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

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Purpose: Clinically relevant secondary variants were identified in parents enrolled with a child with developmental delay and intellectual disability.

Methods: Exome/genome sequencing and analysis of 789 "unaffected" parents was performed.

Results: Pathogenic/likely pathogenic variants were identified in 21 genes within 25 individuals (3.2%), with 11 (1.4%) participants harboring variation in a gene defined as clinically actionable by the American College of Medical Genetics and Genomics. These 25 individuals self-reported either relevant clinical diagnoses (5); relevant family history or symptoms (13); or no relevant family history, symptoms, or clinical diagnoses (7). A limited carrier screen was performed yielding 15 variants in 48 (6.1%) parents. Parents were also analyzed as mate pairs ($n = 365$) to identify cases in which both parents were carriers for the same recessive disease, yielding three such cases (0.8%), two of which had children with the relevant recessive disease. Four participants had two findings (one carrier and one noncarrier variant). In total, 71 of the 789 enrolled parents (9.0%) received secondary findings.

Conclusion: We provide an overview of the rates and types of clinically relevant secondary findings, which may be useful in the design and implementation of research and clinical sequencing efforts to identify such findings.

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Key Words: ACMG; Clinical Sequencing Exploratory Research Consortium; disease risk; genomic sequencing; secondary findings

INTRODUCTION

Whole-exome and whole-genome sequencing have proven to be powerful tests for identifying clinically relevant genetic variation. 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 be returned, and the necessary extent of patient consent, education, and genetic counseling. The American College of Medical Genetics and Genomics (ACMG) released recommendations about the interpretation of variants in genes considered to be clinically actionable, including those that confer a high risk of cancer or heart disease. The ACMG recommends that these be sought and provided to patients that consent to receive such results.[1,2] Recommendations related to use of specific gene lists and approaches for returning secondary findings were intended to be used in clinical contexts, although it is also important to examine them in translational research contexts.

Through a study that was part of the Clinical Sequencing Exploratory Research (CSER) Consortium,[3] we assessed the utility of whole-exome/whole-genome sequencing to identify genetic causes of developmental delay, intellectual disability (DD/ID), and related congenital anomalies. We have sequenced affected probands from 455 families, and have identified DD/ID-related pathogenic/likely pathogenic (P/LP) variants in 29% of cases.[4] As our DD/ID study includes proband–parent trios, we have the ability to assess secondary findings in a sizable cohort of adults.[4]

We use the term "secondary findings" throughout this work to describe variation identified via proactive searching[5] and report rates and types of secondary findings in context of reported symptoms or family history. 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. We demonstrate the utility of dissemination of such findings in a cohort of parent study participants, and highlight this through case-study analyses.

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MATERIALS AND METHODS

Study participant population

There was no public recruitment for this study. Parent and child (n = 455 families) participants 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 preferences and consent

We developed the Preferences Instrument for Genomic Secondary Results[6] to elicit parents' preferences for receiving categories of secondary results. This instrument divides secondary findings into 13 disease categories (**Figure 1**). Results were returned only to participants who opted to receive secondary findings. Decisions regarding disclosure of secondary findings solely in the proband were based on a combination of parent preferences for themselves and medical relevance to the proband during childhood. In the case of adopted probands, preferences were solicited from the adoptive parents on behalf of the proband.

Phenotyping

At enrollment, a genetic counselor generated a three-generation pedigree based on information provided by the parents/guardians of the proband. Parents' health records were not available to the study nor was a physical exam performed. The genetic counselor asked questions related to family history of cancer and sudden/unusual deaths of adults (e.g., cardiac arrest). Cascade sequencing was not conducted as part of this study. We have (i) retained the language used by the participant to describe their phenotypes or family histories and (ii) included any reported information that is plausibly related to the phenotype of concern.

Return of results

Participants who received secondary findings were scheduled for private disclosure with a medical geneticist and genetic counselor. The clinical significance of findings was addressed and documents detailing variant information and relevant resources were provided. Secondary findings were not by default placed in the participant's medical record and no formal referrals to relevant specialists were made. If the participants chose to share results with their health-care provider, formal referrals were coordinated.

