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Exome and Genome Sequencing in Adults with Undiagnosed Disease: A Prospective Cohort Study

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Abstract

Background: Exome and genome sequencing have been demonstrated to increase diagnostic yield in pediatric populations, improving treatment options and providing risk information for relatives. There are limited studies examining the clinical utility of these tests in adults, who currently have limited access to this technology.

Methods: Patients from adult and cancer genetics clinics across Toronto, Ontario, Canada were recruited into a prospective cohort study evaluating the diagnostic utility of exome and genome sequencing in adults. Eligible patients were 18 years of age and suspected of having a hereditary disorder but had received previous uninformative genetic test results. In total, we examined the diagnostic utility of exome and genome sequencing in 47 probands and 34 of their relatives who consented to participate and underwent exome or genome sequencing.

Results: Overall, 17% (8/47) of probands had a pathogenic or likely pathogenic variant identified in a gene associated with their primary indication for testing. The diagnostic yield for patients with a cancer history was similar to the yield for patients with a non-cancer history [4/18 (22%)

vs. 4/29 (14%)). An additional 24 probands (51%) had an inconclusive result. Secondary findings were identified in 10 patients (21%); three had medically actionable results.

Conclusions: This study lends evidence to the diagnostic utility of exome or genome sequencing in an undiagnosed adult population. The significant increase in diagnostic yield warrants the use of this technology. The identification and communication of secondary findings may provide added value when using this testing modality as a first line test.

INTRODUCTION

Genetic disorders contribute a significant proportion of morbidity and mortality, cumulatively affecting up to 1-2% of adults[1]. This range may be an underestimate, given that adult genetic diseases can be challenging to diagnose because they present later in life, are often overlooked in the differential diagnosis, and have variable penetrance and clinical features that can overlap with common adult co-morbidities[2–4]. Standard investigations consist of multiple tests and procedures, costing the healthcare system tens of thousands of dollars per patient[5,6]. Despite extensive testing, many patients remain undiagnosed or misdiagnosed and do not receive appropriate management.

Genomic sequencing technologies have been largely successful in diagnosing rare disorders in pediatrics, where they have increased diagnostic yield up to 30-70% in populations with suspected forms of hereditary neurological diseases and seizures[7,8]. Exome sequencing (ES) and genome sequencing (GS) have been shown to alter medical management by informing treatment options for patients and providing reproductive risk information for their families[5,9,10]. The few studies that have assessed ES in adults have reported lower diagnostic yields, ranging from 18% to 27%[11–13] and provide limited evidence on the clinical utility of results.

In Canada, clinical ES is not currently offered by laboratories, lagging behind the United States (US)[14–16]. In the province of Ontario, patients must receive approval for clinical ES through the Ministry of Health and Long Term Care (MOHLTC) on a case by case basis; once approved, testing is paid for by the MOHLTC and performed out of province. The limited evidence of clinical utility of ES and GS in adults has, up until recently, led to reduced access to this technology in this population.

Thousands of Canadian adults are seen annually by medical geneticists, many of whom will not receive a diagnosis using current genetic testing practices and may have no further options for additional genetic investigations. These patients may benefit from ES or GS, which may provide a diagnosis and inform targeted management. We sought to apply ES/GS in a clinically heterogeneous (e.g. cardiovascular, neurological) adult population who were not eligible for clinical ES. We report on the diagnostic yield of ES/GS in this population.

METHODS

Patient Recruitment

We recruited patients from four genetics clinics in Toronto, Ontario; The Fred A. Litwin Family Centre in Genetic Medicine, the Familial Breast Clinic at the Marvella Koffler Breast

Centre, Princess Margaret Cancer Centre, and the Familial Gastrointestinal Cancer Registry. Recruitment began in March 2013 and ended in November 2018. Patients 18 years with a suspected hereditary disorder but prior negative or inconclusive genetic test results (e.g. gene panels) were eligible and identified by their clinician. Inclusion of relatives, affected or unaffected, was recommended but not required.

Consent was obtained for access to relevant data including contact information, demographics, family history (pedigree), medical records related to the primary indication, and to conduct ES or GS and analysis. Participants also consented to having their sample, genomic data and health information stored and potentially shared with other researchers in controlled-access databases and also informed that general research results would be available in open-access databases. Participants were given the option to consent for re-contact for return of secondary findings (SF) and participation in future studies. They also completed a questionnaire that evaluated preferences for receiving SF. A blood sample was obtained at time of consent if an aliquot of DNA was unavailable.

