Genomic sequencing identifies secondary findings in a cohort of parent study participants

Short running title: Secondary and Incidental Findings from WES/WGS

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ABSTRACT

PURPOSE: As part of a CSER project, clinically relevant secondary variants were identified in parents enrolled with their child with developmental delay and intellectual disability. **METHODS:** Exome/genome sequencing and analysis of 789 'unaffected' parents was performed. **RESULTS:** Pathogenic/likely pathogenic variants expected to elevate disease risk were identified in 21 genes within 25 individuals (~3.2%). Eleven (1.4%) of these participants harbored variation in a gene defined as clinically actionable by the ACMG. Of the 25 individuals, five carried a variant consistent with a previous clinical diagnosis; 13 were consistent with observed symptoms and/or family history; seven reported no symptoms or family history of disease related to the variant. A limited carrier status search was performed for *HBB*, *HEXA* and *CFTR*, yielding 15 variants in 48 (6.1%) parents. Recessive OMIM disease genes were analyzed on a mate-pair basis; cases in which both parents were carriers for variants in the same gene were identified, leading to one finding (*ATP7B*). In total, we identified secondary variants in 71 of the 789 enrolled parents (9.0%). **CONCLUSION:** We provide an overview of the rates and types of clinically relevant secondary findings and may be useful in the design and implementation of research and clinical sequencing efforts.

Key words: Secondary findings; genomic sequencing; disease risk; CSER; ACMG

INTRODUCTION

Whole exome and genome sequencing (WES/WGS) have proven to be powerful tests for identifying clinically relevant variants, including those unrelated to the primary indication for testing. The existence of secondary and incidental findings has catalyzed debate regarding the types of findings that should be sought by sequencing labs, the circumstances in which certain types of variants should or should not be returned, and the necessary extent of patient consent, education, and genetic counseling. The American College of Medical Genetics and Genomics (ACMG) have released recommendations about the interpretation of genes that it considers to be clinically actionable, including those that pose high risk of cancer and heart disease. The ACMG suggests that these be sought and provided to patients that are willing to receive such results [1,2]. Recommendations made by the ACMG related to the use of specific gene lists and the approach for returning secondary findings were intended to be used in clinical versus research contexts. However, to assess the clinical impact of these recommendations and establish realistic expectations as to the rates and types of secondary findings, it is important to examine them in translational research contexts.

Through a translational study that was part of the Clinical Sequencing Exploratory Research (CSER) Consortium [3], we assessed the utility of WES/WGS to identify genetic causes of developmental delay, intellectual disability (DD/ID), and related congenital anomalies. To date, we have sequenced affected probands from 455 families, and have identified DD/ID-related pathogenic/likely pathogenic (P/LP) variants in 29% [4]. As our DD/ID study focuses on trios, i.e., DD/ID-affected children and both biological parents,

we have the ability to assess secondary findings – in this case unrelated to DD/ID – in a sizable cohort of adults.

We and others have investigated the impact of returning secondary findings while considering bioethical implications, patient-reported health history, and psychosocial outcomes [3,5-7], most of which are not discussed in this report. We focus on the rates and types of secondary findings in our cohort and how they relate to reported symptoms or family history. We use the term 'secondary findings' throughout the manuscript to describe variation identified via proactive searching [8] among four distinct categories: carrier status information in *CFTR, HEXA,* and *HBB;* OMIM genes in which both parents harbored a potentially pathogenic variant for a recessive disorder; genes included on the ACMG list as being associated with clinically actionable information [1,2]; and previously reported pathogenic/likely pathogenic (P/LP) variation in a gene not on the ACMG gene list. Beyond general overviews, we also discuss specific case studies that provide details related to clinical referral after return of results and patient follow-up.

We report a strategy and framework for identifying secondary genetic variation in a way that is analytically feasible and manageable within a clinical lab setting. We also demonstrate the utility of dissemination of such findings in a cohort of parent study participants. Our experiences and data suggest the value of genomic sequencing in a clinical setting not only for disease patients, but also for those not currently exhibiting an overt disease phenotype.

MATERIALS AND METHODS

Study participant population

There was no public recruitment for this study. Parents of children participating in the CSER project at the HudsonAlpha Institute for Biotechnology [4] were enrolled at North Alabama Children's Specialists in Huntsville, AL. Consent was obtained for study participation and publication of data generated by this study. Review boards at Western Institutional Review Board (20130675) and the University of Alabama at Birmingham (X130201001) approved and monitored this study.

