivity.

Figure 1
The minor A-allele of rs3803300 in AKT1 (151 A-carriers vs 716 G/G genotypes) is associated with a decreased paranoid reactivity to social stress.



4. DISCUSSION

4.1. Findings

Previous work has demonstrated the importance of social stress in the paranoia continuum (Bentall and Fernyhough, 2008) as well as a familial component in moderating paranoid reactivity to the social environment (Lataster et al., 2010). However, efforts to investigate the relevance of interactions between social stress and genetic variability on psychosis phenotypes have been limited (van Winkel et al., 2008b).

The present study therefore investigated possible interactions between social stress and relevant SNPs in 5 samples across the paranoia continuum and found that, in each of the five subsamples, social stress was associated with paranoid reactivity in daily life, confirming earlier findings (Myin-Germeys et al., 2005b; Simons et al., 2009). Of the 33 selected SNPs, one SNP in AKT1 and five SNPs in MHC showed significant, replicated interactions in the healthy controls and general population twins. Confirmation of this interaction was found for rs3803300 in AKT1 in the psychotic patient group as well as in the unaffected sibling sample. The interaction of the 5 SNPs

in MHC with social stress was not confirmed in either psychosis samples, however these SNPs did show significant interactions with social stress in the depressive patients group. The interactions between social stress and rs3803300 in AKT1 as well as the MHC SNPs, were confirmed in the overall sample with Bonferroni corrected statistical significance, validating their relevance across the paranoia continuum.

4.2. AKT1 and the environment

AKT1 has previously been shown to interact with environmental factors associated with the development of psychosis. Nicodemus and colleagues (Nicodemus et al., 2008) found three SNPs in AKT1 (rs3803300, rs2494735 and rs1130233) that showed significant associations with serious obstetric complications in individuals with schizophrenia spectrum diagnoses. Another SNP in AKT1 (rs2494732) has been implicated in increasing the risk for psychosis following cannabis use (Di Forti et al., 2012b; van Winkel and GROUP Investigators, 2011). Decreased AKT activation has also been shown to be associated with an increased vulnerability to social defeat stress in mice (Krishnan et al., 2008). Mice susceptible to social defeat stress were compared with unsusceptible mice, and although similar total levels of AKT were observed, susceptible mice showed a significant decrease in AKT phosphorylation. Although these findings were used as a model for depression, similar decreases in AKT phosphorylation have been observed in schizophrenia patients (Emamian et al., 2004; Sei et al., 2007). Moreover, in a recent human study (Balog et al., 2012), several loci associated with schizophrenia at genome-wide significance (Schizophrenia Psychiatric GWAS Consortium, 2011), were associated with decreased phosphorylated AKT1 levels.

The finding of the present study cannot directly be linked to AKT1 phosphorylation or protein levels; nevertheless this study emphasizes the potential role of AKT1 in moderating environmental influences on the development of psychotic symptoms.

4.3. Stress, the MHC and BDNF

A second finding, although less convincing than the AKT1 finding, was an interaction between social stress and SNPs in MHC. SNPs in the MHC region have repeatedly been associated with the risk for psychotic disorders in recent GWAS (Li et al., 2010; Shi et al., 2009; Stefansson et al., 2009), however the knowledge about their role on intermediate phenotypes or clinical characteristics of these disorders, is very limited (Debnath et al., 2012). Since genes in the MHC region are implicated in reaction to stress and infection and in neurodevelopment, they form a conceivable junction between organism and environment, also with potential relevance in psychosis

(Debnath et al., 2012; Van Winkel et al., 2010b). Given that the MHC x stress interaction could not be replicated in the psychosis and the unaffected relatives sample, further confirmation of this GxE finding is needed.

No evidence was found for variation in the $BDNF^{Val66Met}$ genotype to be associated with paranoid reactivity to social stress. This is not in agreement with earlier findings (Simons et al., 2009). Simons and colleagues did find an interaction between social stress and $BDNF^{Val66Met}$, however their sample comprised only the general population female twins, also used in this study (Simons et al., 2009), and therefore, the BDNF finding may be seen as gender or sample specific .

4.4. Limitations and strenghts

Several methodological limitations have to be considered. First, the replication sample used in this study was a general population twin sample. As this sample consisted of only women, SNPs specifically associated with paranoia or paranoid reactivity in males could have been missed. However, there was no *a priori* hypothesis about gender differences and in analyses of the mixed gender samples, potential confounding by gender was statistically controlled for.

Second, the separate sample sizes are relatively small (see table 1). Therefore, false-negative findings are likely, on the other hand, together with the replication strategy, inferences made from the positive findings are strengthened. Lastly, paranoia and social stress were examined using self-report ESM. However, earlier studies have shown the validity of this approach (Jacobs et al., 2007).

An important strength of this study is the use of the replication strategy. As a high number of individual measurements increases the power, findings may be specific for the sample. By using a replication approach across different samples, the risk of false positive findings was highly diminished. Another important strength is the ecological validity of the ESM method (Myin-Germeys et al., 2009). More classical interview-based measurements are limited in capturing subtle fluctuations in paranoia in response to daily social stressors.

5. CONCLUSION

In conclusion, the present study identified potentially interesting gene-environment interactions underlying paranoid reactivity to the social environment. In addition, this study also represents a next step in the search for gene-environment interactions in psychiatric disorders, as crossing diagnostic boundaries to disentangle the interplay between genes and environment in ecological valid phenotypes or mechanisms, may promote a better understanding of various psychiatric disorders.

REFERENCES

American Psychiatric Association, 2000. Diagnostic and statistical manual of mental disorders, Washington, DC: Author.

Appel, K., Schwahn, C., Mahler, J., Schulz, A., Spitzer, C., Fenske, K., Stender, J., Barnow, S., John, U., Teumer, A., Biffar, R., Nauck, M., Volzke, H., Freyberger, H.J., Grabe, H.J., 2011. Moderation of adult depression by a polymorphism in the FKBP5 gene and childhood physical abuse in the general population. Neuropsychopharmacology 36(10), 1982-1991.

Balog, Z., Kiss, I., Keri, S., 2012. Five new schizophrenia loci may converge on the same cellular mechanism: the AKT pathway. The American journal of psychiatry 169(3), 335.

Bentall, R.P., Fernyhough, C., 2008. Social predictors of psychotic experiences: specificity and psychological mechanisms. Schizophr Bull 34(6), 1012-1020.

Bevilacqua, L., Carli, V., Sarchiapone, M., George, D.K., Goldman, D., Roy, A., Enoch, M.A., 2012. Interaction between FKBP5 and childhood trauma and risk of aggressive behavior. Arch Gen Psychiatry 69(1), 62-70.

Binder, E.B., Bradley, R.G., Liu, W., Epstein, M.P., Deveau, T.C., Mercer, K.B., Tang, Y., Gillespie, C.F., Heim, C.M., Nemeroff, C.B., Schwartz, A.C., Cubells, J.F., Ressler, K.J., 2008. Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. JAMA 299(11), 1291-1305.

Bradley, R.G., Binder, E.B., Epstein, M.P., Tang, Y., Nair, H.P., Liu, W., Gillespie, C.F., Berg, T., Evces, M., Newport, D.J., Stowe, Z.N., Heim, C.M., Nemeroff, C.B., Schwartz, A., Cubells, J.F., Ressler, K.J., 2008. Influence of child abuse on adult depression: moderation by the corticotropin-releasing hormone receptor gene. Arch Gen Psychiatry 65(2), 190-200.

Calboli, F.C., Tozzi, F., Galwey, N.W., Antoniades, A., Mooser, V., Preisig, M., Vollenweider, P., Waterworth, D., Waeber, G., Johnson, M.R., Muglia, P., Balding, D.J., 2010. A genome-wide association study of neuroticism in a population-based sample. PLoS One 5(7), e11504.

Collip, D., Nicolson, N.A., Lardinois, M., Lataster, T., Van Os, J., Myin-Germeys, I., 2011a. Daily cortisol, stress reactivity and psychotic experiences in individuals at above average genetic risk for psychosis. Psychological Medicine 41, 2305-2315.

Collip, D., Nicolson, N.A., Lardinois, M., Lataster, T., van Os, J., Myin-Germeys, I., 2011b. Daily cortisol, stress reactivity and psychotic experiences in individuals at above average genetic risk for psychosis. Psychological Medicine 41(11), 2305-2315.

Collip, D., van Winkel, R., Peerbooms, O., Lataster, T., Thewissen, V., Lardinois, M., Drukker, M., Rutten, B.P., Van Os, J., Myin-Germeys, I., 2011c. COMT Val158Met-stress interaction in psychosis: role of background psychosis risk. CNS Neurosci Ther 17(6), 612-619.

Combs, D.R., Penn, D.L., Chadwick, P., Trower, P., Michael, C.O., Basso, M.R., 2007. Subtypes of paranoia in a nonclinical sample. Cogn Neuropsychiatry 12(6), 537-553.

Cordell, H.J., Clayton, D.G., 2005. Genetic association studies. Lancet 366(9491), 1121-1131.

Debnath, M., Cannon, D.M., Venkatasubramanian, G., 2012. Variation in the major histocompatibility complex [MHC] gene family in schizophrenia: Associations and functional implications. Progress in neuro-psychopharmacology & biological psychiatry.

Decoster, J., van Os, J., Myin-Germeys, I., De Hert, M., van Winkel, R., 2012. Genetic Variation Underlying Psychosis-inducing Effects of Cannabis: Critical Review and Future Directions. Current Pharmaceutical Design.

Delespaul, P., 1995. Assessing schizophrenia in daily life: the experience sampling method. University of Limburg: Maastricht.

DeYoung, C.G., Cicchetti, D., Rogosch, F.A., 2011. Moderation of the association between childhood maltreatment and neuroticism by the corticotropin-releasing hormone receptor 1 gene. J Child Psychol Psychiatry 52(8), 898-906.

Di Forti, M., Iyegbe, C., Sallis, H., Kolliakou, A., Falcone, M.A., Paparelli, A., Sirianni, M., La Cascia, C., Stilo, S.A., Marques, T.R., Handley, R., Mondelli, V., Dazzan, P., Pariante, C., David, A.S., Morgan, C., Powell, J., Murray, R.M., 2012. Confirmation that the AKT1 (rs2494732) genotype influences the risk of psychosis in cannabis users. Biological psychiatry 72(10), 811-816.

Emamian, E.S., Hall, D., Birnbaum, M.J., Karayiorgou, M., Gogos, J.A., 2004. Convergent evidence for impaired AKT1-GSK3beta signaling in schizophrenia. Nat Genet 36(2), 131-137.

Ferreira, M.A., O'Donovan, M.C., Meng, Y.A., Jones, I.R., Ruderfer, D.M., Jones, L., Fan, J., Kirov, G., Perlis, R.H., Green, E.K., Smoller, J.W., Grozeva, D., Stone, J., Nikolov, I., Chambert, K., Hamshere, M.L.,

Nimgaonkar, V.L., Moskvina, V., Thase, M.E., Caesar, S., Sachs, G.S., Franklin, J., Gordon-Smith, K., Ardlie, K.G., Gabriel, S.B., Fraser, C., Blumenstiel, B., Defelice, M., Breen, G., Gill, M., Morris, D.W., Elkin, A., Muir, W.J., McGhee, K.A., Williamson, R., MacIntyre, D.J., MacLean, A.W., St, C.D., Robinson, M., Van Beck, M., Pereira, A.C., Kandaswamy, R., McQuillin, A., Collier, D.A., Bass, N.J., Young, A.H., Lawrence, J., Ferrier, I.N., Anjorin, A., Farmer, A., Curtis, D., Scolnick, E.M., McGuffin, P., Daly, M.J., Corvin, A.P., Holmans, P.A., Blackwood, D.H., Gurling, H.M., Owen, M.J., Purcell, S.M., Sklar, P., Craddock, N., 2008. Collaborative genome-wide association analysis supports a role for ANK3 and CACNA1C in bipolar disorder. Nat Genet 40(9), 1056-1058.

Freeman, D., 2008. Studying and treating schizophrenia using virtual reality: a new paradigm. Schizophr Bull 34(4), 605-610.

Freeman, D., McManus, S., Brugha, T., Meltzer, H., Jenkins, R., Bebbington, P., 2010. Concomitants of paranoia in the general population. Psychol Med, 1-14.

Geschwind, N., Peeters, F., Drukker, M., Van Os, J., Wichers, M., 2011. Mindfulness training increases momentary positive emotions and reward experience in adults vulnerable to depression: a randomized controlled trial. Journal of Consulting and Clinical Psychology 79(5), 618-628.

Grabe, H.J., Schwahn, C., Mahler, J., Appel, K., Schulz, A., Spitzer, C., Fenske, K., Barnow, S., Freyberger, H.J., Teumer, A., Petersmann, A., Biffar, R., Rosskopf, D., John, U., Volzke, H., 2012. Genetic epistasis between the brain-derived neurotrophic factor Val66Met polymorphism and the 5-HTT promoter polymorphism moderates the susceptibility to depressive disorders after childhood abuse. Prog Neuropsychopharmacol Biol Psychiatry 36(2), 264-270.

Heim, C., Bradley, B., Mletzko, T.C., Deveau, T.C., Musselman, D.L., Nemeroff, C.B., Ressler, K.J., Binder, E.B., 2009. Effect of Childhood Trauma on Adult Depression and Neuroendocrine Function: Sex-Specific Moderation by CRH Receptor 1 Gene. Front Behav Neurosci 3, 41.

Henquet, C., van Os, J., Kuepper, R., Delespaul, P., Smits, M., Campo, J.A., Myin-Germeys, I., Psychosis reactivity to cannabis use in daily life: an experience sampling study. Br J Psychiatry 196(6), 447-453.

Hosang, G.M., Uher, R., Keers, R., Cohen-Woods, S., Craig, I., Korszun, A., Perry, J., Tozzi, F., Muglia, P., McGuffin, P., Farmer, A.E., 2010. Stressful life events and the brain-derived neurotrophic factor gene in bipolar disorder. J Affect Disord 125(1-3), 345-349.