Sequencing and Analysis

ES was performed at one of several sequencing platforms in downtown Toronto. All sequencing was performed using next generation sequencing on the Illumina HiSeq 2500 platform (Illumina, San Diego, California, USA). GS was carried out at The Centre for Applied Genomics beginning in 2018. Bioinformatic and data processing from the raw sequence reads were analyzed using the in-house pipeline developed by JLE at MSH (Mount Sinai Hospital). The raw data was also analyzed using third party software - the VariantX Genomic Intelligence platform V. 2.6.0.2 (<https://www.variantyx.com>) that uses proprietary algorithms for analysis of sequence variants. Reanalysis from raw data for inconclusive cases was performed for a subset of cases by the Broad Institute. See supplementary information for detailed information on sequencing and capture methods, data processing and analysis tools (online supplementary file 1).

Reporting

A written report was generated for each proband and issued to the referring clinician who returned results to the patient. The report included variants classified as pathogenic, likely pathogenic, or of uncertain significance (VUS) occurring in genes that matched or were related to the clinical phenotype(s), using ACMG/AMP (American College of Medical Genetics and Genomics/Association for Molecular Pathology) guidelines[17]. Cases were classified as positive if the variant identified was likely pathogenic or pathogenic or as inconclusive if a VUS was identified or only one pathogenic/likely pathogenic variant was identified in a gene associated with autosomal recessive (AR) disease. Alternatively, a report was called inconclusive if a pathogenic/likely pathogenic variant was identified in a gene that was not considered diagnostic or related to the patients' phenotype.

Secondary results—We did not intentionally analyze data for SF. However, if a pathogenic or likely pathogenic variant was identified in a gene within the phenotypic filters but did not directly match the presentation, the results were reported as SF to patients who consented to receiving them (online supplementary table 1). Secondary findings were not

limited to the 59 genes recommended by the ACMG[18]. As part of the study, patients also completed a questionnaire on their preferences for receiving SF (online supplementary file 2). They were asked about their interest in receiving SF for diseases with varying levels of penetrance (e.g. <5%, 5-10% and so on) and types of genetic variants/conditions.

Costs

Costs for prior genetic investigations were obtained from MOHLTC documents in clinical charts. If unavailable, laboratories were contacted to obtain the cost of the specific test.

RESULTS

Cohort enrollment and description

In total, 47 probands and 34 relatives were consented, underwent sequencing and received results. ES was performed on 39 probands and GS on 8 probands. GS became available in 2018 and offered to families who were recommended for GS by their referring clinician.

Approximately 77% (36/47) of the probands were female (mean age 47.32). Patients had a wide variety of indications. The most commonly observed phenotypes included cancer, neurological, and cardiovascular/connective tissue abnormalities (table 1 and figure 1).

In 23 cases, only the proband was sequenced; proband plus one first-degree relative (16 families), trios (6 families) or quartet families (2 families). Out of the 34 relatives included in the study, 22 were affected and 12 were unaffected. On average, 49 760 variants were identified per proband in the ES cases (online supplementary tables 1 and 2).

Diagnostic yield

Overall, 17% (8/47) of probands who underwent ES or GS had a pathogenic or likely pathogenic variant identified in a gene that was consistent with their primary indication (table 2). The correlation between the pathogenic or likely pathogenic variant and the phenotype was confirmed with the referring clinician.

The diagnostic yield for patients with a cancer history was similar to the yield in patients with a non-cancer history (22% or 4/18 vs. 14% or 4/29) ($p=0.46$, Fisher's exact test).

The majority of the findings were associated with pathogenic variants in autosomal dominant (AD) genes (seven out eight positive cases). One patient had one likely pathogenic variant and one VUS in *COL6A2*, which is associated with autosomal dominant and recessive presentations. Among the nine pathogenic or likely pathogenic variants, four were expected to produce a premature stop codon due to a frameshift or nonsense variant, one patient harbored a variant in the canonical splice donor site and the other four corresponded to missense variants classified as pathogenic or likely pathogenic variants. Of note, 7/8 positive cases were identified by ES and only 1/8 was identified through GS (*SCN5A*; c.3823G>A), which was a missense variant and therefore would have been identified by ES.

