ARTICLE

Preconception Carrier Screening by Genome Sequencing: Results from the Clinical Laboratory

Sumit Punj,[1] Yassmine Akkari,[1,9,10] Jennifer Huang,[1,10] Fei Yang,[1] Allison Creason,[1] Christine Pak,[1] Amiee Potter,[1] Michael O. Dorschner,[2,3] Deborah A. Nickerson,[3] Peggy D. Robertson,[3] Gail P. Jarvik,[3,4] Laura M. Amendola,[4] Jennifer Schleit,[2] Dana Kostiner Simpson,[5] Alan F. Rope,[5] Jacob Reiss,[6] Tia Kauffman,[6] Marian J. Gilmore,[5] Patricia Himes,[5] Benjamin Wilfond,[7,8] Katrina A.B. Goddard,[6] and C. Sue Richards[1,*]

Advances in sequencing technologies permit the analysis of a larger selection of genes for preconception carrier screening. The study was designed as a sequential carrier screen using genome sequencing to analyze 728 gene-disorder pairs for carrier and medically actionable conditions in 131 women and their partners (n = 71) who were planning a pregnancy. We report here on the clinical laboratory results from this expanded carrier screening program. Variants were filtered and classified using the latest American College of Medical Genetics and Genomics (ACMG) guideline; only pathogenic and likely pathogenic variants were confirmed by orthologous methods before being reported. Novel missense variants were classified as variants of uncertain significance. We reported 304 variants in 202 participants. Twelve carrier couples (12/71 couples tested) were identified for common conditions; eight were carriers for hereditary hemochromatosis. Although both known and novel variants were reported, 48% of all reported variants were missense. For novel splice-site variants, RNA-splicing assays were performed to aid in classification. We reported ten copy-number variants and five variants in non-coding regions. One novel variant was reported in *F8*, associated with hemophilia A; prenatal testing showed that the male fetus harbored this variant and the neonate suffered a life-threatening hemorrhage which was anticipated and appropriately managed. Moreover, 3% of participants had variants that were medically actionable. Compared with targeted mutation screening, genome sequencing improves the sensitivity of detecting clinically significant variants. While certain novel variant interpretation remains challenging, the ACMG guidelines are useful to classify variants in a healthy population.

Introduction

Traditionally, carrier screening has focused on specific disorders that are known to have a higher prevalence in certain ethnic populations. More recently, lower sequencing costs coupled with higher accuracy of next generation sequencing-based methodologies have made it affordable for clinical laboratories to offer screening for substantially more conditions.[1–4] Both autosomal-recessive and X-linked conditions, which comprise a typical carrier-screening panel, are often observed in individuals with no family history of the condition. Therefore, for a healthy couple, offering pan-ethnic, expanded carrier screening is appropriate, particularly in a culturally and genetically heterogeneous population such as the United States. Many professional societies have developed their own practice guidelines on expanded carrier screening, in recognition of its increasing popularity. Furthermore, the American College of Medical Genetics and Genomics, American Congress of Obstetricians and Gynecologists, National Society of Genetic Counselors, Society of Maternal-Fetal Medicine, and the Perinatal Quality Foundation have collaborated to issue a joint statement for healthcare providers and clinical laboratory personnel to educate and guide them on the use of this screening approach.[5]

Massively parallel sequencing or next-generation sequencing (NGS) has provided the technical means to not only screen the full gene, but also analyze multiple genes and multiple individuals simultaneously, as compared to the targeted mutation panel approach of traditional carrier screening. However, given the rapid pace of its application, there is a paucity of information on the downstream impact of NGS in the healthcare system and in routine medical care.

To this end, the NextGen study (Figure S1A), a part of the National Human Genome Research Institute's Clinical Sequencing Exploratory Research consortium (CSER), was focused on exploring the possibility of using genome sequencing as part of a preconception expanded carrier screening program from a variety of contexts. The multidisciplinary team generated evidence on a variety of goals to achieve this overarching objective including evaluating the clinical utility of genome sequencing (GS) in this clinical scenario, exploring critical interactions between

[1]Department of Molecular and Medical Genetics, Knight Diagnostic Laboratories, Oregon Health & Science University, Portland, OR 97239, USA; [2]Pathology, Division of Medical Genetics, University of Washington, Seattle, WA 98195, USA; [3]Genome Sciences, Division of Medical Genetics, University of Washington, Seattle, WA 98195, USA; [4]Department of Medicine, Division of Medical Genetics, University of Washington, Seattle, WA 98195, USA; [5]Department of Medical Genetics, Kaiser Permanente Northwest, Portland, OR 97227, USA; [6]Center for Health Research, Kaiser Permanente Northwest, Portland, OR 97227, USA; [7]Department of Pediatrics, Division of Bioethics, University of Washington, Seattle, WA 98195, USA; [8]Treuman Katz Center for Pediatric Bioethics, Seattle Children's Hospital, Seattle, WA 98101, USA
[9]Present address: Legacy Laboratory Services, Legacy Health, Portland, OR 97232, USA
[10]These authors contributed equally to this work
*Correspondence: richarsu@ohsu.edu
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individuals, providers, and laboratories that influence the implementation of clinical sequencing programs, and identifying and addressing barriers to integration of genomic and health data for clinical decision making. The study was designed as a randomized controlled trial with GS and analysis of a pre-selected list of 728 gene-disorder pairs (genes known to be associated with human disorders) for autosomal-recessive and X-linked conditions[6] as well as 148 genes[7,8] for conditions that are considered medically actionable. We reported known as well as novel variants that for this study were defined as those not previously reported in affected individuals. Here, we describe the analytic pipeline and the clinical laboratory results for subjects who received GS as part of the NextGen study.

Subjects and Methods

Selection of Participants and Study Design

All female participants were members of the Kaiser Permanente Northwest (KPNW) integrated healthcare delivery health management system. Our study is based on the sequential model and not the couple-based model of carrier screening. Females were first sequenced and if at least one positive carrier result was disclosed to the participant, her male partner was invited to join the GS arm of the study following consent (Figure S1B). To be eligible to participate in this experimental randomized controlled trial,[9] the female participants must have satisfied three criteria: (1) planning a pregnancy in the near future, (2) had a carrier screening test, usually cystic fibrosis (MIM: 219700), ordered by a clinician that was resulted and completed, and (3) not pregnant at the time of consent. All women who consented to participate in the study filled out a baseline survey including demographic information prior to being randomly assigned into the GS arm or the usual care arm of the study (Figure S1A). At KPNW, all participants (i.e., females and males) in the GS arm had a pre-test consent visit with a genetic counselor before their blood draw. Blood samples were sent to the CLIA laboratories at Illumina Clinical Services Laboratory and Oregon Health & Science University's (OHSU) Knight Diagnostic Laboratories for GS and variant confirmation, respectively, while secondary analysis was performed at the University of Washington. Positive carrier results were discussed with a genetic counselor for all participants during post-test counseling (Figure S1B). We sequenced a total of 202 participants: 131 females and 71 male partners (i.e., 71 couples). This study was reviewed and approved by the Institutional Review Board (IRB) at Kaiser Permanente Northwest, the University of Washington, and OHSU ceded IRB authority to KPNW. All participants received full written and IRB-approved consent and could withdraw at any time during the study without consequences.

Genes Analyzed

Within the GS arm of the study, only variants in pre-selected genes that were determined by the NextGen Return of Results Committee (RORC)[6] were chosen for analysis (Figure S1). These genes included those for carrier screening and medically actionable findings. For carrier screening, the 728 gene-disorder pairs, which comprised autosomal-recessive and X-linked conditions, were categorized into lifespan limiting (177 genes), serious (406 genes), mild (93 genes), unpredictable (41 genes), and adult onset (11 genes). For medically actionable (also called secondary or additional) findings,[10] we used an expanded list compared with the most recent ACMG list.[11] This list was comprised of 121 genes for autosomal-dominant conditions, 23 genes for autosomal-recessive conditions, and 4 genes for X-linked conditions. The selection process for these genes has been previously published[7] and was based on their clinical validity and the clinical utility of medically actionable genes. The analytical validity of these genes was one of the metrics that was assessed in this study. The genes are listed in the Supplemental Note. All participants who consented to the study received results for at least the 177 conditions that were categorized as lifespan limiting.[6] The remaining categories of carrier conditions were optional and were returned only if requested. The National Center for Biotechnology Information (NCBI)-curated reference sequences ("NM and NP categories") were used for variant analysis. In addition, variants in the promoter region of *CFTR* (MIM: 602421) were also analyzed.

Sequencing

Genome sequencing was performed at the Illumina Clinical Services Laboratory. Briefly, genomic DNA was extracted from the participant's blood and processed for sequencing using the Illumina TruSeq DNA LT kit. The DNA sample was sequenced on a HiSeq 2000 or 2500 (Illumina, version 3 chemistry) with 100 base pair, paired-end reads. The sequenced fragments were assessed for quality and aligned to the NCBI reference genome (GRCh37/hg19) to generate BAM files. The BAM files were subsequently sent to the University of Washington for secondary analysis.

Bioinformatics Pipeline (Secondary Analysis)

For compatibility with the University of Washington's data analysis pipeline, FASTQ files, with the original read sequences, were generated from the BAM files received from the Illumina Clinical Services Laboratory. The reads were re-aligned to the NCBI GRCh37/hg19 reference sequence with the Burrows-Wheeler Aligner (v. 0.7.6a).[12] The aligned read data were subject to further analysis using tools from the Genome Analysis Tool Kit (GATK)[13] by removal of duplicate reads (Picard MarkDuplicates v.1.96), indel realignment (GATK RealignerTargetCreater and IndelRealigner v.2.6), and base-quality recalibration (GATK BaseRecalibrator v.2.6).

Single-Nucleotide Variants (SNV) and Small Insertion and Deletions (Indel)

SNVs and indel variants were called by the GATK UnifiedGenotyper v.2.6, followed by the GATK VariantAnnotation and VariantFiltration (to flag low-quality calls). Variant quality terms, QUAL and QD, were assigned by UnifiedGenotyper and the VariantFiltration tool assigned a "PASS" to all variants with a QUAL score $>$ 100 and a QD (QUAL score normalized by allele depth) score $>$ 5. Further annotation was performed by SeattleSeqAnnotation138,[14] using a local cache database that also served the website. These annotations that are included on the website are dbSNP[15] and clinical association data as well as scores from PolyPhen,[16] GERP,[17] CADD,[18] Grantham,[19] protein-protein interactions,[20] microRNAs from miRbase,[21] and population frequencies from NHLBI GO Exome Sequencing Project (ESP)[22] and Exome Aggregation Consortium (ExAC).[23] Also present were University of California Santa Cruz (UCSC) Browser[24] annotations: repeats, chimp alleles, CpG islands, and KEGG pathways. Additional annotations that are not part of the standard

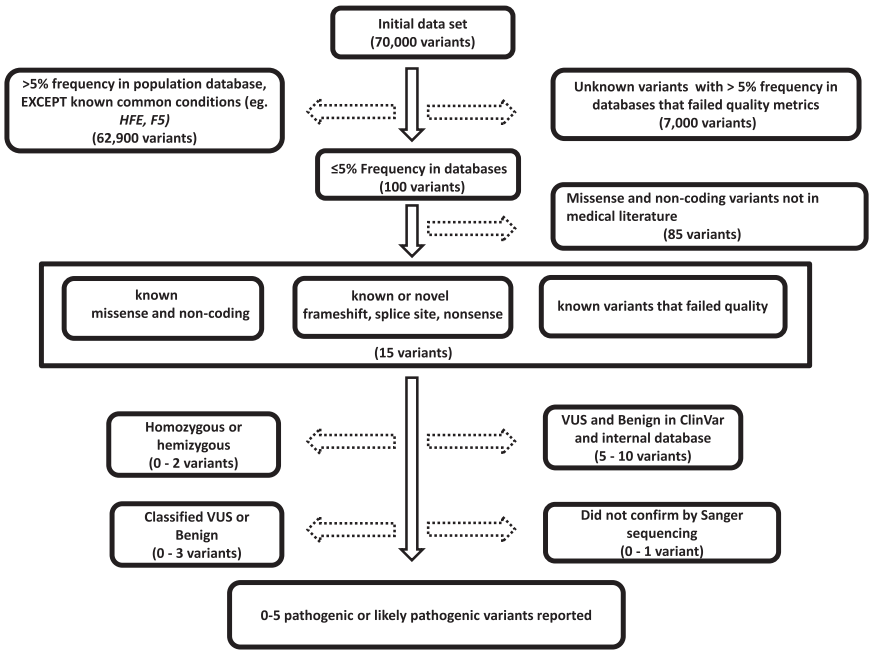

Figure 1. Schematic Representative of Variant Filtering.
The solid arrows are variants that were prioritized and stippled arrows indicate the variants that were discarded in the analysis. The numbers of variants depicted are averages per person.

SeattleSeqAnnotation138 software suite were included to further support variant interpretation. These additional tools were SIFT[25] and SPIDEX for splicing,[26] data from ClinVar[27] and the professional version of Human Gene Mutation Database,[28] and population frequencies in 1000 Genomes Project,[29] ICR1000 UK exomes,[30] and 500 local exomes. The Human Genome Variation Society (HGVS)[31] notations were used for the nomenclature of SNVs and indels in exons.

Structural Variant Analysis of the Sequencing Data

Structural variants were called by LUMPY[32] and augmented by CNVnator[33] for the entire genome. For the RORC-selected genes, regions were defined that included 2,000 nucleotides upstream and downstream of the first and last exon in the longest transcript. Overlapping reads that included one or more exons of RORC-selected genes were then analyzed for structural variants. Quality metrics were applied by CNVnator and LUMPY to filter for signal type paired-end and/or split-read. Finally, structural variants in the BAM files were manually curated using the Integrated Genome Viewer (IGV).[34] Pathogenicity of copy-number variants (CNVs) were based on a laboratory-developed guideline that included consideration of the pathogenic mechanism of the variants, classifications in the population databases such as the Database of Genomic Variants (DGV)[35] and the human disease databases such as ClinVar and DECIPHER,[36] medical literature review, the variant frequency, and the consequence of the CNV; i.e., a deletion of several exons resulting in the remaining flanking exons being out of frame, or loss of the translation start site. Novel duplications were generally not reported due to lack of functional evidence of their effect on gene product or location, unless located in a well-studied gene (e.g., *DMD* [MIM: 300377]).

Tertiary Analysis and Variant Confirmation for SNVs and CNVs

The variants that were called and annotated at the University of Washington were sent via a secure site to the OHSU CLIA-laboratory for variant filtering, interpretation, confirmation, and reporting (Figure S1B). Only those SNVs and CNVs that were classified as either pathogenic or likely pathogenic were confirmed by an orthologous methodology (Sanger sequencing for SNVs; gene-focused array or multiplex PCR for CNVs) before reporting the results to the clinician and participant. Based on the frequency of the variant in the population databases (1000 Genomes Project, ESP, and ExAC), a 5% threshold for variant frequency was used for initially filtering variants (Figure 1). The 2015-ACMG guidelines[37] were used for variant interpretation and classification for SNVs and small indels. Confirmation for SNVs and small indels was performed, in both directions, by capillary electrophoresis-based Sanger sequencing (CE).[38] If a SNV was detected in a gene that was also known to have a pseudogene, long-range PCR followed by nested PCR was performed before CE. Structural variant confirmation for *HBA2* (MIM: 141850) deletion variants was performed by a multiplex polymerase chain reaction method.[39] Other structural variant confirmations were performed on a clinically validated gene-focused array, CytoSure Medical Research Exome Array (Oxford Gene Technology), according to the manufacturer's instructions. Finally, for splice-site variants, splicing analysis was performed by extracting RNA from whole blood, converting the mRNA to cDNA (SuperScript II Reverse Transcriptase, ThermoFisher Scientific), and sequencing the product by CE. Disorders associated with trinucleotide repeats were not analyzed. All reported variants were submitted to ClinVar.[27]

Clinical Report

The clinical reporting was performed in two phases to enable post-test genetic counseling and survey completion. The first phase included only the carrier results. For participants who chose to receive medically actionable (secondary) findings, an additional report containing positive results, if any, was provided at a later date. A sample clinical report is available in the Supplemental Note.