Ising, M., Depping, A.M., Siebertz, A., Lucae, S., Unschuld, P.G., Kloiber, S., Horstmann, S., Uhr, M., Muller-Myhsok, B., Holsboer, F., 2008. Polymorphisms in the FKBP5 gene region modulate recovery from psychosocial stress in healthy controls. Eur J Neurosci 28(2), 389-398.

Jacobs, N., Myin-Germeys, I., Derom, C., Delespaul, P., van Os, J., Nicolson, N.A., 2007. A momentary assessment study of the relationship between affective and adrenocortical stress responses in daily life. Biological psychology 74(1), 60-66.

Jokela, M., Lehtimaki, T., Keltikangas-Jarvinen, L., 2007. The influence of urban/rural residency on depressive symptoms is moderated by the serotonin receptor 2A gene. Am J Med Genet B Neuropsychiatr Genet 144B(7), 918-922.

Juhasz, G., Dunham, J.S., McKie, S., Thomas, E., Downey, D., Chase, D., Lloyd-Williams, K., Toth, Z.G., Platt, H., Mekli, K., Payton, A., Elliott, R., Williams, S.R., Anderson, I.M., Deakin, J.F., 2011. The CREB1-BDNF-NTRK2 pathway in depression: multiple gene-cognition-environment interactions. Biol Psychiatry 69(8), 762-771.

Krishnan, V., Han, M.H., Mazei-Robison, M., Iniguez, S.D., Ables, J.L., Vialou, V., Berton, O., Ghose, S., Covington, H.E., 3rd, Wiley, M.D., Henderson, R.P., Neve, R.L., Eisch, A.J., Tamminga, C.A., Russo, S.J., Bolanos, C.A., Nestler, E.J., 2008. AKT signaling within the ventral tegmental area regulates cellular and behavioral responses to stressful stimuli. Biological psychiatry 64(8), 691-700.

Lataster, T., Collip, D., Lardinois, M., van Os, J., Myin-Germeys, I., 2010. Evidence for a familial correlation between increased reactivity to stress and positive psychotic symptoms. Acta Psychiatr Scand 122(5), 395-404.

Lataster, T., Valmaggia, L., Lardinois, M., van Os, J., Myin-Germeys, I., 2012. Increased stress reactivity: a mechanism specifically associated with the positive symptoms of psychotic disorder. Psychological Medicine, 1-12.

Lesch, K.P., 2004. Gene-environment interaction and the genetics of depression. J Psychiatry Neurosci 29(3), 174-184.

Li, T., Li, Z., Chen, P., Zhao, Q., Wang, T., Huang, K., Li, J., Li, Y., Liu, J., Zeng, Z., Feng, G., He, L., Shi, Y., 2010. Common variants in major histocompatibility complex region and TCF4 gene are significantly associated with schizophrenia in Han Chinese. Biol Psychiatry 68(7), 671-673.

Lincoln, T.M., Peter, N., Schafer, M., Moritz, S., 2009. Impact of stress on paranoia: an experimental investigation of moderators and mediators. Psychol Med 39(7), 1129-1139.

Luijk, M.P., Velders, F.P., Tharner, A., van Ijzendoorn, M.H., Bakermans-Kranenburg, M.J., Jaddoe, V.W., Hofman, A., Verhulst, F.C., Tiemeier, H., 2010. FKBP5 and resistant attachment predict cortisol reactivity in infants: gene-environment interaction. Psychoneuroendocrinology 35(10), 1454-1461.

Moffitt, T.E., Caspi, A., Rutter, M., 2005. Strategy for investigating interactions between measured genes and measured environments. Archives of General Psychiatry 62(5), 473-481.

Moritz, S., Burnette, P., Sperber, S., Kother, U., Hagemann-Goebel, M., Hartmann, M., Lincoln, T.M., 2010. Elucidating the Black Box From Stress To Paranoia. Schizophr Bull.

Myin-Germeys, I., Delespaul, P., van Os, J., 2005a. Behavioural sensitization to daily life stress in psychosis. Psychol Med 35(5), 733-741.

Myin-Germeys, I., Marcelis, M., Krabbendam, L., Delespaul, P., van Os, J., 2005b. Subtle fluctuations in psychotic phenomena as functional states of abnormal dopamine reactivity in individuals at risk. Biol Psychiatry 58(2), 105-110.

Myin-Germeys, I., Oorschot, M., Collip, D., Lataster, J., Delespaul, P., van Os, J., 2009. Experience sampling research in psychopathology: opening the black box of daily life. Psychological Medicine 39(9), 1533-1547.

Nakamura, Y., Ito, Y., Aleksic, B., Kushima, I., Yasui-Furukori, N., Inada, T., Ono, Y., Ozaki, N., 2010. Influence of HTR2A polymorphisms and parental rearing on personality traits in healthy Japanese subjects. J Hum Genet 55(12), 838-841.

Nicodemus, K.K., Marenco, S., Batten, A.J., Vakkalanka, R., Egan, M.F., Straub, R.E., Weinberger, D.R., 2008. Serious obstetric complications interact with hypoxia-regulated/vascular-expression genes to influence schizophrenia risk. Mol Psychiatry 13(9), 873-877.

O'Donovan, M.C., Craddock, N., Norton, N., Williams, H., Peirce, T., Moskvina, V., Nikolov, I., Hamshere, M., Carroll, L., Georgieva, L., Dwyer, S., Holmans, P., Marchini, J.L., Spencer, C.C., Howie, B., Leung, H.T., Hartmann, A.M., Moller, H.J., Morris, D.W., Shi, Y., Feng, G., Hoffmann, P., Propping, P., Vasilescu, C., Maier, W., Rietschel, M., Zammit, S., Schumacher, J., Quinn, E.M., Schulze, T.G., Williams, N.M., Giegling, I., Iwata, N., Ikeda, M., Darvasi, A., Shifman, S., He, L., Duan, J., Sanders, A.R., Levinson, D.F., Gejman, P.V., Cichon, S., Nothen, M.M., Gill, M., Corvin, A., Rujescu, D., Kirov, G., Owen, M.J., Buccola, N.G., Mowry, B.J., Freedman, R., Amin, F., Black, D.W., Silverman, J.M., Byerley, W.F., Cloninger, C.R., 2008. Identification of loci associated with schizophrenia by genome-wide association and follow-up. Nat Genet 40(9), 1053-1055.

Oorschot, M., Kwapil, T., Delespaul, P., Myin-Germeys, I., 2009. Momentary assessment research in psychosis. Psychological Assessment 21(4), 498-505.

Peerbooms, O., Rutten, B.P., Collip, D., Lardinois, M., Lataster, T., Thewissen, V., Rad, S.M., Drukker, M., Kenis, G., van Os, J., Myin-Germeys, I., van Winkel, R., 2012. Evidence that interactive effects of COMT and MTHFR moderate psychotic response to environmental stress. Acta Psychiatr Scand 125(3), 247-256.

Roy, A., Gorodetsky, E., Yuan, Q., Goldman, D., Enoch, M.A., 2010. Interaction of FKBP5, a stress-related gene, with childhood trauma increases the risk for attempting suicide. Neuropsychopharmacology 35(8), 1674-1683.

Salo, J., Jokela, M., Lehtimaki, T., Keltikangas-Jarvinen, L., 2011. Serotonin receptor 2A gene moderates the effect of childhood maternal nurturance on adulthood social attachment. Genes Brain Behav 10(7), 702-709.

Schizophrenia Psychiatric GWAS Consortium, 2011. Genome-wide association study identifies five new schizophrenia loci. Nature genetics 43(10), 969-976.

Schulze, T.G., Detera-Wadleigh, S.D., Akula, N., Gupta, A., Kassem, L., Steele, J., Pearl, J., Strohmaier, J., Breuer, R., Schwarz, M., Propping, P., Nothen, M.M., Cichon, S., Schumacher, J., Rietschel, M., McMahon, F.J., 2009. Two variants in Ankyrin 3 (ANK3) are independent genetic risk factors for bipolar disorder. Mol Psychiatry 14(5), 487-491.

Sei, Y., Ren-Patterson, R., Li, Z., Tunbridge, E.M., Egan, M.F., Kolachana, B.S., Weinberger, D.R., 2007. Neuregulin1-induced cell migration is impaired in schizophrenia: association with neuregulin1 and catechol-o-methyltransferase gene polymorphisms. Molecular psychiatry 12(10), 946-957.

Shi, J., Levinson, D.F., Duan, J., Sanders, A.R., Zheng, Y., Pe'er, I., Dudbridge, F., Holmans, P.A., Whittemore, A.S., Mowry, B.J., Olincy, A., Amin, F., Cloninger, C.R., Silverman, J.M., Buccola, N.G., Byerley, W.F., Black,

D.W., Crowe, R.R., Oksenberg, J.R., Mirel, D.B., Kendler, K.S., Freedman, R., Gejman, P.V., 2009. Common variants on chromosome 6p22.1 are associated with schizophrenia. Nature 460(7256), 753-757.

Shifman, S., Bhomra, A., Smiley, S., Wray, N.R., James, M.R., Martin, N.G., Hettema, J.M., An, S.S., Neale, M.C., van den Oord, E.J., Kendler, K.S., Chen, X., Boomsma, D.I., Middeldorp, C.M., Hottenga, J.J., Slagboom, P.E., Flint, J., 2008a. A whole genome association study of neuroticism using DNA pooling. Mol Psychiatry 13(3), 302-312.

Shifman, S., Johannesson, M., Bronstein, M., Chen, S.X., Collier, D.A., Craddock, N.J., Kendler, K.S., Li, T., O'Donovan, M., O'Neill, F.A., Owen, M.J., Walsh, D., Weinberger, D.R., Sun, C., Flint, J., Darvasi, A., 2008b. Genome-wide association identifies a common variant in the reelin gene that increases the risk of schizophrenia only in women. PLoS Genet 4(2), e28.

Simons, C.J., Wichers, M., Derom, C., Thiery, E., Myin-Germeys, I., Krabbendam, L., van Os, J., 2009. Subtle gene-environment interactions driving paranoia in daily life. Genes Brain Behav 8(1), 5-12.

Snijders, T.A.B., Bosker, R.J., 1999. Multilevel analysis: an introduction to basic and advanced multilevel modeling. SAGE Publications Ltd, London.

StataCorp LP, 2012. Stata/MP 11.2 for Windows, Stata/MP 11.2 for Windows ed, College Station, TX.

Stefansson, H., Ophoff, R.A., Steinberg, S., Andreassen, O.A., Cichon, S., Rujescu, D., Werge, T., Pietilainen, O.P., Mors, O., Mortensen, P.B., Sigurdsson, E., Gustafsson, O., Nyegaard, M., Tuulio-Henriksson, A., Ingason, A., Hansen, T., Suvisaari, J., Lonnqvist, J., Paunio, T., Borglum, A.D., Hartmann, A., Fink-Jensen, A., Nordentoft, M., Hougaard, D., Norgaard-Pedersen, B., Bottcher, Y., Olesen, J., Breuer, R., Moller, H.J., Giegling, I., Rasmussen, H.B., Timm, S., Mattheisen, M., Bitter, I., Rethelyi, J.M., Magnusdottir, B.B., Sigmundsson, T., Olason, P., Masson, G., Gulcher, J.R., Haraldsson, M., Fossdal, R., Thorgeirsson, T.E., Thorsteinsdottir, U., Ruggeri, M., Tosato, S., Franke, B., Strengman, E., Kiemeney, L.A., Melle, I., Djurovic, S., Abramova, L., Kaleda, V., Sanjuan, J., de Frutos, R., Bramon, E., Vassos, E., Fraser, G., Ettinger, U., Picchioni, M., Walker, N., Toulopoulou, T., Need, A.C., Ge, D., Yoon, J.L., Shianna, K.V., Freimer, N.B., Cantor, R.M., Murray, R., Kong, A., Golimbet, V., Carracedo, A., Arango, C., Costas, J., Jonsson, E.G., Terenius, L., Agartz, I., Petursson, H., Nothen, M.M., Rietschel, M., Matthews, P.M., Muglia, P., Peltonen, L., St Clair, D., Goldstein, D.B., Stefansson, K., Collier, D.A., 2009. Common variants conferring risk of schizophrenia. Nature 460(7256), 744-747.

Tandon, R., Nasrallah, H.A., Keshavan, M.S., 2009. Schizophrenia, "just the facts" 4. Clinical features and conceptualization. Schizophr Res 110(1-3), 1-23.

Thewissen, V., Bentall, R.P., Lecomte, T., Van Os, J., Myin-Germeys, I., 2008. Fluctuations in self-esteem and paranoia in the context of daily life. Journal of Abnormal Psychology 117(1), 143-153.

Tyrka, A.R., Price, L.H., Gelernter, J., Schepker, C., Anderson, G.M., Carpenter, L.L., 2009. Interaction of childhood maltreatment with the corticotropin-releasing hormone receptor gene: effects on hypothalamic-pituitary-adrenal axis reactivity. Biol Psychiatry 66(7), 681-685.

van den Oord, E.J., Kuo, P.H., Hartmann, A.M., Webb, B.T., Moller, H.J., Hettema, J.M., Giegling, I., Bukszar, J., Rujescu, D., 2008. Genomewide association analysis followed by a replication study implicates a novel candidate gene for neuroticism. Arch Gen Psychiatry 65(9), 1062-1071.

van Os, J., Kenis, G., Rutten, B.P., 2010. The environment and schizophrenia. Nature 468(7321), 203-212.

van West, D., Del-Favero, J., Deboutte, D., Van Broeckhoven, C., Claes, S., 2010. Associations between common arginine vasopressin 1b receptor and glucocorticoid receptor gene variants and HPA axis responses to psychosocial stress in a child psychiatric population. Psychiatry Res 179(1), 64-68.