The results from our study can have a broad range of clinical management implications for the patients and their relatives, such as surgical recommendations, regular imaging, reproductive counselling, and medication and diet changes (online supplementary table 3).

Inconclusive cases—Inconclusive cases were defined as those in which a VUS was identified in a gene related to the primary indication or one pathogenic or likely pathogenic variant identified in a gene associated with AR disease. In total, 24 of 47 (51%) probands had an inconclusive result (online supplementary tables 4 and 5).

Secondary or incidental findings—SF were defined as likely pathogenic or pathogenic variants in genes not associated with the primary indication. Ten patients (21%) had a total of 18 SF, of which 3/18 (17%) were in genes associated with diseases considered to be medically actionable (*LDLR*, *APOB* and *MUTYH*) by the ACMG (table 3)[18].

Cancer cases—At the initiation of this study (2013), multigene cancer panels were not yet standard of care or readily available to Ontario patients. For families with a history suggestive of a hereditary cancer syndrome, research ES was offered if first-tier testing was negative or inconclusive. Research ES was available for cancer patients until March 2017 when panels became clinically available in Ontario.

ES for 18 families with a cancer history revealed a causative pathogenic variant in four families, a yield of 22% (table 2). Two families with a history of GI cancers were identified to have an *MSH6* variant (c.3739_3742delACTC)[19] and a *POLE* variant (c.1372T>A) respectively, and two breast cancer families had an *ATM* variant (c.1065+1G>T) and *MSH6* variant (c.3959_3962del) respectively.

Non-cancer cases—Results are described below and presented in table 2 and online supplementary files and tables.

Family 31

The proband in family 31 was a 30-year-old female with early infantile-onset seizure disorder, global developmental delay and multiple other abnormalities (see online supplementary table 2 and online supplementary file 3 for more details). Previous workup had been extensive but uninformative, and included a 170-gene neurodevelopmental disorders panel.

ES identified a previously described heterozygous pathogenic variant in *KCNQ2*, c.1687G>A (p. Asp563Asn) in the proband. The variant was not identified in the proband's mother (her father was unavailable for testing).

Pathogenic variants in *KCNQ2* are associated with a spectrum of phenotypes ranging from benign familial neonatal epilepsy (BFNE) to early infantile or neonatal epileptic encephalopathy (EIEE or NEE)[20]. Features include encephalopathy which may result in moderate to severe global developmental impairment in addition to swallowing difficulties, strabismus, nystagmus, recurrent respiratory infections, microcephaly, and dysmorphic features[20,21]. Although the *KCNQ2* variant did not explain all of the proband's features,

it explained most and is likely contributory to her history. Of note, the neurodevelopmental disorders panel conducted in 2015 did not include *KCNQ2*.

Family 39

Family 39 comprised of two sisters in their 60s with a personal and family history suggestive of periodontal Ehlers-Danlos syndrome (pEDS, formerly EDS type VIII). Clinical features included skin fragility with atrophic scars and easy bruising, loss of all teeth in the third or fourth decade, and gastrointestinal symptoms (irritable bowel syndrome, colitis). The family history was consistent with autosomal dominant inheritance. Both sisters underwent an EDS panel that included 18 genes associated with various types of EDS, which was uninformative.

ES identified a heterozygous pathogenic variant in *C1S*, c.949_951delGTG (p. Val317del) in both sisters, confirming the diagnosis of pEDS. Of note, a variant that results in the same protein change was previously reported in the literature and segregated in four affected relatives with pEDS with clinical features consistent with these patients[22]. The *C1S* gene had been not associated with pEDS when the sisters had undergone panel testing in 2016, and therefore *C1S* was not on the EDS panel.

Family 57

The proband in family 57, age 55, had a clinical diagnosis of Charcot-Marie Tooth disease-axonal neuropathy type. She reported a history of muscle issues beginning in childhood, severe hypotonia, and congenital dislocation of the hip. Over the course of her workup, she revealed that she had undergone an electromyography (EMG) in childhood, which revealed chronic neurogenic process along with a strong predominant myopathic process. These findings suggested a congenital myopathy. The proband also had a history of aortic dilatation and other features including inguinal hernias and subluxation that were suggestive of a connective tissue disorder. On testing of a panel of connective tissue genes, she was found to carry a variant of unknown clinical significance in *COL3A1* (c.2702A>G). Skin biopsy was arranged for biochemical collagen analysis and the results were negative, thus ruling out a diagnosis of vascular-type EDS. Extensive genetic testing including an aortic root dilation panel and spinal muscular atrophy panel were all uninformative.

