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Title: Expanded carrier screening for monogenic disorders: Where are we now?

Running head: Expanded carrier screening for monogenic disorders

Authors: Davit Chokoshvili[1], MSc, MA, Danya Vears[1], PhD, Pascal Borry[1], PhD

[1] Centre for Biomedical Ethics and Law, Department of Public Health and Primary Care, University of Leuven. Belgium

Address for correspondence:
Davit Chokoshvili
University of Leuven
Department of Public Health and Primary Care
Centre for Biomedical Ethics and Law
Kapucijnenvoer 35
3000 Leuven
Belgium
E-mail: davit.chokoshvili@kuleuven.be
Tel: +32 16 37 95 17
Fax: +32 16 3 73368

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What is already known about this topic?

- Expanded carrier screening for reproductive purposes has been available to prospective parents since 2010, primarily through direct-to-consumer genetic testing companies
- The market of expanded carrier screening is rapidly growing, with more than 200000 tests being performed annually in the US alone

What does this study add?

- This study provides a comprehensive review of the expanded carrier screening tests currently available, focusing on test characteristics such as the number and nature of disorders included and variant interpretation strategies used
- Appreciable differences have been identified across providers, with the number of disorders screened for ranging from 41 to 1700. In addition, providers differed considerably in terms of the mutations screened and/or variant interpretation/reporting strategies.

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ABSTRACT

Background

Expanded carrier screening (ECS), which can identify carriers of a large number of recessive disorders in the general population, has grown in popularity and is now widely accessible to prospective parents. This article presents a comprehensive overview of the characteristics of currently available ECS tests.

Methods

To identify relevant ECS providers, we employed a multi-step approach, which included online searching, review of the recent literature, and consultations with researchers familiar with the current landscape of ECS.

Results

As of January 2017, there were sixteen providers of ECS tests: 13 commercial companies, two medical hospitals and one academic diagnostic laboratory. We observed drastic differences in the characteristics of ECS tests, with the number of conditions ranging from 41 to 1700. Only three conditions (Cystic fibrosis, Maple syrup urine disease 1b, and Niemann-Pick disease) were screened for by all providers. Where the same disease gene was included by multiple providers, substantial differences existed in the mutations screened and/or variant interpretation/reporting strategies.

Conclusion

Given the importance of carrier screening results in reproductive decision-making, the observed heterogeneity across ECS panels is concerning. Efforts should be made to ensure that clear and concrete criteria are in place to guide the development of ECS panels.

Key words: Expanded carrier screening, genetic testing, reproductive genetics, consumer genomics, direct-to-consumer testing

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INTRODUCTION

Carrier screening is a form of reproductive genetic testing which aims to identify healthy individuals with a single mutated copy of a gene associated with a recessive (autosomal or X-linked) disorder. Such individuals, known as carriers, are typically unaffected, but they may be at risk of having a child with the disorder. In particular, there is a one-in-four chance in each pregnancy that a couple will conceive an affected child where both members of the couple carry a faulty copy of a gene associated with the same autosomal recessive disorder or where the female member is the carrier of an X-linked recessive disorder.[1] Due to the recessive pattern of inheritance, most carrier couples have no family history suggestive of the disorder and are therefore unaware of their reproductive risk.[2,3] As a consequence, it is common for couples to only find out about their carrier status after giving birth to an affected child.[4] Such couples could benefit from carrier screening, ideally before conception, to identify their reproductive risks and inform decisions.[5] If identified preconceptionally, couples can choose to pursue artificial reproduction through pre-implantation genetic diagnosis or using egg or sperm from a non-carrier donor. They can also decide to forego pregnancy with the current partner and, for example, adopt a child instead. Where carrier status is identified in an ongoing pregnancy, the options are limited to deciding on the prenatal diagnosis and termination of pregnancy if the fetus is found to be affected.[6] Alternatively, some carrier couples may choose not to alter their reproductive plans and use their test results for information purposes, such as to emotionally and logistically prepare for the possibility of having an affected child. Where effective therapeutic interventions for the identified condition exist, awareness of carrier status may also allow for reducing morbidity and mortality in an affected child, by initiating medical treatment early on in life.[7]