Sequencing and variant information

Further details regarding whole-exome/whole genome sequencing, read alignment, variant calling, filtering, classification, and validation can be found in our previous report[4] and in the **Supplementary Methods** online. Briefly, we searched for: P/LP variation in ACMG genes;[1,2] P/LP variation in ClinVar outside of ACMG genes; recessive variation in individuals who harbored two or more P/LP variants in the same gene; variation in which both parents of a pair harbored P/LP variation for the same recessive disorder (defined in OMIM); and carrier status information in *CFTR*, *HEXA*, and *HBB*. Only P/LP variants were returned.

Data sharing

Identified variants in parent participants have been shared through ClinVar and dbGaP, with consent. Additional information is provided in the **Supplementary Methods** online.

RESULTS

Demographics of study population

Of 455 enrolled families, 424 included at least one parent, and both parents were available for 365 families. Demographics

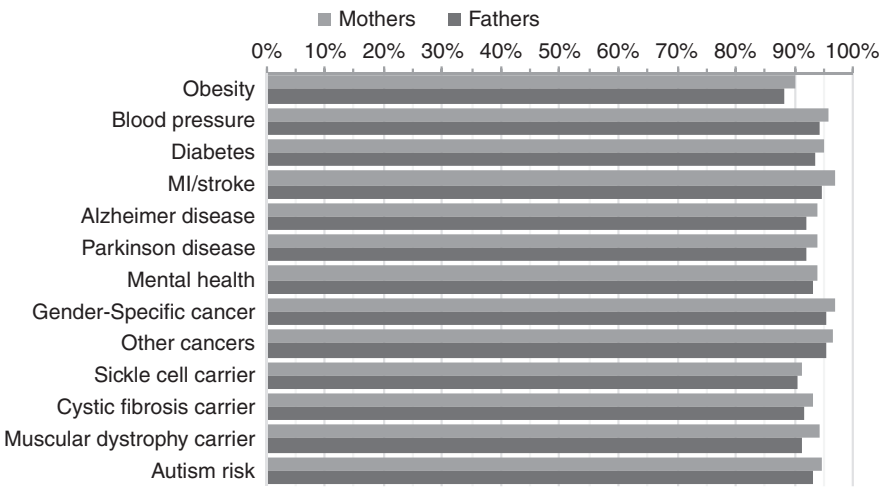

Figure 1 Participant preferences for receipt of secondary genetic findings. Participant preferences were assessed for return of genetic variation across a number of different disease categories. An overwhelmingly large majority (85%) of study participants chose to receive any identified secondary variant,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

Table 1 Demographics of parent participants enrolled in the HudsonAlpha Clinical Sequencing Exploratory Research Consortium 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	67 (8.5%)	28 (7.6%)	39 (9.2%)
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 (4.0%)	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 (15.4%)	67 (18.2%)	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%)

Race, ethnicity, education are 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
G542*	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
G40*	1

for the 789 parent participants are reported in Table 1. The study population had a mean age of 41 years and included 422 females and 367 males; 80.5% self-reported to be of European ancestry ("White"), 8.5% as African American ("Black"), and 8.2% as "Other or Multiracial." More than 25% had a high school diploma or less, and 34.5% reported some college education (Table 1).

Patient preferences

One goal of our study was to understand preferences as they relate to receiving secondary findings across various disease categories.[6] Eighty-five percent of parents requested all secondary findings, while 1.6% declined to receive all findings. The most frequently requested category was risk for gender-specific cancers (breast, ovarian, testicular, and prostate; 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 relevant to cystic fibrosis (*CFTR*, MIM 219700), β-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) (refs. 7–9). We observed eight P/LP variants in *CFTR* across 35 individuals (4.4% of parent cohort), four *HEXA* variants across five individuals (0.6%), and three *HBB* variants across eight individuals (1%) (Table 2; **Supplementary Table S2** online). Additionally, we searched for cases in which parental "mate pairs" were both carriers for variants in a gene associated with a recessive disorder that was not relevant to the proband's developmental disability (i.e., was truly "secondary" relative to the reason for study enrollment). This analysis led to three returnable results, including a parent pair with recessive mutations in each of *OCA2* (MIM 203200), *FYCO1* (MIM 610019), and *ATP7B* (MIM 277900) (**Supplementary Table S2** online). For the former two cases (*OCA2* and *FYCO1*), the enrolled probands inherited both alleles and were affected by the given disease (see below),

while the latter family (*ATP7B*) did not have any currently affected children.