ES initially revealed a variant of uncertain significance, c.2417 G>A (p.Cys806Tyr) in the *COL6A2* gene. Follow-up clinical testing identified a likely pathogenic variant, c.1273_1280 dupCGCAGGGG (p. Asp428Alafs*120) in *COL6A2*, which was filtered out due to a low-quality score but found upon re-inspection of exome data with the Variantx software.

Pathogenic variants in *COL6A2* are associated with collagen type VI-related disorders, which represent a clinical spectrum of disorders that range from Bethlem myopathy at the mild end and Ullrich congenital muscular dystrophy at the severe end, both of which can display dominant or recessive inheritance. The proband's history appeared consistent with a mild form of Ullrich congenital muscular dystrophy, which is characterized by congenital weakness, hypotonia, proximal joint contractures and hyperlaxity of distal joints. However, she did not report a history of joint contractures.

Family 84

Family 84 had a striking history of early-onset strokes. The proband, age 29 presented with a stroke at age 27. The proband's brother had 2 strokes, at ages 28 and 33. Their father and paternal uncle both had a history of multiple strokes before age 50. The family was of Ashkenazi Jewish ancestry.

Multiple investigations had been conducted in the family, including *NOTCH3* and *TREX1* sequencing and a 10-gene hemiplegia/stroke panel – all of which was uninformative.

The affected proband and paternal uncle underwent GS, which identified a shared heterozygous pathogenic variant in *SCN5A*, c.3823G>A, (p.Asp1275Asn). Follow-up testing of the proband's affected brother and father confirmed the presence of this variant in them. Pathogenic *SCN5A* variants are associated with varying phenotypes, ranging from dilated cardiomyopathy to Brugada syndrome. *SCN5A* variants are also associated with atrial fibrillation, which increases the risk of strokes. This specific variant has been described previously in a 14-year-old male with atrial arrhythmia and a stroke[23]. Although none of the four relatives described above have been documented to have atrial arrhythmia, most have first degree atrioventricular block with or without left atrial enlargement. Based on our current knowledge, this variant likely explains the family history of early-onset strokes.

Secondary findings cases (non-primary findings)

Family 31

The proband in family 31 (described above) was identified to have a likely pathogenic variant in *LDLR*, c.1085A>C (p. Asp362Ala), that was not detected in her mother. Pathogenic variants in *LDLR* are associated with familial hypercholesterolemia, and is included in the medically actionable genes recommended for return in clinical ES by ACMG[18]. This SF suggests that the proband is at significantly increased risk to develop severely elevated LDL cholesterol levels and subsequent cardiovascular sequelae. The early identification could allow for proper management and improved clinical outcomes for the proband, as well as cascade testing for her siblings and other relatives.

Family 37

The proband was identified as having three SF; a pathogenic variant in each *GJB2*, *HADHA*, and *HFE*. Therefore, she is a carrier of non-syndromic hearing loss and deafness (DFNB1), Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency and hereditary hemochromatosis. These results may have implications for family planning for the proband and relatives.

Family 81

ES identified multiple SF in the proband, including two trans pathogenic variants in the *MUTYH* gene, associated with *MUTYH*-adenomatous polyposis.

Of note, her presenting features included a history of GI issues, namely IBS, Celiac and Crohn's disease with recurrent episodes of fevers, vomiting, diarrhea, abdominal cramping

and hand swelling. Given that colonic polyps may cause some of these symptoms, her GI history may be explained by the *MUTYH* variants. Further investigations, such as a colonoscopy, could provide additional information. She was also identified to be a carrier of the common 35delG variant in *GJB2*, Pendred syndrome (*SLC26A4*) and fructose intolerance (*ALDOB*).

Diagnostic Odyssey and Costs

The number of prior genetic investigations for the eight diagnosed cases ranged from 2 to 11 tests, at an average cost of \$9178.14 per case (total cost of \$73 425.12 CAD) (online supplementary table 6). This cost is higher than costs reported for paediatric populations and likely reflective of adult patients undergoing genetic investigations[5]. For the cohort of diagnosed cases, we estimate that the cost of ES would have been roughly \$2937.50 CAD per patient for exome or \$4500 CAD for genome (estimated using costs from the literature and commercial laboratories).