Patient consent and preferences

We developed the Preferences Instrument for Genomic Secondary Results (PIGSR)[5] to elicit parents' preferences for receiving categories of secondary results. This instrument divides secondary findings into 13 distinct disease categories (Figure 1). In order to study the effect of timing on expressed preferences, families were randomized into two groups: group one recorded their preferences to receive genomic results at the time of enrollment and group two recorded their preferences immediately prior to their return of results visit. Individuals that were randomized into group one were given the option to change their preferences at the return of results appointment.

Whole exome and genome sequencing

Blood samples were sent for sequencing at the HudsonAlpha Genomic Services Laboratory (http://gsl.hudsonalpha.org). Genomic DNA was isolated from peripheral blood and WES (Nimblegen v3) or WGS was conducted to a mean depth of 71X or 35X,

respectively, with >80% of bases covered at 20X. WES was conducted on Illumina HiSeq 2000 or 2500 platforms; WGS was performed using Illumina HiSeq X. In total, WES was performed for 240 parents and WGS for 549 parents.

Further details regarding read alignment, variant calling, variant validation, and return of results to participants can be found in our previous report [4].

Variant classification

Through a combination of automated annotation, filtration, and prioritization and manual curation, variants were analyzed and placed into one of five categories including benign, likely benign, uncertain significance (VUS), likely pathogenic (LP), and pathogenic (P). Only variants found to be P/LP were eligible for return as a secondary finding. Our study began prior to publication of the formal classification system proposed by the ACMG [9]. However, for this publication, we have updated pathogenicity scores and assigned ACMG evidence codes to all returned variants. Key annotations (e.g., allele frequencies, PubMed identifiers, and computational inferences of variant effect) used to support the disease relevance of each variant are supplied in Table S1.

Data sharing

All variants identified in parent participants have been shared in ClinVar [10] under the submitter study name: CSER-HudsonAlpha. Sequencing data for the participants who consented to such sharing have been submitted to dbGaP (phs001089.v2.p1) [11].

RESULTS

Demographics of study population

Of the 455 enrolled families, 424 included at least one parent that was sequenced, and both biological parents were sequenced in 365 families. Demographics for the 789 biological parents enrolled are reported in Table 1. Overall, the study population had a mean age of 41 years and consisted of 422 females and 367 males. 80% are self-reported to be of European ancestry ("White"), 9% self-reported as African-American ("Black"), and 8% reported "Other" or more than one ancestry ("Multiracial"). Four percent self-reported as Hispanic or Latino. Over 25% of participating parents had a high school diploma or less, while 34% reported having some college education (Table 1).

Patient Preferences

In addition to returning secondary findings to individuals in hopes of positively impacting their long-term health, another goal of our study was to understand patient preferences as they relate to receipt of secondary findings in various disease-defined categories [5]. 85% of parents requested all secondary findings, while 1.6% declined to receive any secondary findings. The most frequently requested sub-category was risk for gender-specific cancers (breast and ovarian cancer in women; testicular and prostate cancer in men) (n=584; 96.1%). The least frequently requested result was risk for developing obesity (n=542; 89.2%) (Figure 1).

Carrier status findings

We conducted a limited carrier screen for variants that are relevant to cystic fibrosis (*CFTR*, MIM:219700), beta-thalassemia (*HBB*, MIM:613985), sickle cell disease (*HBB*, MIM:603903), and Tay-Sachs disease (*HEXA*, MIM:272800), which are among the most common Mendelian diseases (average carrier risk is 1/40) [12-14]. Eight variants in *CFTR* were identified in 35 individuals (4.4% of total parent population), four *HEXA* variants were found in five individuals (0.6%), and three *HBB* variants were found in eight individuals (1%) (Table 2; Table S2). Additionally, we searched for cases in which mate pairs were both carriers for variants in a gene associated with a recessive disorder. This analysis led to returnable findings for one of the 365 parental pairs, in which both parents harbored variation in *ATP7B* associated with autosomal recessive Wilson disease (MIM: 277900) (Table S2).