Van Winkel, R., Esquivel, G., Kenis, G., Wichers, M., Collip, D., Peerbooms, O., Rutten, B., Myin-Germeys, I., Van Os, J., 2010. REVIEW: Genome-wide findings in schizophrenia and the role of gene-environment interplay. CNS Neurosci Ther 16(5), e185-192.

van Winkel, R., GROUP Investigators, 2011. Family-based analysis of genetic variation underlying psychosis-inducing effects of cannabis: sibling analysis and proband follow-up. Arch Gen Psychiatry 68(2), 148-157.

van Winkel, R., Henquet, C., Rosa, A., Papiol, S., Fananas, L., De Hert, M., Peuskens, J., van Os, J., Myin-Germeys, I., 2008a. Evidence that the COMT(Val158Met) polymorphism moderates sensitivity to stress in psychosis: an experience-sampling study. Am J Med Genet B Neuropsychiatr Genet 147B(1), 10-17.

van Winkel, R., Stefanis, N.C., Myin-Germeys, I., 2008b. Psychosocial stress and psychosis. A review of the neurobiological mechanisms and the evidence for gene-stress interaction. Schizophr Bull 34(6), 1095-1105.

White, M.J., Lawford, B.R., Morris, C.P., Young, R.M., 2009. Interaction between DRD2 C957T polymorphism and an acute psychosocial stressor on reward-related behavioral impulsivity. Behav Genet 39(3), 285-295.

Wichers, M., Schrijvers, D., Geschwind, N., Jacobs, N., Myin-Germeys, I., Thiery, E., Derom, C., Sabbe, B., Peeters, F., Delespaul, P., Van Os, J., 2009. Mechanisms of gene-environment interactions in depression: evidence that genes potentiate multiple sources of adversity. Psychological Medicine 39, 1077-1086.

Wigman, J.T., van Nierop, M., Vollebergh, W.A., Lieb, R., Beesdo-Baum, K., Wittchen, H.U., van Os, J., 2012. Evidence that psychotic symptoms are prevalent in disorders of anxiety and depression, impacting on illness onset, risk, and severity--implications for diagnosis and ultra-high risk research. Schizophr Bull 38(2), 247-257.

Wilson, S.T., Stanley, B., Brent, D.A., Oquendo, M.A., Huang, Y.Y., Haghighi, F., Hodgkinson, C.A., Mann, J.J., 2012. Interaction between tryptophan hydroxylase I polymorphisms and childhood abuse is associated with increased risk for borderline personality disorder in adulthood. Psychiatr Genet 22(1), 15-24.

Yang, C., Xu, Y., Sun, N., Ren, Y., Liu, Z., Cao, X., Zhang, K., 2010. The combined effects of the BDNF and GSK3B genes modulate the relationship between negative life events and major depressive disorder. Brain Res 1355, 1-6.

Chapter Seven

General Discussion

The aim of this thesis was to explore the research possibilities, in the post-GWAS era, to unravel the genetics of psychosis and integrating it with existing knowledge on the role of environmental factors assumed to play a causal role in the development of psychosis. In this final chapter I will discuss the research findings of this thesis in the light of the ongoing and future genetic research in psychosis. What are the options, besides bigger genome-wide studies?

1. Endophenotypes

Although the endophenotype concept was proposed in the 1960s (John and Lewis, 1966), it caught the widespread attention of psychiatry researchers only in the beginning of this century. The renaissance of the idea inspired Gottesman and Gould to broaden the initial definition of "internal phenotypes discoverable by a biochemical test or microscopic examination", by launching five widely cited and used criteria (Gottesman and Gould, 2003); (i) association with the disorder in the general population, (ii) heritable, (iii) state-independent, (iv) cosegregation with the disorder within families and (v) non-affected family members display the endophenotype in a higher degree than the general population. Any measurable component fulfilling these criteria can be a valid and useful endophenotype. The general idea was that endophenotypes may be less genetically complex and closer to the actual underlying biology, thus facilitating the identification of genes causally implicated in the onset of a given disorder (Gottesman and Gould, 2003).

So far, mainly neurocognitive measures or deficits (Greenwood et al., 2011; Gur et al., 2007), structural and functional imaging parameters (Brown and Thompson, 2010; Khadka et al., 2013; Prasad and Keshavan, 2008), stress-reactivity (Lataster et al., 2012; Myin-Germeys and van Os, 2007) and electrophysiological parameters (Light et al., 2012; Rissling and Light, 2010) were proposed as endophenotypes for psychotic disorders. In **Chapter Two** of this thesis I argue why, amongst the suggested candidate endophenotypes, the P300-amplitude is a very good endophenotype for schizophrenia. This theorem was recently strengthened by a well-conducted study of Light and colleagues (Light et al., 2012). Out of a battery of 18 neurophysiologic and neurocognitive measures, P300-amplitude was shown to be the candidate-endophenotype with the biggest differences between patients and controls, the most state independence and the most long-term stability, together with Mismatch Negativity (MMN) and oculomotor antisaccade (Light et al., 2012). The usefulness of P300 as endophenotype is further strengthened when taking into account the heritability of around 80% (Bestelmeyer et al., 2009; Hall et al., 2009).

Although endophenotypes are described as promising to inform the "gene-to-phene gap" in psychotic disorders, there are few studies that used endophenotypes for a (partly) agnostic, genetic association approach (Greenwood et al., 2012; Greenwood et al., 2013) and even none for the very promising P300 measure. Therefore, the study described in **Chapter Two**, was conducted. Out of 193 SNPs in 67 genes, four SNPs displayed a possible association with the P300 amplitude in patients with psychosis, with rs1045642 in ABCB1 as the most convincing finding. This replicates an earlier finding of ABCB1 rs1045642 implication in the P300 amplitude in healthy volunteers (Liu et al., 2009). ABCB1 gene encodes for P-glycoprotein that has a known function in ATP-driven cellular excretion of drugs (Moons et al., 2011) and THC (Bonhomme-Faivre et al., 2008). In view of the potential relevance for the observed deficits in directed attention and working memory in schizophrenia, further translational research could be aimed to unravel the exact molecular mechanism underlying the association between ABCB1 and P300 amplitude. Also the associations between P300 amplitude and rs1625579 in miR-137, rs6265 in BDNF and rs1572899 in DISC-1, which of course still await confirmation, may generate some interesting hypotheses for further (translational) research. All three genes are implicated in the complex and crucial processes of neurodevelopment (Favalli et al., 2012; Shaikh et al., 2011; Wright et al., 2013), subtle deficits of which could be reflected by abnormal or inefficient P300 generation.

Since P300 is one of the most established intermediate phenotypes of schizophrenia and a first attempt to unravel its genetic underpinnings (**Chapter Two**) seems to be fruitful, further efforts with regard to other electrophysiological paradigms (e.g. MMN, P50, LDAEP, ...) could form an interesting complementary strategy. Due to the fact that electrophysiological parameters directly reflect the reaction of the organism to its environment, they also form potential clues for subsequent GxE research.

2. GxE

In the etiology of psychotic disorders, genetic effects may not be detectable by looking for main effects of SNPs, but only by considering higher order gene-gene or gene-environment interactions (GxE) (Cichon et al., 2009; Duncan and Keller, 2011a; van Os et al., 2010; Van Winkel et al., 2010b). This thesis was focused on the latter and tried to deal with challenges pertinent to GxE research.

2.1. Which E?

Epidemiological research provided us already with several environmental factors that are unequivocally associated with the prevalence of psychotic disorders. Meta-analytic

evidence links paternal age (Matheson et al., 2011; Miller et al., 2011), complications during pregnancy and delivery (Matheson et al., 2011; Mittal et al., 2008), urbanicity (Vassos et al., 2012), childhood trauma (Matheson et al., 2013; Varese et al., 2012), migration (Bourque et al., 2011; Veling, 2013) and cannabis use (more details in **Chapter Five**) with an increased risk for psychosis. Although in most cases causality is plausible, the exact mechanisms are still to be elucidated, taking into account that the investigated environmental factor can be nothing more than a proxy for another, more delineated causal factor (March et al., 2008) and that only a minority of all exposure leads to disorder. Such a differential reaction to a potentially harmful exposure, may point to an interaction between individual genetic sensitivity and the implicated environmental factor. The most suitable environmental factors for molecular GxE studies are those for which (i) neuroscientific findings suggest a potential mechanism (Caspi and Moffitt, 2006) and (ii) epidemiological evidence shows an increased sensitivity in people with a genetic predisposition (familial or psychometric) for psychosis (van Os et al., 2008a). The use of these criteria before performing GxE research with a certain 'E', can constitute a first filter for false positive findings. Thus, in **Chapter Five**, cannabis use is extensively argumented as an ideal candidate 'E' for GxE research, which was deployed in **Chapter Three** and **Chapter Four**.

Because of the complex interplay between nature en nurture, gene-environment correlation (rGE) can never be ruled out entirely (van Os et al., 2008a). Therefore, for each 'E' in GxE it remains important to be aware of, and control for, the possibility of rGE. For the BDNF Val66Met polymorphism (**Chapter Three**) no association between genotype and cannabis use could be detected. In **Chapter Four**, longitudinal evidence makes a possible rGE between cannabis use and AKT1 rs2497432 very unlikely.

In GxE research, high-quality measurements of the 'E' is crucial, but not always so easy to perform. In **Chapter Three** and **Chapter Four**, cannabis use was well documented using the CIDI-interview and urine analysis. Although some concerns can still be raised (van Os et al., 2008a), not every 'E' is suitable for such a clear-cut approach, certainly not if exposure (e.g. social stress) is more subtle during daily life. In this case, as argumented in **Chapter Six,** the Experience Sampling Method (ESM) can be a very useful instrument. The findings of **Chapter Six** reveal a glimpse of the possibilities of ESM in GxE research.

2.2. Which G?

In **Chapter Five** of this thesis the limitations of candidate gene approaches (cGxE)(Collins et al., 2011; Duncan and Keller, 2011a) are illustrated with reference to the 'Gene x Cannabis' research in psychosis. The big challenge for future GxE research

(in psychosis) remains combining the agnostic detection of disorder-associated genetic variance from GWAS with the hypothesis-based approach from epidemiological and neurobiological studies (van Os and Rutten, 2009). Also in **Chapter Five,** some possible strategies are suggested. One interesting strategy could be to use a broad range of SNPs with either a reliable association to the disorder or a plausible relation to the underlying biology by which the environmental factor may exert its effects (Van Winkel et al., 2010b). The first attempt that used this strategy (van Winkel and GROUP Investigators, 2011) resulted in the identification of the AKT1 rs2497432 x cannabis interaction, which was given further credibility with the study reported in **Chapter Four**. This finding, which was robust across different samples and study designs, may support the claim that the described strategy may be fruitful. In **Chapter Six,** the strategy was further refined by using a SNP selection with SNPs emerging from GWAS of relevant phenotypes on the one hand, and candidate SNPs emerging from previous GxE studies on the other hand.

Two other, more advanced strategies outlined in **Chapter Five**, are very promising for the future: (i) gene-selections based on biological pathways discovered by so-called 'gene ontology analyses' (Holmans et al., 2009; Poelmans et al., 2011; Weng et al., 2011) or 'convergent functional genomics' (Ayalew et al., 2012) and (ii) Gene-Environment Wide Interaction Studies (GEWIS)(Khoury and Wacholder, 2009).

2.3. Which outcome/phenotype?

2.3.1. Clinical diagnosis

In the available GxE research in schizophrenia/psychosis, most research focused on interactions that predict a clinical diagnosis (Caspi et al., 2005; Nicodemus et al., 2008; van Winkel and GROUP Investigators, 2011). The employed statistical strategy in papers using clinical disorders as outcome require a strict distinction between 'normal' and 'affected'. Although GxE findings based on such a distinction can be very informative (e.g. the confirmation of the AKT1 rs2497432 x cannabis interaction in **Chapter Four**), it ignores the heterogeneity and the dimensional nature of psychotic disorders, as elaborated in **Chapter One**. This thesis advocates the use of disease characteristics, subclinical symptoms or endophenotypes as outcome in GxE research.

2.3.2. Disease characteristic

It has long been suggested that modifiers of the age at onset of psychosis may offer important clues to the etiology of the illness (DeLisi, 1992). The known clinical and prognostic relevance (Crespo-Facorro et al., 2007; Rabinowitz et al., 2006) seems to underline the key role of age at onset in the heterogeneity of psychotic disorders. Also

gender could play a central role in the observed heterogeneity, possibly even by influencing age at onset (Aleman et al., 2003; van der Werf et al., 2012). Since there is evidence that cannabis use (De Hert et al., 2011; Large et al., 2011), as well as genetic factors (Hare et al., 2010) affects age at onset, examining gene-by-cannabis interaction is a logical GxE approach in the search for modifiers of age at onset. Therefore, the GxE found in **Chapter Three** of this thesis; a sex-specific interaction between BDNF Val66Met and cannabis use, predicting age at onset of psychosis, may be a promising finding. Although possibly subject to the limitations of cGxE, the tested hypothesis was based on 'building blocks' delivered by neuroscience, enhancing its *a priori* probability. The results and possible interpretations on their turn, can fuel new neuroscientific research (Caspi and Moffitt, 2006).