Secondary variants in individuals reporting a relevant clinical diagnosis

P/LP variants were found in five individuals with a self-reported previous clinical diagnosis but in whom a specific genetic cause was unknown. A 35-year-old woman was found to harbor a heterozygous missense variant in *SLC4A1* (spherocytosis, MIM 612653), and had family history of related disease (**Table 3**; **Supplementary Table S1** online). We identified three missense variants (two likely in *cis*) in *SLC22A5* in a 37-year-old woman with recessive systemic primary carnitine deficiency (MIM 212140). Finally, a canonical splice donor site (D1) variant affecting *PKD2* was identified in a 36-year-old woman with polycystic kidney disease (MIM 613095). This individual also reported a family history of disease (**Table 3**; **Supplementary Table S1** online).

Secondary genetic variation related to cardiovascular disease was identified in two individuals with a previous clinical diagnosis and a family history of cardiovascular phenotypes. One 30-year-old woman reported to have experienced cardiomyopathy postpartum, had a paternal family history of arrhythmia, and stated that 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). Although *DSG2* has not per se been associated with peripartum cardiomyopathy, we find it probable that the variant explains her disease history. The clinical symptoms of peripartum cardiomyopathy are similar to those of dilated cardiomyopathy[10] and other genetic variants associated with dilated cardiomyopathy are thought to be risk factors for peripartum cardiomyopathy.[11] In a 52-year-old man with hypertrophic cardiomyopathy and arrhythmia, we identified missense variation in *ANK2,* a gene associated with ankyrin-B-related cardiac arrhythmia and long QT syndrome (MIM 600919). It is unknown whether this individual presents with long QT intervals. Additionally, although not clearly related to *ANK2* variation, this individual also reported that his father had ischemic heart disease.

Finally, six of the eight parents carrying P/LP variation in *HBB* reported having sickle cell or thalassemia trait at time of enrollment (**Table 2**; **Supplementary Table S2** online).

Secondary variants in individuals reporting relevant symptoms and/or family history

We identified secondary variants in 13 individuals with no previous diagnosis or genetic testing despite the manifestation of disease and/or family history (**Table 3**; **Supplementary Table S1** online). Given information provided at time of enrollment, six of these cases (*CLCN1*, *MFN2*, *BRCA1*, *BRCA2*, *BARD1*, and *PMS2*; **Table 3**) would have met criteria for genetic consultation and testing via standard clinical guidelines.[12,13] Given additional phenotypic information acquired at return of results, two additional cases (*SCN4A* and *HARS*; **Table 3**) would have met such criteria.[14,15] These eight cases are described below.

A heterozygous missense variant in *CLCN1* was identified in a 29-year-old woman who reported leg cramps and restless legs beginning in childhood. Variation in *CLCN1* associates with myotonia congenita (MIM 160800) characterized by muscle stiffness. 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 presentation of symptoms, including "stiffness" that occurred "especially in cold [temperatures]." In a separate case, a heterozygous missense variant in *MFN2* (Charcot–Marie–Tooth disease type 2A2A, MIM 609260) was identified in a 35-year-old woman who reported balance difficulties and weakness since childhood that have progressed to severe cramping, myalgia, and numbness most prominently in lower extremities. Her family history is notable for neuromuscular disorder, with similar symptoms present in her brother, father, paternal grandmother, and paternal aunt. Though a clinician has not formally evaluated her, she reported that her brother was diagnosed with Charcot–Marie–Tooth.