Traditionally, carrier screening was available for a small number of recessive disorders with a relatively high prevalence in the general population or among select ethnic groups, such as the Ashkenazi Jewish.[8,9] However, the continuous improvements in molecular genetics and increasing characterization of genetic disorders, has lead to the development of expanded carrier screening (ECS) tests for a large number of conditions.[10] Adding genes to ECS tests is now technically easy and inexpensive, which has resulted in commercially available ECS panels screening for hundreds of recessive disorders.[11]

There are two major advantages of ECS, compared to the traditional ethnicity-based approach to carrier screening. First, ECS identifies carriers in the general population, thus potentially benefiting at-risk couples regardless of their ethnic background. Second, ECS is a more cost-effective approach to identifying carriers, with the cost of a typical ECS not exceeding that of carrier screening tests for specific monogenic disorders.[12,13] Due to these advantages, ECS has grown in popularity and the number of ECS tests performed annually has been estimated at 200 000 in the US.[14] However, to date, the expansion of ECS panels has mainly been driven by technology, which has been met with considerable criticism. Authors have argued that screening for the highest number of recessive disorders may not necessarily promote reproductive autonomy, as carrier status information may be less helpful for couples at risk of conceiving a child with a mild, low-penetrant or phenotypically variable disease.[5,15,16] Because of this, professional medical organizations have advocated for clear medical criteria to be used for the development of ECS panels, as opposed to including as many disorders as technically possible.[5,12,16] For example, in a 2015 joint statement, several US genetic and reproductive health organizations recommended limiting ECS to serious health conditions and discouraged screening for disorders predominantly manifesting themselves in adulthood (such as alpha 1 antitrypsin deficiency) or conditions characterized by low penetrance and variable expressivity

(e.g. MTHFR deficiency and hereditary hemochromatosis).[12] Similarly, professional recommendations emphasize the importance of well-established genotype-phenotype correlations for disease-associated variants and require the existence of sufficient clinical evidence to report a variant as pathogenic.[5,12]

While recent professional recommendations addressing ECS panel composition offer valuable guidance in the development of ECS tests, it is unclear whether ECS providers follow these recommendations in practice. Moreover, some recommendations lack specificity and can be subject to interpretation. Providers of ECS may hold different views on whether a particular disorder should be characterized as severe, or how to define whether a genotype-phenotype correlation is clear. This complexity means that at present, decisions regarding the inclusion of disorders on ECS, and whether to categorize a particular variant as pathogenic, primarily lie with individual ECS providers.[11] Given the subjective nature of decisions surrounding the development of ECS tests, the aim of this study was to comprehensively review and compare ECS tests, including: the list and nature of recessive disorders screened for by different providers, characteristics of disease-specific mutation panels and/or novel variant interpretation and reporting practices.

METHODS

In order to identify potentially relevant providers of ECS, three complementary strategies were employed during the period of August-December 2016. First, an online search was performed using the following search string: "(carrier OR reproductive OR preconception OR preconceptional) AND (screening OR test OR testing) AND (purchase OR buy OR order)". The search was performed independently by two researchers (DC and DV) using a de-personalized Google search to execute the query. Both researchers reviewed the first 30 pages

(300 entries) of the results, beyond which results were deemed to be repetitive and of no additional value. This process identified 27 providers of ECS services.

Second, we identified five additional ECS providers by reviewing recent academic literature on carrier screening and consumer genetic testing. These providers were either based in non-English speaking countries, or did not actively advertise their ECS services through their websites and thus were not identified in the online search. Third, we consulted with colleagues from VU University Medical Centre Amsterdam, who had carried out a systematic review of cystic fibrosis carrier screening offers, which identified two additional providers.

Using these combined search strategies, we identified 34 ECS providers. However, as the goal of this study was to review the characteristics of ECS tests, we excluded intermediary companies and medical clinics offering ECS services developed by external laboratories. We also excluded carrier screening offers primarily targeted at specific ethnic groups (e.g. Ashkenazi Jewish screening programs), and research-oriented ECS initiatives (e.g. preconception carrier screening offer from University of Groningen, the Netherlands), as these tests are currently not accessible to the general public. Finally, we excluded one testing company whose website did not contain information regarding the genes on the company's ECS panel. This resulted in 16 ECS providers. The available information about their ECS tests, web-based content, information brochures and/or hypothetical test reports was archived and downloaded for subsequent analysis. Archiving took place on 19 January 2017.

