ORIGINAL ARTICLE

Further delineation of the phenotype of chromosome 14q13 deletions: (positional) involvement of *FOXC1* appears the main determinant of phenotype severity, with no evidence for a holoprosencephaly locus

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Received 9 February 2012

Accepted 2 April 2012

ABSTRACT

Background Deletions including chromosome 14 band q13 have been linked to variable phenotypes. With current molecular methods the authors aim to elucidate a genotype–phenotype correlation by accurately determining the size and location of the deletions and the associated phenotype.

Methods Here the authors report the molecular karyotyping and phenotypic description of seven patients with overlapping deletions including chromosome 14q13.

Results The authors show that deletions including 14q13 result in a recognisable phenotype mainly due to haploinsufficiency of two genes (*NKX2-1*, *PAX9*). *FOXC1* (on chromosome band 14q12) involvement seems to be the main determinant of phenotype severity. The patients in this study without *FOXC1* involvement and deletions of up to 10 Mb have a relatively mild phenotype. The authors cannot explain why some patients in literature with overlapping but smaller deletions appear to have a more severe phenotype. A previously presumed association with holoprosencephaly could not be confirmed as none of the patients in this series had holoprosencephaly.

Conclusions *FOXC1* appears the main determinant of the severity of phenotypes resulting from deletions including 14q13. The collected data show no evidence for a locus for holoprosencephaly in the 14q13 region, but a locus for agenesis of the corpus callosum cannot be excluded.

INTRODUCTION

With the advent of array-based molecular karyotyping more precise delineation of copy number variants has become the standard. This technical advance has stimulated a search for genotype–phenotype correlations in patients with deletions in the same chromosomal region with variable phenotypes. Illustrative examples of this type of research are studies on deletions in chromosome 19p13.12¹ and 18q.² Genotype–phenotype relations are important for several reasons: (1) when a deletion is detected in a neonate, young child or even prenatally, the parents can be informed about the expected phenotype of their child, and treatment/surveillance can be adapted to the expected

phenotype and (2) candidate genes for monogenic disorders may be found.

Deletions including 14q13 have been described repeatedly in literature.^{3–11} In several of these publications, a 14q13 deletion has been linked to holoprosencephaly (HPE) that led to the definition of a holoprosencephaly locus (HPE8).^{3–8–10} Deletions of the *NKX2-1* (*TITF1*) gene dominate the phenotype of the remaining publications.^{5–7–11–12} However, clinical data are often sparse and most of these publications rely on conventional karyotyping or focus on only one or two genes (*NKX2-1*, *PAX9*) in the deletion. Recently, two papers have appeared describing additional patients with a deletion including 14q13 and a severe phenotype,^{13–14} although the patients in the latter paper¹⁴ have much larger deletions including chromosome band 14q12 and *FOXC1*.

Here, we present the molecular karyotyping and the phenotypic description of seven patients with overlapping 14q13 deletions, and compare the results with patients reported in the literature.

MATERIALS AND METHODS

Molecular karyotyping

For patients 1, 2, 3, 6 and 7 DNA was extracted from whole blood by a Gentra Puregene DNA purification Kit (Gentra Systems, Minneapolis, USA), following the manufacturer's instructions. The Affymetrix GeneChip Human Mapping 262K *NspI* array (Affymetrix, Santa Clara, California, USA) was performed following the manufacturer's instructions and data were analysed as described previously.¹⁵

For patients 4 and 5, array-CGH was performed by means of the '180k Cytosure ISCA v2 array', 'Oxford Gene Technology', according to standard procedures.

CLINICAL REPORTS

In total, seven patients were included of whom three (patients 4, 6 and 7) were published previously.^{5–11–12} Three patients with a deletion in the 14q13 region from the laboratories in Leiden (The Netherlands) and two from Leuven (Belgium) (of which one was previously published¹²) were included in the study. In addition, authors from

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papers describing patients with 14q13 deletions and a severe phenotype were contacted in order to obtain DNA. The DNA of one of the sisters described by Iwatani *et al*⁵ and patient 1 from the paper by Carré *et al*¹¹ were obtained.

A detailed clinical description of the patients is provided below and summarised in table 1. We have also included patients from literature with a deletion in 14q13. Patients from literature were included when the size of the deletion was narrowed down using molecular methods, no other chromosomal aberrations were present and basic clinical information was available. Patients from literature with *FOXC1* deletions were excluded from this table (see Discussion).