Secondary variation in individuals with a previous clinical diagnosis

P/LP variants were found in five individuals with a previous clinical diagnosis noted in their personal medical history. However, the specific genetic cause for their condition was unknown. A 35-year-old female individual who had previously been clinically diagnosed with spherocytosis (MIM: 612653) was found to harbor a heterozygous missense variant in *SLC4A1*. This individual's father and two of her children were also noted to have spherocytosis though these individuals were not enrolled and sequenced in our study (Table 3; Table S1). In another case, we identified three missense variants (two of which are likely in *cis*) in *SLC22A5* in a 37-year-old female who had been

clinically diagnosed with autosomal recessive systemic primary carnitine deficiency (MIM: 212140). The third case was in a 36-year-old female with polycystic kidney disease (MIM: 613095) in whom a variant affecting a canonical splice donor site (D1) of *PKD2* was identified (Table 3; Table S1). This individual reported strong family history of the disease, with her brother, mother, two nieces, maternal uncle, maternal aunt, and maternal grandmother all reportedly exhibiting symptoms associated with polycystic kidney disease.

We also identified secondary genetic variation affecting cardiac genes in two individuals with a previous clinical diagnosis and that had family history of cardiovascular phenotypes. One 30-year-old female individual reported to have experienced cardiomyopathy postpartum, had a paternal family history of arrhythmia, and her paternal uncle suffered two heart attacks prior to age 40. She was found to harbor a frameshift variant in *DSG2,* a gene associated with arrhythmogenic right ventricular dysplasia and dilated cardiomyopathy (MIM: 610193, MIM: 612877). In a second case, we identified missense variation in *ANK2,* a gene associated with ankyrin-B-related cardiac arrhythmia and long QT syndrome (MIM: 600919), in a 52-year-old male with a clinical diagnosis of arrhythmia. This individual also reported that his father passed from ischemic heart disease. While we could not obtain samples from the affected relatives of either of these two individuals, we find it probable that the variants we identified explain at least some of the disease history in these families. Finally, six of the eight parents that were identified as carriers for variation in *HBB* had clinical notes indicating

they exhibited sickle cell or thalassemia trait (Table 2; Table S2).

Secondary genetic variation in symptomatic individuals and/or those that have family history of disease

We found secondary variants in 13 individuals who had no previous genetic testing despite the manifestation of disease and/or significant family history (Table 3; Table S1). Given information provided at the time of enrollment, six of these cases (*CLCN1, MFN2, BRCA1, BRCA2, BARD1, PMS2;* Table 3) would have met criteria to justify genetic consultation and testing via standard clinical recommendations [15,16]. Given additional phenotypic information acquired at return of results, two additional cases (*SCN4A*, *HARS;* Table 3) would have met such criteria [17,18]. These eight cases are described below.

A heterozygous missense variant in *CLCN1* was identified in a 29 year-old female who reported leg cramps and restless legs in childhood and still presents with occasional cramps. Variation in *CLCN1* is associated with myotonia congenita (MIM:160800), which is characterized by muscle stiffness and inability to relax muscles after voluntary contraction, phenotypes that are exacerbated by colder temperatures. Her mother was diagnosed with myotonia congenita when she was 10 years old and her maternal grandfather had a muscle biopsy performed in his 30s due to symptoms, including "stiffness" that occurred "especially in cold". In a separate case, a heterozygous missense variant in *MFN2,* a gene associated with axonal Charcot-Marie-Tooth (CMT) Disease type 2A2A (MIM: 609260), was identified in a 35-year-old female who reported

experiencing balance difficulties and weakness since childhood that has progressed to severe cramping, myalgia, and numbness, most prominently in her legs and feet. These symptoms are exacerbated by exercise and relieved by rest. Her family history is notable for neuromuscular disorder, with similar symptoms present in her brother, father, paternal grandmother, and paternal aunt. Though she has not been formally evaluated by a clinician for these symptoms, her brother has been clinically diagnosed with CMT.