Data from the 16 providers were input in a Microsoft Excel document and analyzed using descriptive statistics. As some providers used different names for the same disorder and, occasionally, denoted the same gene by different gene aliases, entries in the Excel document were manually reviewed for accuracy. Where synonymous diseases or gene aliases were identified, entries were aggregated to ensure that they were treated as the same.

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RESULTS

Comparison of ECS Panels among Various Providers

The general overview of the current ECS landscape is summarized in Table 1. The majority of the ECS providers (13/16) were private genetic testing companies, with only two medical hospitals and one diagnostic laboratory offering internally-developed ECS tests. Additionally, most (13/16) providers of ECS tests were based in the US, with only three operating elsewhere. The number of recessive disorders included on ECS panels was between 41 and 1792, while the number of genes ranged from 40 to 1556. Of note, several providers offered an option to screen for a subset of disorders. For example, consumers of *Natera*, together with the ordering physician, could choose to purchase one of the five *Horizon*™ panels screening for up to 270+ disorders, with *Horizon*™ 274 being the most comprehensive test option. Screening for a subset of disorders was also possible with other providers, including *EGL Genetics*, *Genaware*, *Good Start Genetics*, *GenPath*, *Integrated Genetics*, *Mount Sinai Hospital*, and *Progenity.* In most cases, providers offered smaller, standardized panels of so-called "(Ashkenazi) Jewish" disorders. However, some used other criteria to differentiate among subpanels, such as GenPath Diagnostics who screened for a subset of ~70 disorders described as 'clinically manageable' (i.e. where effective therapeutic interventions exist). Additionally, several providers had standard panels of <20 disorders, consisting exclusively of disorders for which carrier screening has been recommended by a major professional organization, such as the American College of Medical Genetics and Genomics (ACMG) or the American College of Obstetricians and Gynecologists (ACOG).

Table 2 shows the list of recessive genes and their associated disorders screened for by the highest number of ECS providers (see supplementary material for a complete list of genes screened for by at least one provider). Although the providers collectively screened for approximately 1700 recessive genes (supplementary material), only three genes, *BCKDHB, CFTR,* and *SMPD1* were included by all 16 providers (Table 2). Only 167 genes (approximately 10%) were included by at least half of the providers (8/16), while more than 1000 genes were screened for by a single provider (supplementary material). Among the genes screened for by the highest number of providers, most were associated with autosomal recessive disorders. However, some X-linked disorders, such as fragile X syndrome and ornithine transcarbamylase deficiency, were also commonly included. Clinical characteristics of the included disorders varied substantially. For example, the same number of providers (15) screened for Tay-Sachs disease, a lethal disorder typically resulting in death during infancy, as phenylketonuria, a chronic condition treatable through dietary interventions. (Table 2). To a lesser extent, providers also screened for disorders inherited in an autosomal dominant manner. For example, familial hypercholesterolemia (*LDLR* gene) and factor V Leiden thrombophilia (*F5* gene), both of which typically result in morbidity in homozygous individuals, were included on ECS panels, by four and three providers, respectively (Supplementary material).

We also looked at whether providers screened for the three disorders (alpha 1 antitrypsin deficiency, hereditary hemochromatosis, and MTHFR deficiency) for which carrier screening is currently considered of unclear clinical value and therefore discouraged by several professional organizations.[12] The majority of providers (12/16) included at least one of the three disorders on their standard panels, while two providers routinely screened for all three (Table 3). The approach adopted by *Counsyl* was different from other providers, as the company had included alpha 1 antitrypsin deficiency on their Family Prep™ ECS test, but performed

screening for MTHFR deficiency and hereditary hemochromatosis only upon consumer request.

Comparison of Screening Strategies and Mutation Panels among ECS Providers

We observed that even where the same gene was screened for by multiple providers, there were significant differences in the list of included pathogenic variants and/or approaches to interpreting novel variants, if non-targeted sequencing was used. To illustrate these differences, we compared mutation panels and/or variant interpretation strategies, where available, for the three recessive disorders screened for by all 16 providers of ECS (Maple syrup urine disease type 1B, cystic fibrosis and other CFTR-related disorders, and Niemann-Pick disease A/B).