Results

Participant Choices for Results

The average participant age was 32 years for females (range: 21–46 years) and 34 years for males (range: 24–50 years). White/non-Hispanic participants comprised 78% of study participants. While most (93%) participants opted for receiving all categories of carrier results, some participants (~7%) did not want to know their carrier status for unpredictable or adult-onset onset conditions.

Table 1. Carrier Findings for Well-Known Disease-Associated Variants within Introns

Gene	Variant	Disorder	Condition Category	Variant Category	Classification
PTS (MIM: 612719)	NM_000317.2 (c.84_291A>G)	BH4-deficient hyperphenylalaninemia (MIM: 261640)	serious	known	likely pathogenic
GAA (MIM: 606800)	NM_001079804.2 (c.−32−13T>G)	glycogen storage disease II (MIM: 232300)	serious	known	pathogenic
GJB2 (MIM: 121011)	NM_004004.5 (c.−22−2A>C)	deafness and hearing loss (MIM: 220290)	mild	known	pathogenic
	NM_004004.5 (c.−23+1G>A)			known	pathogenic
PYGM (MIM: 608455)	NM_005609.3 (c.425−26A>G)	McArdle disease (MIM: 232600)	unpredictable	known	likely pathogenic

These variants are not in the canonical splice-site within introns. An *a priori* knowledge of the mutation spectrum in a gene is not required for using GS-based carrier testing. The variants are annotated according to the Human Genome Variation Society (HGVS)[28] recommended nomenclature. The variants in *GAA* and *GJB2* are located in the introns that are upstream (5′ in the coding strand) of the translational start codon (where the adenine position in ATG start codon is +1).

Almost every participant (99%) requested the return of medically actionable findings.

Sequencing Performance

For GS, the average depth of sequencing was 38.5× with an average of 79.5% of reads covered at a depth of at least 30×. *SMN1* ([MIM: 600354]; spinal muscular atrophy [MIM: 253300, 253550, 253400, 271150]) and *IKBKG* ([MIM: 300248]; X-linked hypohidrotic ectodermal dysplasia with immune deficiency [MIM: 300291] and anhidrotic ectodermal dysplasia with immune deficiency, osteopetrosis, and lymphedema [MIM: 300301]) were consistently sequenced at less than 10× coverage. This was due to the close proximity of these genes to their respective pseudogenes, thereby resulting in poor mapping quality of the sequence reads. Furthermore, specific bioinformatic strategies would be required for variant detection of these regions by NGS.[40] Therefore, variant interpretation for those gene-disorder pairs that were sequenced at less than 10× depth were not returned due to insufficient coverage.

Variant Filtering and Confirmation

On average, approximately 70,000 variants were detected in the 728 gene-disorder pairs for each participant. Based on the ACMG guidelines' stand-alone criterion for a benign classification, those variants with an average frequency of >5% in the population databases were classified benign, with the exception of the known clinically significant variants, GenBank: NM_000410.3 (c.845G>A [p.Cys282Tyr]) and GenBank: NM_000410.3 (c.187C>G [p.His63Asp]), variants in *HFE* (MIM: 613609), and the factor V (*F5*) (MIM: 612309) Leiden variant (GenBank: NM_000130.4; c.1601G>A [p.Arg534Gln]). The GenBank: NM_000410.3 (c.187C>G [p.His63Asp]) variant that has a frequency of 10.6% in the ExAC database was reported only if that participant's partner had consented to be tested and carried a heterozygous, GenBank: NM_000410.3 (c.845G>A [p.Cys282Tyr]) variant in *HFE*. Additionally, well-described, common variants (such as GenBank: NM_004004.5; c.109G>A [p.Val37Ile] in *GJB2* [MIM: 121011] that has a frequency of 7.2% in the East Asian population) that met the criteria for pathogenicity albeit with high prevalence (>5%) in a specific ethnic population were retained. Based on the 5% threshold for filtering variants, approximately 98% of all variants were classified as benign.

For each participant, an average of four missense variants were identified that had a population frequency below the disease allele frequency for that respective gene, but with no published records in the medical literature at the time of analysis. These novel missense variants were classified as variants of uncertain significance (VUS) and were not reported.

We reported a total of 304 variants in this study. Approximately 92% (280/304) of variants passed the NGS variant quality filter (described in Subjects and Methods) and were confirmed by Sanger sequencing (CE), while 8.5% (26/304) of the variants did not pass the quality filter by NGS but met the criteria for pathogenicity and were confirmed by CE. In contrast, three variants were labeled as false positive calls from NGS-based analysis. These variants, which included two SNVs detected by NGS analysis pipeline and one CNV detected by the CNV analysis software, satisfied the criteria for pathogenicity but were not confirmed by CE and exon-centric aCGH, respectively, and therefore not reported.

Carrier Status and Types of Variants Reported

Among the 202 participants' samples analyzed, 78% received at least one positive carrier result. The average number of variants was 1.5 per individual, with a range of 0 to 5 variants and a mode of 1 variant per individual. We have reported all types of variants (Table S1) with known missense variants comprising the majority of a specific type of variant (48%). Table S2 lists all variants reported in this study. While most variants were reported within coding regions of the genes or affecting the canonical splice-site at the intron/exon junctions, we have reported five well-known pathogenic, non-coding variants that were not in the canonical splice site (Table 1).

Table 2. Classification of Putative Splice Variants before and after mRNA Analysis

Gene	Variant	Disorder	Condition Category	Variant Category	Initial Classification	Final Classification
CEP290 (MIM: 610142)	NM_025114.3 (c.6645+1G>A)	ciliopathies (MIM: 615991, 610188, 611755, 611134, 610189)	lifespan limiting	novel	likely pathogenic	likely pathogenic
CEP290 (MIM: 610142)	NM_025114.3 (c.6818+1_6818+2insGG)	ciliopathies (MIM: 615991, 610188, 611755, 611134, 610189)	lifespan limiting	novel	likely pathogenic	pathogenic
ERCC2 (MIM: 126340)	NM_000400.3 (c.594+2_594+5delTGAG)	trichothiodystrophy (MIM: 601675)	serious	novel	likely pathogenic	pathogenic
LRPPRC (MIM: 607544)	NM_133259.3 (c.469+1G>A)	Leigh syndrome, French-Canadian type (MIM: 220111)	lifespan limiting	novel	likely pathogenic	likely pathogenic
FAH (MIM: 613871)	NM_000137.2 (c.81+2T>A)	tyrosinemia type I (MIM: 276700)	serious	novel	likely pathogenic	VUS[a]

These genes are expressed in leukocytes. All individuals were heterozygotes.
[a]The assay results did not indicate a splicing defect in *FAH*; however, allele drop-out analysis was not performed, and further confirmation is necessary to determine the functional consequence of the GenBank: NM_000137.2 (c.81+2T>A) variant.

Approximately 64% of variants (195 of 304 total variants reported) were "distinct" (i.e., every reported variant counted only once). Within this distinct category, approximately 22% (44/195) were novel variants. The majority of the novel variants were classified as likely pathogenic, with the exception of some novel splice-site variants (Table 2) and CNVs (Table 3). For the genes that were expressed in blood (listed in Table 2), RNA-based splice-site analysis was performed on novel variants that were initially classified as likely pathogenic. RNA analysis was used as evidence to re-interpret the classification of the variant to determine whether the variant caused an in-frame exon skipping, out-of-frame exon skipping, intron retention, or no splicing defect. Thus, two variants were upgraded to pathogenic and one was downgraded to a VUS (Table 2).

Five individuals were identified as silent carriers for α-thalassemia (MIM: 604131); however, only four samples could be confirmed by multiplex PCR because there was no DNA available to confirm the fifth sample. Additionally, five other individuals harbored a CNV in a gene on either the carrier list or the medically actionable list (Table 3). Therefore, approximately 5% (10/202) of all participants were carriers of at least one clinically significant CNV.

Condition Categories Reported

The reported conditions comprised approximately 18% (134/728) of all the carrier gene-disorder pairs[6] analyzed in this study. Due to the small population size (n = 202) and ethnicity bias (78% were of European descent), there were variants and conditions that were observed multiple times (Table 4). For some disorders, the calculated carrier frequency in this study was higher than the estimated carrier frequency in the general population (Table 4). As expected, the common variants for *HFE*-associated hereditary hemochromatosis (MIM: 235200) comprised approximately 20% (40/202) of all heterozygotes in the study and 13% (40/304) of all variants reported.

Carrier Couples

Each couple's data were analyzed to determine whether they were carriers for the same condition as their respective partner. Not surprisingly, we identified carrier couples for a few common conditions: eight carrier couples for hereditary hemochromatosis (MIM: 235200), with each partner a carrier of either GenBank: NM_000410.3 (c.845G>A [p.Cys282Tyr]) or GenBank: NM_000410.3 (c.187C>G [p.His63Asp]), two couples for alpha-1 anti-trypsin deficiency (MIM: 613490), and one couple each for Factor V Leiden (MIM: 227400) and non-syndromic hearing loss ([MIM: 220290]; *GJB2*). We did not identify a carrier couple for any rare autosomal-recessive disorder. Three females were carriers for X-linked conditions, of which one was hemophilia A (MIM: 306700), a serious condition; the other two conditions were categorized as mild conditions (Table 5).

Medically Actionable (Secondary)

Based on our expanded medically actionable gene list (148 genes), additional findings were reported in 3.5% of participants (7 of 202); however, considering only the updated ACMG v2.0 list for secondary findings (59 genes),[11] the proportion of participants with such findings would be 2.9% (Table 6). The difference is due to the absence of *SERPINA1* (MIM: 107400) on the ACMG list. Although two participants were found to be compound heterozygotes in *HFE* (GenBank: NM_000410.3; c.845G>A [p.Cys282Tyr]; GenBank: NM_000410.3; c.187C>G [p.His63Asp]) for hereditary hemochromatosis, only homozygotes for the GenBank: NM_000410.3 (c.845G>A [p.Cys282Tyr]) variant in *HFE* would have been considered medically actionable. This study did not identify any individual who was homozygous for GenBank: NM_000410.3 (c.845G>A [p.Cys282Tyr]). In addition, we identified a male with a pathogenic variant, GenBank: NM_000059.3 (c.4965C>G [p.Tyr1655Ter]), in *BRCA2* (MIM: 600185). This variant was considered a carrier finding for Fanconi anemia (MIM: 605724) as well as a medically actionable

Table 3. CNVs Identified by NGS in Carriers and Confirmed by an Orthogonal Method

Gene	Deletion Variant	Disorder	Condition Category	Variant Category	Classification	No. of Heterozygotes
HBA2 (MIM: 141850)	whole gene (NM_000517.4)	alpha thalassemia (MIM: 604131)	carrier list: lifespan limiting	known	pathogenic	5[a]
FANCA (MIM: 607139)	exons 18–28 (NM_000135.3)	Fanconi anemia (MIM: 227650)	carrier list: serious	novel	likely pathogenic	1
TBCE (MIM: 604934)	exons 3–4 (NM_001079515.2)	hypoparathyroidism-retardation-dysmorphism-syndrome (MIM: 241410)	carrier list: serious	novel	pathogenic	1
INVS (MIM: 243305)	5′ UTR, exons 1 and 2 (NM_014425.4)	nephronophthisis 2 (MIM: 602088)	carrier list: lifespan limiting	novel	likely pathogenic	1
PROM1 (MIM: 604365)	5′ UTR and exon 1 (NM_006017.2)	retinitis pigmentosa 41 (MIM: 612095)	carrier list: mild	novel	likely pathogenic	1
BRCA1 (MIM: 113705)	5′ UTR exons 1–11 (NM_007300.3)	hereditary breast and ovarian cancer (MIM: 604370)	medically actionable	known	pathogenic	1

Variants were confirmed by high-density microarray, except the whole gene deletion of *HBA2*, which was confirmed by multiplex PCR (see Subjects and Methods).
[a]One variant was not confirmed because additional DNA was unavailable for confirmatory testing.

finding for an increased risk of male breast and prostate cancer (MIM: 114480 and 176807, respectively).

Moreover, we identified an incidental finding in an individual with mild hearing loss who harbored two variants, GenBank: NM_004004.5 (c.35delG [p.Gly12Valfs*2]; c.101T>C [p.Met34Thr]) in *GJB2* (MIM: 121011) for nonsyndromic hearing loss and deafness (DFNB1A [MIM: 220290]) (Table 6). This finding was not included in the medically actionable findings.

Discussion

One of the goals of this exploratory study was to learn more about the clinical utility of using GS for carrier screening in a clinical setting. To this end, a broad selection of gene/disorder pairs that would impact carrier status was analyzed. GS coupled with this large selection of gene/disorder pairs allowed us to increase the sensitivity of capturing most clinically significant variants. Recent studies have highlighted the advantages[41] and controversies[42] surrounding expanded carrier screening using an NGS-based approach. It is expected, as observed with this study, that clinically significant variants for nonserious and reduced penetrance, adult-onset conditions will be detected with high frequency. While the inclusion of mild or reduced penetrant conditions (hereditary hemochromatosis and factor V Leiden) may not be considered appropriate for clinical carrier screening, from a research perspective, it posits an unbiased approach to gather informative data on carrier status while offering autonomy of the participant's choices. As shown in our study, most participants requested results for all condition categories after appropriate genetic counseling.

In this study, the male partner of female carriers of pathogenic and likely pathogenic variants were invited to join the GS arm of the study. Again, only pathogenic and likely pathogenic variants in the genes reported for the female participant were reported to the male partner. This was designed to avoid prenatal diagnosis based on VUS results.

The NGS technology is advancing rapidly. Additionally, there is a simultaneous effort to improve and standardize the variant interpretation process.[37,43] To our knowledge, there are currently very few studies reported to use the 2015-ACMG guidelines for variant interpretation to analyze genomic data in individuals with no clinical phenotype. For novel variants that predicted a null effect in genes where loss-of-function is an established mechanism of disease, it was challenging to predict genotype-phenotype correlation. To add to the complexity of classifying novel variants in the absence of phenotype, it is also challenging to classify novel variants in a gene that is associated with clinical heterogeneity. For example, one participant was a carrier for a novel variant, GenBank: NM_020366.3 (c.1116delA [p.Lys372Asnfs*3]) in *RPGRIP1* (MIM: 605446). *RPGRIP1* pathogenic variants are associated with both Leber congenital amaurosis (MIM: 613826) and cone-rod dystrophy 3 (MIM: 608194), but it was not possible to predict for which condition the participant was a carrier. Thus, in our experience, the ability to accurately classify variants and predict outcomes is more challenging in a healthy population than in an affected individual and is less robust than in individuals presenting with an adverse phenotype.[44] While evidence based on phenotype is not a strong consideration in favor of pathogenicity according to the ACMG variant interpretation guidelines, sometimes highly specific phenotypic information does provide important evidence for interpreting variants associated with single-gene disorders.