We also identified cancer risk variants in a number of individuals who report a history of disease within their family. In one case, we identified a frameshift variant in *BRCA1* (familial breast/ovarian cancer, MIM: 604370) in a 40-year-old male whose mother was diagnosed with breast cancer in her thirties. In another case, variation in a canonical acceptor splice site of *BRCA2* (familial breast/ovarian cancer, MIM: 612555) was identified in a 38-year-old female who had a history of breast cancer on both sides of the family: a paternal grandmother diagnosed at an unknown age, as well as a maternal grandfather diagnosed with bilateral breast cancer at 60 years of age (Figure 2A); transmission was not determined because none of these relatives were sequenced as part of this study. A frameshift variant in *BARD1* (MIM:114480) was identified in a 33-year-old female whose grandmother had bladder, lung, and peritoneal cancer as well as a great-grandmother diagnosed with breast cancer in her fifties. Additionally, a frameshift variant in *PMS2* (hereditary nonpolyposis colorectal cancer; MIM: 614337) was identified in a 43-year-old male with family history of colon cancer: the individual's father diagnosed in his sixties and his paternal aunt in her forties. This individual also

had a paternal aunt and grandmother who were diagnosed with breast cancer in their sixties and fifties, respectively (Figure 2B). After receipt of this finding, the study participant followed-up with a colonoscopy, which was found to be negative, although he reports that he will continue periodic assessment.

Secondary variants were also found in two symptomatic individuals who were not aware that their symptoms were unusual and thus never had a clinical or genetic evaluation prior to study enrollment (Table 3). In one case, a 28-year-old female was found to harbor a pathogenic missense variant in *SCN4A*, implicated in hyperkalemic periodic paralysis and paramyotonia congenita (MIMs:170500; 168300), two related neuromuscular disorders characterized by intermittent muscle weakness and/or myotonia. At study enrollment, she did not self-report any symptoms relevant to neuromuscular phenotypes. However, after receiving the *SCN4A* result, she reported a history of discomfort during exercise that began at approximately five years of age. She noted that her legs become painfully stiff during exercise and that her throat “locks up” after drinking cold liquids. Additionally, she reported that her eyelids “stick” and “become heavy” throughout the day. She reports that her mother displays similar phenotypes. This individual has plans to follow-up with a neurologist. In a second case, a 41-year-old male was found to harbor pathogenic variation in *HARS,* a gene implicated in Charcot-Marie-Tooth disease (MIM:616625) and is characterized by gait difficulties and distal sensory impairment caused by peripheral neuropathy. At return of results this individual indicated that he had always been clumsy and that he was discharged from

military boot camp due to his inability to march in formation. He also stated that he often wears out shoes because of feet shuffling.

Identification of genetic risk factors in individuals who are currently asymptomatic and have no family history of disease

We also identified genetic risk factors in individuals who are currently asymptomatic and have no family history of variant-associated disease (Table 3). Two unrelated individuals, a 52-year-old female and a 50-year-old male, were found to harbor variation in *SCN5A* (Long QT syndrome, MIM:603830) and *DSG2* (dilated cardiomyopathy, MIM:618277), respectively. Screening and follow-up with a cardiologist was recommended for both of these individuals. A 31-year-old male study participant was found to harbor a missense variant in *ACTN1*, which is associated with a bleeding disorder (MIM:615193). This individual was referred to hematology for blood count and functional platelet studies. Finally, P/LP variants were identified in four participants with no family history of cancer, including one in each of *MSH2*, *BARD1*, *BRCA2*, and *RET* (Table 3; Table S1). Notably, a missense variant (C609Y) in *RET* was identified in a 52-year-old male participant who reported no personal or family history of *RET*-associated cancer. This variant is associated with multiple endocrine neoplasia type 2A (MIM:171400), medullary thyroid carcinoma (MTC; MIM:155240), and/or Hirschsprung's disease (MIM: 142623). The C609Y variant has been observed in numerous affected patients and families and has been designated as level B risk from the American Thyroid Association (level D is highest risk), with expected age of onset of less than 30 years [19,20].

Information related to prophylactic thyroidectomy varies as some reports state that it should occur as early as age five [20,21], while others suggest thyroidectomy be performed at 10-15 years of age [22]. More recent studies indicate individuals harboring *RET* C609Y may have onset of MTC as late as age 50, much later than previously noted [23]. Following return of this secondary finding, the father harboring *RET* C609Y and his family were referred to cancer genetic counseling. Furthermore, his 11 year-old daughter, (not enrolled into our study as she did not exhibit DD/ID), was reported to have Hirschsprung's disease and is likely to have *RET* C609Y transmitted from her father. It was suggested that the Hirschsprung's-affected daughter be tested for the same *RET* variant, and that both the father and his daughter begin tumor screening.