Although we did not conduct a full economic analysis, our results suggest that the use of a single test, ES or GS in adults could identify a diagnosis. This could reduce the number of tests per patient and the time to diagnosis, representing a potentially a cost-effective approach.

DISCUSSION

Using ES or GS in a population of adults with suspected hereditary conditions, we observed a diagnostic yield of 17%, with a similar yield detected in cancer cases compared to non-cancer cases [22% vs. 14%]. The overall diagnostic yield is comparable to the yield of ES previously detected in an adult population[12] and as anticipated, lower than the yield reported in paediatrics[24,25].

Posey et al. conducted a retrospective study of 486 adults with heterogeneous presentations who had undergone ES[12]. A molecular diagnosis was identified in 85 patients, giving a yield of 17%, comparable to our results. It is important to note that there are some *methodological* and *population* distinctions between our study and Posey et al. Unlike our sample, ES was performed on the proband only for the entire cohort in Posey et al. Additionally, more than half of their participants (52%) were between the ages of 18 and 30 years. Whereas in our study, less than a third of our participants (13/47 or 28%) were between the ages of 18 and 35 and more than half (25 or 53%) were over 45. Another key distinction is in the distribution of clinical presentations of the referrals. While cancer referrals were rare (47/486) in Posey et al., 18/47 or 38% of our participants were referred for a cancer history. With regards to the non-cancer referrals, our population was mainly composed of patients with connective tissue/cardiovascular or neurological abnormalities, whereas abnormalities of the nervous system, musculature, and skeletal system were the most frequent phenotype classes in Posey et al. In our sample, diagnostic yield was highest for cerebrovascular abnormalities, cancer, and neurological phenotypes (1/2 or 50%, 4/18 or 22%, and 2/9 or 22%, respectively). In Posey et al., the highest yield was reported in patients with neurodevelopmental abnormalities (28%, 53/191) whereas the yield for cancer was less than 5%.

With regards to hereditary cancers, gene panels now offered routinely in Ontario may render ES less useful in this population. For example, *MSH6*, *POLE* and *ATM* are typically included in comprehensive cancer panels, and therefore the pathogenic variants detected in our study would likely be identified by standard of care testing if the families were to undergo investigations today. However, these families either previously tested negative for standard of care testing or were not eligible for testing at the time of evaluation. ES was able to fill a diagnostic gap. For example, family 21 with the *MSH6* variant would not have met criteria for Lynch syndrome testing at the time based on family history alone[19].

Currently, genetic testing for most adult patients with a suspicion of a hereditary condition consists of phenotype-driven gene panels. Panels have been established to be cost-effective approaches in various clinical settings, including hereditary cancers and epilepsy, among others[26–28]. However, many patients undergoing investigations remain undiagnosed. All the cases with positive results in our study would not have been identified by standard genetic testing available in Toronto at the time of analysis.

The decline in ES/GS costs in combination with the improvements in quality, accuracy and uniformity in coverage make a strong case for the transition from panels to ES and eventually GS. However, the large-scale adoption for routine clinical testing would have to consider diagnostic yield, turnaround time and the technical limitations of each test. In general, ES is cheaper, requires less analysis and comes with a shorter turnaround time than GS. However, ES is limited in its ability to detect large insertions and deletions, deep intronic mutations and trinucleotide repeat expansions. For these reasons, GS is expected to have higher diagnostic yield; [29] however, initial data suggests that increase may be modest[29]. Whether GS will indeed have a higher yield and be cost-effective remains to be established. These questions deserve further consideration.

Approximately half of the probands had VUS identified. Over time, improvements in our understanding of human disease genes and pathogenicity may lead to reclassification of these variants. There is growing literature suggesting that reanalysis may enhance the diagnostic yield of ES, lead to management changes for patients and that it may be cost-effective. However, the process requires significant resource investment on the part of the laboratory professionals and clinicians, who may have to recontact patients in some cases[30]. Reanalysis and recontact of patients will need to be balanced with other clinical demands and priorities. Furthermore, improvements in conveying phenotype information from the clinic to the diagnostic laboratory, in addition to the application of data sharing modalities, may further increase the yield.