We also identified cancer risk variants in individuals who report a family history of cancer. We identified a frameshift variant in *BRCA1* (familial breast/ovarian cancer, MIM 604370) in a 40-year-old man whose mother was diagnosed with breast cancer in her 30s. In another case, a canonical splice acceptor variant of *BRCA2* (familial breast/ovarian cancer, MIM 612555) was identified in a 38-year-old woman who had a history of breast cancer on both sides of the family: paternal grandmother (unknown age) and maternal grandfather (age 60). A frameshift variant in *BARD1* (Breast cancer, MIM 114480) was identified in a 33-year-old woman whose maternal grandmother had bladder, lung, and peritoneal cancer as well as a great-grandmother diagnosed with breast cancer in her 50s. Additionally, a frameshift variant in *PMS2* (hereditary nonpolyposis colorectal cancer; MIM 614337) was identified in a 43-year-old man with a family history of colon cancer—father (60s) and paternal aunt (40s). This individual also had a paternal aunt and grandmother who were diagnosed with breast cancer in their 60s and 50s, respectively. After receipt of this finding, the study participant followed up with a colonoscopy, the results of which were negative. He reports that he will continue periodic assessment.

Secondary variants were also identified in two symptomatic individuals who were not aware that their symptoms were unusual and thus never had clinical or genetic evaluation (**Table 3**). At enrollment, neither individual reported relevant phenotypes to the variants identified. In one case, a 28-year-old woman was found to harbor a pathogenic missense variant in *SCN4A*, implicated in hyperkalemic periodic paralysis and paramyotonia congenita (MIM 170500, 168300), neuromuscular disorders characterized by intermittent muscle weakness and/or myotonia. At results return, she reported a history of painful stiffness during exercise that began at approximately age 5 and that her throat "locks up"

Table 3 Secondary findings of enrolled parents segregated into "Relevant clinical diagnoses", "Relevant family history or symptoms", and "No relevant family history, symptoms or clinical diagnosis"

Age (M/F)	Gene	Variant info	Associated phenotype (MIM)	Phenotypes or family history reported by parent participants[a]
Relevant clinical diagnoses reported (0.6% total population)				
35 (F)	*SLC4A1*	V488M	Spherocytosis, type 4 (612653)	Clinically diagnosed with spherocytosis; two daughters and father with spherocytosis
37 (F)	*SLC22A5*	A142S; T440M, R488H	Carnitine deficiency, systemic primary (212140)	Clinically diagnosed with carnitine deficiency
36 (F)	*PKD2*	c.1319+1G > A	Polycystic kidney disease 2 (613095)	Clinically diagnosed with polycystic kidney disease; mother, brother, two nieces, maternal aunt, uncle, and grandmother with polycystic kidney disease
30 (F)	*DSG2*	V986fs	Cardiomyopathy, dilated, 1BB; arrhythmogenic right ventricular dysplasia 10 (612877; 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, ankyrin-B-related, long QT syndrome 4 (600919)	Clinically diagnosed with hypertrophic cardiomyopathy and arrhythmia; father died with ischemic heart disease
Relevant family history or symptoms reported (1.6% of total population)				
29 (F)	*CLCN1*	F413C	Myotonia congenita, dominant (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
35 (F)	*MFN2*	W740S	Charcot–Marie–Tooth disease, axonal, type 2A2A (609260)	History of muscle wasting in back, lower extremities; brother clinically diagnosed with Charcot–Marie–Tooth, 30s; multiple family members affected with "unspecified muscle disorder"
40 (M)	*BRCA1*	G1756fs	Breast/ovarian cancer, familial 1 (604370)	Mother with breast cancer, 30s
38 (F)	*BRCA2*	c.8488-1G > A	Breast/ovarian cancer, familial 2 (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, type 4 (614337)	Father (60s) and paternal aunt (40s) had colon cancer; paternal aunt (60s) and grandmother (50s) with breast cancer
28 (F)	*SCN4A*	T1313M	Paramyotonia congenita (168300)	At enrollment, no report of neuromuscular phenotypes. At return of results, indicated that she had muscle stiffness but always thought she was "easily fatigued" and had "low stamina"; mother displays similar symptoms
41 (M)	*HARS*	R137Q	Charcot–Marie–Tooth, axonal, type 2W (616625)	At enrollment, no report of neuromuscular phenotypes. At return of results, indicated that he had Charcot–Marie–Tooth associated phenotypes. Always thought he was "just clumsy"
32 (F)	*KCNQ1*	R366W	Long QT syndrome 1 (192500)	Father with coronary artery disease with triple bypass, early 50s, paternal aunt with early-onset stroke, late 30s
47 (M)	*KCNQ1*	P7S	Long QT syndrome 1 (192500)	Mother "fainted" and "hit the floor"; was told this impact prevented cardiac arrest
39 (M)	*MYBPC3*	E542Q	Hypertrophic cardiomyopathy 4; dilated cardiomyopathy 1MM (115197; 615396)	"Leaky heart valve"; Dad has pacemaker and mom has "leaky heart valve," 60s
30 (M)	*DDX41*	D140fs	Susceptibility to familial myeloproliferative/ lymphoproliferative neoplasms (616871)	Paternal cousin with lymphoma "unspecified," age unknown
37 (F)	*MC4R*	C271Y	Obesity, autosomal dominant (601665)	Obese (body mass index: 41)