Our finding of a variant in an X-linked condition, which was particularly impactful for a pregnancy outcome, illustrates the advantage of phenotypic information

Table 4. Genes with Variants Reported as Pathogenic or Likely Pathogenic More than Once

Gene	Variants Reported	Disorder	Condition Category	Variant Category	Classification	No. of heterozygotes (n = 202)	Frequency of Disorder in Study (n = 202)	Carrier Frequency (%)[a]
HFE (MIM: 613609)	NM_000410.3 (c.845G>A [p.Cys282Tyr])	hereditary hemochromatosis (MIM: 235200)	adult onset	Known	pathogenic	30	20%	6%–13%
	NM_000410.3 (c.187C>G [p.His63Asp])[b]			Known	pathogenic	10		
GJB2 (MIM: 121011)	NM_004004.5 (c.35delG [p.Gly12Valfs*2])	nonsyndromic, hearing loss (MIM: 220290)	mild	Known	pathogenic	3	9%	2.3%
	NM_004004.5 (c.35dupG [p.Val13Cysfs])			Known	pathogenic	1		
	NM_004004.5 (c.109G>A [p.Val37Ile])			Known	likely pathogenic	4		
	NM_004004.5 (c.−23+1G>A)			Known	pathogenic	1		
	NM_004004.5 (c. 101T>C [p.Met34Thr])			Known	likely pathogenic	4		
	NM_004004.5 (c.269T>C [p.Leu90Pro])			Known	pathogenic	3		
	NM_004004.5 (c.416G>A [p.Ser139Asn])			Known	likely pathogenic	1		
	NM_004004.5 (c.−22−2A>C)			Known	pathogenic	1		
F5 (MIM: 612309)	NM_000130.4 (c.1601G>A [p.Arg534Gln])	factor V Leiden thrombophilia (MIM: 227400)	unpredictable	known	pathogenic	17	8%	3%–8%
SERPINA1 (MIM: 107400)	NM_001127700.1 (c.1096G>A [p.Glu366Lys])	alpha-1 antitrypsin deficiency (MIM: 613490)	adult onset	known	pathogenic	6	6%	2.4%–4.8%
	NM_001127700.1 (c.863A>T [p.Glu288Val])			known	pathogenic	6		
ABCA4 (MIM: 601691)	NM_000350.2 (c.6089G>A [p.Arg2030Gln])	cone rod dystrophy 3 (MIM: 604116); Stargardt disease (MIM: 248200)	mild	known	pathogenic	1	4%	2%
	NM_000350.2 (c.1964T>G [p.Phe655Cys])			known	likely pathogenic	2		
	NM_000350.2 (c.4139C>T [p.Pro1380Leu])			known	pathogenic	1		
	NM_000350.2 (c.2588G>C [p.Gly863Ala])			known	likely pathogenic	2		
	NM_000350.2 (c.5882G>A [p.Gly1961Glu])			known	pathogenic	3		

(*Continued on next page*)

Table 4. ***Continued***

Gene	Variants Reported	Disorder	Condition Category	Variant Category	Classification	No. of heterozygotes (n = 202)	Frequency of Disorder in Study (n = 202)	Carrier Frequency (%)[a]
CYP21A2[c] (MIM: 613815)	NM_000500.8 (c.1360C>T [p.Pro454Ser])	congenital adrenal hyperplasia (MIM: 201910)	serious	known	pathogenic	3	4%[d]	1.6%–6%[e]
	NM_000500.8 (c.844G>T [p.Val282Leu])			known	pathogenic	3		
	NM_000500.8 (c.955C>T [p.Gln319Ter])			known	pathogenic	2		
CFTR (MIM: 602421)	NM_000492.3 (c.1521_1523delCTT [p.Phe508delPhe])	cystic fibrosis (MIM: 219700)	serious	known	pathogenic	6	3%	4%
SPG7 (MIM: 602783)	NM_003119.3 (c.1529C>T [(p.Ala510Val))	spastic paraplegia 7 (MIM: 607259)	adult onset	known	pathogenic	3	2%	0.9%–1.5%
	NM_003119.3 (c.1045G>A [p.Gly349Ser])			known	pathogenic	2		

All variants use the HGVS nomenclature.
[a]Source: GeneReviews or Genetics Home Reference.
[b]The H63D variant in *HFE* was analyzed and reported only if the partner was a carrier of GenBank: NM_000410.3 (c.845G>A [p.Cys282Tyr]).
[c]The coding DNA nomenclature is used instead of the protein sequence nomenclature to avoid the discrepancy associated with the latter when using the hg19 genomic reference sequence; the coding DNA is also used for nomenclature for splice/intronic variants; one participant was homozygous for the *SERPINA1* GenBank: NM_001127700.1 (c.1096G>A [p.Glu366Lys]) allele and was excluded from the table.
[d]Combined classic and non-classic with 3% carrying a variant for the non-classic form and 1% carrying a variant for the classic form.
[e]Combined frequency for classic and non-classic form; most of these variants were observed more than once.

for variant interpretation. In a female participant, we reported a heterozygous, nonsense variant in *F8* (MIM: 300841), which is associated with hemophilia A (MIM: 306700). This variant was novel and the limited evidence satisfied the criteria for only a likely pathogenic classification. The participant, who was already pregnant at the time of receiving this result, opted for prenatal testing. Her male fetus was found to harbor this variant, and subsequently, the newborn developed a complication, an acute subgaleal hemorrhage that is associated with the severe form hemophilia A. The prior knowledge that this infant was suspected to be affected with hemophilia A helped guide the immediate treatment plan (red cell and platelet transfusions with anti-hemophilic factor/von Willebrand factor complex) to avoid a fatal outcome. The additional evidence on phenotype prompted variant re-classification to pathogenic. Being novel, this variant would not have been detected on a targeted mutation panel. Overall, we conclude that the 2015-ACMG variant interpretation guideline is a powerful tool for systematic and organized classification for rare and novel variants that are detected by GS.

The variant classification process is continually evolving and this may explain the discrepancies in the carrier status results between our study and those from a previous study by Bell et al.[45] In the latter study, the authors focused on 448 recessive disorders in 104 individuals and reported a carrier burden of 2.8 per individual. In contrast, we analyzed more gene/disorder pairs (728) but reported 1.5 clinically significant variants per individual. The databases used for variant interpretation in the Bell et al. study[45] were limited compared with the currently available resources and the criteria for selecting pathogenicity of variants were also different. Of note, more than 70% (76/104) of individuals in the Bell et al. study[45] were either affected or known carriers of severe pediatric conditions, but in our study, only 3% of participants were previously known to be carriers for cystic fibrosis (MIM: 219700).

We also identified 3.5% of participants with a variant in a gene on our medically actionable list. While our list of genes (148 genes) was more extensive than that recommended by ACMG[11,46] (59 genes), all of the medically actionable findings detected in this study were in genes on the ACMG list, except *SERPINA1*. This result is consistent with that of a previous report[7] that used an expanded medically actionable findings list. Considering only the ACMG list (i.e., excluding *SERPINA1*), our rate of secondary findings also remains consistent with that of another study on 2,000 exomes.[47]

It is important to note that the lack of ethnic diversity and the small size of our individual cohort represented some limitations to our study. Although the study was designed to be offered to a pan-ethnic population, the data from this study were biased toward the fact that the majority of our participants were of European descent. Furthermore, data from 202 participants have limited

Table 5. Carrier Females for X-Linked Disorders

Gene	Variant	Disorder	Condition Category	Variant Category	Classification
F8 (MIM: 300841)	NM_000132.3 (c.3144G>A [p.Trp1048Ter])	hemophilia A (MIM: 306700)	serious	novel	pathogenic
TRAPPC2 (MIM: 300202)	NM_001128835.2 (c.12G>A [p.Trp4Ter])	X-linked spondyloepiphyseal dysplasia tarda (MIM: 313400)	mild	novel	likely pathogenic
G6PD (MIM: 305900)	NM_001042351.2 (c.376A>G [p.Asn126Asp]); NM_001042351.2 (c.202G>A[a] [Val68Met])	G6PD deficiency (MIM: 300908)	mild	known	pathogenic

[a]The A-haplotype in *G6PD* comprises two variants, GenBank: NM_001042351.2 (c.376A>G [p.Asn126Asp]) and GenBank: NM_001042351.2 (c.202G>A [p.Val68Met]) that are present in *cis*.

statistical power for analysis of carrier frequency. The small sample size may be a factor in the observed carrier frequency of certain conditions above the expected value. For example, in *SPG7* (MIM: 602783), which is associated with spastic paraplegia 7 (MIM: 607259) that has a reported prevalence of 2–6:100,000 (GeneReviews in Web Resources), the GenBank: NM_003119.3 (c.1529C>T [p.Ala510Val]) variant has an allele frequency of 0.0025 in the ExAC database; however, in our study, it was observed three times. While the frequency in our study suggested a variant of uncertain significance, we classified it as pathogenic based on other published evidence for its pathogenicity.[43]

Compared with mutation screening panels that were traditionally designed to target certain ethnic populations, NGS technologies are much better at detecting rare and novel pathogenic variants in a pan-ethnic population. Some of the current drawbacks of GS include the inability to detect mosaicism because of low read depth, high data storage, and generating a large number of VUSs. However, within existing NGS platforms, GS can address some of the limitations of a capture or amplicon-based sequencing approach. GS can bypass the disadvantages of PCR-based library preparation, which can be a source of introducing variant artifacts and PCR biases resulting in a non-uniform representation of the DNA library. It is a superior method for determining structural variation in the genome because the exact breakpoint in the DNA sequence can be identified. Finally, from a cost perspective, it can overcome the continuous need to re-design and validate clinical targeted gene panels when new pathogenic variants are identified in non-coding regions.

Current clinical NGS applications still do not have the sensitivity and specificity to detect all types of variants,

Table 6. Secondary and Incidental Findings in Participants

Gene	Variant	Disorder	Condition Category	Variant Category	Classification
BRCA1 (MIM: 113705)	NM_007300.3 (c.2071delA [p.Arg691Aspfs*10])	hereditary breast and ovarian cancer (MIM: 604370, 612555)	medically actionable	known	pathogenic
	NM_007300.3 (c.3485delA [p.Asp1162Valfs*48])			known	pathogenic
	NM_007300.3 (c.(?_-30)_(4185+1_4186-1)del)			known	pathogenic
BRCA2[a] (MIM: 600185)	NM_000059.3 (c.4965C>G [p.Tyr1655Ter])		medically actionable and serious (carrier)	known	pathogenic
SERPINA1[b] (MIM: 107400)	NM_001127700.1 (c.1096G>A [p.Glu366Lys])	alpha-1 antitrypsin deficiency (MIM: 613490)	medically actionable	known	pathogenic
APC (MIM: 611731)	NM_000038.5 (c.1042C>T [p.Arg348Ter])	*APC*-associated polyposis conditions (MIM: 175100)	medically actionable	known	pathogenic
GJB2[c] (MIM: 121011)	NM_004004.5 (c.35delG [p.Gly12Valfs*2]); NM_004004.5 (c.101T>C [Met34Thr])	nonsyndromic hereditary hearing loss (DFNB1) (MIM: 220290)	carrier list: mild	known	pathogenic
SMAD3 (MIM: 603109)	NM_005902.3 (c.484G>T [p.Glu162Ter])	Loeys-Dietz syndrome type 3 1C (MIM: 613795)	medically actionable	novel	likely pathogenic

These results are based on screening 130 female and 69 male participants; 3 individuals did not opt-in to receive these results.
[a]Carrier for Fanconi anemia.
[b]Homozygous GenBank: NM_001127700.1 (c.1096G>A [p.Glu366Lys]) (PI*ZZ).
[c]Incidental finding for the GenBank: NM_004004.5 (c.35delG [p.Gly12Valfs*2]); NM_004004.5 (c.101T>C [p.Met34Thr]) variants in one individual presumed to be in *trans*.

such as those causing triplet repeat disorders (e.g., fragile X [MIM: 300624]) and regions of the genome with high homology (pseudogenes). Due to the inherent limitations of the short-read approach in NGS platforms that are currently utilized in most clinical laboratories, multiple methodologies would need to be used to support detection of the full range of variant classes. However, these challenges will be short-lived because the implementation of long-read DNA sequencing (third generation) technologies[48] coupled with advances in bioinformatic pipelines for detecting short tandem repeats[49] and copy number variation[50] is imminent in the clinical laboratory. NGS is a paradigm shift in the rate at which carrier status is determined for several hundred disorders simultaneously. It may soon replace other methodologies and become a unifying platform for performing most molecular genetic tests.

Accession Numbers

The accession number for the sequence data reported in this paper is dbGap: phs00927.

Supplemental Data

Supplemental Data include one figure, two tables, and Supplemental Note (clinical report) and can be found with this article online at https://doi.org/10.1016/j.ajhg.2018.04.004.

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Web Resources

ClinVar, https://www.ncbi.nlm.nih.gov/clinvar/
dbGaP, https://www.ncbi.nlm.nih.gov/gap
GenBank, https://www.ncbi.nlm.nih.gov/genbank/
GeneReviews, Casari, G., and Marconi, R. (1993). Spastic Paraplegia 7. https://www.ncbi.nlm.nih.gov/books/NBK1107/
OMIM, http://www.omim.org/
SeattleSeq Annotation 138, http://snp.gs.washington.edu/SeattleSeqAnnotation138/

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Supplemental Data

Preconception Carrier Screening by Genome Sequencing: Results from the Clinical Laboratory

Sumit Punj, Yassmine Akkari, Jennifer Huang, Fei Yang, Allison Creason, Christine Pak, Amiee Potter, Michael O. Dorschner, Deborah A. Nickerson, Peggy D. Robertson, Gail P. Jarvik, Laura M. Amendola, Jennifer Schleit, Dana Kostiner Simpson, Alan F. Rope, Jacob Reiss, Tia Kauffman, Marian J. Gilmore, Patricia Himes, Benjamin Wilfond, Katrina A.B. Goddard, and C. Sue Richards

Supplemental Note: Clinical Report

Carrier Results: Four Known Pathogenic Variants Detected.

Gene	Inheritance	Disease	Prevalence	Variant	Classification
SLC26A4	Autosomal Recessive	Pendred Syndrome/ Non-syndromic Hearing Loss DFNB4 with enlarged vestibular aqueduct	1/500[A]	c.1246A>C, (p.Thr416Pro)	Pathogenic
SPG7	Autosomal Recessive	Spastic Paraplegia 7	2-6/100,000[++]	c.1045G>A, (p.Gly349Ser)	Pathogenic
HBA2	Autosomal Recessive	Alpha Thalassemia	1-5/10,000[+++]	$-\alpha^{3.7}$ (α^{+}- thalassemia)	Pathogenic
HFE	Autosomal Recessive	Hereditary Hemochromatosis	1/200 – 1/1000+	c.845G>A (p.Cys282Tyr)	Pathogenic

+: GeneReviews; ++: Genetics Home Reference; +++: orphan.net – varies with population; A- Generalized prevalence of all deafness and hearing loss

Interpretation:

A sample from this individual was referred to our laboratory for analysis of Next-Generation Genome Sequencing (NGS) and Sanger confirmation of variants identified in carrier screening for: (1) conditions with significantly shortened lifespan; (2) serious conditions; (3) mild conditions; (4) conditions with unpredictable outcomes: and (5) conditions that begin as adults.

One known heterozygous missense variant, c.1246A>C (p.Thr416Pro) (NM_000441.1), was detected in exon 10 of the *SLC26A4* gene of this individual by NGS. This study indicated that this individual is a carrier (i.e. not affected) of a pathogenic variant in the *SLC26A4* gene. The *SLC26A4* gene encodes an anion transporter known as pendrin (OMIM#: 605646). Pathogenic variants in the *SLC26A4* gene are associated with autosomal recessive Pendred Syndrome/ Non- syndromic Hearing Loss DFNB4 with enlarged vestibular aqueduct (MIM#: 274600;600791). Individuals affected with Pendred syndrome experience prelingual onset of severe-to-profound bilateral sensorineural hearing impairment with vestibular dysfunction, temporal bone abnormalities, and enlarged thyroid (goiter). DFNB4 is characterized as nonsyndromic sensorineural hearing impairment, vestibular dysfunction, and enlarged vestibular aqueduct without thyroid defects (GeneReviews: Fatemeh *et al*., 2014, http://www.ncbi.nlm.nih.gov/books/NBK1467/). The prevalence of all hearing loss is 1/500 with a carrier frequency of 1/12. This condition is autosomal recessive and is considered a mild condition. We have confirmed this finding in our laboratory using Sanger sequencing.