Patient 1

Patient 1 is a 7-year-old girl, the first child of non-consanguineous parents, born at a gestational age of 42 weeks with a birth weight of 3600 g (0 SD score, SDS). Pregnancy was uneventful; the delivery was complicated by a decreased fetal heart rate but Apgar scores were normal. The neonatal screening programme showed hypothyroidism for which she was treated accordingly. Since the age of 2 years she displayed a movement disorder described as choreoathetosis and she has a delayed motor development. She showed class I dental malocclusion with absence of 55, 65 and 85, as well as permanent agenesis of 15–17, 25–27, 36–37 and 45–47. There was a discrepancy between her performat IQ (76) and verbal IQ (105). She attends a regular primary school with a 1-year delay. Physical examination at 7 years of age showed no dysmorphisms apart from deeply-set eyes and widely spaced teeth. Her growth parameters are normal. In the past, *NKX2-1* sequencing showed normal results.

Patient 2

Patient 2 was a 1-week-old male neonate, the first child of non-consanguineous parents, born after a term pregnancy with normal birth weight. The diagnosis of hypothyroidism was established by the neonatal screening programme. He suffered from recurrent episodes of pulmonary insufficiency and possible pneumonias. His motor development is delayed and restlessness was observed at a young age as well as ataxia, which severely impair his motor skills. Otherwise, his development is normal for his age. Dental examination showed absence of six primary elements and permanent agenesis of 11 elements.

NKX2-1 mutation analysis was included in the suggested investigation but he was lost to follow-up. Physical examination at the ages of 3 and 5 years did not reveal any major dysmorphic features (figure 1). His father reported that the patient experiences sleeping difficulties with short periods of sleeping during the whole night.

Patient 3

Patient 3 was born as the first child of healthy non-consanguineous parents. In pregnancy, the mother was on paroxetine (20 mg/day). Because of this, a detailed ultrasound scan at 20 weeks of gestation was performed. This detected essentially the same complex cardiac anomaly as postnatal investigations (see below). Amniocentesis showed a normal male karyotype. A 22q11 deletion was excluded using the fluorescent *in situ* hybridisation technique.

He was born after 36.3 weeks of gestation, with a birth weight of 2590 g, after an emergency caesarean section because of pre-eclampsia and deterioration of fetal condition. Postnatal cardiac examination showed a complex congenital heart malformation: an interrupted arterial arch type B, bicuspid

aortic valve, subvalvular and valvular aortic stenosis, hypoplasia of the annulus of the aorta, and a ventricular septum defect (left ventricular outflow tract obstruction).

Physical examination at the age of 2 weeks showed minor facial dysmorphisms: short palpebral fissures, bilateral earpits, pointed and indented chin, with vertical creases.

At 1 month of age, poor gain of weight necessitated a hybrid Norwood procedure (bilateral banding of the pulmonary arteries and stenting of the ductus arteriosus). After successful recovery, the patient was discharged. His growth and development in this period were unremarkable. At the age of 4 months, his condition was sufficient to undergo a Ross–Konno procedure. The reconstruction was successful; the operation was however complicated by a massive lung bleeding, sadly resulting in peri-operative death. Postmortem studies were not performed.

Because of a first degree relative with aortic aneurysm, his father had a cardiac ultrasound with normal results.

NOTCH1 mutation analysis was performed and no mutations were found.

Patient 4

Patient 4 was a female neonate previously described by Devriendt *et al*¹² born after an uncomplicated term pregnancy with a birth weight of 3400 g (0 SDS). She was admitted with severe respiratory distress and subclinical hypothyroidism was diagnosed. She developed mild choreoathetotic movements and decreased balance control which did not prevent her from winning several sporting competitions. She experienced recurrent pulmonary infections in the past year. Her intelligence is normal but she has trouble processing information and keeping up with a high pace. She attended regular primary school until the 5th year, after which she changed to special education. Physical examination at the age of 10–15 years showed normal growth parameters (weight 51.2 kg (0.9 SDS), height 159.9 cm (0.6 SDS) and head circumference 54.8 cm (0.6 SDS)) and several missing dental elements.

Patient 5

Patient 5 was a woman in her thirties, the first child of non-consanguineous parents born after an uncomplicated term pregnancy with a birth weight of 3600 g (+0.5 SDS). A perforated lung was diagnosed neonatally. Hypothyroidism was diagnosed in childhood. She suffers from recurrent respiratory infections/chronic bronchitis. Her developmental milestones were delayed (walking at 2 years of age) and she has mild intellectual disability. She attended special education. She has short stature (159 cm, –2 SDS) and has a normal head circumference of 53.9 cm. She has a movement disorder and falls frequently. There is an absence of elements 55, 65, 75 and 85 as well as permanent agenesis of 5–8 in all quadrants.

Patient 6

Patient 6 is the eldest of the two sisters described by Iwatani *et al*.⁵ She was born to non-consanguineous parents at a gestational age of 41 weeks with a birth weight of 3150 g (+0.1 SDS). She was admitted recurrently with respiratory difficulties, failure to thrive, severe feeding difficulties and microcephaly. Her development was severely delayed with absence of head control. She died of respiratory failure at the age of 13 years. Her younger sister had a similar clinical picture and died of respiratory failure at the age of 3 years.