DISCUSSION

The ACMG estimated that WES/WGS has the potential to reveal secondary findings in genes relevant to variety of actionable phenotypes like heart arrhythmias and cancers, for ~1% of sequenced individuals [1,2]. Our rate of identification for variation harbored within ACMG genes is approximately 1.4%, which is consistent with the 1%-3.4% reported by other research and clinical laboratories [9,24,25]. The overall identification of secondary findings in 9% of our cohort, however, varies considerably from that reported by other groups due in large part to study design, especially, but not exclusively, the extent to which carrier status is analyzed. Our study assessed carrier status for only three genes, *HBB, HEXA*, and *CFTR*, and led to the identification of P/LP variants in ~6.1% of enrolled parent participants. Elimination of variation from these genes would reduce

our total secondary finding yield by approximately two-thirds (3.2% non-carrier); in contrast, had we assessed all of the genes known to associate with recessive disease [26], the overall secondary finding yield would increase dramatically, as would the burden of analysis [27,28].

Patient preferences

The question of whether patients and research participants need to be offered choices about receiving secondary findings has been debated in recent years, especially following the release of the 2013 ACMG recommendations on managing secondary findings. A number of studies have documented that most participants want to receive most secondary findings, and in most cases, want all results that could be provided. This trend is consistent between studies asking this question as a hypothetical [29-32], as well as those asking to inform actual return of results [33-36]. Consistent with these previous studies, the vast majority of parents participating in our study chose to receive all 13 categories of potential secondary results. However, a minor but substantial fraction of participants (15.2%) declined at least one category of secondary results; 1.6% declined all such results. One of the secondary findings listed in Table 3 was not returned because the parent had declined that category of secondary findings (obesity, MIM: 601665; *MC4R*).

Challenges associated with variant interpretation

One of the most challenging tasks when analyzing secondary findings is interpretation of

genetic variation, particularly for variants that have not been previously described in scientific literature or in clinical genetic databases. Moreover, clinical variant databases can contain rare variation described as disease causal even when the variation is benign [37]. Interpretation is made even more challenging when an individual harbors potential disease-associated variation but does not present with the associated phenotype or have a family history of the disease. Missense variants that have not been previously described are even more difficult to evaluate, even when the missense variation is known to lead to disease and are computationally predicted to be damaging. Detailed family histories were obtained when possible, and all variants were analyzed in light of known phenotypic information. That said, ACMG evidence codes were assigned and variants that were deemed to be P/LP were offered for return to parent participants regardless of any particular phenotype or family history.

Utility of secondary findings

The secondary genetic findings that we identified may be of great utility to the parent participants. For five individuals, we were able to confirm, and genetically refine, a previous clinical diagnosis (Table 3), including two individuals with cardiovascular phenotypes (*ANK2, DSG2*), and one individual each with polycystic kidney disease *(PKD2*), spherocytosis (*SLC4A1*), or carnitine deficiency (*SLC22A5*). Such information may prove useful for future clinical management and/or in discussions with family members, as four of these five individuals reported knowledge of similarly-affected relatives.

Secondary genetic findings were also identified in 13 individuals who reported strong family history or symptoms of a variant-relevant disease. As described in detail in the results section, it is evident that for a number of these cases, genetic counseling and testing could/should have been ordered clinically based on their presentation of symptoms and/or family history. Further, we identified secondary genetic variants in four individuals whom we believed to be at increased risk of disease, but report minimal family history of disease (two cases of *KCNQ1, MYBPC3, DDX41*). Through participation in our study, these individuals now understand their risk or cause of disease, and in some cases, the likely culprit of their family's disease history (Table 3). Finally, we identified secondary genetic variation in seven individuals who report neither symptoms nor family history of disease (*MSH2, RET, BARD1, BRCA2, ACTN1, SCN5A, DSG2*). Nevertheless, these study participants appear to be at increased risk of disease and have been referred to follow-up with an appropriate specialist (Table 3).