2.3.3. (Subclinical) Symptom level

Convincing research is available to support an etiological continuity in the psychosis continuum, for genetic as well as environmental factors (van Os et al., 2009). Therefore, as circumstantially motivated in **Chapter One,** investigating the etiological and pathophysiological mechanisms, with for instance GxE studies, along the entire continuum may help to increase the knowledge about underlying causes of psychosis. Clinically relevant psychosis seems to be marked by several upper ends of different continua, creating many interesting research hypotheses, all based on relevant characteristics, properties and/or (subclinical) symptoms of the disorder. Earlier GxE research already focused on stress-reactivity (Collip et al., 2011; van Winkel et al., 2008a), cognition (Henquet et al., 2006), positive psychotic symptoms (Henquet et al., 2009b; Henquet et al., 2006), schizotypy (van Winkel and GROUP Investigators, 2011) and paranoia (Simons et al., 2009). In **Chapter Four** of this thesis, the earlier finding of an AKT1 rs2497432 – cannabis interaction associated with schizotypy, validating the same interaction associated with clinical diagnosis of schizophrenia, is confirmed. In **Chapter Six** of this thesis, GxE between a broad selection of SNPs (G) and ESM-documented social stress (E) is examined along the paranoia continuum. The rationale and the advantages of the SNP selection and the ESM measures are described above. With an elegant replication design, variation in AKT1 rs3803300 was detected as a potential moderator of paranoid reactivity to social stress. Also genetic variation in the Major Histocompatibility Complex (MHC), with 5 SNPs detected to interact with social stress, may be of relevance along the paranoia continuum.

2.3.4. Endophenotypes

As mentioned before, endophenotypes and more particular electrophysiological endophenotypes, may form potential clues for GxE research. No environmental factors are unequivocally known to influence P300 amplitude, so no GxE design could be added to **Chapter Two** of this thesis. But with regard to this topic, an interesting study illustrates the possibilities of endophenotypes in GxE research; schizophrenia risk alleles in the TCF4 gene were found to interact with smoking behavior in predicting the P50 sensory gating response (Quednow et al., 2012). Nicotine use, excessively present in patients with psychosis, might thus be considered in future research to genetic risk factors.

3. Next step?

Ideally, epidemiological GxE findings should be replicated independently and followed up in "experimental ecogenetic" paradigms to validate them and to rule out rGE (van Os et al., 2008a). Also integration in "functional enviromics" paradigms to further unravel underlying biological mechanisms, would be an interesting next step (van Os et al., 2008a). The findings of **Chapters Two** (ABCB1, DISC1, BDNF and miR137 involvement in P300 generation), **Chapter Three** (sex-specific interaction between BDNF Val66Met and cannabis use, moderating age at onset of psychosis), **Chapter Four** (AKT1 rs2497432 – cannabis interaction associated with schizophrenia and schizotypy) and **Chapter Six** (AKT1 rs3803300 and MHC implication in paranoid reactivity to social stress) of this thesis can provide the neuroscience community with new hypotheses to test in translational (animal) research. Such a translational approach would combine epidemiological and biological/molecular research efforts in trying to explain the pathophysiological processes underlying schizophrenia, or better; to explain the cross-diagnostical processes of suspiciousness, salience attribution, disorganization, disinhibition, … (van Os, 2009). After all, the most important lesson learned in genetic research so far, is that the current nosological concepts in the clinic will likely have to make place for more (multi)dimensional and mechanistic diagnoses (Owen, 2012).

4. Challenges for the field

The main challenges of future gene x cannabis research listed in **Chapter Five** of this thesis, can be generalized to GxE research at large. Some of them are already mentioned and interpreted above; (i) improved gene-selection, (ii) high-quality

measurement of the environmental exposure, (iii) collaborative (multi-center) efforts to increase sample sizes, (iv) developing statistical strategies to deal with the large number of statistical tests and associated low power for detecting (genome-wide) significant GxE signals and (v) efforts for true replication of positive 'new' findings.

However, inspired by a critical review of GxE research so far (Duncan and Keller, 2011a) and influenced by several media reports during my own PhD trajectory about scientific fraud, it has become obvious that there are more than only methodological challenges for the GxE (and other) researchers. The current focus of funding agencies and universities on the number of publications researchers are ought to achieve (preferably in high impact journals) nourishes (i) publication bias for underpowered, positive findings and replication attempts (with the associated high false discovery rates)(Duncan and Keller, 2011a), (ii) undisclosed multiple testing (Munafo and Flint, 2009) or (iii) even flagrant forms of fraud. It also may hamper the very necessary multi-centered, collaborative research efforts. Hopefully, the awareness of such mechanisms inherent to 'modern' science, will grow fast.

REFERENCES

Aleman, A., Kahn, R.S., Selten, J.P., 2003. Sex differences in the risk of schizophrenia: evidence from meta-analysis. Archives of General Psychiatry 60(6), 565-571.

Ayalew, M., Le-Niculescu, H., Levey, D.F., Jain, N., Changala, B., Patel, S.D., Winiger, E., Breier, A., Shekhar, A., Amdur, R., Koller, D., Nurnberger, J.I., Corvin, A., Geyer, M., Tsuang, M.T., Salomon, D., Schork, N.J., Fanous, A.H., O'Donovan, M.C., Niculescu, A.B., 2012. Convergent functional genomics of schizophrenia: from comprehensive understanding to genetic risk prediction. Molecular psychiatry 17(9), 887-905.

Bestelmeyer, P.E., Phillips, L.H., Crombie, C., Benson, P., St Clair, D., 2009. The P300 as a possible endophenotype for schizophrenia and bipolar disorder: Evidence from twin and patient studies. Psychiatry Res 169(3), 212-219.

Bonhomme-Faivre, L., Benyamina, A., Reynaud, M., Farinotti, R., Abbara, C., 2008. Disposition of Delta tetrahydrocannabinol in CF1 mice deficient in mdr1a P-glycoprotein. Addict Biol 13(3-4), 295-300.

Bourque, F., van der Ven, E., Malla, A., 2011. A meta-analysis of the risk for psychotic disorders among first- and second-generation immigrants. Psychological Medicine 41(5), 897-910.

Brown, G.G., Thompson, W.K., 2010. Functional brain imaging in schizophrenia: selected results and methods. Curr Top Behav Neurosci 4, 181-214.

Caspi, A., Moffitt, T.E., 2006. Gene-environment interactions in psychiatry: joining forces with neuroscience. Nat Rev Neurosci 7(7), 583-590.

Caspi, A., Moffitt, T.E., Cannon, M., McClay, J., Murray, R., Harrington, H., Taylor, A., Arseneault, L., Williams, B., Braithwaite, A., Poulton, R., Craig, I.W., 2005. Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. Biol Psychiatry 57(10), 1117-1127.

Cichon, S., Craddock, N., Daly, M., Faraone, S.V., Gejman, P.V., Kelsoe, J., Lehner, T., Levinson, D.F., Moran, A., Sklar, P., Sullivan, P.F., 2009. Genomewide association studies: history, rationale, and prospects for psychiatric disorders. Am J Psychiatry 166(5), 540-556.

Collins, A.L., Kim, Y., Sklar, P., O'Donovan, M.C., Sullivan, P.F., 2011. Hypothesis-driven candidate genes for schizophrenia compared to genome-wide association results. Psychol Med, 1-10.

Collip, D., van Winkel, R., Peerbooms, O., Lataster, T., Thewissen, V., Lardinois, M., Drukker, M., Rutten, B.P., Van Os, J., Myin-Germeys, I., 2011. COMT Val158Met-stress interaction in psychosis: role of background psychosis risk. CNS Neurosci Ther 17(6), 612-619.

Crespo-Facorro, B., Pelayo-Teran, J.M., Perez-Iglesias, R., Ramirez-Bonilla, M., Martinez-Garcia, O., Pardo-Garcia, G., Vazquez-Barquero, J.L., 2007. Predictors of acute treatment response in patients with a first episode of non-affective psychosis: sociodemographics, premorbid and clinical variables. J Psychiatr Res 41(8), 659-666.

De Hert, M., Wampers, M., Jendricko, T., Franic, T., Vidovic, D., De Vriendt, N., Sweers, K., Peuskens, J., van Winkel, R., 2011. Effects of cannabis use on age at onset in schizophrenia and bipolar disorder. Schizophr Res 126(1-3), 270-276.

DeLisi, L.E., 1992. The significance of age of onset for schizophrenia. Schizophr Bull 18(2), 209-215.

Duncan, L.E., Keller, M.C., 2011. A Critical Review of the First 10 Years of Candidate Gene-by-Environment Interaction Research in Psychiatry. Am J Psychiatry 168(10), 1041-1049.

Favalli, G., Li, J., Belmonte-de-Abreu, P., Wong, A.H., Daskalakis, Z.J., 2012. The role of BDNF in the pathophysiology and treatment of schizophrenia. Journal of psychiatric research 46(1), 1-11.

Gottesman, II, Gould, T.D., 2003. The endophenotype concept in psychiatry: etymology and strategic intentions. Am J Psychiatry 160(4), 636-645.

Greenwood, T.A., Lazzeroni, L.C., Murray, S.S., Cadenhead, K.S., Calkins, M.E., Dobie, D.J., Green, M.F., Gur, R.E., Gur, R.C., Hardiman, G., Kelsoe, J.R., Leonard, S., Light, G.A., Nuechterlein, K.H., Olincy, A., Radant, A.D., Schork, N.J., Seidman, L.J., Siever, L.J., Silverman, J.M., Stone, W.S., Swerdlow, N.R., Tsuang, D.W., Tsuang, M.T., Turetsky, B.I., Freedman, R., Braff, D.L., 2011. Analysis of 94 candidate genes and 12 endophenotypes for schizophrenia from the Consortium on the Genetics of Schizophrenia. Am J Psychiatry 168(9), 930-946.

Greenwood, T.A., Light, G.A., Swerdlow, N.R., Radant, A.D., Braff, D.L., 2012. Association analysis of 94 candidate genes and schizophrenia-related endophenotypes. PLoS One 7(1), e29630.

Greenwood, T.A., Swerdlow, N.R., Gur, R.E., Cadenhead, K.S., Calkins, M.E., Dobie, D.J., Freedman, R., Green, M.F., Gur, R.C., Lazzeroni, L.C., Nuechterlein, K.H., Olincy, A., Radant, A.D., Ray, A., Schork, N.J., Seidman, L.J., Siever, L.J., Silverman, J.M., Stone, W.S., Sugar, C.A., Tsuang, D.W., Tsuang, M.T., Turetsky, B.I., Light, G.A., Braff, D.L., 2013. Genome-wide linkage analyses of 12 endophenotypes for schizophrenia

from the Consortium on the Genetics of Schizophrenia. The American journal of psychiatry 170(5), 521-532.

Gur, R.E., Calkins, M.E., Gur, R.C., Horan, W.P., Nuechterlein, K.H., Seidman, L.J., Stone, W.S., 2007. The Consortium on the Genetics of Schizophrenia: neurocognitive endophenotypes. Schizophr Bull 33(1), 49-68.

Hall, M.H., Schulze, K., Rijsdijk, F., Kalidindi, S., McDonald, C., Bramon, E., Murray, R.M., Sham, P., 2009. Are auditory P300 and duration MMN heritable and putative endophenotypes of psychotic bipolar disorder? A Maudsley Bipolar Twin and Family Study. Psychol Med 39(8), 1277-1287.

Hare, E., Glahn, D.C., Dassori, A., Raventos, H., Nicolini, H., Ontiveros, A., Medina, R., Mendoza, R., Jerez, A., Munoz, R., Almasy, L., Escamilla, M.A., 2010. Heritability of age of onset of psychosis in schizophrenia. Am J Med Genet B Neuropsychiatr Genet 153B(1), 298-302.

Henquet, C., Rosa, A., Delespaul, P., Papiol, S., Fananas, L., van Os, J., Myin-Germeys, I., 2009. COMT ValMet moderation of cannabis-induced psychosis: a momentary assessment study of 'switching on' hallucinations in the flow of daily life. Acta Psychiatr Scand 119(2), 156-160.

Henquet, C., Rosa, A., Krabbendam, L., Papiol, S., Fananas, L., Drukker, M., Ramaekers, J.G., van Os, J., 2006. An experimental study of catechol-o-methyltransferase Val158Met moderation of delta-9-tetrahydrocannabinol-induced effects on psychosis and cognition. Neuropsychopharmacology 31(12), 2748-2757.

Holmans, P., Green, E.K., Pahwa, J.S., Ferreira, M.A., Purcell, S.M., Sklar, P., Owen, M.J., O'Donovan, M.C., Craddock, N., 2009. Gene ontology analysis of GWA study data sets provides insights into the biology of bipolar disorder. Am J Hum Genet 85(1), 13-24.

John, B., Lewis, K.R., 1966. Chromosome variability and geographic distribution in insects. Science 152(3723), 711-721.

Khadka, S., Meda, S.A., Stevens, M.C., Glahn, D.C., Calhoun, V.D., Sweeney, J.A., Tamminga, C.A., Keshavan, M.S., O'Neil, K., Schretlen, D., Pearlson, G.D., 2013. Is Aberrant Functional Connectivity A Psychosis Endophenotype? A Resting State Functional Magnetic Resonance Imaging Study. Biological psychiatry.

Khoury, M.J., Wacholder, S., 2009. Invited commentary: from genome-wide association studies to gene-environment-wide interaction studies--challenges and opportunities. Am J Epidemiol 169(2), 227-230; discussion 234-225.

Large, M., Sharma, S., Compton, M.T., Slade, T., Nielssen, O., 2011. Cannabis use and earlier onset of psychosis: a systematic meta-analysis. Arch Gen Psychiatry 68(6), 555-561.

Lataster, T., Valmaggia, L., Lardinois, M., van Os, J., Myin-Germeys, I., 2012. Increased stress reactivity: a mechanism specifically associated with the positive symptoms of psychotic disorder. Psychological Medicine, 1-12.

Light, G.A., Swerdlow, N.R., Rissling, A.J., Radant, A., Sugar, C.A., Sprock, J., Pela, M., Geyer, M.A., Braff, D.L., 2012. Characterization of neurophysiologic and neurocognitive biomarkers for use in genomic and clinical outcome studies of schizophrenia. PLoS One 7(7), e39434.

Liu, J., Kiehl, K.A., Pearlson, G., Perrone-Bizzozero, N.I., Eichele, T., Calhoun, V.D., 2009. Genetic determinants of target and novelty-related event-related potentials in the auditory oddball response. Neuroimage 46(3), 809-816.