Providers differed in their approaches to screening, with some employing targeted genotyping and others using sequencing as the primary screening strategy (Table 4). Some providers (e.g. *Counsyl* and *Natera*) used a combination of both strategies, or offered a choice between these strategies to the ordering healthcare professional (e.g. *EGL Laboratory*). Where mutation panels were used, the number of variants screened for varied considerably, with differences being the most prominent in *CFTR*, where the number of variants included ranged from 28 to more than 600. Among those providers who employed sequencing, we identified different approaches to reporting of novel variants. For example, *Counsyl* had a policy to limiting reporting to pathogenic and likely pathogenic variants, whereas *Baby Genes* also communicated variants of uncertain significance 'that result in non-synonymous protein changes but have no known clinical association'.

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DISCUSSION

In this review of currently available ECS offers, we have found considerable discrepancies across various ECS tests. One of the most striking differences among ECS panels was in the number of recessive genes screened, which ranged from 40 to above 1500. Only three recessive disease genes were screened for by all sixteen providers and the majority of genes were included by less than half of them. This suggests that the providers may have used quite different criteria to develop their ECS panels. Reducing the differences to achieve greater harmonization among ECS panels would therefore require that the providers use consistent disease inclusion criteria. One approach that has been gaining acceptance in the context of ECS is inclusion of recessive disorder genes based on the severity of their associated phenotypes. In 2014, Lazarin and colleagues proposed a method for categorizing disorders on the spectrum of severity ranging from 'mild' to 'profound'.[17] Using severity as a key criterion for including disorders in ECS may be a sound strategy for several reasons. First, as demonstrated in recent studies, it is feasible to achieve a severity-based taxonomy for genetic conditions, with medical professionals and laypersons holding similar views regarding the severity of a particular disorder.[18,19] Second, couples at risk of having an affected child often decide not to alter their reproductive plans when they perceive the disorder to be mild, indicating that screening for mild disorders may be of limited utility in the context of reproductive decision-making.[20] Third, taking steps to prevent the birth of a child with a mild disorder, for example, through prenatal diagnosis and termination of an affected pregnancy, may be refused by providers of reproductive healthcare on legal or moral grounds, making it problematic for couples who wish to utilize these options.[21] Although, in principle, carrier couples may still benefit from their carrier status information by emotionally and logistically preparing for the possibility of having an affected child, these benefits may be less prominent where the identified disorder is of a mild nature.[22] However,

categorizing recessive disorders by severity can also be complicated by the fact that some disorders are associated with multiple phenotypes that may vary in their clinical characteristics.[23] Among the ECS panels analyzed in our study, we observed that smaller panels tended to be designed in the way that maximizes the reliability of positive test results. For example, *23andMe* and *VU University Medical Centre Amsterdam*, who screen for 40 and 50 genes, respectively, utilized targeted genotyping for known pathogenic variants in relatively common recessive disorders. Additionally, neither provider screened for the three disorders currently considered of unclear clinical value due to their variable expressivity and low penetrance (alpha 1 antitrypsin deficiency, MTHFR deficiency, and hereditary hemochromatosis). In contrast, larger ECS panels typically aimed at maximizing the reliability of negative test results, employing more comprehensive targeted mutation panels and/or non-targeted sequencing. The principal advantage of more comprehensive ECS tests is that couples receiving a negative test result can be highly confident that they are definitely not at risk of having a child with one of the screened disorders.[23] However, this approach may also lead to an increased proportion of false positive results, where some couples identified as carriers through the test will not be at risk of having an affected child. For example, in hereditary hemochromatosis, genotype-phenotype correlations are very low, with the penetrance estimated at <10%.[12] This means that the majority of couples where both members are identified as 'carriers' of hereditary hemochromatosis do, in effect, receive false positive results. Importantly, the issue of low penetrance is not unique to disorders with unclear clinical value in carrier screening. For example, in cystic fibrosis (*CFTR* gene), where screening is widely recommended, there is conflicting evidence regarding the pathogenicity of some variants, such as L997F (c.2991G>C).[24] Studies have suggested that a substantial proportion of individuals who are compound heterozygotes for L997F and a 'classical' pathogenic mutation may never present with symptoms, while others may develop a phenotype ranging from a mild CFTR-

related disorder to classical cystic fibrosis.[25] Consequently, carrier screening for L997F has been contested, as reproductive couples in which one partner screens positive for L997F and the other partner is found to be a carrier of another CFTR-related mutation will be confronted with uncertain results.[25,26] While couples with uncertain carrier screening results may benefit from post-test genetic counseling to assist them to make reproductive choices with the information at hand, genetic counseling is not routinely offered by all providers of ECS tests.[11]