Table 3 Continued

Age (M/F)	Gene	Variant info	Associated phenotype (MIM)	Phenotypes or family history reported by parent participants[a]
No relevant family history, symptoms, or clinical diagnosis reported (0.9% of total population)				
52 (F)	*SCN5A*	T1303M	Long QT syndrome 3 (603830)	Recommended to have cardiovascular evaluation
50 (M)	*DSG2*	E1020fs	Cardiomyopathy, dilated, 1BB; arrhythmogenic right ventricular dysplasia 10 (612877; 610193)	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, type 1 (120435)	Recommended to follow up and have colonoscopy
36 (F)	*BARD1*	Y404*	Breast cancer susceptibility (114480)	Recommended to discuss with physician and cancer genetic counselor
47 (M)	*BRCA2*	V220fs	Breast/ovarian cancer, familial 2 (612555)	Recommended to have self- and clinical breast exams; discuss with cancer genetic counselor
52 (M)	*RET*	C609Y	Medullary thyroid carcinoma (155240); susceptibility to Hirschsprung disease 1 (142623)	Recommended to follow up and test daughter with Hirschsprung disease

M, male; F, female.
[a]We have (i) retained the language used by the participant and (ii) included any reported family history that is plausibly related to the phenotype of concern.

after drinking cold liquids. Additionally, she reported that her eyelids "stick" and "become heavy" throughout the day. She noted that her mother displays similar phenotypes. This individual plans to follow up with a neurologist. In a second case, a 41-year-old man was found to harbor pathogenic variation in *HARS,* associated with Charcot–Marie–Tooth disease Type 2W (MIM 616625) characterized by gait difficulties and sensory impairment caused by peripheral neuropathy. At return of results, he indicated that he was "clumsy," was discharged from military boot camp due to his inability to march in formation, and often wears out shoes because he shuffles his feet.

Secondary variants in individuals reporting no relevant symptoms or family history of disease