Patient 7

Patient 7 was previously published as patient 1 in the paper by Carré *et al*.¹¹ She was born at term and presented with mild

Table 1 Clinical data of the patients in the current series and published patients with 14q deletions

Case	Present series							Breedveld <i>et al</i> ¹⁶ and Guala <i>et al</i> ¹⁷				Kannasaran <i>et al</i> ²⁰				Shimojima <i>et al</i> ²¹		Caliebe <i>et al</i> ¹³
	1	2	3	4	5	6	7	Petek <i>et al</i> ⁷	F	M	10 y (16 y)	Devos <i>et al</i> ¹⁸	Visser <i>et al</i> ¹⁹	Pt1	Pt4	Pt6	F	M
Gender	F	M	M	F	F	F	F	M				M	M	F	F	F	F	M
Age at diagnosis (last examination)	7 y	3 y (4 y)	1 m (2 w)	0 (10–15 y)	30–35 y	10–15 y	0 (5–10 y)	2 y 5 m	10 y (16 y)			(44 y)	(16 y)	(6 m)	(6 y)	(28 m)	9 m (5 y)	(17 m)
Height (SDS)	–1.4	N/A	N/A	–1	–3.5	–3.4	N/A	–1.1	N/A			N/A	+0.3	–2	N/A	N/A	N/A	–2.5
OFC (SDS)	–0.9	N/A	N/A	–1	–2	N/A	N/A	–3.6	N/A			N/A	+0.6	<–2	N/A	N/A	N/A	–1.5
Developmental delay	Mild	–	–	Mild	Moderate	Severe	Severe	Severe	Unlikely			–	Mild	+	Severe	Severe	Severe	Severe
Speech delay	–	–	N/A	–	+	+	+	+	N/A			–	–	N/A	+	N/A	N/A	N/A
Motor delay	+	+	N/A	–	+	+	+	+	N/A			+	–	N/A	+	N/A	N/A	+
Deleted genes with phenotypic relevance	N,P,G	N,P	P	N,P,G	N,P,G	N,P,G,F	N,P,G	N,P,G	N,P			N,P	N,PAS3	N,P,G	N,P,G	N,P,G	G	G
Choreoathetosis	+	+	N/A	+	+	N/A	+	N/A	+			+	–	N/A	N/A	N/A	N/A	N/A
Hypothyroidism	+	+	–	+	+	+	+	+	+			+	–	–	N/A	+	–	N/A
Pulmonary problems	–	+	N/A	+	+	+	+	+	N/A			+	–	N/A	+	N/A	N/A	+
Oligo/hypodontia	+	+	N/A	+	+	N/A	N/A	+	+			+	–	N/A	N/A	N/A	N/A	N/A
Congenital heart defect	–	N/A	+	–	–	N/A	N/A	N/A	N/A			N/A	–	N/A	N/A	N/A	N/A	N/A
Brain anomalies	– (MRI)	N/A	– (US)	–	(MRI)	N/A	ACC (MRI)	ACC (MRI)	N/A			– (MRI)	– (CT)	ACC	ACC	ACC	– (MRI)	– (MRI)
Dysmorphisms	–	–	+	–	–	+	N/A	†	–			N/A	–	N/A	N/A	N/A	–	**
Other manifestations	–	Ataxia	–	–	–	S	–	Bilateral optic atrophy	–			–	–	–	–	Neonatal seizures	Intractable epilepsy, mild brain atrophy	Epilepsy, IUGR, caesarean section at 33+3 weeks
Size of deletion (Mb)	3.7	0.7	0.1	9.9	7.0	17.9	15.4	2.9–5.4	1.2–1.3			0.5–1.2	0.2	7.4–11.5	8.8–9.2	6.9–8.7	2.2	2.0

For some patients age ranges are given to ensure anonymity as no patient consent could be obtained.

*Cerebral atrophy, ventricle dilatation and a cyst of the cavum septum pellucidum.

†Short palpebral features and bilateral ear pits.

‡A round face, hypertelorism, small mandible, high arched palate, hypertrichosis, single transverse palmar creases and contractures lower legs.

§Failure to thrive, feeding difficulties and moderate hearing loss.

¶Hypertelorism, epicanthic folds, a long and flat philtrum, laterally placed, hypoplastic mamillae, second degree hypospadias, bifid scrotum and bilateral cryptorchidism.

**Frontal bossing, a small face with a pointed chin, a small haemangioma on the right ear, a deep philtrum and a small penis.

ACC, agenesis of the corpus callosum; F, female; F, FOXG1; G, GARNL1; IUGR, intrauterine growth restriction; M, male; m, months; Mb, megabases; N/A, not assessed; N, NKX2-1/TTF1; OFC, occipital frontal circumference; P, PAX9; US, antenatal ultrasound; y, years; +, present; –, absent.