March, D., Hatch, S.L., Morgan, C., Kirkbride, J.B., Bresnahan, M., Fearon, P., Susser, E., 2008. Psychosis and place. Epidemiol Rev 30, 84-100.

Matheson, S.L., Shepherd, A.M., Laurens, K.R., Carr, V.J., 2011. A systematic meta-review grading the evidence for non-genetic risk factors and putative antecedents of schizophrenia. Schizophr Res 133(1-3), 133-142.

Matheson, S.L., Shepherd, A.M., Pinchbeck, R.M., Laurens, K.R., Carr, V.J., 2013. Childhood adversity in schizophrenia: a systematic meta-analysis. Psychological Medicine 43(2), 225-238.

Miller, B., Messias, E., Miettunen, J., Alaraisanen, A., Jarvelin, M.R., Koponen, H., Rasanen, P., Isohanni, M., Kirkpatrick, B., 2011. Meta-analysis of paternal age and schizophrenia risk in male versus female offspring. Schizophr Bull 37(5), 1039-1047.

Mittal, V.A., Ellman, L.M., Cannon, T.D., 2008. Gene-environment interaction and covariation in schizophrenia: the role of obstetric complications. Schizophr Bull 34(6), 1083-1094.

Moons, T., de Roo, M., Claes, S., Dom, G., 2011. Relationship between P-glycoprotein and second-generation antipsychotics. Pharmacogenomics 12(8), 1193-1211.

Munafo, M.R., Flint, J., 2009. Replication and heterogeneity in gene x environment interaction studies. Int J Neuropsychopharmacol 12(6), 727-729.

Myin-Germeys, I., van Os, J., 2007. Stress-reactivity in psychosis: evidence for an affective pathway to psychosis. Clinical psychology review 27(4), 409-424.

Nicodemus, K.K., Marenco, S., Batten, A.J., Vakkalanka, R., Egan, M.F., Straub, R.E., Weinberger, D.R., 2008. Serious obstetric complications interact with hypoxia-regulated/vascular-expression genes to influence schizophrenia risk. Mol Psychiatry 13(9), 873-877.

Owen, M.J., 2012. Implications of genetic findings for understanding schizophrenia. Schizophr Bull 38(5), 904-907.

Poelmans, G., Pauls, D.L., Buitelaar, J.K., Franke, B., 2011. Integrated genome-wide association study findings: identification of a neurodevelopmental network for attention deficit hyperactivity disorder. Am J Psychiatry 168(4), 365-377.

Prasad, K.M., Keshavan, M.S., 2008. Structural cerebral variations as useful endophenotypes in schizophrenia: do they help construct "extended endophenotypes"? Schizophr Bull 34(4), 774-790.

Quednow, B.B., Brinkmeyer, J., Mobascher, A., Nothnagel, M., Musso, F., Grunder, G., Savary, N., Petrovsky, N., Frommann, I., Lennertz, L., Spreckelmeyer, K.N., Wienker, T.F., Dahmen, N., Thuerauf, N., Clepce, M., Kiefer, F., Majic, T., Mossner, R., Maier, W., Gallinat, J., Diaz-Lacava, A., Toliat, M.R., Thiele, H., Nurnberg, P., Wagner, M., Winterer, G., 2012. Schizophrenia risk polymorphisms in the TCF4 gene interact with smoking in the modulation of auditory sensory gating. Proceedings of the National Academy of Sciences of the United States of America 109(16), 6271-6276.

Rabinowitz, J., Levine, S.Z., Hafner, H., 2006. A population based elaboration of the role of age of onset on the course of schizophrenia. Schizophr Res 88(1-3), 96-101.

Rissling, A.J., Light, G.A., 2010. Neurophysiological measures of sensory registration, stimulus discrimination, and selection in schizophrenia patients. Curr Top Behav Neurosci 4, 283-309.

Shaikh, M., Hall, M.H., Schulze, K., Dutt, A., Li, K., Williams, I., Walshe, M., Constante, M., Broome, M., Picchioni, M., Toulopoulou, T., Collier, D., Stahl, D., Rijsdijk, F., Powell, J., Murray, R.M., Arranz, M., Bramon, E., 2011. Effect of DISC1 on the P300 Waveform in Psychosis. Schizophr Bull.

Simons, C.J., Wichers, M., Derom, C., Thiery, E., Myin-Germeys, I., Krabbendam, L., van Os, J., 2009. Subtle gene-environment interactions driving paranoia in daily life. Genes Brain Behav 8(1), 5-12.

van der Werf, M., Hanssen, M., Kohler, S., Verkaaik, M., Verhey, F.R., van Winkel, R., van Os, J., Allardyce, J., 2012. Systematic review and collaborative recalculation of 133 693 incident cases of schizophrenia. Psychological Medicine, 1-8.

van Os, J., 2009. A salience dysregulation syndrome. The British journal of psychiatry : the journal of mental science 194(2), 101-103.

van Os, J., Kenis, G., Rutten, B.P., 2010. The environment and schizophrenia. Nature 468(7321), 203-212.

van Os, J., Linscott, R.J., Myin-Germeys, I., Delespaul, P., Krabbendam, L., 2009. A systematic review and meta-analysis of the psychosis continuum: evidence for a psychosis proneness-persistence-impairment model of psychotic disorder. Psychol Med 39(2), 179-195.

van Os, J., Rutten, B.P., 2009. Gene-environment-wide interaction studies in psychiatry. Am J Psychiatry 166(9), 964-966.

van Os, J., Rutten, B.P., Poulton, R., 2008. Gene-environment interactions in schizophrenia: review of epidemiological findings and future directions. Schizophr Bull 34(6), 1066-1082.

Van Winkel, R., Esquivel, G., Kenis, G., Wichers, M., Collip, D., Peerbooms, O., Rutten, B., Myin-Germeys, I., Van Os, J., 2010. REVIEW: Genome-wide findings in schizophrenia and the role of gene-environment interplay. CNS Neurosci Ther 16(5), e185-192.

van Winkel, R., GROUP Investigators, 2011. Family-based analysis of genetic variation underlying psychosis-inducing effects of cannabis: sibling analysis and proband follow-up. Arch Gen Psychiatry 68(2), 148-157.

van Winkel, R., Henquet, C., Rosa, A., Papiol, S., Fananas, L., De Hert, M., Peuskens, J., van Os, J., Myin-Germeys, I., 2008. Evidence that the COMT(Val158Met) polymorphism moderates sensitivity to stress in psychosis: an experience-sampling study. Am J Med Genet B Neuropsychiatr Genet 147B(1), 10-17.

Varese, F., Smeets, F., Drukker, M., Lieverse, R., Lataster, T., Viechtbauer, W., Read, J., van Os, J., Bentall, R.P., 2012. Childhood adversities increase the risk of psychosis: a meta-analysis of patient-control, prospective- and cross-sectional cohort studies. Schizophr Bull 38(4), 661-671.

Vassos, E., Pedersen, C.B., Murray, R.M., Collier, D.A., Lewis, C.M., 2012. Meta-analysis of the association of urbanicity with schizophrenia. Schizophr Bull 38(6), 1118-1123.

Veling, W., 2013. Ethnic minority position and risk for psychotic disorders. Curr Opin Psychiatry 26(2), 166-171.

Weng, L., Macciardi, F., Subramanian, A., Guffanti, G., Potkin, S.G., Yu, Z., Xie, X., 2011. SNP-based pathway enrichment analysis for genome-wide association studies. BMC Bioinformatics 12, 99.

Wright, C., Turner, J.A., Calhoun, V.D., Perrone-Bizzozero, N., 2013. Potential Impact of miR-137 and Its Targets in Schizophrenia. Front Genet 4, 58.

Chapter Eight

Valorisation

1. Societal need

Psychotic disorders are estimated to be among the health states with the highest disability (Salomon et al., 2013). Patients, caregivers and professional helpers are making big efforts to treat the disease and lessen the impact on quality of life, but many of them are confronted with the limitations of their efforts, interventions and care. Many of them have to deal with feelings of powerlessness and have to cope with permanent disabilities.

Fortunately, since the 1950s, antipsychotic medication is available. Although it underwent already some improvements, important shortcomings still remain; side-effects and/or insufficient effectiveness. The general pharmacodynamic mechanism consists of influencing dopaminergic signaling in the brain, which disturbance is believed to be a common final pathophysiological pathway in psychosis (Lau et al., 2013). However, this final pathway does not represent the etiology of the disorder.

We have already considerable knowledge about genetic and environmental risk factors for developing psychotic disorder, but how these factors interact and via which precise neurobiological mechanisms they mediate their harmful effects, is not yet understood (Keshavan et al., 2008; Tandon et al., 2008).

Fully understanding the exact etiology of psychosis would make it possible to develop new and better prevention and treatment strategies. This is the ultimate goal of genetic researchers in psychosis. Our DNA builds the smallest elements in the network of our brain, so detecting disease-specific variations in the DNA may lead us to the discovery of molecular brain pathways, that really matter.

Since the basic knowledge of the etiology of psychotic disorders is still limited, and possible leverage points to intervene are still hidden, the scientific need is quite obvious. The search for prevention strategies, early detection strategies and better treatment options to lessen the personal, societal and economical harm caused by the disease, mainly run aground on the still remaining scientific need to elucidate the etiology.

Once more is known about the etiology, pharmaceutical research and development groups can start their search for new pharmacological compounds. Also healthcare organizations and policy makers will be able to think about the implementation of prevention strategies, more efficient early detection programs and more personalized treatment protocols. And hopefully, patients and their families will have more guidance in dealing with increased genetic risk, preventing disease development, coping with symptoms and preventing relapse.

2. Critical analysis

Alas, psychotic disorders are very complex and multifactorial. So, the 'code' is not easily cracked. Brute force strategies to unravel the genetic code of schizophrenia, e.g. GWAS, has only had limited success so far. Some possible explanations are provided in the introduction and the general discussion of this dissertation. Some alternatives, suggested in the literature, are listed.

But also the refined, purely hypothesis-driven strategies that are already used, e.g. candidate SNP GxE studies, have important shortcomings, as extensively substantiated in chapter five, based on the example of research on genes x cannabis interactions in psychosis.
This critical analysis is followed by the listing of some guidelines which can be used by researchers who are designing new GxE studies or by funding agencies which have to decide which study protocols to sponsor:

Large samples

- Collaborative efforts

Appropriate gene selection

- Caution with (single) candidate gene approaches
- Possibility for broad SNP range approaches: SNPs associated with disorder or underlying biology by which the environmental factor may exert its effects
- Genes implicated in underlying pathways detected by gene ontology analyses
- GEWIS and subsequent pathway detection

Adequate replication

- Importance of replication in clinical phenotype
- Efforts for true replication of reported findings

The use of this guidelines is not limited to genes x cannabis use in psychosis, but can be expanded to all GxE research in complex, multifactorial (psychiatric) disorders.

3. Proof of concept

In this dissertation some suggested, alternative methodologies in genetic research are applied. The studies show some very interesting results, which are discussed in the specific chapters. In addition, some of these studies can be seen as proofs of concept.

The successful application of the alternative strategies for genetic research invites other scientists to use these strategies in bigger samples and with more extensive ways of gene-selection.

3.1. Electrophysiological endophenotypes

Although endophenotypes are described as promising to inform the "gene-to-phene gap" in psychotic disorders, there are few studies that used endophenotypes for a (partly) agnostic, genetic association approach (Greenwood et al., 2012; Greenwood et al., 2013) and even none for the very promising P300-wave. Since P300 is one of the most established intermediate phenotypes of schizophrenia and a first attempt, in this dissertation, to unravel its genetic underpinnings seems to be fruitful, further efforts to replicate and extend this approach are justified. Also the use of other electrophysiological paradigms (e.g. MMN, P50, LDAEP, ...) could form an interesting complementary strategy.

3.2. Experience Sampling Method in GxE research

As stated before (Moffitt et al., 2005), several strategies can improve the quality of environmental data; prospective instead of retrospective, proximal instead of distal and cumulative, repeated measures instead of snapshot measures. The latter because it provides more precise, sensitive, and reliable measurement of the environmental risk factor. The Experience Sampling Method (ESM), also described in this dissertation, is an elegant diary method that assesses thoughts, emotions and (social) context in the flow of daily life and meets the above mentioned criteria for optimal measurement of the environment (Myin-Germeys et al., 2009). The ecological validity of the ESM method is outcompeting this of more classical interview-based measurements, that are limited in capturing subtle fluctuations of the studied phenotype (e.g. paranoia) in response to daily life stressors (e.g. social stress). Referring to the results of the study in this dissertation, a broader use of ESM data in GxE approaches can be advocated.

3.3. Crossing diagnostic boundaries with GxE research

Studying psychosis with a binary distinction between 'ill' and 'not ill', ignores the heterogeneity and the dimensional nature of psychotic disorders, as elaborated in the general introduction.

As described in chapter six of this thesis, crossing diagnostic boundaries to disentangle the interplay between genes and environment in ecological valid phenotypes, characteristics or mechanisms, may promote a better understanding of various

psychiatric disorders. Given the interesting results of the mentioned study, this innovative strategy deserves to be propagated.

4. Dissemination

4.1. Publication

Given the stage of the genetic research in psychosis and the fact that most of the results in this thesis still need replication, the most important stakeholders for this type of research are other (future) scientists. Also the above mentioned innovative approaches first need to be adopted and applied by other researchers.
Therefore, dissemination of the work in this dissertation will mainly be limited to publication in scientific journals or presentation at research congresses. See the publication list at the end of this booklet for more details.

4.2. Employing acquired skills within network

The combination of clinical work in the University Psychiatric Centre KU Leuven, collecting data in a prospective twin survey and in an electrophysiological lab and performing research activities in Maastricht University, gave me the opportunity to acquire a lot of skills. It also creates a lot of opportunities for cross-pollination at several levels between several clinical and research groups.
Therefore, after finishing my PhD-trajectory, I will be glad to keep contact with all the people and groups I met. It would be an honor to be able to participate in the further analyses of the collected data. And I will certainly try to employ my acquired skills to help promoting the quality of scientific research, and its output, in the whole network.