One known heterozygous missense variant, c.1045G>A (p.Gly349Ser) (NM_003119.2), was detected in exon 8 of the *SPG7* gene of this individual by NGS. This study indicated that this individual is a carrier (i.e. not affected) of a pathogenic variant in the *SPG7* gene. The *SPG7* gene encodes paraplegin, a component of the m-AAA protease, and an ATP-dependent proteolytic complex of the mitochondrial inner membrane that degrades misfolded proteins and regulates ribosome assembly (MIM# 607259). The disorder, spastic paraplegia 7 is characterized by progressive bilateral lower limb weakness and spasticity, and usually presents in early adulthood. Additional features such as hyperreflexia in the arms, sphincter disturbances, spastic dysarthria, dysphagia, pale optic disks, ataxia, nystagmus, strabismus, decreased hearing, scoliosis, pes cavus, motor and sensory neuropathy, and amyotrophy may be observed (Gene Reviews, Casari G. and Marconi R.: http://www.ncbi.nlm.nih.gov/books/NBK1107/). This condition is autosomal recessive and is considered an adult-onset condition. We have confirmed this finding in our laboratory using Sanger sequencing.

One known whole-gene deletion variant of *HBA2* was detected in this individual by NGS. This study indicated that this individual is a silent carrier (i.e., not affected) of a pathogenic variant for the alpha thalassemia disorder. *HBA1* and *HBA2* genes encode the α-globin chains in the hemoglobin molecule. Hemoglobin, the molecule that carriers oxygen in the red blood cells, is a tetramer that comprises two α-globin and two β-globin chains. The duplicate copies of the α- globin genes (*HBA1* and *HBA2*) are on the same chromosome; therefore a normal individual carries four copies of the α-globin gene (2 on each chromosome; αα/αα). Pathogenic variants in α-globin genes can result in complete deletion of one or more copies of the gene or non-deletion variants (GeneReviews: Origa R *et al*., last update 2013). The loss of α-globin chains as a result of these pathogenic variants leads to α-thalassemia (alpha- thalassemia). Pathogenic variants in both genes on the same chromosome (in *cis*) are associated with α^0 – thalassemia and variants affecting only one gene are associated with α^+ – thalassemia. An individual's genotype can determine if they are: silent carriers (-α/αα); alpha- thalassemia trait (--/αα or – α/- α); HbH disease (--/-α) and Hb Bart's hydrops foetalis (--/--). Clinical symptoms can range from asymptomatic (silent carriers) to severely affected (Hb Bart's hydrops foetalis). α-thalassemia is a common disorder and its prevalence varies among various ethnic groups. According to the WHO, the carrier frequency of α^+ – thalassemia (Heterozygous and homozygous) is approximately 5% in the American population (Modell B and Darlison M, 2008 http://www.who.int/bulletin/volumes/86/6/06-036673-table-T1.html). Alpha – thalassemia is autosomal recessive and is considered a life-span limiting condition. We have confirmed this variant by a multiplex PCR assay.

A well-known heterozygous missense variant, c.845G>A (p.Cys282Tyr) (NM_000410.3), was detected in exon 4 of the *HFE* gene of this individual by NGS. This study indicated that this individual is a carrier (ie., not affected) of a pathogenic variant in the *HFE* gene which causes Hereditary Hemochromatosis (HH, MIM#: 235200). *HFE*- associated Hereditary Hemochromatosis (*HFE*-HH) is a common disorder that is characterized by inappropriately high absorption of iron by the gastrointestinal mucosa. (GeneReviews: Seckington *et al*., 2015, http://www.ncbi.nlm.nih.gov/books/NBK1440/). The prevalence of

HFE-HH is 1/200 to 1/1,000, and the carrier frequency is between 1/8 and 1/16. This is an autosomal recessive disorder and is considered an adult-onset condition.

It is important to understand that next generation genome sequencing is a screening test. This individual could carry a variant not detected by this test, or in genes that are not analyzed (see test limitations). In addition, only known pathogenic and likely pathogenic variants are reported.

Recommendations:
In general, we recommend that the reproductive partner of an individual who is a carrier for an autosomal recessive condition be tested. Genetic counseling is recommended.

Evidence for Variant Interpretations:
c.1246A>C (p.Thr416Pro) in Exon 10 of the *SLC26A4* gene (NM_000441.1, chr7:107330665) is interpreted as Pathogenic.
The c.1246A>G (p.Thr416Pro) missense variant in the *SLC26A4* gene is one of the most common pathogenic variants associated with Pendred Syndrome and Non-syndromic Hearing Loss DFNB4 with enlarged vestibular aqueduct as it has been previously reported in multiple affected individuals (Van Hauwe *et al*. 1998, Ladsous M *et al*., 2014). This indicates that the prevalence of this variant is significantly higher in cases compared with controls. Several *in vitro* functional studies have demonstrated that this variant has a damaging effect on the protein's intracellular localization and function (Rotman-Pikielny P *et al*., 2002; Yoon JS *et al*., 2008; Scott DA *et al*., 2000). This variant was also shown to co-segregate with disease in multiple families (Van Hauwe *et al*. 1998). The frequency of this variant is either absent or below that of the disease allele frequency (Exome Sequencing Project [ESP] = 0.021%, 1000 Genomes = NA, ExAC = 0.0021). Multiple *in silico* algorithms predict this variant to have a deleterious effect GERP = 5.10; CADD = 19.47; PolyPhen = 1; SIFT = 0). Finally, several reputable diagnostic laboratories have reported this variant as Pathogenic. Therefore, this collective evidence supports the classification of the c.1246A>C (p.Thr416Pro) as a recessive Pathogenic variant. We have confirmed this variant by Sanger sequencing.

c.1045G>A (p.Gly349Ser) in Exon 8 of the *SPG7* gene (NM_003119.2 3, chr16: 89598369) is interpreted as Pathogenic.
The c.1045G>A (p.Gly349Ser) variant has been observed in a compound heterozygous state with truncating and missense variants, including the p.A510V variant, in several affected individuals (Choquet K *et al.*, 2016; Bonn *et al.* 2010; Brugman et al. 2008; van Gassen KL *et al*., 2012; Schlipf NA et al., 2011). Moreover, this variant is located in the AAA_core (ATPase) domain of the protein, and using a yeast complementation assay system, Bonn *et al*. (2010) showed this variant inhibited respiratory growth in yeast. The frequency of the c.1045G>A (p.Gly349Ser) variant is below that of the disease allele frequency (absent in 1000 Genome and 0.17% in Exome Sequencing Project [ESP]). This variant was also shown to co-segregate with disease in two families (Choquet K *et al.*, 2016; Bonn *et al.* 2010). Finally, computational algorithms predict this variant to be damaging to the protein (GERP = 5.85; CADD = 29.7; PolyPhen = 1; SIFT = 0). A reputable clinical laboratory has recently classified this variant as Pathogenic. Together, this evidence is consistent with a Pathogenic classification. We have confirmed this variant by Sanger sequencing.

The 3.7kb deletion allele of the *HBA2* gene is interpreted as Pathogenic (α^+ – thalassemia silent carrier).

Individuals who are silent carriers of α – thalassemia have only three functional copies of the α-globin gene. Because the two genes are located between homologous elements within this locus, there are several possible breakpoints for deletions. The most common deletions associated with α^+ – thalassemia heterozygous (silent carrier) are the 3.7 kb deletion or the 4.2 kb deletions (Harteveld CL and Higgs DR, 2010), both of which involve deletion of the *HBA2* gene (GeneReviews: Origa R *et al*., last update 2013). Clinically, these individuals can either be asymptomatic or present with a mild reduction in mean corpuscular volume (MCV), moderate microcytosis and hypochormia (Galanello R and Cao A, 2011). We have confirmed this variant by a multiplex PCR assay.

c.845G>A, p.Cys282Tyr in Exon 4 of the *HFE* gene, (NM_000410.3, chr6: 26093141) is interpreted as Pathogenic.

The c.845G>A (p.Cys282Tyr) missense variant is widely recognized as one of the two most common disease-causing variants in the *HFE* gene. Cys282Tyr homozygotes account for 80-85% of typical patients with Hereditary Hemochromatosis (HH). However, the majority of individuals who are homozygous for this variant do not develop the disease (GeneReviews: Seckington *et al*., 2015, http://www.ncbi.nlm.nih.gov/books/NBK1440/; Ramrakhiani S, Bacon BR., 1998, Morrison ED *et al*., 2003). In summary, this variant c.845G>A (p.Cys282Tyr) meets our criteria for a recessive Pathogenic classification. We have confirmed this finding in our laboratory using Sanger sequencing.

Method:

Next-generation genome sequencing was performed in the Illumina CLIA laboratory. Genomic DNA was prepped with TruSeq DNA LT and then sequenced on a HiSeq 2000 or 2500 (Illumina, version 3 chemistry) with 100bp paired-end reads. Resulting sequences were aligned to the human genome reference (hg19) using the Burrows-Wheeler Aligner (BWA) and variants identified with the Genome Analysis Toolkit (GATK) at the University of Washington (UW). A modified version of the SeattleSeq tool was used to annotate variants found within a defined set of colon cancer and actionable genes. OHSU laboratory analyzed the annotated variant list to identify pathogenic variants in the attached gene list for evaluation of carrier status. For confirmation studies, genomic DNA was extracted in our laboratory using the Puregene extraction method, and pathogenic variants were confirmed by custom designed Sanger sequencing. The sequence was assembled and analyzed in comparison to the published reference sequence for each gene in which a pathogenic variant was identified. Only known pathogenic and likely pathogenic variants were confirmed and reported.

The NGS data was also assessed for the average depth of coverage and data quality threshold values:

Mean Depth of Coverage[1]	42.7X
Quality threshold[2]	87.73%

[1]Mean depth of coverage refers to the sequence mean read depth across the genome.

[2]The quality threshold refers to the percentage of the genome where read depth was at least 30X coverage to permit high quality variant base calling, annotation and evaluation. Average quality threshold is 87.73% at >=30X of the genome, indicating that a small portion of the target region may not be covered with sufficient depth or quality to call variant positions confidently.

***HBA2* – gene deletion assay:**

A multiplex PCR assay, as described by Liu YT *et al*., (2000) was used to determine deletions at the alpha thalassemia locus.

List of Carrier Status Genes Covered at Less Than 10X Read Depth:

1. Survival Motor Neuron 1: *SMN1*
2. Hemoglobin, alpha 2: *HBA2*

Limitations:

1. This assay has limited ability to detect large deletions or duplications as well as small insertions and deletions.
2. This test also has limited ability to detect mosaicism.
3. This test does not provide complete coverage of all coding exons.
4. Noncoding regions may have limited information and limited ability to interpret.
5. The assay does not detect variants located:
    a. in regions of insufficient coverage,
    b. in regions containing paralogous genes or pseudogenes,
    c. in regions where the reference genome is inaccurate or contains gaps and insertions,
    d. in regions of high GC content
6. All identified variants of uncertain significance are not reported.
7. Genes not associated clinically with Mendelian disorders at the time this test was performed were not analyzed.
8. Genes not analyzed.

References:

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Genes and Associated Diseases:

CATEGORY: LIFESPAN LIMITING

AARS2- COMBINED OXIDATIVE PHOSPHORYLATION DEFICIENCY 8, ***ABCA12***- ICHTHYOSIS CONGENITA, HARLEQUIN FETUS, ***ACAD9***- MITOCHONDRIAL COMPLEX I DEFICIENCY DUE TO ACAD9 DEFICIENCY, ***ACE***- RENAL TUBULAR DYSGENESIS, ***ACOX1***- PEROXISOMAL ACYL-COA OXIDASE DEFICIENCY, ***AGPS***- RHIZOMELIC CHONDRODYSPLASIA PUNCTATA,3, ***AGT***- RENAL TUBULAR DYSGENESIS, ***AGTR1***- RENAL TUBULAR DYSGENESIS, ***ALG1***- CONGENITAL DISORDER OF GLYCOSYLATION,IK, ***AMACR***- BILE ACID SYNTHESIS DEFECT,CONGENITAL,4, ***AMT***- GLYCINE ENCEPHALOPATHY, ***ARX***- CORPUS CALLOSUM AGENESIS OF WITH ABNORMAL GENITALIA; EPILEPTIC ENCEPHALOPATHY,EARLY INFANTILE,1; LISSENCEPHALY, X-LINKED, 2, ***ATP7A***- MENKES DISEASE, ***ATPAF2***- MITOCHONDRIAL COMPLEX V (ATP SYNTHASE) DEFICIENCY, NUCLEAR TYPE 1, ***B9D1***- MECKEL SYNDROME 9, ***B9D2***- MECKEL SYNDROME 10, ***BCS1L***- GRACILE SYNDROME; MITOCHONDRIALCOMPLEX III DEFICIENCY, ***C5ORF42***- JOUBERT SYNDROME 17, ***CC2D2A***- COACH SYNDROME; JOUBERT SYNDROME 9; MECKEL SYNDROME, TYPE 6, ***CDKL5***- EPILEPTIC ENCEPHALOPATHY,EARLY INFANTILE,2, ***CEP290***- JOUBERT SYNDROME 5; LEBER CONGENITAL AMAUROSIS 10; MECKEL SYNDROME, TYPE 4, ***CHRNA1***- MULTIPLE PTERYGIUM SYNDROME, LETHAL, ***CHRND***- MULTIPLE PTERYGIUM SYNDROME, LETHAL, ***CHRNG***- MULTIPLE PTERYGIUM SYNDROME, LETHAL, ***CLN6***- CEROID LIPOFUSCINOSIS, NEURONAL,6, ***CNTN1***- MYOPATHY, CONGENITAL, COMPTON-NORTH, ***COQ6***- COENZYME Q10 DEFICIENCY, PRIMARY, 6, ***COQ9***- COENZYME Q10 DEFICIENCY, PRIMARY, 5, ***COX10***- LEIGH SYNDROME, ***COX15***- CARDIOENCEPHALOMYOPATHY, FATAL INFANTILE, DUE TO CYTOCHROME C OXIDASE DEFICIENCY 2, ***CPT1A***- CARNITINE PALMITOYLTRANSFERASE DEFICIENCY 1A, ***CRTAP***- OSTEOGENESIS IMPERFECTA, TYPE VII, ***CTSD***- CEROIDLIPOFUSCINOSIS,NEURONAL, 10, ***DCX***- LISSENCEPHALY1, ***DGUOK***- MITOCHONDRIAL DNA DEPLETION SYNDROME 3, ***DNAJC19***- 3-METHYLGLUTACONIC ACIDURIA,V, ***DOK7***- FETAL AKINESIA DEFORMATION SEQUENCE, ***DOLK***- CONGENITAL DISORDER OF GLYCOSYLATION, IM, ***DSP***- EPIDERMOLYSIS BULLOSA, LETHAL ACANTHOLYTIC, ***EFEMP2***- CUTIS LAXA, TYPE IB, ***EIF2AK3***- MULTIPLE EPIPHYSEAL DYSPLASIAWITH EARLY ONSET DIABETES MELLITUS, ***ENPP1***- ARTERIAL CALCIFICATION, GENERALIZED,OF INFANCY, ***ERBB3***- LETHAL CONGENITAL CONTRACTURE SYNDROME 2, ***ERCC6***- CEREBROOCULOFACIOSKELETAL SYNDROME1; COCKAYNE SYNDROME,B, ***ERCC8***- COCKAYNE SYNDROME,A, ***ESCO2***- ROBERTS SYNDROME, ***ETFA***- MULTIPLE ACYL-COA DEHYDROGENASE DEFICIENCY, ***ETFB***- MULTIPLE ACYL-COA DEHYDROGENASE DEFICIENCY, ***ETFDH***- MULTIPLE ACYL-COA DEHYDROGENASE DEFICIENCY, ***ETHE1***- ETHYLMALONIC ENCEPHALOPATHY, ***FANCB***- FANCONI ANEMIA, COMPLEMENTATION GROUP B, ***FAM20C***- RAINE SYNDROME, ***FARS2***-