Owing to the challenges raised by unclear clinical significance of some variants, professional medical organizations recommend that carrier screening be limited to variants with clearly established genotype-phenotype correlations.[5,12] Accordingly, the use of non-targeted sequencing in the context of carrier screening has been controversial, as this approach may routinely identify novel variants, many of which could be of unclear clinical significance.[22,27,28] On the other hand, some novel variants, such as those occurring at canonical splice sites, could be clearly pathogenic with a highly predictable health impact.[29] These may be particularly common in individuals from geographically isolated areas or with ethnic backgrounds where genotype data is sparse. Therefore, when applied under rigorous laboratory protocols and manual curation of all novel potentially relevant pathogenic variants, non-targeted sequencing can reliably identify some additional carrier couples.[23] Among the ECS providers analyzed in our study, we found that half of them (8/16) employed non-targeted sequencing, either as the primary or as a complementary strategy to identify carriers. In this regard, we found substantial differences in variant reporting practices, with some limiting reporting to clearly/likely pathogenic variants, while others routinely reporting variants of uncertain significance. These differences have implications for the results received by consumers of ECS services. An individual may receive positive, negative, or indeterminate carrier status result for the same

variant in a recessive disease gene, depending on the provider through which they pursue testing.[30]

Finally, the present review of ECS panels also revealed that some providers offered tests that may identify individuals who are undergoing screening as either being affected, or being at risk of developing a genetic disease. This primarily refers to screening for X-linked recessive disorders which may identify affected women, and to autosomal recessive disorders where affected (homozygous or compound heterozygous) individuals may not have come to medical attention due to the late-onset or relatively mild nature of the disorder. Furthermore, some ECS panels also included a handful of autosomal dominant disorders, such as familial hypercholesterolemia and factor V Leiden thrombophilia, where routine identification of potentially affected individuals is even more likely. These findings suggest that the traditional distinction between carrier screening and diagnostic or predictive genetic testing has become blurred. It is important to ensure that individuals undergoing ECS are adequately informed of a potential health-related finding and have access to quality genetic counseling, as well as follow-up diagnostic tests and clinical care, where necessary.[15]

Given the significance of carrier status information it is essential that test results communicated to prospective parents are reliable, correctly estimating their reproductive and/or health risks. Of particular importance is devising criteria for interpreting and reporting novel variants, since an increasing number of providers are moving away from using targeted genotyping panels and are adopting non-targeted sequencing. Additionally, efforts should be made to reduce discrepancies among ECS panels by applying consistent criteria for including genetic disorders. The professional medical community has an important role to play by providing continuous guidance and updated recommendations regarding ECS test characteristics. However, this alone may not be sufficient, since some providers of ECS may choose not to follow such

recommendations, as evidenced in the present study. Therefore, closer collaboration between the professional medical community and the providers of ECS might be necessary in order to develop best testing practices.

LIMITATIONS

Despite our multi-step approach to identifying all the relevant providers of ECS, it is possible that some providers were not included in the present study. This is primarily due to the fact that the market of ECS tests is still in its early stages and is undergoing expansion, with a growing number of new providers entering the field. Some of these providers may be operating in specific geographical areas and could have their websites in a language other than English, which makes their identification particularly challenging. As providers of ECS tests often used different disease names and, to a lesser extent, different gene aliases in the description of their ECS panels, it is possible that the data used in our study contained some errors. However, we made efforts to minimize the possibility of such errors by carefully reviewing the entries for the genes/diseases and comparing ambiguous items with the entries in authoritative databases such as ClinVar and Online Mendelian Inheritance in Man (OMIM).

CONCLUSION

In this study, we have compared ECS tests offered by sixteen providers. We found substantial differences in terms of both panel size and the lists of recessive disorder genes included on ECS panels. Furthermore, where multiple providers screened for the same gene, their approaches

varied in the lists of specific disease-associated variants (in targeted genotyping) and variant interpretation/reporting practices (in non-targeted sequencing).

Given the significance of ECS test results in reproductive decision-making and, increasingly, in personal medical care, such drastic differences among providers are concerning. Efforts should be made to achieve a greater harmonization of ECS panels by using consistent criteria for the inclusion of genes and disorders. Additionally, guidance is needed towards developing clear standards for variant interpretation and reporting practices. This can be best accomplished through a close collaboration between the professional genetic community and the providers of ECS tests.