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Figure 1 Face of patient 2, aged 3 years and 5 months, showing no apparent dysmorphisms. Note that the apparent smooth philtrum is secondary to his tendency to grimace during posing.

hypothyroidism and infant respiratory distress syndrome for which she needed ventilation for 8 days. She was dependent on oxygen for the first 3 months of life. At 7 months of age, her head circumference was 38.1 cm (−3.5 SDS). Atactic movements and psychomotor delay were observed during the first year of life. At the age of around 5 years she was severely intellectually disabled.

RESULTS OF MOLECULAR KARYOTYPING

Array analysis in these seven patients showed 14q13 deletions varying in size from 105 kb to 16.96 Mb (table 2, figure 2). Three deletions were proven to be de novo, a fourth was most likely de novo, two were not tested and one was due to a familial insertional translocation.

DISCUSSION

Impact of *FOXG1* on the 14q13 deletion phenotype

In this series, seven patients with a deletion in chromosome region 14q13 are described (table 1, figure 2). There appear to be two groups of patients: those who suffer from severe intellectual disability and multiple congenital anomalies, and those whose phenotype is milder and largely determined by haploinsufficiency of *NKX2-1* and *PAX9*.

Two of our patients were severely intellectually disabled (patients 6 and 7). Obtaining the DNA and extending the previously performed investigations allowed us to explain the severe intellectual disability in patient 6 and her sister⁵ by the deletion of *FOXG1*. Haploinsufficiency of *FOXG1* causes a congenital variant of Rett syndrome, with poor reactivity, hypotonia and severe microcephaly.^{23 24} Patient 7 also showed severe intellectual disability, yet retained *FOXG1*. The most likely explanation for the difference between this patient and those with mild intellectual disability is the size of the deletion (15.5 Mb). Especially the distal part of this deletion is quite rich in genes. Additionally, Kortüm *et al*²⁵ have convincingly argued that there are long range *cis*-regulatory elements of *FOXG1* in the 1 Mb downstream of *FOXG1*. Although this deletion is just outside the suggested region, a positional effect on *FOXG1* cannot be excluded.

We have searched the literature for patients with deletions including chromosome region 14q13. We included papers in which the size of the deletion was narrowed down using molecular methods and basic clinical information was available. Since haploinsufficiency of *FOXG1* results in a well-described phenotype, we decided to exclude patients with a clear deletion of *FOXG1* from table 1. Indeed, it is our opinion that inclusion of these patients in genotype–phenotype correlation studies confounds the ‘true’ phenotype of patients with a 14q13 deletion. A recent paper¹⁴ which claims to describe the phenotype of patients with a 14q deletion is therefore not representative for patients whose 14q13 deletion does not include *FOXG1*.

Most of the reported patients with a deletion in the 14q region have a severe phenotype. In some cases, this is sufficiently explained by haploinsufficiency of *FOXG1* as in the recent publication by Torgyekes *et al*,¹⁴ and patient 5 in the paper by

Table 2 Size, location and number of genes in the chromosome 14 deletions of each patient

Case ID (DECIPHER code)	Platform	Start position*	End position*	Size	No of genes*	Additional findings (if any)	Status of inheritance (origin if known)
1 (264287)	Affymetrix 262K <i>Nspl</i>	35.199.722	38.886.152	3.69 Mb	20		Most likely de novo (p)†
2 (250393)	Affymetrix 262K <i>Nspl</i>	36.010.235	36.727.597	717 kb	5		de novo (m)
3 (264289)	Affymetrix 262K <i>Nspl</i>	36.114.849	36.219.911	105 kb	4	Maternally inherited deletion on 11q	de novo (p)
4	OGT 180k	34.077.461	43.971.142	9.89 Mb	34	An additional duplication on chromosome 1q of 264 kb was found containing the <i>IPP</i> and <i>MAST2</i> genes	De novo
5	OGT 180k	33.670.261	40.648.438	6.98 Mb	34		Not tested
6 (264290)	Affymetrix 262K <i>Nspl</i>	24.383.933	41.340.975	16.96 Mb	54	An additional duplication on chromosome 10q of 454 kb containing <i>NRG3</i> was also found. On chromosome 8q a 2.27 Mb duplication was found which has been described as a genomic variant in the Japanese population ²²	Familial insertional translocation‡
7 (264291)	Affymetrix 262K <i>Nspl</i>	29.701.682	45.144.380	15.44 Mb	59		Not tested

*Ensembl release 54—May 2009, Genome build NCBI36.

†SNP-array from father was only available.