We also identified P/LP variants in individuals that are currently asymptomatic and report no relevant family history (**Table 3**). Two unrelated individuals, a 52-year-old woman and a 50-year-old man, were found to harbor variation in *SCN5A* (long QT syndrome, MIM 603830) and *DSG2* (dilated cardiomyopathy, MIM 612877), respectively. A 31-year-old man was found to harbor a missense variant in *ACTN1*, associated with a bleeding disorder (MIM 615193). Finally, P/LP cancer-associated variants were identified in four participants with no personal or family history, including one in each of *MSH2*, *BARD1*, *BRCA2*, and *RET* (**Table 3**; **Supplementary Table S1** online). Notably, a pathogenic missense variant (C609Y) in *RET,* associated with multiple endocrine neoplasia type 2A (MIM 171400), medullary thyroid carcinoma (MTC; MIM 155240), and/or Hirschsprung disease (MIM 142623), was identified in a 52-year-old man who reported no history of *RET*-associated cancer. C609Y has been observed in many medullary thyroid carcinoma–affected individuals and indicates level B risk according to the American Thyroid Association (D is the highest risk level), with an expected age of onset of less than 30 years.[16,17] Recommendations for C609Y carriers vary but often include prophylactic thyroidectomy at a young age.[18,19] However, more recent studies indicate *RET* C609Y may have lower penetrance or later onset of medullary thyroid carcinoma than previously noted,[20,21] consistent with the observation of no related cancers in this family. Interestingly, while C609Y was not transmitted to the enrolled, developmentally delayed proband, the family reported that they have another daughter who has Hirschsprung disease and is therefore likely to have inherited C609Y. The family was referred for genetic counseling to test for the variant in the Hirschsprung-affected daughter and it was recommended that both the father and daughter follow up with oncologists.

Secondary findings in DD/ID-affected children

For three enrolled children, we identified secondary variation not inherited from a parent. Two individuals whose biological parents were not available harbored pathogenic variation in *CFTR* (Phe508del) and *BRCA2* (Leu579*), respectively. Also, a

6-year-old girl harbored a pathogenic *de novo* variant in *FBN1* (Asn2144Ser). At time of analysis, this proband did not exhibit Marfan phenotypes (MIM 154700), with exception of crowded teeth and scoliosis. In three additional probands, compound heterozygous variation associated with recessive disease was identified. Two P/LP variants, one inherited from each carrier parent, in *OCA2* (oculocutaneous albinism type II, MIM 203200) were identified in an 11-year-old boy and his 6-year-old brother; both presented with albinism. In a third case, a 9-year-old girl with cataracts was found to have inherited a P/LP variant from each carrier parent in *FYCO1*, a gene associated with cataract 18 (MIM 610019).

DISCUSSION

The ACMG estimated that secondary findings in genes relevant to a defined list of actionable phenotypes would be found in ~1% of sequenced individuals.[1,2] We observed variation in ACMG-defined genes in 1.4% of parent participants, consistent with that estimate and the 1–5.6% reported by other laboratories.[22–25]

Our study assessed carrier status in all participants for only three genes—*HBB*, *HEXA*, and *CFTR*—leading to the identification of P/LP variation in ~6.1% of parent participants. These genes were selected based on their anticipated frequencies in the population sampled and our desire to balance yield with analytical and cost burden. Had we assessed all genes known to associate with recessive disease,[26] the burden of analysis would have increased substantially.[27,28] Further, expanded carrier screening and discovery efforts would have increased Sanger validation costs and the time required from genetic counselors and medical geneticists for return of results. Thus, while our choice of genes as targets for carrier analysis was semiarbitrary, it imposed minimal analytical burden and led to a substantial but manageable yield relevant to a few of the most prevalent Mendelian diseases.

One additional more comprehensive carrier status strategy we used was to search within both parents of a parental pair for P/LP variants in the same gene (expanding beyond *CFTR*, *HBB*, and *HEXA* to include all genes associated with recessive disease in OMIM). Of the 365 parental pairs enrolled, recessive disease risk (i.e., 25% for their children) was identified in three (0.8% of parental pairs). This rate is likely to grow in the future as additional evidence accrues on the pathogenicity of variants in genes causing recessive disorders.[22] The treatment of parental pairs as units of analysis for carrier status is an effective way to minimize analytical and cost burden and yet effectively capture those carrier results likely to have the greatest potential impact.

Copy-number variation (CNV) was not explored in parents as a source of secondary findings. This decision was driven by the considerable manual scrutiny that is required to evaluate the technical quality of CNVs, the costs and challenges of CNV validation, and the relative lack of robust CNV population frequency data, particularly for smaller events. Analyses of CNVs as secondary variation may be of interest to future efforts to increase the yield of medically relevant information from sequencing data.