Challenges of returning unexpected variants to families

Many of the parents enrolled in this study have gone through a diagnostic odyssey, including previous genetic testing and physician consultations to identify the cause of their child's DD/ID. Individuals who carried P/LP secondary variants therefore required counseling and recommendations for clinical follow-up regarding their secondary findings, in addition to information regarding the care and well-being of their affected children. Returning genetic information relevant to a new or unexpected disease risk may be particularly problematic when no results are found relevant to the primary

indication for testing; in our study ~56% of the 71 parents that harbored one or more secondary findings (the second finding being carrier status) did not receive a primary result for their enrolled DD/ID-affected child. The lack of primary result may increase the shock value of a secondary finding. A parent may expect the conversation to revolve around their child's health but instead spends time discussing the meaning of their own genetic result. This fact highlights that the potential financial, emotional, and clinical implications of secondary findings should be clearly addressed in the informed consent discussion prior to sequencing so that families are sufficiently aware of all the possible outcomes of this type of testing.

Conclusions

Our study describes the identification and return of secondary variation to parents who received genomic sequencing in hopes of receiving a genetic diagnosis for their DD/ID-affected child. Although the return of secondary genetic variation has been a subject of considerable debate [38-40], a large majority of parent participants in this study opted to receive all identified secondary findings, regardless of disease category, suggesting that participants are generally open to receiving genetic information that may be relevant to their health. This study demonstrates the utility of returning secondary variants, as it may facilitate preventative screening for individuals who are genetically predisposed to cardiovascular disease and/or cancer. This information can also be useful to individuals who have been clinically diagnosed with a specific condition but for whom the causal genetic variant has been unknown. We have also shown that secondary genetic

information may lead to clinical diagnosis in individuals who have experienced symptoms related to a disorder not previously diagnosed. Some individuals also described significant family history that would have justified genetic evaluation and possible testing regardless of their participation in this study. Finally, our study describes a framework for identifying secondary genetic variation in a broad yet manageable, manner, including a limited yet productive carrier screen by focusing on common recessive diseases. The methods and results related to secondary variation identification may be of use to other research and clinical laboratories that are conducting genomic sequencing.

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Conflict of interest. The authors declare no conflicts of interests.

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Figure and Figure Legends

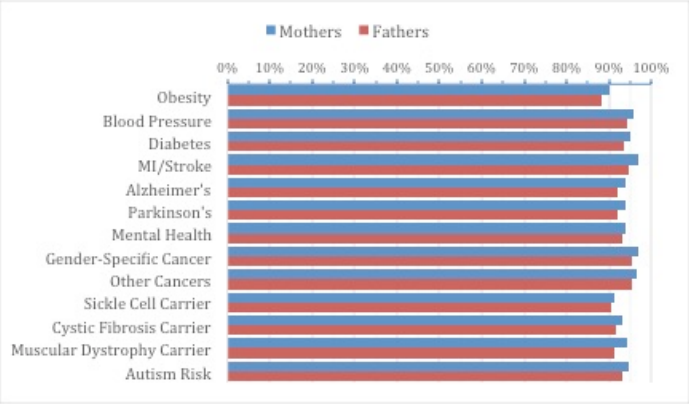

Figure 1. Participant preferences for receipt of secondary genetic findings. Participant preferences were assessed for return of genetic variation across a number of different disease categories. An overwhelmingly large majority (85%) of study participants chose to receive any identified secondary variant, regardless of disease association (n=789).