COMBINED OXIDATIVE PHOSPHORYLATION DEFICIENCY 14, ***FBLN5***- CUTIS LAXA,IA, ***PKHD1***- POLYCYSTIC KIDNEY DISEASE, AUTOSOMAL RECESSIVE, ***FGFR2***- ANTLEY-BIXLER SYNDROME WITHOUT GENITAL ANOMALIES OR DISORDERED STEROIDOGENESIS, ***FH***- FUMARASE DEFICIENCY, ***FKTN***- MUSCULAR DYSTROPHY-DYSTROGLYCANOPATHY (CONGENITAL WITH BRAIN AND EYE ANOMALIES), TYPE A, ***FOXP3***- IMMUNODYSREGULATION, POLYENDOCRINOPATHY, AND ENTEROPATHY, ***GALC***- KRABBE DISEASE, ***GFM1***- COMBINED OXIDATIVE PHOSPHORYLATION DEFICIENCY 1, ***GLB1***- GM1-GANGLIOSIDOSIS, TYPE I, ***GLE1***- LETHAL CONGENITAL CONTRACTURE SYNDROME1, ***GNPTAB***- MUCOLIPIDOSIS II ALPHA/BETA, ***GUSB***- MUCOPOLYSACCHARIDOSIS VII, ***HBA1***- ALPHA THALASSEMIA, ***HBA2***- ALPHA THALASSEMIA, ***HEXA***- TAY-SACHS DISEASE, ***HIBCH***- 3-HYDROXYISOBUTYRYL-COA HYDROLASE DEFICIENCY, ***HSD17B4***- D-BIFUNCTIONAL PROTEIN DEFICIENCY, ***HSPG2***- DYSSEGMENTAL DYSPLASIA, SILVERMAN-HANDMAKER TYPE, ***HYLS1***- HYDROLETHALUS SYNDROME 1, ***IDUA***- HURLER SYNDROME, ***IGHMBP2***- SPINAL MUSCULAR ATROPHY, DISTAL,1, ***IKBKG***- ECTODERMAL DYSPLASIA, ANHIDROTIC, WITH IMMUNODEFICIENCY, OSTEOPETROSIS, AND LYMPHEDEMA, ***INSR***- DONOHUE SYNDROME, ***INVS***- NEPHRONOPHTHISIS 2, ***ITGA6***- EPIDERMOLYSIS BULLOSA JUNCTIONALIS WITH PYLORIC ATRESIA, ***ITGB4***- EPIDERMOLYSIS BULLOSA JUNCTIONALIS WITH PYLORIC ATRESIA, ***KLHL40***- NEMALINE MYOPATHY 8, ***LAMA3***- EPIDERMOLYSIS BULLOSA, JUNCTIONAL, NONHERLITZ, ***LAMB2***- PIERSON SYNDROME, ***LAMB3***- EPIDERMOLYSIS BULLOSA, JUNCTIONAL, NONHERLITZ, ***LAMC2***- EPIDERMOLYSIS BULLOSA, JUNCTIONAL,NONHERLITZ, ***LBR***- GREENBERG DYSPLASIA, ***LIAS***- PYRUVATE DEHYDROGENASE LIPOIC ACID SYNTHETASE DEFICIENCY, ***LIFR***- STUVE-WIEDEMANN SYNDROME, ***LMNA***- RESTRICTIVE DERMOPATHY, LETHAL, ***LRPPRC***- LEIGH SYNDROME,FRENCH CANADIAN, ***MKS1***- BARDET-BIEDL SYNDROME 13; MECKEL SYNDROME,1, ***MOCS1***- MOLYBDENUM COFACTOR DEFICIENCY, COMPLEMENTATION GROUP A, ***MOCS2***- MOLYBDENUM COFACTOR DEFICIENCY, COMPLEMENTATION GROUP B, ***MPL***- AMEGAKARYOCYTIC THROMBOCYTOPENIA,CONGENITAL, ***MRPS22***- COMBINED OXIDATIVE PHOSPHORYLATION DEFICIENCY 5, ***MYO5A***- ELEJALDE DISEASE; GRISCELLI SYNDROME,1, ***NDUFA11***- MITOCHONDRIAL COMPLEX I DEFICIENCY, ***NDUFAF2***- MITOCHONDRIAL COMPLEX I DEFICIENCY, ***NDUFAF3***- MITOCHONDRIAL COMPLEX I DEFICIENCY, ***NDUFAF4***- MITOCHONDRIAL COMPLEX I DEFICIENCY, ***NDUFAF6***- LEIGH SYNDROME, ***NDUFB3***- MITOCHONDRIAL COMPLEX I DEFICIENCY, ***NDUFS1***- MITOCHONDRIAL COMPLEX I DEFICIENCY, ***NDUFS2***- MITOCHONDRIAL COMPLEX I DEFICIENCY, ***NDUFS4***- LEIGH SYNDROME, ***NDUFS6***- MITOCHONDRIAL COMPLEX I DEFICIENCY, ***NDUFS7***- LEIGH SYNDROME, ***NDUFS8***- LEIGH SYNDROME, ***NDUFV1***- MITOCHONDRIAL COMPLEX I DEFICIENCY, ***NFU1***- MULTIPLE MITOCHONDRIAL DYSFUNCTIONS SYNDROME 1, ***NPHP3***- RENAL-HEPATIC-PANCREATIC DYSPLASIA 1, ***OFD1***- OROFACIODIGITAL SYNDROME I; SIMPSON-GOLABI-BEHMEL SYNDROME, TYPE 2, ***OSTM1***- OSTEOPETROSIS, AUTOSOMAL RECESSIVE 5, ***P3H1***- OSTEOGENESIS IMPERFECTA,VIII, ***PC***- PYRUVATE CARBOXYLASE DEFICIENCY, ***PDHA1***- PYRUVATE DEHYDROGENASE E1-ALPHA DEFICIENCY, ***PDHB***- PYRUVATE DEHYDROGENASE E1-BETA DEFICIENCY, ***PDSS1***- COENZYME Q10 DEFICIENCY, PRIMARY, 2, ***PDSS2***- COENZYME Q10 DEFICIENCY, PRIMARY, 3, ***PEX1***- PEROXISOME BIOGENESIS DISORDER 1A (ZELLWEGER), ***PEX7***- RHIZOMELIC CHONDRODYSPLASIA PUNCTATA, TYPE 1, ***PLA2G6***- NEURODEGENERATION WITH BRAIN IRON ACCUMULATION 2A, ***PLEC***- EPIDERMOLYSIS BULLOSA SIMPLEX WITH MUSCULAR DYSTROPHY, ***POMT1***- MUSCULAR DYSTROPHY-DYSTROGLYCANOPATHY (CONGENITAL WITH BRAIN AND EYE ANOMALIES), TYPE A, 1, ***POR***- ANTLEY-BIXLER SYNDROME WITH GENITAL ANOMALIES AND DISORDERED STEROIDOGENESIS, ***PPT1***- NEURONAL CEROID LIPOFUSCINOSIS 1, ***PRF1***- HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS, FAMILIAL, 2, ***PRKAG2***- GLYCOGEN STORAGE DISEASE OF HEART,LETHAL CONGENITAL, ***PROP1***- PITUITARY HORMONE DEFICIENCY, COMBINED, 2, ***PSAP***- COMBINED SAPOSIN DEFICIENCY; KRABBE DISEASE,ATYPICAL,DUE TO SAPOSIN A DEFICIENCY; METACHROMATIC LEUKODYSTROPHY DUE TO SAPOSIN B DEFICIENCY, ***PTH1R***- CHONDRODYSPLASIA, BLOMSTRAND TYPE, ***RARS2***- PONTOCEREBELLAR HYPOPLASIA, TYPE 6, ***REN***- RENAL TUBULAR DYSGENESIS, ***RMND1***- COMBINED OXIDATIVE PHOSPHORYLATION DEFICIENCY 11, ***RPGRIP1L***- MECKEL SYNDROME, TYPE 5, ***RRM2B***- MITOCHONDRIAL DNA DEPLETION SYNDROME 8A (ENCEPHALOMYOPATHIC TYPE WITH RENAL TUBULOPATHY), ***SARS2***- HYPERURICEMIA, PULMONARY HYPERTENSION, RENAL FAILURE, AND ALKALOSIS SYNDROME, ***SCO1***- MITOCHONDRIAL COMPLEX IV DEFICIENCY, ***SCO2***- CARDIOENCEPHALOMYOPATHY, FATAL INFANTILE, DUE TO CYTOCHROME C OXIDASE DEFICIENCY 1, ***SDHA***- CARDIOMYOPATHY,

DILATED, 1GG; LEIGH SYNDROME; MITOCHONDRIAL RESPIRATORY CHAIN COMPLEX II DEFICIENCY, ***SLC17A5***- INFANTILE SIALIC ACID STORAGE DISEASE, ***SLC25A22***- EPILEPTIC ENCEPHALOPATHY,EARLY INFANTILE,3, ***SLC25A3***- MITOCHONDRIAL PHOSPHATE CARRIER DEFICIENCY, ***SLC26A2***- ACHONDROGENESIS, TYPE IB; ATELOSTEOGENESIS,II, ***SLC35D1***- SCHNECKENBECKEN DYSPLASIA, ***SMN1***- SPINAL MUSCULAR ATROPHY 1; SPINAL MUSCULAR ATROPHY 2; SPINAL MUSCULAR ATROPHY 3; SPINAL MUSCULAR ATROPHY 4, ***SMPD1***- NIEMANN-PICK TYPE A, ***SNAP29***- CEREBRAL DYSGENESIS, NEUROPATHY, ICHTHYOSIS, AND PALMOPLANTAR KERATODERMA SYNDROME, ***STRA6***- MICROPHTHALMIA, SYNDROMIC 9, ***SUCLG1***- MITOCHONDRIAL DNA DEPLETION SYNDROME 9 (ENCEPHALOMYOPATHIC TYPE WITH METHYLMALONIC ACIDURIA), ***SURF1***- LEIGH SYNDROME, ***TAZ***- BARTH SYNDROME, ***TCIRG1***- OSTEOPETROSIS,AR1, ***TCTN3***- OROFACIODIGITAL SYNDROME IV, ***TK2***- MITOCHONDRIAL DNA DEPLETION SYNDROME,MYOPATHIC, ***TMEM231***- JOUBERT SYNDROME 20; MECKEL SYNDROME 11, ***TMEM237***- JOUBERT SYNDROME 14, ***TMEM70***- MITOCHONDRIAL COMPLEX V (ATP SYNTHASE) DEFICIENCY, NUCLEAR TYPE 2, ***TPP1***- CEROID LIPOFUSCINOSIS, NEURONAL, 2, ***TSEN54***- PONTOCEREBELLAR HYPOPLASIA, TYPE 5; PONTOCEREBELLAR HYPOPLASIA TYPE 2A; PONTOCEREBELLAR HYPOPLASIA TYPE 4, ***TSFM***- COMBINED OXIDATIVE PHOSPHORYLATION DEFICIENCY 3, ***TSPYL1***- SUDDEN INFANT DEATH WITH DYSGENESIS OF THE TESTES SYNDROME, ***UBA1***- SPINAL MUSCULAR ATROPHY, X-LINKED 2, ***UBR1***- JOHANSON BLIZZARD SYNDROME, ***VPS33B***- ARTHROGRYPOSIS, RENAL DYSFUNCTION, AND CHOLESTASIS 1, ***WAS***- WISKOTT ALDRICH SYNDROME; NEUTROPENIA, SEVERE CONGENITAL, X-LINKED; THROMBOCYTOPENIA 1, ***ZMPSTE24***- RESTRICTIVE DERMOPATHY, LETHAL

CATEGORY: SERIOUS

AAAS- ACHALASIA-ADDISONIANISM-ALACRIMA-SYNDROME, ***ABCB11***- CHOLESTASIS, PROGRESSIVE FAMILIAL INTRAHEPATIC2, ***ABCB7***- ANEMIA, SIDEROBLASTIC, AND SPINOCEREBELLAR ATAXIA, ***ABCC8***- FAMILIAL HYPERINSULINEMIA, ***ABCD1***- ADRENOLEUKODYSTROPHY, ***ABHD5***- CHANARIN-DORFMAN SYNDROME(ICHTHYOTIC NEUTRAL LIPID STORAGE DISEASE), ***ACADM***- MEDIUM-CHAIN ACYL-COA DEHYDROGENASE DEFICIENCY, ***ACADVL***- VERY LONG-CHAIN ACYL-COA DEHYDROGENASE DEFICIENCY, ***ACO2***- INFANTILE CEREBELLAR-RETINAL DEGENERATION, ***ADA***- SEVERE COMBINED IMMUNODEFICIENCY, T CELL-NEGATIVE, ***ADAMTS13***- THROMBOTIC THROMBOCYTOPENIC PURPURA,CONGENITAL, ***ADAMTS2***- EHLERS DANLOS SYNDROME, VII, ***ADAMTSL2***- GELEOPHYSIC DYSPLASIA, ***ADGRV1***- USHER SYNDROME, IIC, ***AFG3L2***- SPASTIC ATAXIA 5, ***AGA***- SPARTYLGLYCOSAMINURIA, ***AGK***- SENGERS SYNDROME, ***AGL***- GLYCOGEN STORAGE DISEASE III, ***AGXT***- HYPEROXALURIA, PRIMARY, TYPE I, ***AHI1***- JOUBERT SYNDROME 3, ***AIPL1***- LEBER CONGENITAL AMAUROSIS 4, ***AIRE***- AUTOIMMUNE POLYENDOCRINE SYNDROME, TYPE I, +/- REVERSIBLE METAPHYSEAL DYSPLASIA, ***ALDH3A2***- SJOGREN-LARSSON SYNDROME, ***ALDH5A1***- SUCCINIC SEMI ALDEHYDE DEHYDROGENASE DEFICIENCY, ***ALG6***- CONGENITAL DISORDER OF GLYCOSYLATION IC, ***ALMS1***- ALSTROM SYNDROME, ***ALOX12B***- ICHTHYOSIS, CONGENITAL, 2, ***ALPL***- HYPOPHOSPHATASIA, INFANTILE, ***ALS2***- AMYOTROPHIC LATERAL SCLEROSIS 2,JUVENILE; PRIMARY LATERAL SCLEROSIS,JUVENILE, ***AP3B1***- HERMANSKY-PUDLAK SYNDROME 2, ***APTX***- ATAXIA,EARLY ONSET,WITH OCULOMOTOR APRAXIA AND HYPOALBUMINEMIA, ***ARL13B***- JOUBERT SYNDROME 8, ***ARL6***- BARDET-BIEDL SYNDROME 3, ***ARSA***- METACHROMATIC LEUKODYSTROPHY, ***ARSB***- MUCOPOLYSACCHARIDOSIS VI, ***ARSE***- CHONDRODYSPLASIA PUNCTATA 1, ***ASL***- ARGININOSUCCINIC ACIDURIA, ***ASPA***- CANAVAN, ***ASS1***- CITRULLINEMIA TYPE 1, ***ATM***- ATAXIA TELANGIECTASIA, ***ATP13A2***- KUFOR-RAKEB SYNDROME, ***ATP5E***- MITOCHONDRIAL COMPLEX V (ATP SYNTHASE) DEFICIENCY, NUCLEAR TYPE 3, ***ATP6V0A2***- CUTIS LAXA II, ***ATP7B***- WILSON DISEASE, ***ATP8B1***- CHOLESTASIS, ROGRESSIVE FAMILIAL INTRAHEPATIC 1, ***ATR***- SECKEL SYNDROME 1, ***ATRX***- THALASSEMIA/MENTAL ETARDATION SYNDROME,NONDELETION, ***AVP***- DIABETES INSIPIDUS, NEUROHYPOPHYSEAL, ***B4GALT1***- CONGENITAL DISORDER OF GLYCOSYLATION, IID, ***BBS1***- BARDET-BIEDL SYNDROME 1, ***BBS10***- BARDET-BIEDL SYNDROME 10, ***BBS12***- BARDET-BIEDL SYNDROME 12, ***BBS2***- BARDET-BIEDL SYNDROME 2, ***BBS4***- BARDET-BIEDL SYNDROME 4, ***BBS5***- BARDET-BIEDL SYNDROME 5, ***BBS7***- BARDET-BIEDL SYNDROME 7, ***BCKDHA***- MAPLE SYRUP URINE DISEASE IA, ***BCKDHB***- MAPLE SYRUP URINE DISEASE, CLASSIC, IB, ***BRCA2***- FANCONI ANEMIA, COMPLEMENTATION GROUP D1, ***MPC1***- MITOCHONDRIAL PYRUVATE CARRIER DEFICIENCY, ***BTD***- BIOTINIDASE DEFICIENCY, ***BTK***- AGAMMAGLOBULINEMIA, ***C10ORF2***-