4.3. Coaching young researchers

Coaching young researchers will be a good way to preserve own skills and to further disseminate the above mentioned innovative approaches in genetic research.

5. Concern

As already mentioned in the general discussion, I am concerned about the strong trend to valorization of (fundamental) science. I agree that cooperation between university, industry, clinic and public policy should be encouraged. But, since I do not expect a lucrative or quickly implementable scientific breakthrough in psychosis research in the near future, I am afraid that the quality of research will suffer under the perceived need of quantity and that the so much needed collaborative efforts will be hampered.

REFERENCES

Greenwood, T.A., Light, G.A., Swerdlow, N.R., Radant, A.D., Braff, D.L., 2012. Association analysis of 94 candidate genes and schizophrenia-related endophenotypes. PLoS One 7(1), e29630.

Greenwood, T.A., Swerdlow, N.R., Gur, R.E., Cadenhead, K.S., Calkins, M.E., Dobie, D.J., Freedman, R., Green, M.F., Gur, R.C., Lazzeroni, L.C., Nuechterlein, K.H., Olincy, A., Radant, A.D., Ray, A., Schork, N.J., Seidman, L.J., Siever, L.J., Silverman, J.M., Stone, W.S., Sugar, C.A., Tsuang, D.W., Tsuang, M.T., Turetsky, B.I., Light, G.A., Braff, D.L., 2013. Genome-wide linkage analyses of 12 endophenotypes for schizophrenia from the Consortium on the Genetics of Schizophrenia. The American journal of psychiatry 170(5), 521-532.

Keshavan, M.S., Tandon, R., Boutros, N.N., Nasrallah, H.A., 2008. Schizophrenia, "just the facts": what we know in 2008 Part 3: neurobiology. Schizophr Res 106(2-3), 89-107.

Lau, C.I., Wang, H.C., Hsu, J.L., Liu, M.E., 2013. Does the dopamine hypothesis explain schizophrenia? Rev Neurosci 24(4), 389-400.

Moffitt, T.E., Caspi, A., Rutter, M., 2005. Strategy for investigating interactions between measured genes and measured environments. Archives of General Psychiatry 62(5), 473-481.

Myin-Germeys, I., Oorschot, M., Collip, D., Lataster, J., Delespaul, P., van Os, J., 2009. Experience sampling research in psychopathology: opening the black box of daily life. Psychological Medicine 39(9), 1533-1547.

Salomon, J.A., Vos, T., Hogan, D.R., Gagnon, M., Naghavi, M., Mokdad, A., Begum, N., Shah, R., Karyana, M., Kosen, S., Farje, M.R., Moncada, G., Dutta, A., Sazawal, S., Dyer, A., Seiler, J., Aboyans, V., Baker, L., Baxter, A., Benjamin, E.J., Bhalla, K., Bin Abdulhak, A., Blyth, F., Bourne, R., Braithwaite, T., Brooks, P., Brugha, T.S., Bryan-Hancock, C., Buchbinder, R., Burney, P., Calabria, B., Chen, H., Chugh, S.S., Cooley, R., Criqui, M.H., Cross, M., Dabhadkar, K.C., Dahodwala, N., Davis, A., Degenhardt, L., Diaz-Torne, C., Dorsey, E.R., Driscoll, T., Edmond, K., Elbaz, A., Ezzati, M., Feigin, V., Ferri, C.P., Flaxman, A.D., Flood, L., Fransen, M., Fuse, K., Gabbe, B.J., Gillum, R.F., Haagsma, J., Harrison, J.E., Havmoeller, R., Hay, R.J., Hel-Baqui, A., Hoek, H.W., Hoffman, H., Hogeland, E., Hoy, D., Jarvis, D., Karthikeyan, G., Knowlton, L.M., Lathlean, T., Leasher, J.L., Lim, S.S., Lipshultz, S.E., Lopez, A.D., Lozano, R., Lyons, R., Malekzadeh, R., Marcenes, W., March, L., Margolis, D.J., McGill, N., McGrath, J., Mensah, G.A., Meyer, A.C., Michaud, C., Moran, A., Mori, R., Murdoch, M.E., Naldi, L., Newton, C.R., Norman, R., Omer, S.B., Osborne, R., Pearce, N., Perez-Ruiz, F., Perico, N., Pesudovs, K., Phillips, D., Pourmalek, F., Prince, M., Rehm, J.T., Remuzzi, G., Richardson, K., Room, R., Saha, S., Sampson, U., Sanchez-Riera, L., Segui-Gomez, M., Shahraz, S., Shibuya, K., Singh, D., Sliwa, K., Smith, E., Soerjomataram, I., Steiner, T., Stolk, W.A., Stovner, L.J., Sudfeld, C., Taylor, H.R., Tleyjeh, I.M., van der Werf, M.J., Watson, W.L., Weatherall, D.J., Weintraub, R., Weisskopf, M.G., Whiteford, H., Wilkinson, J.D., Woolf, A.D., Zheng, Z.J., Murray, C.J., 2013. Common values in assessing health outcomes from disease and injury: disability weights measurement study for the Global Burden of Disease Study 2010. Lancet 380(9859), 2129-2143.

Tandon, R., Keshavan, M.S., Nasrallah, H.A., 2008. Schizophrenia, "just the facts" what we know in 2008. 2. Epidemiology and etiology. Schizophr Res 102(1-3), 1-18.

Chapter Nine

Epilogue

1. Summary
2. Samenvatting (*Summary in Dutch*)
3. Dank (*Acknowledgements*)
4. Curriculum Vitae
5. List of publications

1. SUMMARY

A lot of research efforts has already been made to unravel the underlying genetics of schizophrenia. Genome Wide Association Studies (GWAS) gave the scientific world a few good findings but were not able to capture the whole complexity of the disease. This thesis, *Breaking down schizophrenia, into phenes, genes and environment*, explores the further possibilities to unravel the genetics of psychosis, by leaving the concept of schizophrenia and focusing on separate disease characteristics, (subclinical) symptoms and endophenotypes. Also the methodology of simple genetic association was exchanged for the more ecologically valid model of gene-environment interaction (GxE).

Chapter One describes the concept of schizophrenia and questions its validity in the light of recent knowledge. Instead of a binary distinction between being diagnosed or not, the psychosis continuum, from subclinical psychotic experiences to symptoms requiring treatment, can offer a more promising approach for scientific research. Like for a phenomenological continuum, there is also evidence available for an etiological and pathophysiological continuity. Since only a better understanding of the underlying molecular pathophysiological mechanisms can provide scientists with new clues to develop new and better biological, therapeutic interventions and since the genetic association studies in schizophrenia, given their limitations, seem to approach their maximum return, other research strategies are needed.
The concept of intermediate phenotype is outlined and decreased P300 amplitude is put forward as a valid candidate. Also the importance of the environment in a possible shift on the psychosis continuum is stressed. For researchers, properly measuring the environment is a big challenge, in which the introduced Experience Sampling Method (ESM) can be an elegant solution.
To complete the introduction, gene-environment interactions are announced as a more ecological valid way to look at the complex phenomenon of psychosis. Exploring the application of GxE, together with the application of the endophenotype approach, in genetic research in psychosis is the aim of this thesis.

Chapter Two assesses decreased P300 as a valid endophenotype for schizophrenia and looks for its genetic underpinnings. To do so, a range of 193 single nucleotide polymorphisms (SNPs) were examined for their association with P300 amplitude in 336 patients diagnosed with schizophrenia. The SNP selection was based on previous evidence of association with schizophrenia, involvement in dopamine or

endocannabinoid signaling or involvement in the regulation of environmental influences. 4 SNPs of the selection show significant association with P300 amplitude; rs1045642 in ABCB1, rs1572899 in DISC1, rs6265 in BDNF and rs1625579 in miR137. Especially the ABCB1-SNP seems to be a relevant finding since it has the most convincing association with P300 amplitude, but also because this finding is in line with that of an earlier study in healthy individuals. ABCB1 encodes for P-glycoprotein, which has a known function in ATP-driven cellular excretion.
The underlying biology of the found associations, as well as the specific significance for psychosis, remains to be elucidated. Anyway, further efforts to understand the molecular mechanisms underlying P300-generation, seem to be of big interest for psychosis research.

The next three chapters discuss the differential effects of cannabis on the development of psychosis, based on the GxE theory. In **Chapter Three** a candidate-gene approach is used to examine the association between cannabis use and BDNF Val66Met genotype on the one hand, and age at onset of psychosis on the other, taking into account the possibility of a sex-specific GxE. Age at onset is an important disease characteristic; both clinically (in predicting the course of the illness) and etiologically. Analyses in a sample of 585 patients with a psychotic disorder show an interaction between cannabis use and BDNF in association with age at onset in female patients. In the total sample, cannabis use before illness onset is associated with a 2.7 years earlier age at onset. However, in female patients the effect of cannabis use can only be interpreted when taking into account the interaction with BDNF Val66Met; Met carrying female patients with cannabis use before onset have a 7 years earlier age at onset. These results suggest complex interactions between BDNF, cannabis and estrogen (e.g. in the hippocampal structures), which may be a subject for further research.
Chapter Four describes a study that adds more credibility to the existence of an interesting GxE with rs2494732 in AKT1, suggested to contribute to the differential sensitivity to the psychotogenic effects of cannabis. Data of two samples; (i) 598 unaffected siblings of 689 patients and (ii) 533 patients with psychosis, are used in a follow-up and a case-only replication analysis. The follow-up part of the study finds that the interaction between cannabis use and AKT1 rs2494732 significantly predicts levels of schizotypy after three years. Only siblings with both persistent cannabis use and the rs2494732 C-allele show increased schizotypy scores. The case-only part could not find a statistically significant association between AKT1 genotype and a history of cannabis use, but the direction of association and the effect sizes were comparable to

those in the original report. A combined analysis (n=1222) of the discovery sample and the replication sample did confirm the association between rs2494732 AKT1 genotype and cannabis use (history as well as frequency of use).

Chapter Five provides a critical review of the available evidence suggesting a GxE with cannabis in psychosis. Molecular studies in this matter are only valid and relevant if epidemiological findings suggest that psychosis liability contributes to differential sensitivity to the effects of cannabis. Familial risk as well as psychometric risk is used as a proxy for psychosis liability in epidemiological studies. The available studies do find a differential sensitivity to cannabis, by *a priori* liability, to develop (subclinical) psychotic symptoms, but evidence for differential sensitivity at the level of cognition and neurobiological alterations is less consistent, respectively less abundant. In molecular GxE studies few (if any) interactions have been consistently replicated, although the reported interaction with AKT1 is promising. Several reasons for the apparent lack of consistent replication are listed; problems with gene selection, underpowered samples, lack of adequate attempts for replication and 'pseudo-replications', and considerable heterogeneity in research designs and outcome measures. Based on this observations, some recommendations are made for future research.

Finally and in the spirit of the above-described etiological continuity in the psychosis continuum, **Chapter Six** examines GxE in paranoia, a core symptom of psychotic disorders. The results of a large ESM-study (n=876), exploring underlying genetics of paranoid reactivity to social stress, are presented. For this study, 33 SNPs emerging from previous GWAS or GxE studies, are selected and tested for their interaction with perceived social stress in predicting paranoia. A replication strategy within two samples of healthy individuals is followed-up in an at-risk (unaffected siblings of psychotic patients) and two patient samples; (i) psychotic patients and (ii) formerly depressed patients with residual symptoms. An interaction between rs3803300 in AKT1 and social stress was detected, replicated and confirmed in the at-risk sample and the psychotic patient group. 5 SNPs in the Major Histocompatibility Complex (MHC) were replicated and confirmed in the depressed patients group. The interactions between social stress and rs3803300 in AKT1 as well as the MHC SNPs, were confirmed in the overall sample with Bonferroni corrected statistical significance, validating their relevance across the paranoia continuum. Besides the identification of potentially interesting GxE underlying paranoia, this study also proposes a next step in the search for gene-environment interactions in psychiatric disorders by crossing diagnostic boundaries with ecological valid phenotypes.

Chapter Seven discusses the findings of this thesis in the light of the ongoing genetic research in psychosis and provides some directions for future research, which are further elaborated in **Chapter Eight.**

2. SAMENVATTING

Al heel wat onderzoek probeerde om de onderliggende genetica van schizofrenie te ontrafelen. *Genome Wide Association Studies* (GWAS) leverden enkele goede bevindingen op, maar waren niet in staat om de volledige complexiteit van de aandoening te vatten. Dit proefschrift, *Breaking down schizophrenia, into phenes, genes and environment*, verkent de verdere mogelijkheden om de genetica van psychose te ontcijferen. Dit onder meer door het concept 'schizofrenie' te verlaten en in te zoomen op afzonderlijke ziektekenmerken, (subklinische) symptomen en endofenotypes. Ook de methode van het enkelvoudig, genetisch associatie-onderzoek wordt ingeruild voor de zoektocht naar, ecologisch meer valide, gen-omgevingsinteracties (GxE).

Hoofdstuk Eén beschrijft het concept van schizofrenie en stelt, in het licht van de huidige kennis, zijn validiteit in vraag. Het omruilen van het onderscheid tussen 'ziek' en 'niet ziek' voor een psychose-continuüm, biedt een meerbelovende benadering voor wetenschappelijk onderzoek. Niet enkel voor het bestaan van een fenomenologische continuïteit (gaande van subklinische psychotische ervaringen tot symptomen die behandeling behoeven), maar ook van een etiologische en pathofysiologische continuïteit, bestaat er reeds wetenschappelijk bewijs. Omdat het ontwikkelen van nieuwe biologische behandelingen enkel kan wanneer we de onderliggende moleculaire, etiologische en pathofysiologische mechanismen beter begrijpen, en omdat genetische associatiestudies in schizofrenie hun maximale rendement lijken te benaderen, zijn er andere onderzoeksstrategieën nodig.
Het concept 'endofenotype' wordt geschetst en een verminderde P300 amplitude wordt vooruitgeschoven als een goede kandidaat. Ook het belang van de omgeving binnen het psychose-continuüm wordt benadrukt. Het accuraat meten van omgevings-variabelen vormt echter een grote uitdaging, waarbij de *Experience Sampling Method* een elegante oplossingkan bieden. Deze methode wordt voorgesteld.
De algemene introductie wordt afgerond met het voorstellen van GxE als een ecologisch meer valide model om het complexe fenomeen van psychose te bestuderen. Naast het toepassen van de endofenotype benadering, vormt ook het verkennen van de toepassing van GxE binnen het genetisch onderzoek naar psychose, het doel van dit proefschrift.