Overall, we anticipate these results will have direct medical consequences for patients and their relatives. In addition to providing a molecular diagnosis and triggering medical management changes, results can inform reproductive risk for relatives. Thus, this study provides evidence for the clinical utility of ES and GS for adults and therefore offers support for the use of ES or GS in this population.

SF from ES provide an opportunity to inform disease prevention for patients and their relatives, preventing significant morbidity. All of the SF identified in our study could have

implications for the probands and relatives (e.g. family planning). More than one fifth of our participants (10/47) had a SF, with 6.4% having a medically actionable result, as defined by ACMG[18]. The frequency of actionable findings is consistent with the literature, where the frequency of actionable variants is in the range of 1-4%. Notably, SF were not actively sought for most of the families in our study- in which case, the frequency may have differed. Across the literature, the frequency of SF depends largely on the context, population and list of genes used, with frequencies in the lower end of the range identified when only the ACMG list is used[31–34].

A major limitation in this study was sample size and clinical heterogeneity. This limited our ability to determine the diagnostic yield by phenotype. A larger cohort, especially of non-cancer patients is needed to further evaluate yield across different disease areas and to better understand the clinical utility of ES or GS for adults and guide recommendations for eligibility.

ES and GS were also offered late in the diagnostic odyssey; most patients had undergone multiple investigations at the time of referral, including extensive genetic and biochemical testing. Therefore, the yield of GS and ES may indeed be higher and potentially more cost-effective if offered earlier in the diagnostic trajectory for adult patients. In addition, bias of ascertainment is likely to have factored in to the overall yield because patients were recruited at the discretion of the referring physician; if the patient had prior clinical ES testing requests rejected from the MOHLTC and if exhaustive testing was uninformative.

CONCLUSION

To our knowledge, this is one of the first studies investigating ES and GS in a cohort of adult patients with heterogeneous indications in Canada. From our early experience, ES or GS for adults was able to provide a molecular diagnosis for a proportion of patients, albeit less than is observed in paediatric populations. However, in many cases, the results ended a long and expensive diagnostic odyssey. In summary, ES or GS in this cohort of adults with suspected hereditary conditions led to an increased diagnostic yield over standard-of-care genetic testing and may support widespread adoption of this technology. Future studies should also focus on quantifying costs and assess the cost-effectiveness of ES and GS in adult populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1:
Distribution of primary results by phenotype

Table 1:

Clinical characteristics of probands

Characteristic	No. of probands (%)
Sex	
Male	11 (23%)
Female	36 (77%)
Age at enrollment	
18-25	1 (2.1%)
26-35	12 (26%)
36-44	9 (19%)
>45	25 (53%)
Main presentation	
Cancer	18 (38%)
Neurological	9 (19%)
Connective tissue	6 (13%)
Cardiovascular	3 (6.4%)
Musculoskeletal	3 (6.4%)
Cerebrovascular	2 (4.3%)
Ophthalmological	1 (2.1%)
Renal	1 (2.1%)
Multiple congenital anomalies and gastrointestinal issues	1 (2.1%)
Metabolic	1 (2.1%)
Endocrinological	1 (2.1%)
Immunological	1 (2.1%)
Type of test	
Exome	39 (83%)
Genome	8 (17%)
Result	
Positive	8 (17%)
Inconclusive	24 (51%)

	10 (21%)	Secondary
15 (32%)	Negative	
No. of probands		Characteristic

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Table 2:
Summary of cases with pathogenic or likely pathogenic variants consistent with phenotype