‡Karyotype of the mother: 46,XX, inv ins (8;14) (8pter→8q24.3: 14q13.3→14q12: 8q24.3→8qter; 14pter→14q12: 14q13.3→14qter). m, maternal; p, paternal.

Figure 2 Graphical representation of the region spanned by the deletions of each of the patients. The first seven patients are those described in this paper. Below, the patients from relevant papers from literature are depicted. Thick bars represent the minimal size of the deletion, and thinner bars represent the maximal size of the deletion. The colour represents the severity of the phenotype: mild (green), moderate (orange) or severe (red). Since patient 3 died at a young age, disease severity could not be assessed. The dark box represents the putative regulatory region for *FOXG1*, and the white box represents the remaining candidate region for agenesis of the corpus callosum (see Discussion). The size and location from the deletions in literature depicted here are determined using the marker data reported in literature. Please note that there is a discrepancy between the microscopic karyotype of patient 1 of Kamnasaran *et al* (2001)²⁰ (46,XX,del(14)(q11.2q21.1)) and the results of marker analysis for this patient as presented in Kamnasaran *et al* (2005).⁹

Kamnasaran *et al.*²⁰ The severity of the phenotype of patients 4 and 6 described in the latter paper might well be explained by the positional effect of these deletions on *FOXG1* as these deletions span the region with putative *cis*-regulatory elements²⁵ (figure 2).

Only three patients in the literature with a severe phenotype cannot be explained by *FOXG1*-related deletions. The first of these patients was reported by Petek *et al.*⁷ Unfortunately, it was not possible to obtain DNA from this patient to determine the size of the deletion. This patient has a phenotype with severe intellectual disability and bilateral optic atrophy. Most of the deletion of this patient overlaps with deletions from our patients who have a much milder phenotype. The only gene outside the common deleted region is *NPAS3*, which might be a gene relevant for developmental delay, but is unlikely to be the single cause of the severe phenotype of this patient since the patient presented by Visser *et al.*¹⁹ with a deletion of the first exon of *NPAS3* has an IQ of 76. It is likely that this deletion will preclude translation and therefore lead to haploinsufficiency of *NPAS3*. A mutation in another gene or a copy number variant elsewhere might be the cause of the phenotype in the patient reported by Petek *et al.*⁷ The presence of a recessive mutation in the second copy of one of the deleted genes cannot be excluded.

The other two patients with a severe phenotype and no *FOXG1* involvement share a highly similar deletion and

phenotype. The first was published by Shimojima *et al.*²¹ and was reported to be severely intellectually disabled and had intractable epilepsy. The second patient was reported by Caliebe *et al.*¹³ and was diagnosed with severe developmental delay and epilepsy. At the age of 3 years, he was diagnosed with pneumonia. In the further course he developed symptoms of haemophagocytic lymphohistiocytosis. He died due to organ failure. This patient showed severe intrauterine growth retardation and was delivered by caesarean section at 33+3 weeks of gestation. A small placenta was found and he needed 10 days of ventilation after birth. An influence of the aforementioned on his phenotype cannot be excluded. The deletions of these patients include *GARNL1*. It has been suggested^{21 26} that haploinsufficiency of *GARNL1/TULIP1* might be the cause of developmental delay and epilepsy. Indeed, heterozygous mutations were found in 4/140 of such patients, although these mutations were all inherited from healthy parents. Since three of our patients with mild to moderate developmental delay and no epilepsy had a deletion of this gene, we consider it unlikely that mutations in *GARNL1* are an important cause of severe intellectual disability. However, it is striking that these two patients share almost the same deletion and have comparable phenotypes. Then again, the deletion of our patient 5 completely overlaps (and is 5 Mb larger) and she had moderate intellectual disability and no epilepsy. Imprinting effects can be ruled out as

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a possible cause, since the patient reported by Petek *et al*⁷ had a deletion on the maternal allele and the patient reported by Shimojima *et al*²¹ had a deletion on the paternal allele. Alternatively, the combination of the deletion and a (relatively) frequently occurring SNP in this region might cause a severe phenotype.

Impact of NKX2-1 and PAX9 on the 14q13 deletion phenotype

In contrast to these patients with a severe phenotype, our patient 2 had no developmental delay, patients 1 and 4 had mild developmental delay and patient 5 had moderate developmental delay. These patients have partially overlapping deletions ranging from 717 kb to 10 Mb. It appears that haploinsufficiency of the genes in these deletions does not lead to severe intellectual disability.