Hoofstuk Twee beoordeelt 'verminderde P300 amplitude' als een geldig endofenotype voor schizofrenie. Van 193 *single nucleotide polymorphisms* (SNPs) wordt het

statistische verband met de P300 amplitude van 336 patiënten met schizofrenie onderzocht. De selectie van SNPs werd gebaseerd op eerder onderzoek die deze SNPs verband met schizofrenie, met dopaminerge of endocannabinoïde signaaloverdracht of met moleculaire regulatie van omgevingsinvloeden. 4 SNPs in 4 verschillende genen toonden een statistisch significant verband met P300 amplitude; rs1045642 in ABCB1, rs1572899 in DISC1, rs6265 in BDNF en rs1625579 in miR137. Vooral de ABCB1 SNP lijkt een relevante bevinding. Niet alleen omwille van het meest overtuigende verband met P300 amplitude, maar ook omdat ditzelfde verband eerder al beschreven werd bij gezonde proefpersonen. Het ABCB1-gen codeert het P-glycoproteïne, een eiwit betrokken bij ATP-gedreven cellulaire excretie.
Wat de bevindingen biologisch gezien betekenen, ook specifiek binnen psychose, moet nog uitgeklaard worden. Hoe dan ook, verdere inspanningen om de moleculaire mechanismen achter de P300-golf te begrijpen, lijken van groot belang voor psychose onderzoek.

De volgende drie hoofdstukken behandelen, gebaseerd op de GxE theorie, de gedifferentieerde effecten van cannabis binnen de ontwikkeling van psychose. In **Hoofdstuk Drie** wordt een kandidaatgen benadering gebruikt om het verband tussen cannabis gebruik en BDNF Val66Met genotype aan de ene kant, en leeftijd van eerste psychose aan de andere kant te onderzoeken, rekening houdende met de mogelijkheid van een geslachtsspecifieke GxE. Leeftijd van eerste psychose is een ziektekenmerk dat zowel klinisch (voorspellend voor het verloop van de aandoening) als voor etiologisch onderzoek van belang kan zijn. Analyses bij 585 patiënten met een psychotische aandoening tonen een interactie tussen cannabisgebruik en BDNF die de leeftijd van eerste psychose bij vrouwelijke patiënten beïnvloedt. In de volledige steekproef is cannabisgebruik vóór de eerste psychose geassocieerd met een 2,7 jaar vroeger begin van de aandoening. Echter, bij de vrouwelijke patiënten kan het effect van cannabisgebruik enkel geïnterpreteerd worden wanneer ook rekening wordt gehouden met de interactie met het BDNF Val66Met genotype. Vrouwen met het Met-allel die voor het begin van de aandoening cannabis gebruikten, maakten 7 jaar vroeger dan de andere vrouwelijke patiënten hun eerste psychose mee. Deze resultaten suggereren complexe interacties tussen BDNF, cannabis en oestrogeen (vb; in de hippocampale structuren), die verder onderzoek vereisen.
Hoofdstuk Vier beschrijft een studie die meer geloofwaardigheid verleent aan het bestaan van een reeds beschreven GxE met rs2494732 in AKT1. Deze SNP wordt verondersteld bij te dragen tot de gedifferentieerde gevoeligheid om een psychotische aandoening te ontwikkelen na cannabisgebruik. Gegevens van twee steekproeven, (i)

598 gezonde broers en zussen van 689 patiënten en (ii) 533 patiënten met psychose, worden gebruikt in een *follow-up* studie en een *case-only* studie. Het *follow-up* gedeelte toont dat, bij gezonde broers en zussen, de interactie tussen cannabisgebruik en AKT1 rs2494732 op een significante manier het niveau van schizotypie 3 jaar later, voorspelt. Enkel diegene met zowel blijvend cannabisgebruik als het rs2494732 C-allele vertoonden verhoogde schizotypie scores. Het *case-only* gedeelte kon geen statistisch significant verband vinden tussen AKT1 genotype en een voorgeschiedenis van cannabisgebruik, maar de richting en de effectgrootte van het verband was gelijkaardig aan deze in de eerste studie die het verband beschreef. Een gecombineerde analyse (n=1222) van de steekproef van deze eerste studie en onze steekproef, bevestigde wel het verband tussen AKT1 rs2494732 genotype en cannabisgebruik (zowel het al dan niet gebruiken als de mate waarmee gebruikt werd).
Hoofdstuk Vijf presenteert een *critical review* van de beschikbare literatuur over GxE met cannabis bij psychose. Moleculaire studies in deze zijn enkel valabel en relevant wanneer epidemiogische bevindingen aantonen dat vatbaarheid voor psychose bijdraagt tot de gedifferentieerde gevoeligheid voor cannabis. In epidemiologische studies wordt zowel familiaal risico als psychometrisch risico gebruikt als benadering van vatbaarheid voor psychose. De beschikbare studies vinden wel degelijk een gedifferentieerde gevoeligheid voor cannabis, volgens *a priori* vatbaarheid, om (subklinische) psychotische symptomen te ontwikkelen, maar bewijs voor gedifferentieerde gevoeligheid op het niveau van cognitie en neurobiologische veranderingen is minder consistent, respectievelijk minder voorhanden. Moleculaire GxE studies tonen weinig (of geen) consistente replicaties, hoewel de gerapporteerde interactie met AKT1 veelbelovend lijkt. Verschillende redenen voor dit gebrek aan consistente replicatie worden opgesomd: problemen met genselectie, te kleine steekproeven, gebrek aan adequate replicatiepogingen en 'pseudo-replicaties', en aanzienlijke verscheidenheid in onderzoeksdesigns en uitkomstmaten. Gebaseerd op deze vaststellingen, worden enkele aanbevelingen gemaakt voor toekomstig onderzoek.

Tenslotte en in de geest van de hierboven reeds genoemde etiologische continuïteit in het psychose continuüm, onderzoekt **Hoofdstuk Zes** GxE in paranoia, een kernsymptoom van psychotische aandoeningen. De resultaten van een grote ESM studie (n=876), die de genetica van paranoïde reactiviteit op sociale stress onderzoekt, worden gepresenteerd. Voor deze studie werden 33 SNPS uit eerdere GWAS of GxE studies geselecteerd en getest op hun interactie met ervaren sociale stress in het voorspellen van paranoia. Na een replicatiestrategie in twee steekproeven van

gezonde vrijwilligers werden bevindingen opgevolgd in een groep met gezonde broers en zussen van psychotische patiënten (verhoogd genetisch risico) en 2 groepen van patiënten; (i) psychotische patiënten en (ii) voorheen depressieve patiënten met resterende symptomen. Er werd een interactie tussen rs3803300 in AKT1 en sociale stress gedetecteerd, gerepliceerd en bevestigd in de groep met verhoogd genetisch risico en in de steekproef van psychotische patiënten. 5 SNPs in het *Major Histocompatibility Complex* (MHC) werden gerepliceerd en bevestigd in de steekproef van depressieve patiënten. De interacties tussen sociale stress en zowel rs3803300 in AKT1 als de MHC SNPs, werden bevestigd in de totale steekproef met Bonferroni correctie voor meervoudige statistische toetsen. Hiermee wordt de relevantie van de bevindingen gevalideerd over het volledige paranoiacontinuüm. Deze studie identificeert niet alleen enkele potentieel interessante gen-omgevingsinteracties in paranoia, maar stelt ook een volgende stap voor in de zoektocht naar GxE in psychiatrische aandoeningen; het doorbreken van diagnostische grenzen met ecologisch valide fenotypes.

Hoofdstuk Zeven bespreekt de bevindingen van dit proefschrift in het licht van het actuele genetisch onderzoek inpsychose en biedt enkele oriënterende beschouwingen voor toekomstig onderzoek. Dit wordt ook nog verder uitgewerkt in **Hoofdstuk Acht**.

VEEL. DANK

September 2009 – november 2014; een prachtige etappe in mijn leven. Niet altijd even vlak, maar steeds met mooie hellingen. Niet altijd wind in de rug, maar dikwijls uit de wind gezet. Niet altijd voldoende energie op zak, maar steeds iemand in de buurt met een extra voorraad. De hindernissen onderweg ben ik nu al bijna allemaal vergeten, de mensen die er waren om me te helpen zeker niet. Voor allen een hartelijke dankjewel!

Inez, Marc en Ruud, bedankt om in mij te geloven en van mij de Leuvense PhD-student binnen EU-GEI te maken. Een nieuwe wereld ging voor mij open. Inez, je enthousiasme en gedrevenheid, inspireerden en motiveerden me. Marc, je eenvoud en onbaatzuchtigheid nopen me tot diep respect. Ruud, je rust en geduld, je rechtuit gegeven mening en advies, boden mij de houvast die ik nodig had om alles binnen mijn promotietraject op de rails te houden.

Oprechte dank aan alle leden van de leescommissie en corona. Zonder jullie geen verdediging.

Claudia, we hadden niet te kiezen, maar ik had ook niet beter kunnen kiezen. Samen brainstormen en creëren, samen organiseren en uitvoeren, samen evalueren en bijsturen, samen pleiten en verzuchten, via Skype of op Vijverdal; het was telkens een plezier om samen met jou aan TwinssCan te werken! Dit was natuurlijk nooit zo vlot gelopen zonder de steun en coaching van Ruud, Marieke en Nele. Bedankt om jullie uitgebreide ervaring met ons te delen. Ook tonnen ervaring en engagement bij Debora, Truda en Ron. De gigantische dataverzameling binnen TwinssCan was volledig in de soep gedraaid zonder jullie. En was natuurlijk ook totaal onmogelijk geweest zonder de enthousiaste inzet van Katrien, Jill en Davinia. Dames, jullie waren fantastisch! Omwille van jullie glimlachende ijver, zag ik er nooit tegenop om naar Oost-Vlaanderen te rijden om mee te testen of te komen vergaderen. Oost-Vlaanderen; waar we te gast mochten zijn bij het *East Flanders Prospective Twin Survey* (EFPTS). Cathérine, Evert, Greet, Lut en Ingeborg, bedankt voor de hartelijke ontmoetingen en de goede samenwerking. Ik hoop dat TwinssCan nog veel vrucht mag dragen.

Ik was nooit zoveel onderweg als de laatste jaren, maar dit telkens om leuke en boeiende mensen te ontmoeten. Niet in het minst alle AIO's, postdoc onderzoekers en professoren verbonden aan Divisie 2. Of het nu in Vijverdal was, op research retreat of

op congres; bij een koffie, een pint of een glas wijn; voor een warm gesprek, een interessante discussie of wat gezever; het was genieten. Merci! Ik wou dat veel van deze ontmoetingen wat minder vluchtig geweest waren, maar helaas, ik was dus veel onderweg…
Een extra woord van dank voor Prof. Dr. Jim van Os. Het is duidelijk dat mijn proefschrift op jouw leest is geschoeid. Bedankt voor de theorieën die je met vuur wereldkundig maakt. Mede dankzij jouw joviale begroetingen voelde ik me welkom in Maastricht.

Zonder elektrofysiologie geen onderzoek. Althans niet voor mij. Mijn eerste voorzichtige stappen in de wereld van het wetenschappelijk onderzoek zette ik op het labo elektrofysiologie in Kortenberg. Ik wil dan ook professor Peuskens bedanken om mij wat te laten experimenteren met ERP-paradigma's. De testbatterij die eruit voortkwam geeft me nog steeds de gelegenheid tot heel wat interessante samenwerking. Het feit dat ik er ook best in de buurt bleef om een handje toe te steken wanneer de registratieapparatuur de zoveelste foutmelding gaf, zorgde ervoor dat ik gedurende een drietal jaar aanspraak kon maken op een bureau in de gezelligste gang van het ziekenhuis in Kortenberg. Ignace, Marianne, Ellen, Guy, Liesbeth, Mieke, Lou, Stijn, Jeroen, Jeroen en Jorne; dankzij jullie had ik er een toffe en fascinerende tijd. Tim, ook jij kwam er meer dan eens langs en ook nadien in Sint-Rafaël kon ik meermaals genieten van een geanimeerd gesprek over ons gedeelde lot van de jonge vader die wetenschap en psychiatrische kliniek probeert te combineren.

Een uiterst welgemeende bedanking gaat naar alle vrijwilligers, met of zonder psychotische kwetsbaarheid, die deelnamen aan wetenschappelijk onderzoek. Het vergt dikwijls een grote inspanning, maar ik ben ervan overtuigd dat deze inspanning vroeg of laat zijn vruchten afwerpt.
Wat zeker ook zijn vruchten zal afwerpen, is alle hartverwarmende vrijwilligheid tijdens *Te Gek!? Op de Pedalen*. Langzaamaan wordt de onwetendheid en het taboe rond psychose doorprikt… Het was een grote eer om mee te mogen sleutelen aan dit prachtige evenement. Marc en *Te Gek!?;* keep up the good work!