PERRAULT SYNDROME 5; MITOCHONDRIAL DNA DEPLETION SYNDROME 7 (HEPATOCEREBRAL TYPE), ***C12ORF65***- SPASTIC PARAPLEGIA 55; COMBINED OXIDATIVE PHOSPHORYLATION DEFICIENCY 7, ***CA2***- OSTEOPETROSIS, 3, ***CBS***- HOMOCYSTINURIA DUE TO CYSTATHIONINE BETA-SYNTHASE DEFICIENCY, ***CD40LG***- IMMUNODEFICIENCY WITH HYPER IGM, 1, ***CDH23***- DEAFNESS 12; USHER SYNDROME, ID, ***CEP41***- JOUBERT SYNDROME 15, ***CFP***- PROPERDIN DEFICIENCY, ***CFTR***- CYSTIC FIBROSIS, ***CHKB***- MUSCULAR DYSTROPHY, CONGENITAL, MEGACONIAL TYPE, ***CHM***- CHOROIDEREMIA, ***CHRNE***- CONGENITAL MYASTHENIC SYNDROME, ***CHRNG***- MULTIPLE PTERYGIUM SYNDROME, ESCOBAR, ***CISD2***- WOLFRAM SYNDROME 2, ***CLDN1***- ICHTHYOSIS, LEUKOCYTE VACUOLES, ALOPECIA, AND SCLEROSING CHOLANGITIS, ***CLDN14***- DEAFNESS 29, ***CLDN19***- HYPOMAGNESEMIA,RENAL,WITH OCULAR INVOLVEMENT, ***CLN3***- CEROID LIPOFUSCINOSIS,NEURONAL 3, ***CLN5***- CEROID LIPOFUSCINOSIS, EURONAL, 5, ***CLN8***- CEROID LIPOFUSCINOSIS, NEURONAL, 8, NORTHERN EPILEPSY VARIANT, ***CLRN1***- RETINITIS PIGMENTOSA 61; USHER SYNDROME, III, ***COL17A1***- EPIDERMOLYSIS BULLOSA, JUNCTIONAL, NONHERLITZ, ***COL1A2***- EHLERS DANLOS SYNDROME, CARDIAC VALVULAR, ***COL4A3***- ALPORT SYNDROME, ***COL4A4***- ALPORT SYNDROME, ***COL4A5***- ALPORT SYNDROME, ***COL6A1***- ULLRICH CONGENITAL MUSCULAR DYSTROPHY 1; BETHLEM MYOPATHY 1, ***COL6A2***- ULLRICH CONGENITAL MUSCULAR DYSTROPHY 1; ETHLEM MYOPATHY 1, ***COL6A3***- ULLRICH CONGENITAL MUSCULAR DYSTROPHY 1; BETHLEM MYOPATHY 1, ***COL7A1***- EPIDERMOLYSIS BULLOSA DYSTROPHICA, ***COX20***- MITOCHONDRIAL COMPLEX IV DEFICIENCY, ***CPS1***- CARBAMOYL PHOSPHATE SYNTHETASE 1 DEFICIENCY, HYPERAMMONEMIA, ***CRLF1***- COLD-INDUCED SWEATING SYNDROME 1, ***CRX***- LEBER CONGENITAL AMAUROSIS 7, ***CSTB***- MYOCLONIC EPILEPSY OF UNVERRICHT AND LUNDBORG, ***CTNS***- CYSTINOSIS, NEPHROPATHIC, ***CTSK***- PYCNODYSOSTOSIS, ***CYP17A1***- CONGENITAL ADRENAL HYPERPLASIA, , DUE TO 17-ALPHA-HYDROXYLASE DEFICIENCY, ***CYP21A2***- CONGENITAL ADRENAL HYPERPLASIA ,DUE TO 21-HYDROXYLASE DEFICIENCY, ***D2HGDH***- D-2-HYDROXYGLUTARIC ACIDURIA, ***NROB1***- ADRENAL HYPOPLASIA, CONGENITAL, ***DCAF17***- WOODHOUSE-SAKATI SYNDROME, ***DCLRE1C***- OMENN SYNDROME, ***DDB2***- XERODERMA PIGMENTOSUM, COMPLEMENTATION GROUP E, ***DDC***- AROMATIC L-AMINO ACID DECARBOXYLASE DEFICIENCY, ***DFNB31***- USHER SYNDROME, TYPE IID, ***DFNB59***- DEAFNESS 59, ***DHCR24***- DESMOSTEROLOSIS, ***DHCR7***- SMITH-LEMLI-OPITZ SYNDROME, ***DKC1***- DYSKERATOSIS CONGENITA, ***DLAT***- PYRUVATE DEHYDROGENASE E2 EFICIENCY, ***DLD***- DIHYDROLIPOAMIDE DEHYDROGENASE DEFICIENCY, ***DLL3***- SPONDYLOCOSTAL YSOSTOSIS 1, ***DMD***- MUSCULAR DYSTROPHY, DUCHENNE TYPE; CARDIOMYOPATHY, DILATED, 3B, ***DMP1***- HYPOPHOSPHATEMIC RICKETS, ***DNMT3B***- IMMUNODEFICIENCY CENTROMERIC INSTABILITY FACIAL ANOMALIES SYNDROME, ***DPAGT1***- CONGENITAL DISORDER OF GLYCOSYLATION, IJ, ***DPM1***- CONGENITAL DISORDER OF GLYCOSYLATION, IE, ***DPYD***- DIHYDROPYRIMIDINE DEHYDROGENASE DEFICIENCY, ***DSP***- SKIN FRAGILITY WOOLLY HAIR SYNDROME, ***DTNBP1***- HERMANSKY-PUDLAK SYNDROME 7, ***DYNC2H1***- SHORT-RIB THORACIC DYSPLASIA 3 +/- POLYDACTYLY, ***EARS2***- COMBINED OXIDATIVE PHOSPHORYLATION DEFICIENCY 12, ***EGR2***- HYPERTROPHIC NEUROPATHY OF DEJERINE SOTTAS; NEUROPATHY, CONGENITAL HYPOMYELINATING OR AMYELINATING, ***EMD***- EMERY-DREIFUSS MUSCULAR DYSTROPHY 1, ***EPM2A***- MYOCLONIC EPILEPSY OF LAFORA, ***ERCC2***- TRICHOTHIODYSTROPHY 1, PHOTOSENSITIVE; XERODERMA PIGMENTOSUM,COMPLEMENTATION GROUP D, ***ERCC3***- XERODERMA PIGMENTOSUM, COMPLEMENTATION GROUP B, ***ERCC4***- XERODERMA PIGMENTOSUM, COMPLEMENTATION GROUP F, ***ERCC5***- XERODERMA PIGMENTOSUM, COMPLEMENTATION GROUP G , ***ERCC6***- DE SANCTIS-CACCHIONE SYNDROME, ***ESPN***- DEAFNESS 36, +/- VESTIBULAR INVOLVEMENT, ***ESRRB***- DEAFNESS 35, ***EVC***- ELLIS-VAN CREVELD SYNDROME, ***F8***- HEMOPHILIA A (FACTOR VIII), ***FAH***- TYROSINEMIA, TYPE I, ***FAM126A***- LEUKODYSTROPHY,HYPOMYELINATING, 5, ***FANCA***- FANCONI ANEMIA COMPLEMENTATION GROUP A, ***FBP1***- FRUCTOSE-1,6-BISPHOSPHATASE DEFICIENCY, ***FECH***- PROTOPORPHYRIA, ERYTHROPOIETIC, ***FLNA***- INTESTINAL PSEUDOOBSTRUCTION, NEURONAL, CHRONIC IDIOPATHIC, ***FMR1***- FRAGILE X MENTAL RETARDATION SYNDROME, ***FOXN1***- TCELL IMMUNODEFICIENCY,CONGENITAL ALOPECIA,AND NAIL DYSTROPHY, ***FOXRED1***- LEIGH SYNDROME, ***FRAS1***- FRASER SYNDROME, ***FREM2***- FRASER SYNDROME, ***FUCA1***- FUCOSIDOSIS, ***FXN***- FRIEDREICH ATAXIA 1, ***G6PC***- GLYCOGEN STORAGE DISEASE IA, ***GAA***- GLYCOGEN STORAGE DISEASE II (POMPE), ***GALT***- GALACTOSEMIA, ***GATA1***- ANEMIA, +/- NEUTROPENIA AND/OR PLATELET ABNORMALITIES; THROMBOCYTOPENIA WITH BETA-THALASSEMIA; THROMBOCYTOPENIA, +/- DYSERYTHROPOIETIC ANEMIA, ***GBE1***- GLYCOGEN STORAGE DISEASE

IV, ***GCDH***- GLUTARIC ACIDEMIA I, ***GCSH***- GLYCINE ENCEPHALOPATHY, ***GIPC3***- DEAFNESS 15, ***GJB3***- DEAFNESS 1A, ***GJB6***- DEAFNESS1B, ***GJC2***- LEUKODYSTROPHY,HYPOMYELINATING, 2, ***GLA***- FABRY DISEASE, ***GLB1***- GM1-GANGLIOSIDOSIS, TYPE II, ***GLDC***- GLYCINE ENCEPHALOPATHY, ***GNPTAB***- MUCOLIPIDOSIS III ALPHA/BETA, ***GNS***- MUCOPOLYSACCHARIDOSIS IIID, ***GPR56***- POLYMICROGYRIA, BILATERAL FRONTOPARIETAL, ***GPR98***- USHER SYNDROME TYPE IIC, ***GPSM2***- CHUDLEY-MCCULLOUGH SYNDROME, ***GRHPR***- HYPEROXALURIA, PRIMARY, TYPE II, ***GRXCR1***- DEAFNESS, 25, ***GSS***- GLUTATHIONE SYNTHETASE DEFICIENCY, ***GTF2H5***- TRICHOTHIODYSTROPHY 3, PHOTOSENSITIVE, ***GUCY2D***- LEBER CONGENITAL AMAUROSIS 1, ***HADH***- 3- HYDROXYACYL-COA DEHYDROGENASE DEFICIENCY, ***HADHA***- LONG-CHAIN 3-HYDROXYACYL-COA DEHYDROGENASE DEFICIENCY; MITOCHONDRIAL TRIFUNCTIONAL PROTEIN DEFICIENCY, ***HADHB***- MITOCHONDRIAL TRIFUNCTIONAL PROTEIN DEFICIENCY, ***HAMP***- HEMOCHROMATOSIS, TYPE 2B, ***HARS2***- PERRAULT SYNDROME 2, ***HBB***- BETA THALASSEMIA; SICKLE CELL ANEMIA, ***HESX1***- SEPTOOPTIC DYSPLASIA, ***HFE2***- HEMOCHROMATOSIS, TYPE 2A, ***HGF***- DEAFNESS 39, ***HGSNAT***- MUCOPOLYSACCHARIDOSIS IIIC, ***HLCS***- HOLOCARBOXYLASE SYNTHETASE DEFICIENCY, ***HMGCL***- 3-HYDROXY-3-METHYLGLUTARYL-COA LYASE DEFICIENCY, ***HPRT1***- LESCH-NYHAN SYNDROME, ***HPS3***- HERMANSKY-PUDLAK SYNDROME 3, ***HPS4***- HERMANSKY-PUDLAK SYNDROME 4, ***HPS5***- HERMANSKY-PUDLAK SYNDROME 5, ***HPS6***- HERMANSKY-PUDLAK SYNDROME 6, ***HSD17B10***- MENTAL RETARDATION, SYNDROMIC 10, ***IDS***- MUCOPOLYSACCHARIDOSIS, TYPE II, ***IFT80***- SHORT-RIB THORACIC DYSPLASIA 2 +/- POLYDACTYLY, ***IGF1***- INSULIN-LIKE GROWTH FACTOR I DEFICIENCY, ***IKBKAP***- NEUROPATHY,HEREDITARY SENSORY AUTONOMIC, III; (FAMILIAL DYSAUTONOMIA), ***IKBKG***- INCONTINENTIA PIGMENTI; ECTODERMAL DYSPLASIA, HYPOHIDROTIC,WITH IMMUNE DEFICIENCY, ***IL2RG***- COMBINED IMMUNODEFICIENCY; SEVERE COMBINED IMMUNODEFICIENCY, ***ILDR1***- DEAFNESS 42, ***INPP5E***- JOUBERT SYNDROME 1, ***IQCB1***- SENIOR LOKEN SYNDROME 5, ***ITGB4***- EPIDERMOLYSIS BULLOSA,JUNCTIONAL, NON HERLITZ, ***IVD***- ISOVALERIC ACIDEMIA, ***JAK3***- SEVERE COMBINED IMMUNODEFICIENCY, T CELL-NEGATIVE, B CELL-POSITIVE, NK CELL-NEGATIVE, ***KCNJ1***- BARTTER SYNDROME, ANTENATAL, TYPE 2, ***KCNJ13***- LEBER CONGENITAL AMAUROSIS 16, ***KCNQ1***- JERVELL AND LANGE NIELSEN SYNDROME 1, ***KCTD7***- EPILEPSY, PROGRESSIVE MYOCLONIC, 3, +/- INTRACELLULAR INCLUSIONS, ***KIF7***- ACROCALLOSAL SYNDROME, ***L1CAM***- HYDROCEPHALUS DUE TO CONGENITAL STENOSIS OF AQUEDUCT OF SYLVIUS; MASA SYNDROME, ***LAMA2***- MUSCULAR DYSTROPHY,CONGENITAL MEROSIN DEFICIENT, 1A, ***LAMA3***- LARYNGOONYCHOCUTANEOUS SYNDROME, ***LAMP2***- DANON DISEASE (LYSOSOMAL GLYCOGEN STORAGE DISEASE WITHOUT ACID MALTASE), ***LARGE***- MUSCULAR DYSTROPHY-DYSTROGLYCANOPATHY (CONGENITAL WITH MENTAL RETARDATION), TYPE B, 6, ***LARS***- INFANTILE LIVER FAILURE SYNDROME 1, ***EVC2***- ELLIS-VAN CREVELD SYNDROME, ***LCA5***- LEBER CONGENITAL AMAUROSIS 5, ***LDLR***- HYPERCHOLESTEROLEMIA, FAMILIAL, ***LHFPL5***- DEAFNESS 67, ***LHX3***- PITUITARY HORMONE DEFICIENCY, COMBINED, 3, ***LOXHD1***- DEAFNESS 77, ***LRAT***- LEBER CONGENITAL AMAUROSIS 14, ***LRP2***- DONNAI-BARROW SYNDROME, ***LRP5***- OSTEOPOROSIS PSEUDOGLIOMA SYNDROME, ***LRTOMT***- DEAFNESS 63, ***LYST***- CHEDIAK HIGASHI SYNDROME, ***MAN2B1***- MANNOSIDOSIS, ALPHA B, LYSOSOMAL, ***MARS2***- SPASTIC ATAXIA 3, ***MARVELD2***- DEAFNESS 49, ***MCCC2***- 3-METHYLCROTONYL-COA CARBOXYLASE2 DEFICIENCY, ***MCOLN1***- MUCOLIPIDOSIS TYPE IV, ***MED12***- LUJAN FRYNS SYNDROME, ***MEFV***- FAMILIAL MEDITERRANEAN FEVER, ***MFN2***- CHARCOT-MARIE-TOOTH DISEASE, AXONAL, TYPE 2A2, ***MFSD8***- CEROID LIPOFUSCINOSIS ,NEURONAL,7, ***MGAT2***- CONGENITAL DISORDER OF GLYCOSYLATION, TYPE IIA, ***MKKS***- MCKUSICK-KAUFMAN SYNDROME; BARDET-BIEDL SYNDROME 6, ***MLC1***- MEGALENCEPHALIC LEUKOENCEPHALOPATHY WITH SUBCORTICAL CYSTS 1, ***MMAB***- METHYLMALONICACIDURIA, CBLB TYPE, ***MMACHC***- METHYLMALONIC ACIDURIA AND HOMOCYSTINURIA, CBLC TYPE, ***MOGS***- CONGENITAL DISORDER OF GLYCOSYLATION, TYPE IIB, ***MPI***- CONGENITAL DISORDER OF GLYCOSYLATION, TYPE IB, ***MPV17***- MITOCHONDRIAL DNA DEPLETION SYNDROME,HEPATOCEREBRAL, ***MPZ***- NEUROPATHY, CONGENITAL HYPOMYELINATING OR AMYELINATING, ***MRPS16***- COMBINED OXIDATIVE PHOSPHORYLATION DEFICIENCY 2, ***MTFMT***- COMBINED OXIDATIVE PHOSPHORYLATION DEFICIENCY 15, ***MTM1***- MYOPATHY, CENTRONUCLEAR, ***MTO1***- COMBINED OXIDATIVE PHOSPHORYLATION DEFICIENCY 10, ***MTPAP***- SPASTIC ATAXIA 4, ***MUT***- METHYLMALONIC ACIDURIA DUE TO METHYLMALONYL-COA MUTASE DEFICIENCY, ***MVK***- MEVALONIC ACIDURIA, ***MYO15A***- DEAFNESS 3, ***MYO6***- DEAFNESS 37, ***MYO7A***- DEAFNESS 2; USHER SYNDROME, I, ***NAGS***- N-ACETYLGLUTAMATE SYNTHASE