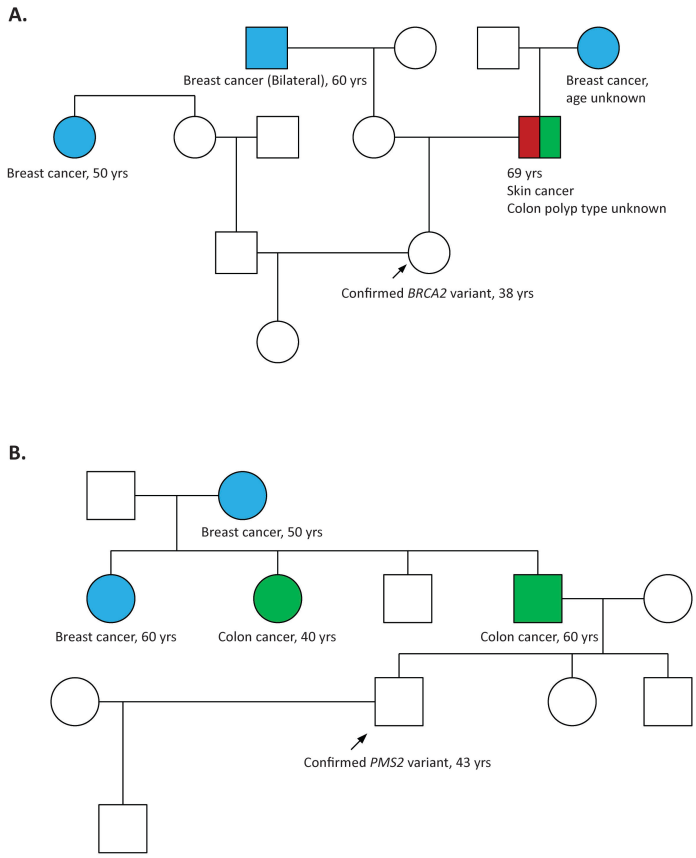

Figure 2. (A) Pedigree for parent participant harboring *PMS2* frameshift variant showing significant family history for colon cancer. (B) Pedigree for parent participant harboring *BRCA2* splice variant showing significant family history for breast cancer.

Table 1. Demographics of parent participants enrolled in the HudsonAlpha CSER project.

	Total, mean (SD) (n=789)	Males, mean (SD) (n=367)	Females, mean (SD) (n=422)
Age	40.95 (9.4)	42.63 (9.67)	39.49 (8.94)
Race *	**Total (% of total)**	**Total (% of males)**	**Total (% of females)**
White	635 (80.5%)	295 (80.4%)	340 (80.6%)
Black or African-American	57 (8.5%)	28 (7.6%)	30 (7.2%)
American Indian/Alaska Native	7 (0.9%)	6 (1.6%)	1 (0.2%)
Other/Multiracial	65 (8.2%)	30 (8.2%)	35 (8.3%)
No Answer	15 (1.9%)	8 (2.2%)	7 (1.7%)
Ethnicity *	**Total (% of total)**	**Total (% of males)**	**Total (% of females)**
Hispanic or Latino	32 (4.1%)	16 (4.4%)	16 (3.8%)
Not Hispanic or Latino	750 (95.1%)	349 (95.1%)	401 (95.0%)
No Answer	7 (0.9%)	2 (0.5%)	5 (1.2%)
Education	**Total (% of total)**	**Total (% of males)**	**Total (% of females)**
Less than High School Diploma	79 (10.0%)	44 (12.0%)	35 (8.3%)
High School Diploma/GED	122 (15.5%)	67 (18.3%)	55 (13.0%)
Some College	272 (34.5%)	113 (30.8%)	159 (37.7%)
Bachelor's Degree	197 (25.0%)	84 (22.9%)	113 (26.8%)
Graduate Degree	118 (15.0%)	58 (15.8%)	60 (14.2%)
No Answer	1 (0.1%)	1 (0.3%)	0 (0.0%)

*Self-reported

Table 2. Unique variants of carrier status in CFTR, HEXA, and HBB

Unique Variant Info	No. of individuals
CFTR (MIM:219700)	**4.4% of total population**
F508del	22
G685fs	3
D1152H	2
G551D	2
G542X	2
R117H	2
c.489+1G>T	1
F342Hfs	1
HEXA (MIM: 272800)	**0.6% of total population**
Y427Ifs	2
c.986+3A>G	1
c.459+5G>A	1
c.1073+1G>A	1
HBB (MIM: 603903; 613985)	**1% of total population**
E7V	6
E27K	1
G40X	1

Table 3. Secondary findings of enrolled parents segregated into "Clinically diagnosed with or without family history", "Notable family history and/or symptomatic", and "Currently asymptomatic with no family history".