All of these patients had a deletion of *NKX2-1* and *PAX9*, which result in a clear phenotype. Haploinsufficiency of *NKX2-1* causes brain-lung-thyroid syndrome, consisting of a triad of extremely variable manifestations. The brain manifestations consist of choreoathetosis which is present in most patients to a variable degree (7/8 in table 1). The lung manifestations range from severe pulmonary insufficiency (7/8 in table 1), sometimes leading to death, to no pulmonary complaints at all (as in our patient 1). The thyroid manifestations (8/8 in table 1) are often detected in congenital screening but sometimes subclinical hypothyroidism is observed. It has previously been stated that chromosome 14q13 deletions show exactly the same phenotype as *NKX2-1* mutations,⁶ but this is not completely accurate. With an increasing size of the deletion the probability of intellectual disability, ranging from mild to severe, increases. In contrast to what has been suggested,¹⁶ deletions of *NKX2-1* do not result in a more severe brain-lung-thyroid-phenotype than mutations in *NKX2-1*.¹¹

Not all patients with *PAX9* haploinsufficiency had oligodontia; however, in none of these the *absence* of oligodontia was explicitly reported. In our series, oligodontia was observed in all patients where the appropriate investigations were performed (4/4), indicating that penetrance of this phenotype is probably high, and that it might have been missed in some of the reported patients. In some cases, it is perhaps because they were too young for oligodontia or permanent agenesis to manifest without performing an orthopantomogram.

Patient 3 appears to be an outlier in our group of patients with regard to his phenotypic manifestations. He had a severe cardiac phenotype but the smallest deletion, including only four genes (three Refseq genes). Since none of the other patients are described with a cardiac phenotype we consider it unlikely that this deletion is the cause of his cardiac problems. Since one of these genes, *NKX2-8*, is highly expressed in early heart progenitor cells,^{27 28} we decided to sequence this gene in patient 3 but did not find any mutations on the other allele. Other causes of his cardiac phenotype such as a 22q11 deletion, a *NOTCH1* mutation or other copy number variations were excluded.

Reviewing the evidence for an HPE locus

Kamnasaran *et al*⁹ have defined an HPE locus (HPE8) mainly using patients described in an earlier paper,²⁰ but none of these patients were actually diagnosed with HPE. Instead, all were diagnosed with agenesis of the corpus callosum (ACC). Although there is a relation between ACC and HPE, and ACC might be a minimal form of HPE, not all cases of ACC should be classified as HPE.²⁹ It is therefore more likely that the HPE8 locus is actually an ACC locus rather than an HPE locus. One

of the patients in our study was diagnosed with ACC but most had normal MRI findings. Considering only those patients where ACC was formally excluded by MRI, a relatively small region of interest remains (as indicated in figure 2). This region contains four genes: *C14orf128*, *ARHGAP5*, *AKAP6* and *NPAS3*. *NPAS3* is partially deleted in patient 58 described by Visser *et al*,¹⁹ who did not have HPE or ACC (as determined by CT scan) (Dr S Kant, personal communication) and in our patient 6, who has a severe phenotype but no HPE. However, since ACC may be missed on a CT scan *NPAS3* remains a candidate gene.

CONCLUSION

Although we cannot exclude that *HPE8* is associated with HPE, the poor a priori evidence for this locus, combined with the results from our studies, provides little support for an HPE locus on chromosome 14q13. However, there might be a locus for ACC containing four genes.

The phenotype of patients with a 14q13 deletion is largely determined by the involvement of *FOXP1*, *NKX2-1* and *PAX9*. From our series and published patients it is clear that deletions involving *FOXP1*, including an 1 Mb upstream region, cause a severe phenotype. In our series, deletions involving *NKX2-1* and *PAX9* with a size of up to 10 Mb result in a clinically recognisable phenotype with mostly mild intellectual disability, implicating that these deletions are relatively well tolerated. However, there are three published patients with a relatively small overlapping deletion with severe phenotypes. No explanation was found for this discrepancy. To improve genotype-phenotype relationships in the future, it is important that clinical descriptions of all patients with copy number variations are made publicly available in databases such as DECIPHER (<http://decipher.sanger.ac.uk>).

Acknowledgements We would like to acknowledge Dr E Petek for his efforts to try and locate his patient with a 14q13 deletion, Dr S Kant for kindly providing information on one of the published patients and Ing. CAJ Bosch for technical assistance.

Contributors GS and EB conceived the study and wrote the paper. YS sequenced the *NKX2-8* gene. AG and CR performed SNP-array data analysis. GS, AC, MH, AH, SALO, AT, HM, MP, KD and EB phenotyped the patients. All authors read, revised and approved the final version of the manuscript.

Competing interests None.

Patient consent Obtained.

Ethics approval Most investigations were performed in the course of routine clinical care. In the remaining cases the molecular karyotyping was performed to delineate the exact size and location of a deletion already found by other methods. No new patient samples were required.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement All CNV information is present in the paper. No additional data will be uploaded to repositories.