DEFICIENCY, ***NBN***- NIJMEGEN BREAKAGE SYNDROME, ***NDP***- NORRIE DISEASE, ***NDRG1***- CHARCOT-MARIE-TOOTH DISEASE, TYPE 4D, ***NDUFA1***- MITOCHONDRIAL COMPLEX I DEFICIENCY, ***NDUFAF5***- MITOCHONDRIAL COMPLEX I DEFICIENCY, ***NDUFS3***- LEIGH SYNDROME, ***NEB***- NEMALINE MYOPATHY 2, ***NEU1***- NEURAMINIDASE DEFICIENCY, ***NEUROG3***- DIARRHEA 4,MALABSORPTIVE, CONGENITAL, ***NHLRC1***- MYOCLONIC EPILEPSY OF LAFORA, ***NMNAT1***- LEBER CONGENITAL AMAUROSIS 9, ***NPC1***- NIEMANN-PICK DISEASE, TYPE C1, ***NPHP1***- JOUBERT SYNDROME 4; NEPHRONOPHTHISIS 1; SENIOR-LOKEN SYNDROME 1, ***NPHP4***- NEPHRONOPHTHISIS 4, ***NPHS1***- NEPHROTIC SYNDROME, TYPE 1, ***NTRK1***- CONGENITAL INSENSITIVITY TO PAINWITH ANHIDROSIS, ***NUBPL***- MITOCHONDRIAL COMPLEX I DEFICIENCY, ***NUP62***- STRIATONIGRAL DEGENERATION, INFANTILE, ***OCRL***- LOWE OCULOCEREBRO RENAL SYNDROME, ***OFD1***- 3-METHYLGLUTACONIC ACIDURIA, TYPE III, ***OTC***- ORNITHINE TRANSCARBAMYLASE DEFICIENCY, ***OTOA***- DEAFNESS 22, ***OTOF***- DEAFNESS 9, ***PAH***- PHENYLKETONURIA, ***PANK2***- NEURODEGENERATION WITH BRAIN IRON ACCUMULATION 1, ***PCDH15***- DEAFNESS 23; USHER SYNDROME, TYPE IF, ***NPHS2***- NEPHROTIC SYNDROME, TYPE 2, ***PDHX***- PYRUVATE DEHYDROGENASE E3-BINDING PROTEIN DEFICIENCY, ***PDP1***- PYRUVATE DEHYDROGENASE PHOSPHATASE DEFICIENCY, ***PLCE1***- NEPHROTIC SYNDROME, TYPE 3, ***PLG***- PLASMINOGEN DEFICIENCY, TYPE I, ***PLOD1***- EHLERS-DANLOS SYNDROME, TYPE VI, ***PLP1***- PELIZAEUS-MERZBACHER DISEASE; SPASTIC PARAPLEGIA 2, ***PMM2***- CONGENITAL DISORDER OF GLYCOSYLATION, TYPE IA, ***PMP22***- HYPERTROPHIC NEUROPATHY OF DEJERINE-SOTTAS, ***PNPO***- PYRIDOXAMINE 5-PRIME-PHOSPHATE OXIDASE DEFICIENCY, ***POU1F1***- PITUITARY HORMONE DEFICIENCY, COMBINED, 1, ***PQBP1***- RENPENNING SYNDROME 1, ***PRPS1***- ARTS SYNDROME, ***BBS9***- BARDET-BIEDL SYNDROME 9, ***PTPRQ***- DEAFNESS 84A, ***PTS***- HYPERPHENYLALANINEMIA, BH4-DEFICIENT, A, ***RAB23***- CARPENTER SYNDROME 1, ***RAB27A***- GRISCELLI SYNDROME, TYPE 2, ***RAB3GAP1***- WARBURG MICRO SYNDROME 1, ***RAB3GAP2***- MARTSOLF SYNDROME, ***RAG1***- OMENN SYNDROME; SEVERE COMBINED IMMUNODEFICIENCY, T CELL-NEGATIVE, B CELL-NEGATIVE, NK CELL-POSITIVE, ***RAG2***- OMENN SYNDROME; SEVERE COMBINED IMMUNODEFICIENCY, T CELL-NEGATIVE, B CELL-NEGATIVE, NK CELL-POSITIVE, ***RAPSN***- FETAL AKINESIA DEFORMATION SEQUENCE, ***RD3***- LEBER CONGENITAL AMAUROSIS 12, ***RDH12***- LEBER CONGENITAL AMAUROSIS 13, ***RDX***- DEAFNESS, 24, ***BLM***- BLOOM SYNDROME, ***RECQL4***- BALLER-GEROLD SYNDROME, ***RELN***- LISSENCEPHALY 2, ***RMRP***- CARTILAGE-HAIR HYPOPLASIA; ANAUXETIC DYSPLASIA, ***RPE65***- LEBER CONGENITAL AMAUROSIS 2; RETINITIS PIGMENTOSA 20, ***RPGRIP1***- LEBER CONGENITAL AMAUROSIS 6; CONE-ROD DYSTROPHY 13, ***RPS6KA3***- COFFIN-LOWRY SYNDROME, ***RS1***- RETINOSCHISIS 1, X-LINKED, JUVENILE, ***SACS***- SPASTIC ATAXIA, CHARLEVOIX-SAGUENAY TYPE, ***SBDS***- SHWACHMAN-DIAMOND SYNDROME, ***SC5D***- LATHOSTEROLOSIS, ***SCNN1A***- PSEUDOHYPOALDOSTERONISM, TYPE I, AUTOSOMAL RECESSIVE, ***SCNN1B***- PSEUDOHYPOALDOSTERONISM, TYPE I, AUTOSOMAL RECESSIVE, ***SCNN1G***- PSEUDOHYPOALDOSTERONISM, TYPE I, AUTOSOMAL RECESSIVE, ***SDHAF1***- MITOCHONDRIAL COMPLEX II DEFICIENCY, ***SEPN1***- RIGID SPINE MUSCULAR DYSTROPHY 1, ***SERAC1***- 3-METHYLGLUTACONIC ACIDURIA WITH DEAFNESS, ENCEPHALOPATHY, AND LEIGH-LIKE SYNDROME, ***SGSH***- MUCOPOLYSACCHARIDOSIS, IIIA, ***SH2D1A***- LYMPHOPROLIFERATIVE SYNDROME,1, ***SIL1***- MARINESCO-SJOGREN SYNDROME, ***SLC12A1***- BARTTER SYNDROME, ANTENATAL, TYPE 1, ***SLC12A6***- AGENESIS OF THE CORPUS CALLOSUM WITH PERIPHERAL NEUROPATHY, ***SLC16A2***- ALLAN-HERNDON-DUDLEY SYNDROME, ***SLC19A3***- THIAMINE METABOLISM DYSFUNCTION SYNDROME 2 (BIOTIN- OR THIAMINE-RESPONSIVE TYPE), ***SLC25A15***- HYPERORNITHINEMIA-HYPERAMMONEMIA-HOMOCITRULLINURIA SYNDROME, ***SLC25A4***- MITOCHONDRIAL DNA DEPLETION SYNDROME 12 (CARDIOMYOPATHIC TYPE), ***SLC26A2***- DIASTROPHIC DYSPLASIA, ***SLC35A1***- CONGENITAL DISORDER OF GLYCOSYLATION, IIF, ***SLC35C1***- CONGENITAL DISORDER O F GLYCOSYLATION, IIC, ***SLC37A4***- GLYCOGEN STORAGE DISEASE IB, ***SLC3A1***- CYSTINURIA, ***SLC6A8***- CEREBRAL CREATINE DEFICIENCY SYNDROME 1, ***SLC9A6***- MENTAL RETARDATION, SYNDROMIC, CHRISTIANSON TYPE, ***SP110***- HEPATIC VENOOCCLUSIVE DISEASE WITH IMMUNODEFICIENCY, ***SPATA7***- LEBER CONGENITAL AMAUROSIS 3, ***ST3GAL5***- AMISH INFANTILE EPILEPSY SYNDROME, ***STAR***- LIPOID CONGENITAL ADRENAL HYPERPLASIA, ***SUCLA2***- MITOCHONDRIAL DNA DEPLETION SYNDROME 5 (ENCEPHALOMYOPATHIC +/- METHYLMALONIC ACIDURIA), ***SUOX***- SULFOCYSTEINURIA, ***TBCE***- HYPOPARATHYROIDISM-RETARDATION-DYSMORPHISM SYNDROME, ***TCTN1***- JOUBERT SYNDROME 13, ***TECTA***- DEAFNESS, 21, ***TGM1***- ICHTHYOSIS, CONGENITAL, 1, ***TH***- SEGAWA SYNDROME, , ***TIMM8A***- MOHR-TRANEBJAERG SYNDROME, ***TMEM138***- JOUBERT SYNDROME 16, ***TMEM216***- JOUBERT SYNDROME 2, ***TMEM67***-

JOUBERT SYNDROME 6, ***TNFRSF11B***- PAGET DISEASE OF BONE 5, JUVENILE-ONSET, ***TNNT1***- NEMALINE MYOPATHY 5, ***TPK1***- THIAMINE METABOLISM DYSFUNCTION SYNDROME 5 (EPISODIC ENCEPHALOPATHY TYPE), ***TREX1***- AICARDI-GOUTIERES SYNDROME 1, ***TRIM37***- MULIBREY NANISM, ***TSHB***- HYPOTHYROIDISM,CONGENITAL,NONGOITROUS, 4, ***TTC21B***- NEPHRONOPHTHISIS-12; SHORT-RIB THORACIC DYSPLASIA-4 +/- POLYDACTYLY, ***TTC8***- BARDET-BIEDL SYNDROME 8, ***TTN***- MYOPATHY,EARLY ONSET,WITH FATAL CARDIOMYOPATHY, ***TULP1***- LEBER CONGENITAL AMAUROSIS 15, ***TYMP***- MITOCHONDRIAL DNA DEPLETION SYNDROME 1 (MNGIE TYPE), ***UGT1A1***- CRIGLER-NAJJAR SYNDROME, TYPE I, ***UQCRQ***- MITOCHONDRIAL COMPLEX III DEFICIENCY, NUCLEAR TYPE 4, ***UROS***- PORPHYRIA,CONGENITAL ERYTHROPOIETIC, ***USH1C***- USHER SYNDROME, IC, ***USH1G***- USHER SYNDROME, TYPE IG, ***USH2A***- USHER SYNDROME, TYPE IIA, ***VDR***- VITAMIN D-DEPENDENT RICKETS, TYPE 2A, ***VLDLR***- CEREBELLAR ATAXIA, MENTAL RETARDATION, AND DYSEQUILIBRIUM SYNDROME 1, ***VPS13B***- COHEN SYNDROME, ***WDR19***- NEPHRONOPHTHISIS 13; SENIOR-LOKEN SYNDROME 8,***WFS1***- WOLFRAM SYNDROME 1, ***XPA***- XERODERMA PIGMENTOSUM, COMPLEMENTATION GROUP A, ***YARS2***- MYOPATHY, LACTIC ACIDOSIS, AND SIDEROBLASTIC ANEMIA 2, ***ZIC3***- VACTERL ASSOCIATION, +/- HYDROCEPHALUS; HETEROTAXY,VISCERAL, 1