Patient preferences

The question of whether patients and research participants need to be offered choices about receiving secondary findings has been debated, especially after the release of ACMG's original secondary findings recommendations in 2013.[1] Studies have documented that most participants want most—and usually all—possible secondary findings. This trend is consistent between studies asking this question as a hypothetical[29–33] or to inform actual return of results.[34–37] Consistent with these previous studies, the vast majority (84.8%) of parents participating in our study chose to receive all categories of secondary results. However, a minor but substantial fraction of participants (15.2%) declined at least one category and 1.6% declined all secondary results. One of the secondary findings listed in **Table 3** was not returned because the parent had declined the relevant category.

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. Even variants previously reported to be pathogenic are often supported only by weak evidence or conversely associated with strong evidence for being benign.[38] 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 disease. That said, in this study, ACMG evidence codes were assigned and variants that were deemed to be P/LP were offered for return regardless of the presence or absence of any particular phenotype or family history. Even for those with indications of disease, the particular phenotypes reported (**Table 3**) are not necessarily directly related to the presence of the given variant. Imprecision and incompleteness of self-reported diseases and family histories and limitations to knowledge of penetrance and expressivity for any given gene, and especially any given variant, all make interpretation more challenging. More precise phenotyping and partnership with referring physicians would be beneficial for laboratories attempting to interpret identified variants.

Utility of secondary findings

The secondary genetic findings that we identified may be of considerable utility to the parent participants. For five individuals, we were able to confirm, and genetically explain, a previous clinical diagnosis (**Table 3**). Such information may prove useful for future clinical management and in discussions with family members that may carry the same variant. Secondary genetic findings were also identified in 13 individuals who reported family history or symptoms that are likely to associate with the detected variant. As described in the results section, genetic counseling and testing could/

should have been offered in eight cases based solely on observed symptoms and/or family history. Additionally, we identified secondary genetic variants in four individuals who have an increased risk of disease with modest but nontrivial evidence for disease (two cases of *KCNQ1*; one case each of *MYBPC3* and *DDX41*). Through participation in our study, these individuals now have a better understanding of their cause or risk of disease and are in position to better manage that disease or risk of disease.

We also identified secondary genetic variation in seven individuals who report neither symptoms nor family history of disease (*MSH2*, *RET*, *BARD1*, *BRCA2*, *ACTN1*, *SCN5A*, and *DSG2*). These study participants appear to be at increased risk of disease and it has been suggested that they follow up with an appropriate specialist (**Table 3**) in the hopes that actions can be taken to screen for, prevent, or mitigate unobserved disease in these individuals.

Finally, we also identified secondary variation in DD/ID affected probands that were not identified in parents, either due to unavailability of parents ($n = 2$) or as a result of the variant arising *de novo* ($n = 1$). Further, three children from two families were found to harbor compound heterozygous variation relevant to an observed disease that was unrelated to their developmental disabilities (i.e., albinism and cataracts).

Challenges of returning unexpected variants to families

Many parents in this study have experienced a diagnostic odyssey in hopes of identifying the cause of their child's developmental disabilities. 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, 51% of the secondary findings identified in the parents were transmitted to the DD/ID-affected proband, and 56% of the 71 parents who harbored a secondary finding did not receive a primary result for their enrolled DD/ID-affected child. The lack of a 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 disease risk and/or an additional, unexpected disease risk relevant to their already affected child. This fact highlights the potential financial, emotional, and clinical implications of secondary findings that should be clearly addressed in the informed consent discussion prior to sequencing so that families are 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 were subject to genomic sequencing for diagnosis of a developmentally delayed child. Although the return of secondary genetic variation has been debated,[39,40] 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 preventive screening for individuals who are genetically predisposed to serious diseases. This information can also be useful to individuals who have been clinically diagnosed with a condition but for which a specific causal explanation is 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, but did not lead to, genetic evaluation independent 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 but productive carrier screen on only a few common Mendelian diseases along with a more comprehensive screen treating parents as mate pairs. The methods and results related to secondary variation identification may be of use to other research and clinical laboratories that are conducting genomic sequencing.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

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DISCLOSURE

The authors declare no conflict of interest.

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