REFERENCES

1. Bonaglia MC, Marelli S, Novara F, Commodoro S, Borgatti R, Minardo G, Memo L, Mangold E, Beri S, Zucca C, Brambilla D, Molteni M, Giorda R, Weber RG, Zuffardi O. Genotype-phenotype relationship in three cases with overlapping 19p13.12 microdeletions. *Eur J Hum Genet* 2010;**18**:1302–9.
2. Feenstra I, Vissers LE, Orsel M, van Kessel AG, Brunner HG, Veltman JA, van Ravenswaaij-Arts CM. Genotype-phenotype mapping of chromosome 18q deletions by high-resolution array CGH: an update of the phenotypic map. *Am J Med Genet A* 2007;**143A**:1858–67.
3. Chen CP, Lee CC, Chen LF, Chuang CY, Jan SW, Chen BF. Prenatal diagnosis of de novo proximal interstitial deletion of 14q associated with cleft lip. *J Med Genet* 1997;**34**:777–8.
4. Devriendt K, Fryns JP, Chen CP. Holoprosencephaly in deletions of proximal chromosome 14q. *J Med Genet* 1998;**35**:612.

5. **Iwatani N**, Mabe H, Devriendt K, Kodama M, Miike T. Deletion of NKX2.1 gene encoding thyroid transcription factor-1 in two siblings with hypothyroidism and respiratory failure. *J Pediatr* 2000;**137**:272–6.
6. **Krude H**, Schutz B, Biebermann H, von Moers A, Schnabel D, Neitzel H, Tonnies H, Weise D, Lafferty A, Schwarz S, Defelice M, von Deimling A, van Landeghem F, DiLauro R, Gruters A. Choreoathetosis, hypothyroidism, and pulmonary alterations due to human NKX2-1 haploinsufficiency. *J Clin Invest* 2002;**109**:475–80.
7. **Petek E**, Plecko-Startinig B, Windpassinger C, Egger H, Wagner K, Kroisel PM. Molecular characterisation of a 3.5 Mb interstitial 14q deletion in a child with several phenotypic anomalies. *J Med Genet* 2003;**40**:e47.
8. **Su PH**, Chen SJ, Lee IC, Wang KL, Chen JY, Hung HM, Lee CF. Interstitial deletion of chromosome 14q in a Taiwanese infant with microcephaly. *J Formos Med Assoc* 2004;**103**:385–7.
9. **Kamnasaran D**, Chen CP, Devriendt K, Mehta L, Cox DW. Defining a holoprosencephaly locus on human chromosome 14q13 and characterization of potential candidate genes. *Genomics* 2005;**85**:608–21.
10. **Segawa Y**, Itokazu N, Hirose A, Nakagawa S, Takashima S. A case of partial 14q with facial features of holoprosencephaly and hydranencephaly. *Pediatr Neurol* 2007;**37**:51–4.
11. **Carre A**, Szinnai G, Castanet M, Sura-Trueba S, Tron E, Broutin-L'Hermite I, Barat P, Goizet C, Lacombe D, Moutard ML, Raybaud C, Raynaud-Ravni C, Romana S, Ythier H, Leger J, Polak M. Five new TTF1/NKX2.1 mutations in brain-lung-thyroid syndrome: rescue by PAX8 synergism in one case. *Hum Mol Genet* 2009;**18**:2266–76.
12. **Devriendt K**, Vanhole C, Matthijs G, de Zegher F. Deletion of thyroid transcription factor-1 gene in an infant with neonatal thyroid dysfunction and respiratory failure. *N Engl J Med* 1998;**338**:1317–18.
13. **Caliebe A**, Martin Subero JI, Muhle H, Gesk S, Janig U, Krause M, Plendl H, Stephani U, Siebert R, Eckmann-Scholz C. A 2 Mb deletion in 14q13 associated with severe developmental delay and hemophagocytic lymphohistiocytosis. *Eur J Med Genet* 2011;**54**:e505–9.
14. **Torgyekes E**, Shanske AL, Anyane-Yeboah K, Nahum O, Pirzadeh S, Blumfield E, Jobanputra V, Warburton D, Levy B. The proximal chromosome 14q microdeletion syndrome: delineation of the phenotype using high resolution SNP oligonucleotide microarray analysis (SOMA) and review of the literature. *Am J Med Genet A* 2011;**155A**:1884–96.
15. **Gijsbers AC**, Lew JY, Bosch CA, Schuurs-Hoeijmakers JH, van Haeringen A, den Hollander NS, Kant SG, Bijlsma EK, Breuning MH, Bakker E, Ruivenkamp CA. A new diagnostic workflow for patients with mental retardation and/or multiple congenital abnormalities: test arrays first. *Eur J Hum Genet* 2009;**17**:1394–402.