ID	Test indication	Sex	Age*	Gene; transcript	Variant (cDNA, protein)	Zygosity	Relatives #T #P	ACMG class	OMIM phenotypes
004	Small bowel cancer; ampullary cancer	F	69	<i>MSH6</i> (NM_000179)	c.3739_3742delACTC, (p.His1248Thrfs*4)	Het	1 (a) 1 (a)	P	Hereditary nonpolyposis colorectal cancer, type 5 (AD); familial endometrial cancer; mismatch repair cancer syndrome (AR)
007	Colorectal cancer, mixed polyposis	F	48	<i>POLE</i> (NM_006231)	c.1372T>A, (p.Tyr458Asn)	Het	1 (a) 1 (a)	LP	Susceptibility to colorectal cancer type 12 (AD); IMAGE-1 syndrome (AR); FLS syndrome (AR)
014	Breast cancer	F	41	<i>ATM</i> (NM_000051)	c.1065+1G>T	Het	- -	LP	Ataxia-telangiectasia (AR); lymphoma, B-cell non-Hodgkin, somatic; lymphoma, mantle cell, somatic; T-cell prolymphocytic leukemia, somatic; susceptibility to breast cancer (AD, Smu)
021	Breast cancer (MSS and IHC intact)	F	36	<i>MSH6</i> (NM_000179)	c.3959_3962del, (p. Ala1320Glu fs*6)	Het	- -	P	Hereditary nonpolyposis colorectal cancer, type 5 (AD); familial endometrial cancer; mismatch repair cancer syndrome (AR)
031	Developmental delay, seizures, microcephaly	F	28	<i>KCNQ2</i> (NM_172107)	c.1687G>A, (p.Asp563Asn)	Het	1 0	P	Early infantile epileptic encephalopathy type 7 (AD); myokymia (AD); benign neonatal seizures type 1 (AD)
039	Periodontal EDS	F	69	<i>C1S</i> (NM_001734)	c.949_951delGTG, (p.Val317del)	Het	1 (a) 1 (a)	P	Ehlers-Danlos syndrome periodontal type 2 (AD); C1s deficiency
057	Clinical diagnosis of Charcot Marie Tooth disease type 2	F	55	<i>COL6A2</i> (NM_001849.3)	c.2417 G>A, (p.Cys806Tyr) c.1273_1280 dupCGCAGGGG (p. Asp428Alafs*120)	Het Het	- -	VUS LP	Congenital myosclerosis(AR); Ullrich congenital muscular dystrophy (AR/AD); Bethlem myopathy 1 (AR/AD)
084	Early-onset strokes	M	29	<i>SCN5A</i> (NM_198056)	c.3823G>A, (p.Asp1275Asn)	Het	1 (a) 1 (a)	P	Familial atrial fibrillation type 10 (AD); Brugada syndrome 1 (AD); dilated cardiomyopathy 1E (AD); nonprogressive heart block, type 1A (AD); Long QT syndrome-3 (AD); sick sinus syndrome 1(AR); familial ventricular fibrillation type 1; susceptibility to sudden infant death syndrome (AR)

* Age at initial referral.

a, affected; p, positive; T, tested

Summary of secondary findings

Table 3:

ID	Test indication	Sex	Gene; transcript	Variant (cDNA, protein)	Zygosity	Relatives #T #P	ACMG class	OMIM phenotypes
019	Achalasia, HL, corneal/retinal anomalies, delayed growth/puberty, gastric dysmotility, dysmorphism	F	<i>APOB</i> (NM_000384)	c.1315C>T; (p.Arg439*)	Het	2 1	P	Familial hypercholesterolemia 2 (AD); hypobetalipoproteinemia (AR)
029	Carotid artery aneurysm; early onset osteoarthritis; scoliosis; widened scars; soft and hyperextensible skin; breast cysts	F	<i>COL7A1</i> (NM_000094)	c.6501G>A; (p.Pro2167Pro)	Het	1 1	LP	EBD inversa (AR); EBD, Bart type (AD); EBD, localisata variant; epidermolysis bullosa dystrophica (AR/AD); epidermolysis bullosa pruriginosa (AR/AD); epidermolysis bullosa, pretibial (AD/AR); toenail dystrophy, isolated (AD); transient bullous of the newborn (AR/AD)
031	Developmental delay, seizures, microcephaly	F	<i>LDLR</i> (NM_000527)	c.1085A>C; (p.Asp362Ala)	Het	1 0	LP	Familial hypercholesterolemia 1 (AD); LDL cholesterol level QTL2 (AD)
037	Polyps; epithelioid inflammatory myofibroblastic sarcoma	F	<i>GJB2</i> (NM_004004)	c.101T>C; (p.Met34Thr)	Het	1 0	P(RP)	Bart-Pumphrey syndrome (AD); Autosomal dominant deafness 3A (AD); Autosomal recessive deafness 1A (AR/DD); Hystrix-like ichthyosis with deafness (AD); Keratitis-ichthyosis-deafness syndrome (AD); Keratoderma, palmoplantar, with deafness (AD); Vohwinkel syndrome (AD)
			<i>HADHA</i> (NM_000182)	c.1528G>C; (p.Glu510Gln)	Het	1 0	P	Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (AR); Acute fatty liver of pregnancy (AR); Maternal HELLP syndrome of pregnancy (AR); Trifunctional protein deficiency (AR)
			<i>HFE</i> (NM_000410)	c.845G>A; (p.Cys282Tyr)	Het	1 1	P (RP)	Hereditary haemochromatosis (AR); Susceptibility to Alzheimer disease (AD); Susceptibility to Porphyria cutanea tarda (AR/AD); Susceptibility to porphyria variegata (AD)
067	Clinical diagnosis of Albright hereditary osteodystrophy; pseudopseudohypoparathyroidism; tachycardia; repetitive movements; developmental delay; hypotonic in neonatal period	F	<i>FANCA</i> (NM_000135)	c.3788_3790 (p.Phe1263del)	Het	1 1	LP	Fanconi anemia, complementation group A (AR)
			<i>CNGB3</i> (NM_019098)	c.1148del (p.Thr383Ilefs*13)	Het	1 1	P	Achromatopsia 3 (AR)
073	Undifferentiated connective tissue disorder or possibly fibromuscular dysplasia; query LDS or vascular EDS	F	<i>CEP164</i> (NM_001271933)	c.381dup (p.Lys128Glnfs*77)	Het	- -	P	Nephronophthisis 15 (AR)
			<i>FLG</i> (NM_002016)	c.7487del (p.Thr2496Asnfs*104)	Het		P	Ichthyosis vulgaris (AD); Susceptibility to atopic dermatitis 2)
077	Pre-menopausal osteoporosis with compression fractures; pulmonary issues;	F	<i>UCP3</i> (NM_003356)	c.427C>T; (p.Arg143*)	Het	- -	LP	Severe obesity with type II diabetes (AR/AD, Multifactorial)