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Ethics approval

Not applicable

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Table 1. An overview of the available ECS offers*

Provider	Type of provider	Country	Number of conditions screened**	Number of genes screened**
23andMe	Genetic testing company	USA	41	40
Baby Genes	Genetic testing company	USA	71	92
Igenomix	Genetic testing company	Spain	633	549
Counsyl	Genetic testing company	USA	113	111
EGL Genetics	Diagnostic laboratory	USA	147	148
VU University Medical Centre Amsterdam	Medical hospital	The Netherlands	50	50
GeneAware	Genetic testing company	USA	158	159
GenPath Diagnostics	Genetic testing company	USA	166	166
Good Start Genetics	Genetic testing company	USA	252	281
Integrated genetics	Genetic testing company	USA	135	136
Macrogen	Genetic testing company	South Korea	1792	1556
Mount Sinai Hospital	Medical hospital	USA	256	279
Natera	Genetic testing company	USA	272	273
Pathway Genomics	Genetic testing company	USA	73	74
Progenity	Genetic testing company	USA	230	226
Recombine	Genetic testing company	USA	314	301

465 * Accurate as of 19 January 2017. Excludes ethnicity-specific and research-oriented ECS offers.

** The highest number of genes/conditions screened for by any given provider. In some cases, testing can be performed for a subset of these genes and/or disorders.

NB: The number of genes may be a more accurate measure for comparing the sizes of ECS panels than the number of conditions. This is because providers may use different approaches to defining a 'condition'. For example, providers A and B may screen for the same 6 genes associated with Neuronal Ceroid-Lipofuscinosis. However, provider A may consider this as a single entry on the list of conditions, whereas provider B may include it as 6 separate entries. On the other hand, the number of genes included on an ECS test, is not subject to the provider's interpretation.

Table 2. Recessive disorder genes screened for by the highest number of ECS providers