Ik werd psychiater dankzij de verschillende teams waar ik deel van mag/mocht uitmaken. Ex-collega's van Sint-Rita, Sint-Agnes, Sint-Monica en van het CIC: ongelofelijk veel respect voor jullie toegewijde zorg en bedankt voor de boeiende tijd samen! Collega's van het PSC, samen werken we in het gezelligste ziekenhuis van het land en het voelt fantastisch om dit op elke donderdagnamiddag/avond opnieuw

bevestigd te zien. Collega's van het MCT en van EPSI/spoed; velen van jullie timmeren al lang aan de weg naar meer crisiszorg op maat. Ik ben heel blij dat ik jullie hierin vanaf januari 2013 mag vergezellen. Ons team en ons enthousiasme blijft groeien. Laat ons er blijven voor gaan!

"Druk, druk, druk," was de laatste jaren heel dikwijls het antwoord. Vrienden, gelukkig zijn jullie er om samen de drukte te doorbreken met momenten van echt leven. Bedankt voor de 'Dinners for Six'. Bedankt voor de fijne weekends. Bedankt voor de supergezellige spelletjesmomenten. Bedankt voor het squashen en fietsen. Bedankt voor de kwis. Bedankt voor het musiceren op woensdagavond. Bedankt voor de culturele avonden en de wereldverbeterende discussies tussen pot en pint. Bedankt voor de etentjes samen. Bedankt voor de helpende hand. Bedankt voor het vertrouwen. Bedankt voor het leven en alles wat erbij hoort!

Mama en papa, ondertussen oma en opa, heel erg bedankt voor alle liefde en alle kansen die jullie me gaven. Ik besef steeds meer wat een onmetelijk geschenk ik van jullie heb meegekregen. Bedankt ook voor alle aanmoedigingen, waardoor uiteindelijk ook zelfs dit proefschrift er kwam. Wie had het nog durven hopen? Meme, bedankt voor je voorbeeld van rustige doorzetting. Dieter, Sofie en Ruben, we zwermden uit over Vlaanderen, maar vinden elkaar steeds beter terug. Ik zie jullie graag.

Lieve en Leo, lieve schoonouders. Bedankt voor alle steun, de gezellige tafelmomenten, de aangename biertjes en natuurlijk ook het jaar onderdak. Pat, Tine, Maud, Flo, Christophe, Karen, Gust en Marie; saaie (schoon)familiemomenten bestaan niet.

Liefste Nathalie, niet te geloven wat we samen al allemaal voor elkaar kregen. Ook dit doctoraat valt daar zeker onder, want zonder jou was dit nooit gelukt. Straks een dikke knuffel! Ik hou van je.
Liefste Warre en Lowie, mijn helden. Van jullie leer ik ongetwijfeld het meest.

4. CURRICULUM VITAE

Jeroen Decoster werd geboren op 16 maart 1982 in Ieper, België. Hij behaalde zijn diploma middelbaar onderwijs in het Sint-Vincentiuscollege te Ieper, richting wetenschappen-wiskunde. In 2000 startte hij met de opleiding geneeskunde aan de KU Leuven om in 2007 met onderscheiding af te studeren.

Nadien vatte hij in het UPC KU Leuven het assistentschap psychiatrie aan en werd hij op 1 oktober 2013 erkend als psychiater. Vanaf 2009 combineerde hij de opleiding tot psychiater met een promotietraject binnen het departement Psychiatrie en Psychologie van de School for Mental Health and Neurosciences, Universiteit Maastricht. Daarnaast behaalde hij in 2012, aan de KU Leuven, het postgraduaatsdiploma in de gedragstherapie.

Momenteel werkt hij als psychiater-psychotherapeut binnen het UPC KU Leuven waar hij de medisch-psychiatrische verantwoordelijkheid draagt voor het Mobiel CrisisTeam (MCT), de EPSI-unit (EPSI: eerste psychiatrische spoed-interventie) en de EPSI-werking op de dienst spoedgevallen. Ook is hij verbonden aan het PSC Sint-Alexius Elsene waar hij mee instaat voor de psychiatrische begeleiding van mensen met een psychotische aandoening, en dit binnen een waaier aan zorgvormen; nacht- en daghospitalisatie, postkuurprogramma, beschut wonen, Assertive Community Treatment (trACTor) en ambulante consultatie.

5. LIST OF PUBLICATIONS

5.1. International journals

Decoster J, De Hert M, Viechtbauer W, Nagels G, Myin-Germeys I, Peuskens J, van Os J, van Winkel R. Genetic association study of the P300 endophenotype in schizophrenia. *Schizophrenia Research;* 2012;141(1):54-9.

Decoster J, van Os J, Myin-Germeys I, De Hert M, van Winkel R. Genetic variation underlying psychosis-inducing effects of cannabis: critical review and future directions. *Current Pharmaceutical Design* 2012;18(32):5015-23.

Decoster J, van Os J, Kenis G, Henquet C, Peuskens J, De Hert M, van Winkel R. Age at onset of psychotic disorder: cannabis, BDNF Val66Met and sex-specific models of gene-environment interaction. *American Journal of Medical Genetics Part B Neuropsychiatric Genetics* 2011; *156B(3); 363-9.*

European Network of National Networks studying Gene-Environment Interactions in Schizophrenia (EU-GEI), van Os J, Rutten BP, Myin-Germeys I, Delespaul P, Viechtbauer W, van Zelst C, Bruggeman R, Reininghaus U, Morgan C, Murray RM, Di Forti M, McGuire P, Valmaggia LR, Kempton MJ, Gayer-Anderson C, Hubbard K, Beards S, Stilo SA, Onyejiaka A, Bourque F, Modinos G, Tognin S, Calem M, O'Donovan MC, Owen MJ, Holmans P, Williams N, Craddock N, Richards A, Humphreys I, Meyer-Lindenberg A, Leweke FM, Tost H, Akdeniz C, Rohleder C, Bumb JM, Schwarz E, Alptekin K, Üçok A, Saka MC, Atbaşoğlu EC, Gülöksüz S, Gumus-Akay G, Cihan B, Karadağ H, Soygür H, Cankurtaran EŞ, Ulusoy S, Akdede B, Binbay T, Ayer A, Noyan H, Karadayı G, Akturan E, Ulaş H, Arango C, Parellada M, Bernardo M, Sanjuán J, Bobes J, Arrojo M, Santos JL, Cuadrado P, Rodríguez Solano JJ, Carracedo A, García Bernardo E, Roldán L, López G, Cabrera B, Cruz S, Díaz Mesa EM, Pouso M, Jiménez E, Sánchez T, Rapado M, González E, Martínez C, Sánchez E, Olmeda MS, de Haan L, Velthorst E, van der Gaag M, Selten JP, van Dam D, van der Ven E, van der Meer F, Messchaert E, Kraan T, Burger N, Leboyer M, Szoke A, Schürhoff F, Llorca PM, Jamain S, Tortelli A, Frijda F, Vilain J, Galliot AM, Baudin G, Ferchiou A, Richard JR, Bulzacka E, Charpeaud T, Tronche AM, De Hert M, van Winkel R, **Decoster J**, Derom C, Thiery E, Stefanis NC, Sachs G, Aschauer H, Lasser I, Winklbaur B, Schlögelhofer M, Riecher-Rössler A, Borgwardt S, Walter A, Harrisberger F, Smieskova R, Rapp C, Ittig S, Soguel-dit-Piquard F, Studerus E, Klosterkötter J, Ruhrmann S, Paruch J, Julkowski D, Hilboll D, Sham PC, Cherny SS, Chen EY, Campbell DD, Li M, Romeo-Casabona CM, Emaldi Cirión A, Urruela Mora A, Jones P, Kirkbride J, Cannon M, Rujescu D, Tarricone I, Berardi D, Bonora E, Seri M, Marcacci T, Chiri L, Chierzi F, Storbini V, Braca M, Minenna MG, Donegani I, Fioritti A, La Barbera D, La Cascia CE, Mulè A, Sideli L, Sartorio R, Ferraro L, Tripoli G, Seminerio F, Marinaro AM, McGorry P, Nelson B, Amminger GP, Pantelis C, Menezes PR, Del-Ben CM, Gallo Tenan SH, Shuhama R, Ruggeri M, Tosato S, Lasalvia A, Bonetto C, Ira E, Nordentoft M, Krebs MO, Barrantes-Vidal N, Cristóbal P, Kwapil TR, Brietzke E, Bressan RA, Gadelha A, Maric NP, Andric S, Mihaljevic M, Mirjanic T. Identifying gene-environment interactions in schizophrenia: contemporary challenges for integrated, large-scale investigations. Schizophrenia Bulletin 2014; Jul;40(4):729-36. doi: 10.1093/schbul/sbu069.

Pishva E, Drukker M, Viechtbauer W, **Decoster J**, Collip D, van Winkel R, Wichers M, Jacobs N, Thiery E, Derom C, Geschwind N, van den Hove D, Lataster T, Myin-Germeys I, van Os J, Rutten BP, Kenis G. Epigenetic genes and emotional reactivity to daily life events: a multi-step gene-environment interaction study. *PLoS One* 2014; Jun 26;9(6):e100935. doi: 10.1371/journal.pone.0100935.

Peerbooms O, **Decoster J**, Rutten BP, van Os J, Kenis G, De Hert M, van Winkel R. No association between MTHFR C677T or A1298C and age at onset of schizophrenia. *American Journal of Medical Genetics Part B Neuropsychiatric Genetics 2010; 153B(7): 1362 – 1363; author reply 1361.*

5.2. Submitted

Decoster J, van Nierop M, Pishva E, Gunter K, Viechtenbauer W, Jacobs N, Thewissen V, Lardinois M, Simons C, Lataster T, Lataster J, Collip D, Oorschot M, Janssens M, Menne-Lothmann C, Hartmann J, Delespaul P, Drukker M, Rutten B, Wichers M, van Os J, Myin-Germeys I, van Winkel R. Paranoia evoked by social stress: evidence for moderation by genetic variation in AKT1.

van Winkel R, **Decoster J**, De Hert M, Drukker M, Kenis G, Genetic Risk and Outcome in Psychosis (GROUP) Investigators. AKT1 rs2494732 and risk of psychosis in cannabis users: case-only analysis of a novel patient sample.

Menne-Lothmann C, **Decoster J**, van Winkel R, Collip D, Van Os J, Rutten B, Delespaul P, De Hert M, Derom C, Thiery E, Jacobs N, Wichers M. Psychological and biological validation of a novel digital social peer evaluation experiment (digi-SPEE).

Laton J, Van Schependom J, Gielen J, **Decoster J**, Moons T, De Keyser J, De Hert M, Nagels G. Single-subject classification of schizophrenia patients based on a combination of oddball and mismatch evoked potential paradigms.

5.3. Conference presentations

Decoster J. Crisispsychiatrie. Crisispsychiater? *Vlaams Geestelijke Gezondheidscongres*, Antwerpen, September 2014.

Decoster J, Myin-Germeys I, van Winkel R. Paranoia bij sociale stress; een kwestie van gen-omgevingsinteractie? *Voorjaarscongres Nederlandse Vereniging voor Psychiatrie*, Maastricht, April 2013.

Decoster J, De Hert M, Viechtenbauer W, Nagels G, Myin-Germeys I, Peuskens J, van Os J, van Winkel R. miR-137 and the P300 waveform in patients with schizophrenia. *International Congress on Schizophrenia Research (ICOSR)*, Orlando, April 2013.

Decoster J, van Nierop M, Pishva E, Kenis G, Viechtenbauer W, van Os J, Myin-Germeys I, van Winkel R. Paranoia in social situations – a matter of gene-environment interaction? *International Congress on Schizophrenia Research (ICOSR)*, Orlando, April 2013.

Decoster J, Nagels G, De Hert M, Myin-Germeys I, Peuskens J, van Os J, van Winkel R. Genetic variation in ABCB1 associated with P300 amplitude in schizophrenia. *Schizophrenia International Research Society (SIRS) Conference*, Florence, April 2012.

Decoster J, van Os J, Myin-Germeys I, De Hert M, van Winkel R. Genetic variation underlying psychosis-inducing effects of cannabis: critical review and future directions. *Schizophrenia International Research Society (SIRS) Conference*, Florence, April 2012.

Decoster J, Collip D, Lataster T, Myin-Germeys I, van Winkel R. Is ZNF804A associated with reactivity to stress in daily life in patients with psychosis? *International Congres on Schizophrenia Research (ICOSR)*, San Diego, April 2011.

Decoster J, Nagels G, De Hert M, Myin-Germeys I, Peuskens J, van Os J, van Winkel R. Genetic underpinnings of decreased P300 amplitude in patients with psychotic disorder. *Annual Research Day, School for Mental Health and Neuroscience*, Maastricht, February 2011.

Decoster J, van Os J, Kennis G, Henquet C, Peuskens J, De Hert M, van Winkel R. Age of onset of psychosis: cannabis, BDNF Val66Met and sex-specific models of gene-environment interaction. In : Early Intervention in Psychiatry, 4 (Suppl 1). *International conference on early psychosis*, Amsterdam, December 2010..

Decoster J, van Os J, Kenis G, Henquet C, Peuskens J, De Hert M, van Winkel R. Age at onset of psychosis in patients with schizophrenia: evidence for a sex-dependent interaction between BDNF val66met genotype and cannabis use. In : Schizophrenia Research, 117 (2-3). *Schizophrenia International Research Society (SIRS) Conference*, Florence, April 2010.

Decoster J, De Hert M, Kenis G, van Os J, van Winkel R. BDNF val66met genotype, male gender and adolescent cannabis use determine age of onset of psychosis: a study in a large sample of psychotic patients. In : Schizophrenia bulletin, 35. *International Congres on Schizophrenia Research (ICOSR)*, San Diego, March 2009. (awarded with the AstraZeneca Poster Award in Psychiatry 2009)

Dat de innerlijke wereld
te grenzeloos doorkliefd wordt door een uitwendige wereld.
Zoals geen begrenzing voelen tussen jezelf en de buitenwereld.
Dat voelt meestal slecht, akelig aan en ook bedreigend met momenten.
Maar na een tijdje heb je dat zelf niet meer door.
(LVM)