16. **Breedveld GJ**, van Dongen JW, Danesino C, Guala A, Percy AK, Dure LS, Harper P, Lazarou LP, van der Linde H, Joosse M, Gruters A, MacDonald ME, de Vries BB, Arts WF, Oostra BA, Krude H, Heutink P. Mutations in TITF-1 are associated with benign hereditary chorea. *Hum Mol Genet* 2002;**11**:971–9.
17. **Guala A**, Falco V, Breedveld G, De Filippi P, Danesino C. Deletion of PAX9 and oligodontia: a third family and review of the literature. *Int J Paediatr Dent* 2008;**18**:441–5.
18. **Devos D**, Vuillaume I, de Beudelievre A, de Martinville B, Dhaenens CM, Cuvelier JC, Cuisset JM, Vallee L, Lemaitre MP, Bourteel H, Hachulla E, Wallaert B, Destee A, Defebvre L, Sablonniere B. New syndromic form of benign hereditary chorea is associated with a deletion of TITF-1 and PAX-9 contiguous genes. *Mov Disord* 2006;**21**:2237–40.
19. **Visser R**, Gijsbers A, Ruivenkamp C, Karperien M, Reeser HM, Breuning MH, Kant SG, Wit JM. Genome-wide SNP array analysis in patients with features of sotos syndrome. *Horm Res Paediatr* 2010;**73**:265–74.
20. **Kamnasaran D**, O'Brien PC, Schuffenhauer S, Quarrell O, Lupski JR, Grammatico P, Ferguson-Smith MA, Cox DW. Defining the breakpoints of proximal chromosome 14q rearrangements in nine patients using flow-sorted chromosomes. *Am J Med Genet* 2001;**102**:173–82.
21. **Shimajima K**, Komoike Y, Tohyama J, Takahashi S, Paez MT, Nakagawa E, Goto Y, Ohno K, Ohtsu M, Oguni H, Osawa M, Higashinakagawa T, Yamamoto T. TULIP1 (RALGAP1) haploinsufficiency with brain development delay. *Genomics* 2009;**94**:414–22.
22. **Harada N**, Takano J, Kondoh T, Ohashi H, Hasegawa T, Sugawara H, Ida T, Yoshiura K, Ohta T, Kishino T, Kajii T, Niikawa N, Matsumoto N. Duplication of 8p23.2: a benign cytogenetic variantN/A. *Am J Med Genet* 2002;**111**:285–8.
23. **Mencarelli MA**, Kleefstra T, Katzaki E, Papa FT, Cohen M, Pfundt R, Ariani F, Meloni I, Mari F, Renieri A. 14q12 microdeletion syndrome and congenital variant of Rett syndrome. *Eur J Med Genet* 2009;**52**:148–52.
24. **Jacob FD**, Ramaswamy V, Andersen J, Bolduc FV. Atypical Rett syndrome with selective FOXP1 deletion detected by comparative genomic hybridization: case report and review of literature. *Eur J Hum Genet* 2009;**17**:1577–81.
25. **Kortum F**, Das S, Flindt M, Morris-Rosendahl DJ, Stefanova I, Goldstein A, Horn D, Klopocki E, Kluger G, Martin P, Rauch A, Roumer A, Saitta S, Walsh LE, Wiczorek D, Uyanik G, Kutsche K, Dobyns WB. The core FOXP1 syndrome phenotype consists of postnatal microcephaly, severe mental retardation, absent language, dyskinesia, and corpus callosum hypogenesis. *J Med Genet* 2011;**48**:396–406.
26. **Schwarzbraun T**, Vincent JB, Schumacher A, Geschwind DH, Oliveira J, Windpassinger C, Ofner L, Ledinegg MK, Kroisel PM, Wagner K, Petek E. Cloning, genomic structure, and expression profiles of TULIP1 (GARNL1), a brain-expressed candidate gene for 14q13-linked neurological phenotypes, and its murine homologue. *Genomics* 2004;**84**:577–86.
27. **Brand T**, Andree B, Schneider A, Buchberger A, Arnold HH. Chicken NKx2-8, a novel homeobox gene expressed during early heart and foregut development. *Mech Dev* 1997;**64**:53–9.
28. **Reecy JM**, Yamada M, Cummings K, Sosic D, Chen CY, Eichele G, Olson EN, Schwartz RJ. Chicken Nkx-2.8: a novel homeobox gene expressed in early heart progenitor cells and pharyngeal pouch-2 and -3 endoderm. *Dev Biol* 1997;**188**:295–311.
29. **Schell-Apacik CC**, Wagner K, Bihler M, Ertl-Wagner B, Heinrich U, Klopocki E, Kalscheuer VM, Muenke M, von Voss H. Agenesis and dysgenesis of the corpus callosum: clinical, genetic and neuroimaging findings in a series of 41 patients. *Am J Med Genet A* 2008;**146A**:2501–11.

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J Med Genet published online May 25, 2012

doi: 10.1136/jmedgenet-2011-100721

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