Screened by	***Gene*** (Associated disorder(s))
16 providers	***BCKDHB*** (Maple yrup urine disease type 1B); ***CFTR*** (Cystic fibrosis and other CFTR-related disorders); ***SMPD1*** (Niemann-Pick disease A/B)
15 providers	***ACADM*** (Medium chain acyl-CoA dehydrogenase deficiency, MCAD); ***ASPA*** (Canavan disease); ***CBS*** (Homocystinuria); ***FAH*** (Tyrosinemia type I); ***FANCC*** (Fanconi anemia group C); ***G6PC*** (Glycogen storage disease Type Ia); ***GAA*** (Glycogen storage disease, type II); **HBB** (Hemoglobinopathies, sickle cell disease); ***HEXA*** (Tay-Sachs disease); ***IKBKAP*** (Familial dysautonomia); ***MCOLN1*** (Mucolipidosis type IV); ***NPC1*** (Niemann-Pick disease type C1); ***PAH*** (Phenylketonuria)
14 providers	***ACADVL*** (Very long-Chain acyl-CoA dehydrogenase deficiency); ***ASS1*** (Citrullinemia, type I); ***BLM/RECQL3*** (Bloom syndrome); ***CLN5*** (Neuronal ceroid lipofuscinosis type 5); ***DLD*** (Dihydrolipoamide dehydrogenase deficiency or Maple syrup urine disease type 3); ***FCYT/PKHD1*** (Polycystic kidney disease, autosomal recessive); ***GALC*** (Krabbe disease); ***GALT*** (Galactosemia); ***GBA*** (Gaucher disease); ***GCDH*** (Glutaric acidemia type 1); ***HADHA*** (Long-Chain 3-Hydroxyacyl-CoA dehydrogenase deficiencyl; Trifunctional protein deficiency); ***IDUA*** (Mucopolysaccharidosis type I or Hurler syndrome); ***PEX7*** (Rhizomelic chondrodysplasia punctata, Type 1); ***PMM2*** (Congenital disorders of glycosylation type 1a); ***PPT1*** (Congenital disorders of glycosylation type 1b); ***SACS*** (Autosomal recessive spastic ataxia of Charlevoix-Saguenay or ARSACS)
13 providers	***AGA*** (Aspartylglucosaminuria); ***ALDH3A2*** (Sjogren-Larsson syndrome); ***ARSA*** (Metachromatic leukodystrophy); ***ASL*** (Argininosuccinic aciduria); ***BCS1L*** (GRACILE syndrome; Mitochondrial comlpex III deficiency, nuclear type 1); ***CLRN1*** (Usher syndrome type 3A); ***CPT2*** (Carnitine palmitoyltransferase deficiency, type 2); ***DHCR7*** (Smith-Lemli-Opitz syndrome); ***GRHPR*** (Primary hyperoxaluria type 2); ***HSD17B4*** (D-bifunctional protein deficiency); ***LAMB3*** (Herlitz junctional epidermolysis bullosa, LAMB3-related); ***NBN*** (Nijmegen breakage syndrome); ***NEB*** (Nemaline myopathy); ***PCDH15*** (Usher syndrome type 1F); ***PEX1*** (Zellweger spectrum disorder); ***SLC12A6*** (Andermann syndrome); ***SLC17A5*** (Sialic acid storage disease or Salla disease); ***SLC37A4*** (Glycogen storage disease Type Ib); ***SMN1*** (Spinal muscular atrophy); ***TMEM216*** (Joubert syndrome type 2); ***TPP1*** (Neuronal ceroid-lipofuscinosis, TPP1-Related); ***TTPA*** (Ataxia with vitamin E deficiency)
12 providers	***AGL*** (Glycogen storage disease, type III); ***AGXT*** (Primary hyperoxaluria, Type 1); ***ALDOB*** (Hereditary fructose intolerance); ***ALPL*** (Hypophosphatasia); ***ATP7B*** (Wlison disease); ***BCKDHA*** (Maple syrup urine disease type 1a); ***BTD*** (Biotinidase deficiency); ***CLN8*** (CLN8-related Neuronal ceroid lipofuscinosis*)*; ***CPT1A*** (Carnitine palmitoyltransferase type 1A deficiency); ***CTNS*** (Cystinosis); ***FKTN*** (Walker-Warburg syndrome; Fukuyama congenital muscular dystrophy); ***GJB2*** (Non-syndromic hearing loss); ***HEXB*** (Sandhoff disease); ***HMGCL*** (HMG-CoA Lyase Deficiency); ***IVD*** (Isovaleric academia); ***MAN2B1*** (Alpha-mannosidosis); ***MEFV*** (Familial mediterranean fever); ***MMAA*** (Methylmalonic aciduria, cblA type); ***MMACHC*** (Methylmalonic aciduria and homocystinuria, type cblC); ***MUT*** (MUT-related Methylmalonic aciduria); ***NPHS1*** (Nephrotic syndrome, type I); ***PCCA*** (Propionic acidemia type 1); ***PCCB*** (Propionic acidemia type 2); ***PDCN/NPHS2*** (Steroid-resistant nephrotic syndrome); ***SGCA*** (Limb-girdle muscular dystrophy type 2D); ***SGCB*** (Muscular dystrophy, limb-girdle, type 2E); ***SLC26A2*** (Skeletal dysplasias; Achondrogenesis type 1B); ***SLC26A4*** (Pendred syndrome)
11 providers	***ABCC8*** (Familial hyperinsulinism/permanent neonatal diabetes); ***ACAT1*** (Beta-ketothiolase deficiency); ***ATM*** (Ataxia-telangiectasia); ***BBS10*** (BBS10-related Bardet-Biedl syndrome); ***CTSK*** (Pycnodysostosis); ***GLB1*** (Mucopolysaccharidosis, type IVB / GM1 Gangliosidosis); ***FMR1*** (Fragile X syndrome); ***GNE*** (Inclusion body myopathy 2, Nonaka type); ***GNPTAB*** (Mucolipidosis type 2/type 3); ***LAMA3*** (LAMA3-related Herlitz junctional epidermolysis bullosa); ***LAMC2*** (LAMC2-related Herlitz junctional epidermolysis bullosa); ***LRPPRC*** (Leigh syndrome, French-Canadian type); ***MLC1*** (Megalencephalic leukoencephalopathy); ***MPI*** (Congenital disorders of glycosylation type 1b); ***NPC2*** (Niemann-Pick disease type C2); ***OTC*** (Ornithine transcarbamylase deficiency); ***SLC22A5*** (Primary carnitine deficiency)