ID	Test indication	Sex	Gene; transcript	Variant (cDNA, protein)	Zygosity	Relatives	ACMG class	OMIM phenotypes			
<hr/>											
severe progressive scoliosis; AVM; unilateral HL											
079	Adult onset progressive ataxia; tachycardia; difficulty maintaining balance; shortness of breath, chest pain; sweating; blurred and double vision	F	PEX14 (NM_004565)	c.298+1G>C	Het	-	-	P	Peroxisome biogenesis disorder 13A, Zellweger syndrome (AR)		
				<hr/>							
081	Neurogenic bladder; fibromyalgia; Crohn's; IBS; Celiac	F	MUTYH (NM_001128425)	c.536A>G, (p.Tyr179Cys)	Het	-	-	P	Adenomas, multiple colorectal (AR), somatic gastric cancer (SMu)		
				c.1187G>A, (p.Gly396Asp)	Het			P			
			ALDOB (NM_000035)	c.178C>T (p.Arg60*)	Het			LP	Fructose intolerance (AR)		
				<hr/>							
084	Early onset strokes	M	GJB2 (NM_004004)	c.35delG, (p.Gly12Valfs*2)	Het			P	Bart-Pumphrey syndrome (AD), Autosomal dominant deafness 3A (AD); Autosomal recessive deafness 1A (AR), Hystrix-like ichthyosis with deafness (AD), Keratitis-ichthyosis-deafness syndrome (AD), Keratoderma, palmoplantar, with deafness (AD), Vohwinkel syndrome (AD)		
				<hr/>							
			SLC26A4 (NM_000441)	c.626G>T, (p.Gly209Val)	Het			P	Autosomal recessive deafness 4, with enlarged vestibular aqueduct (AR); Pendred syndrome (AR)		
				<hr/>							
084	Early onset strokes	M	ERCC3 (NM_000122)	c.325C>T (p.Arg109*)	Het	1	1	LP	Trichothiodystrophy 2, photosensitive (AR); xeroderma pigmentosum, group B (AR)		
				c.901C>T (p.Arg301*)	Het	1	1	LP	Fanconi-Bickel syndrome (AR); diabetes mellitus, noninsulin-dependent (AD)		

a, affected; AVM, arteriovenous malformation; EBD, Epidermolysis bullosa dystrophica; HL, hearing loss; IBS, irritable bowel syndrome; LDL, Low-density lipoprotein; LDS, Loeys—Dietz syndrome; p, positive; RP, reduced penetrance; T, tested.