Age (Male/ Female)	Gene	Variant Info	Associated Phenotype (MIM)	Notes
Clinically diagnosed with or without family history (0.6% total population)				
35- F	*SLC4A1*	V488M	Spherocytosis (612653)	Clinically diagnosed; Two daughters and father with spherocytosis
37- F	*SLC22A5*	A142S; T440M, R488H	Carnitine deficiency (212140)	Clinically diagnosed
36- F	*PKD2*	c.1319+1G>A	Polycystic kidney disease (613095)	Clinically diagnosed with PKD; mother, brother, 2 nieces, maternal aunt, uncle and grandmother with PKD
30-F	*DSG2*	V986Wfs	Cardiomyopathy (618277; 610193)	Clinically diagnosed with postpartum cardiomyopathy; Paternal family history of arrhythmia; paternal uncle with two heart attacks prior to age 40.
52-M	*ANK2*	E1458G	Cardiac arrhythmia, long QT syndrome (600919)	Clinically diagnosed with hypertrophic cardiomyopathy and arrhythmia; father died with ischemic heart disease
Notable family history and/or symptomatic (1.6% of total population)				
29- F	*CLCN1*	F413C	Myotonia congenita (160800)	Leg cramps and restless legs in childhood, still occasionally has cramps; Mother diagnosed with myotonia congenita, 10 years; Maternal grandfather with a muscle biopsy performed in 30s and "stiffness" especially in cold, 30s
35- F	*MFN2*	W740S	Charcot-Marie-Tooth disease (609260)	History of muscle wasting in back, lower extremities; brother clinically diagnosed with CMT, 30s; multiple family members affected with "unspecified muscle disorder"
40- M	*BRCA1*	G1756fs	Familial breast and ovarian cancer (604370)	Mother with breast cancer, 30s
38- F	*BRCA2*	c.8488-1G>A	Familial breast and ovarian cancer (612555)	Maternal grandfather with bilateral breast cancer, 60s; Paternal grandmother with breast cancer, age unknown
33- F	*BARD1*	E652fs	Breast cancer susceptibility (114480)	Maternal great-grandmother with breast cancer, 50s; Maternal grandmother had bladder, lung, and peritoneal cancer, age unknown
43- M	*PMS2*	P246fs	Hereditary nonpolyposis colorectal cancer (614337)	Father (60s) and paternal aunt (40s) had colon cancer, 60s; Paternal aunt (60s) and grandmother (50s) with breast cancer
28- F	*SCN4A*[a]	T1313M	Paramyotonia congenita (168300)	At return of results, indicated that she had muscle stiffness but always thought she was "easily fatigued" and had "low stamina"
41- M	*HARS*[a]	R137Q	Charcot-Marie-Tooth, axonal (616625)	At return of results, indicated that he had CMT-associated phenotypes. Always thought he was "just clumsy"
32- F	*KCNQ1*	R366W	Long QT syndrome (192500; 609621)	Father with coronary artery disease with triple by-pass, early 50s, paternal aunt with early-onset stroke, late 30s
47- M	*KCNQ1*	P7S	Cardiac arrhythmia; Long QT syndrome (192500; 609621)	Mother "fainted" and "hit the floor"-was told this impact prevented cardiac arrest

39- M	*MYBPC3*	E542Q	Hypertrophic cardiomyopathy (115197)	Leaky heart valve; Dad has pace maker and mom has leaky heart valve, 60s
30- M	*DDX41*	D140fs	Familial myeloproliferative neoplasm (616871)	Paternal cousin with lymphoma "unspecified", age unknown
37- F	*MC4R*[b]	C271Y	Obesity (601665)	Overweight
Currently asymptomatic with no family history (0.9% of total population)				
52- F	*SCN5A*	T1303M	Long QT syndrome (603830)	Recommended to have cardiovascular evaluation
50-M	*DSG2*	E1020fs	Dilated cardiomyopathy (618277)	Recommended to have cardiovascular evaluation
31-M	*ACTN1*	V105I	Bleeding disorder, platelet type, 15 (615193)	Recommended to have a complete blood count and functional platelet study
33- M	*MSH2*	Y570fs	Hereditary nonpolyposis colorectal cancer (120435)	Recommended to follow-up and have colonoscopy
36- F	*BARD1*	Y404X	Breast cancer susceptibility (114480)	Recommended to discuss with physician and cancer genetic counselor
47-M	*BRCA2*	V220fs	Familial breast and ovarian cancer (612555)	Recommended to have self- and clinical- breast exams; Discuss with cancer genetic counselor
52- M	*RET*	C609Y	Medullary thyroid carcinoma (155240)	Recommended to follow-up and test daughter with Hirschsprung's disease (also associated with *RET* genetic variation)

[a]Parent participants who harbored the *HARS and SCN4A* variants did not report any muscular-related phenotypes prior to variant identification. At the return of results, both indicated they had clumsiness, and the mother who harbored variation in *SCN4A* indicated fatigue and low stamina. [b]MC4R variant was not returned to participant due to patient preferences