10 providers	***ADA*** (Adenosine deaminase deficiency; ADA-related severe combined immunodeficiency); ***ADAMTS2*** (Ehlers-Danlos syndrome: Type VIIC); ***BBS1*** (BBS1-related Bardet-Biedl Syndrome); ***CAPN3*** (Limb-girdle muscular dystrophy, type 2A); ***CLN3*** (CLN3-related neuronal ceroid lipofuscinosis or Batten disease); ***ETHE1*** (Ethylmalonic encephalopathy); ***HGSNAT*** (Mucopolysaccharidosis type IIIC, Sanfilippo type); ***HLCS*** (Holocarboxylase synthetase deficiency); ***IL2RG*** (X-Linked severe combined immunodeficiency); ***MMAB*** (Methylmalonic aciduria, cblB type); ***MPL*** (Congenital amegakaryocytic thrombocytopenia); ***MTTP*** (Abetalipoproteinemia); ***OPA3*** (3-methylglutaconic aciduria, type III); ***POMGNT1*** (POMGNT1-related muscle-eye-brain disease); ***PYGM*** (Glycogen storage disease, type V); ***RMRP*** (Cartilage-hair hypoplasia); ***TH*** (Segawa syndrome)

Table 3. Providers' practices regarding the inclusion of Alpha 1 antitrypsin deficiency, MTHFR deficiency, and hereditary hemochromatosis on their ECS panels

Provider	Alpha 1 Antitrypsin deficiency (*SERPINA1 gene)*	MTHFR deficiency (*MTHFR gene)*	Hereditary Hemochromatosis *(HFE, HFE2, TFR2 genes)*
23andMe	Not included	Not included	Not included
Baby Genes	Not included	**Included**	Not included
Igenomix	**Included**	**Included**	**Included (HFE, TFR2)**
Counsyl	**Included**	*Not included **	*Not included * (HFE)*
EGL Genetics	**Included**	Not included	**Included (HFE)**
VU University Medical Centre Amsterdam	Not included	Not included	Not included
GeneAware	**Included**	Not included	Not included
GenPath Diagnostics	Not included	Not included	Not included
Good Start Genetics	Not included	**Included**	**Included (HFE2, TFR2)**
Integrated Genetics	Not included	Not included	Not included
Macrogen	Not included	**Included**	**Included (HFE)**
Mount Sinai Hospital	Not included	**Included**	**Included (HFE2,TFR2)**
Natera	Not included	**Included**	**Included (HFE2,TFR2)**
Pathway Genomics	**Included**	Not included	**Included (HFE)**

Progenity	**Included**	Not included	**Included (HFE, HFE2, TFR2)**
Recombine	**Included**	**Included**	**Included (HFE2, TFR2)**

* Not part of the standard screening panel, but can be included if specifically requested by the consumer.

Table 4. Screening strategies and the size of mutation panels for the three genes screened for by all 16 providers

Provider	Maple Syrup Urine Disease Type 1B (BCKDHB gene)	Cystic fibrosis and other CFTR-related disorders (CFTR gene)	Niemann-Pick Disease A/B (SMPD1 gene)
23andMe	TG (2 variants)	TG (28 variants)	TG (3 variants)
Baby Genes	Seq.	Seq.	Seq.
Igenomix	TG (24 variants)	TG (146 variants)	TG (42 variants)
Counsyl	TG (3 variants) + Seq.	TG (99 variants) + Seq.	TG (4 variants) + Seq.
EGL Genetics	TG or Seq.	TG or Seq.	TG or Seq.
VU University Medical Centre Amsterdam	TG	TG	TG
GeneAware	Seq.	CNV + Seq.	Seq.
GenPath Diagnostics	TG (3 variants)	TG (220 variants)	TG (6 variants)
Good Start Genetics	TG + Seq.	TG + Seq.	TG + Seq.
Integrated Genetics	TG	TG (609 variants)	TG
Macrogen	TG (1 variant)	TG (102 variants)	TG (14 variants)
Mount Sinai Hospital	Seq.	TG + Seq.	Seq.
Natera	TG (21 variants) + Seq.	TG (579 variants) + Seq.	TG (50 variants) + Seq.

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Pathway Genomics	TG (3 variants)	TG (82 variants)	TG (5 variants)
Progenity	TG (3 variants)	TG (656 variants)	TG (4 variants)
Recombine	TG (6 variants)	TG (150 variants)	TG (9 variants)

481 TG - Targeted genotyping

482 Seq. - (non-targeted) sequencing

483 CNV - Copy number variation analysis

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