CATEGORY: MILD

ABCA4- CONE ROD DYSTROPHY 3; STARGARDT DISEASE, ***AFF2***- MENTAL RETARDATION, ASSOCIATED WITH FRAGILE SITE FRAXE, ***ALAS2***- ANEMIA, SIDEROBLASTIC, ***ALDOB***- FRUCTOSE INTOLERANCE, HEREDITARY, ***AR***- ANDROGEN INSENSITIVITY SYNDROME; INFERTILE MALE SYNDROME, ***BEST1***- BESTROPHINOPATHY; RETINITIS PIGMENTOSA 50, ***BSND***- BARTTER SYNDROME, TYPE 4A, ***C2ORF71***- RETINITIS PIGMENTOSA 54, ***C8ORF37***- CONE-ROD DYSTROPHY 16, ***CERKL***- RETINITIS PIGMENTOSA 26, ***CIB2***- DEAFNESS 48, ***CNGA1***- RETINITIS PIGMENTOSA 49, ***CNGA3***- ACHROMATOPSIA 2, ***CNGB1***- RETINITIS PIGMENTOSA 45, ***CNGB3***- ACHROMATOPSIA, ***COL11A2***- DEAFNESS 53; OTOSPONDYLOMEGAEPIPHYSEAL DYSPLASIA, ***CRB1***- RETINITIS PIGMENTOSA 12, ***CYP27A1***- CEREBROTENDINOUS XANTHOMATOSIS, ***CYP27B1***- VITAMIN D HYDROXYLATION-DEFICIENT RICKETS, TYPE 1A, ***DHDDS***- RETINITIS PIGMENTOSA 59, ***EDA***- ECTODERMAL DYSPLASIA, HYPOHIDROTIC, ***EDNRB***- ABCD SYNDROME, ***EYS***- RETINITIS PIGMENTOSA 25, ***F11***- FACTOR XI DEFICIENCY, ***FAM161A***- RETINITIS PIGMENTOSA 28, ***FGD1***- AARSKOG-SCOTT SYNDROME, ***G6PD***- ANEMIA, NONSPHEROCYTIC HEMOLYTIC, DUE TO G6PD DEFICIENCY, ***GJB1***- CHARCOT-MARIE-TOOTH DISEASE, 1, ***GJB2***- DEAFNESS 1A, ***GNAT2***- ACHROMATOPSIA 4, ***GP1BA***- BERNARD-SOULIER SYNDROME TYPE A1, ***GP9***- BERNARD-SOULIER SYNDROME TYPE C, ***GPC3***- SIMPSON-GOLABI-BEHMEL SYNDROME, TYPE 1, ***GPR143***- ALBINISM, OCULAR, TYPE I, ***GYS2***- GLYCOGEN STORAGE DISEASE 0, LIVER, ***HPS1***- HERMANSKY-PUDLAK SYNDROME 1, ***IDH3B***- RETINITIS PIGMENTOSA 46, ***IMPG2***- RETINITIS PIGMENTOSA 56, ***ISCU***- MYOPATHY WITH LACTIC ACIDOSIS, HEREDITARY, ***KAL1***- HYPOGONADOTROPIC HYPOGONADISM 1 +/- ANOSMIA, ***LDLRAP1***- HYPERCHOLESTEROLEMIA, ***MERTK***- RETINITIS PIGMENTOSA 38, ***MTTP***- ABETALIPOPROTEINEMIA, ***NR2E3***- ENHANCED S-CONE SYNDROME; RETINITIS PIGMENTOSA 37, ***NRL***- RETINAL DEGENERATION, CLUMPED PIGMENT TYPE, INCLUDED, ***OCA2***- ALBINISM, OCULOCUTANEOUS, TYPE II, ***PDE6A***- RETINITIS PIGMENTOSA 43, ***PDE6B***- RETINITIS PIGMENTOSA 40, ***PDE6C***- CONE DYSTROPHY 4, ***PDE6G***- RETINITIS PIGMENTOSA 57, ***PDE6H***- RETINAL CONE DYSTROPHY 3A, ***PKLR***- PYRUVATE KINASE DEFICIENCY OF RED CELLS, ***PLEKHG5***- SPINAL MUSCULAR ATROPHY, DISTAL, 4, ***POU3F4***- DEAFNESS 2, ***PRCD***- RETINITIS PIGMENTOSA 36, ***PROM1***- RETINITIS PIGMENTOSA 41, ***PRX***- HYPERTROPHIC NEUROPATHY OF DEJERINE-SOTTAS, ***PUS1***- MYOPATHY, LACTIC ACIDOSIS, AND SIDEROBLASTIC ANEMIA 1, ***RGR***- RETINITIS PIGMENTOSA 44, ***RLBP1***- BOTHNIA RETINAL DYSTROPHY, ***RP1***- RETINITIS PIGMENTOSA 1, ***RP2***- RETINITIS PIGMENTOSA 2, ***RPGR***- CONE-ROD DYSTROPHY; MACULAR DEGENERATION, ATROPHIC; RETINITIS PIGMENTOSA, AND SINORESPIRATORY INFECTIONS, +/- DEAFNESS, ***SAG***- OGUCHI DISEASE-1; RETINITIS PIGMENTOSA 47, ***SLC26A4***- DEAFNESS, 4, WITH ENLARGED VESTIBULAR AQUEDUCT; PENDRED SYNDROME, ***SLC26A5***- DEAFNESS, AUTOSOMAL RECESSIVE 61, ***SLC45A2***- ALBINISM, OCULOCUTANEOUS, TYPE IV, ***SLC4A11***- CORNEAL ENDOTHELIAL DYSTROPHY 2; CORNEAL DYSTROPHY AND PERCEPTIVE DEAFNESS, ***SLC7A9***- CYSTINURIA, ***SMPX***- DEAFNESS 4, ***STRC***- DEAFNESS 16, ***STS***- ICHTHYOSIS, ***TMC1***- DEAFNESS 7, ***TMEM126A***- OPTIC ATROPHY 7 +/- AUDITORY NEUROPATHY, ***TMIE***- DEAFNESS 6, ***TMPRSS3***- DEAFNESS 8, ***TPRN***- DEAFNESS 79, ***TRAPPC2***- SPONDYLOEPIPHYSEAL DYSPLASIA TARDA, ***TRIM32***- LIMB-GIRDLE MUSCULAR

DYSTROPHY 2H, ***TRIOBP***- DEAFNESS 28, ***TRMU***- LIVER FAILURE, INFANTILE, TRANSIENT, ***TTPA***- VITAMIN E, FAMILIAL ISOLATED DEFICIENCY OF, ***TULP1***- RETINITIS PIGMENTOSA 14, ***TYR***- ALBINISM, OCULOCUTANEOUS, TYPE IA; ALBINISM, OCULOCUTANEOUS, TYPE IB, ***TYRP1***- ALBINISM, OCULOCUTANEOUS, TYPE III, ***UGT1A1***- GILBERT SYNDROME, ***WNT10A***- ODONTOONYCHODERMAL DYSPLASIA, ***WNT7A***- FIBULAR APLASIA OR HYPOPLASIA, FEMORAL BOWING AND POLY-, SYN-, AND OLIGODACTYLY, ***ZNF469***- BRITTLE CORNEA SYNDROME 1

CATEGORY: LATE ONSET

AMPD1- MYOADENYLATE DEAMINASE DEFICIENCY, ***CP***- ACERULOPLASMINEMIA, ***GNE***- INCLUSION BODY MYOPATHY 2, ***HFE***- HEMOCHROMATOSIS, TYPE 1, ***HGD***- ALKAPTONURIA, ***MAK***- RETINITIS PIGMENTOSA 62, ***MUTYH***- FAMILIAL ADENOMATOUS POLYPOSIS, 2, ***MYO3A***- DEAFNESS 30, ***SERPINA1***- ALPHA-1-ANTITRYPSIN DEFICIENCY, ***SPG7***- SPASTIC PARAPLEGIA 7, ***VPS13A***- CHOREOACANTHOCYTOSIS

CATEGORY: UNPREDICTABLE

ACADS- SHORT-CHAIN ACYL-COA DEHYDROGENASE DEFICIENCY, ***ACADSB***- 2-METHYLBUTYRYL-COA DEHYDROGENASE DEFICIENCY, ***ADCK3***- COENZYME Q10 DEFICIENCY, PRIMARY, 4, ***ANTXR2***- HYALINE FIBROMATOSIS SYNDROME, ***AUH***- 3-METHYLGLUTACONIC ACIDURIA, TYPE I, ***COQ2***- COENZYME Q10 DEFICIENCY, ***CPT2***- CARNITINE PALMITOYLTRANSFERASE DEFICIENCY II, ***DARS2***- LEUKOENCEPHALOPATHY WITH BRAINSTEM AND SPINAL CORD INVOLVEMENT AND LACTATE ELEVATION, ***F5***- COAGULATION FACTOR V, ***FANCC***- FANCONI ANEMIA COMPLEMENTATION GROUP C, ***FGA***- AFIBRINOGENEMIA, CONGENITAL, ***FGB***- AFIBRINOGENEMIA, CONGENITAL, ***FGD4***- CHARCOT-MARIE-TOOTH-DISEASE,4H, ***FGG***- AFIBRINOGENEMIA, CONGENITAL, ***GBA***- GAUCHER DISEASE, TYPE I; GAUCHER DISEASE, TYPE II; GAUCHER DISEASE, TYPE III; GAUCHER DISEASE, TYPE IIIC, ***MED12***- OPITZ-KAVEGGIA SYNDROME, ***MPZ***- HYPERTROPHIC NEUROPATHY OF DEJERINE-SOTTAS, ***MTHFR***- HOMOCYSTINURIA DUE TO DEFICIENCY OF N(5,10)-METHYLENETETRAHYDROFOLATE REDUCTASE ACTIVITY, ***MT-ND5***- LEBER HEREDITARY OPTIC NEUROPATHY; MITOCHONDRIAL MYOPATHY, ENCEPHALOPATHY, LACTIC ACIDOSIS, AND STROKE-LIKE EPISODESLEIGH SYNDROME, ***MT-TF***- MITOCHONDRIAL MYOPATHY, ENCEPHALOPATHY, LACTIC ACIDOSIS, AND STROKE-LIKE EPISODES; MYOCLONIC EPILEPSY ASSOCIATED WITH RAGGED-RED FIBERS, ***MT-TH***- MYOCLONIC EPILEPSY ASSOCIATED WITH RAGGED-RED FIBERS LEBER HEREDITARY OPTIC NEUROPATHY; MITOCHONDRIAL MYOPATHY, ENCEPHALOPATHY, LACTIC ACIDOSIS, AND STROKE-LIKE EPISODES, ***MT-TK***- MYOCLONIC EPILEPSY ASSOCIATED WITH RAGGED-RED FIBERS, ***MT-TL1***- LEBER HEREDITARY OPTIC NEUROPATHY; MYOCLONIC EPILEPSY ASSOCIATED WITH RAGGED-RED FIBERS MITOCHONDRIAL MYOPATHY, ENCEPHALOPATHY, LACTIC ACIDOSIS, AND STROKE-LIKE EPISODES, ***MT-TS1***- MYOCLONIC EPILEPSY ASSOCIATED WITH RAGGED-RED FIBERS MITOCHONDRIAL MYOPATHY, ENCEPHALOPATHY, LACTIC ACIDOSIS, AND STROKE-LIKE EPISODES; DEAFNESS, NONSYNDROMIC SENSORINEURAL, MITOCHONDRIAL, ***MT-TS2***- MYOCLONIC EPILEPSY ASSOCIATED WITH RAGGED-RED FIBERS MITOCHONDRIAL MYOPATHY, ENCEPHALOPATHY, LACTIC ACIDOSIS, AND STROKE-LIKE EPISODES, ***MVK***- HYPER-IGD SYNDROME, ***NAGA***- SCHINDLER DISEASE, TYPE I, ***NR0B1***- 46,XY SEX REVERSAL 2, ***POLG***- MITOCHONDRIAL DNA DEPLETION SYNDROME 4B; SENSORY ATAXIC NEUROPATHY, DYSARTHRIA, AND OPHTHALMOPARESIS; PROGRESSIVE EXTERNAL OPHTHALMOPLEGIA WITH MITOCHONDRIAL DNA DELETIONS 1, ***PROC***- THROMBOPHILIA DUE TO PROTEIN C DEFICIENCY, ***PYGM***- GLYCOGEN STORAGE DISEASE V, ***SGCA***- MUSCULAR DYSTROPHY, LIMB-GIRDLE, TYPE 2D, ***SGCB***- MUSCULAR DYSTROPHY, LIMB-GIRDLE, TYPE 2E, ***SLC22A5***- CARNITINE DEFICIENCY, SYSTEMIC PRIMARY, ***SLC25A13***- CITRULLINEMIA, TYPE II, NEONATAL-ONSET, ***SLC34A2***- PULMONARY ALVEOLAR MICROLITHIASIS, ***TFR2***- HEMOCHROMATOSIS, TYPE 3, ***TTC19***- MITOCHONDRIAL COMPLEX III DEFICIENCY, NUCLEAR TYPE 2

Incidental Findings Results: No known pathogenic variants detected in genes causative of genetic conditions that are medically actionable (incidental findings).

Interpretation:

This individual requested to learn about findings of genetic conditions that are medically actionable (incidental findings; please see attached list). Our analysis did not identify any medically actionable findings in this individual. However, it is important to understand that genome sequencing is a screening test. This individual could carry a variant not detected by this test (see test limitations). In addition, only known pathogenic or likely pathogenic variants are reported.

Recommendations:

Genetic counseling is recommended if the patient has unresolved questions.

ACTIONABLE GENES

ACTA2 : Aortic aneurysm, familial thoracic ; *ACTC1*: Cardiomyopathy, dilated; Cardiomyopathy, familial hypertrophic; Left ventricular noncompaction ; *ACVRL1* : Telangiectasia, hereditary hemorrhagic ; *APC* : Familial adenomatous polyposis ; *APOB*: Familial hypercholesterolemia *few pathogenic variants; *BMPR1A*: Juvenile polyposis syndrome; *BRCA1* : Hereditary breast and ovarian cancer ; *BRCA2* : Hereditary breast and ovarian cancer susceptibility; *CACNA1C*: *SQTS-4*; *CACNA1S*: Malignant hyperthermia susceptibility ; *CACNB2*: *SQTS-5*; *CDC73*: Hyperparathyroidism-jaw tumor syndrome; *CDH1* : Hereditary diffuse gastric cancer ; *CDKN2A*: Melanoma and pancreatic cancer (mild/moderate genetic risk gene) ; *CNBPx*: Myotonic dystrophy 2; *COL3A1*: Ehlers-Danlos syndrome, vascular; *COL5A1*: Ehlers-Danlos syndrome, classic ; *COL5A2*: Ehlers-Danlos syndrome, classic; *DMPK*^{X}: Myotonic dystrophy 1; *DSC2*: Arrhythmogenic right ventricular dysplasia ; *DSG2*: Arrhythmogenic right ventricular dysplasia; Cardiomyopathy, dilated; *DSP*: Arrhythmogenic right ventricular dysplasia; *ENG*: Hereditary Hemorrhagic Telangiectasia; *EPCAM*: Hereditary nonpolyposis colorectal cancer/Lynch syndrome; *FBN1* : Marfan syndrome ; *FH* : Leiomyomatosis and renal cell cancer ; *FLCN*: Birt- Hogg-Dube syndrome ; *GCH1* : Dystonia, DOPA-responsive, with or without hyperphenylalaninemia ; *GREM1*: Hereditary Mixed Polyposis; *HMBS*: Porphyria, acute intermittent; *KCNE1* : LQTS-5; *KCNE2* : LQTS-6; *KCNH2* : LQTS-2, SQTS-1; *KCNQ1* : LQTS-1, SQTS-2; *KIT*: Gastrointestinal stromal tumor; *LDLR*: Hypercholesterolemia, familial ; *LMNA*: Cardiomyopathy; *MAX*: Susceptibility to pheochromocytoma; *MEN1* : Multiple endocrine neoplasia, type 1; *MET*: Renal cell carcinoma, papillary, familial ; *MLH1* : Hereditary nonpolyposis colorectal cancer/Lynch syndrome; *MSH2* : Hereditary nonpolyposis colorectal cancer/Lynch syndrome; *MSH6* : Hereditary nonpolyposis colorectal cancer/Lynch syndrome; *MUTYH* : MYH Associated polyposis ; *MYBPC3*: Cardiomyopathy, dilated; Cardiomyopathy, familial hypertrophic; *MYH11* : Aortic aneurysm, familial thoracic ; *MYH7*: Cardiomyopathy, dilated; Cardiomyopathy, familial hypertrophic; Left ventricular noncompaction; *MYL2*: Cardiomyopathy, familial hypertrophic; *MYL3*: Cardiomyopathy, familial hypertrophic; *MYLK*: Aortic aneurysm, familial thoracic; *NF2* : Neurofibromatosis, type 2 ; *PALB2*: Breast cancer, susceptibility; Pancreatic cancer, susceptibility; *PCSK9*: Familial hypercholesterolemia; *PDGFRA*: Gastrointestinal stromal tumor; *PKP2*: Arrhythmogenic right ventricular dysplasia ; *PLN*: Cardiomyopathy, dilated; Cardiomyopathy, familial hypertrophic; *PMS2* : Hereditary nonpolyposis colorectal cancer/Lynch syndrome; *POLD1*: Colorectal adenomas and carcinomas *only exonuclease domain; *POLE*: Colorectal adenomas and carcinomas *only exonuclease domain; *PRKAG2* : Wolff-Parkinson-White syndrome; Cardiomyopathy, hypertrophic 6 ; *PRKAR1A*: Carney complex, type 1 ; *PRKG1*: Familial thoracic aortic aneurysm *1 variant only c.530C>A, p.Arg177Gln; *PROC*: Thrombophilia due to protein C deficiency; *PROS1*: Thrombophilia due to protein S deficiency; *PRRT2*: Paroxysmal kinesigenic dyskinesia ; *PTCH1*: Basal cell nevus syndrome ; *PTEN* : Cowden syndrome; *RBM20*: Cardiomyopathy, dilated; *RET* : Multiple endocrine neoplasia Type 2 ; *RYR1*: Malignant hyperthermia susceptibility 1; *RYR2*: Arrhythmogenic right ventricular dysplasia; *SCG5*: Hereditary Mixed Polyposis; *SCN5A* : LQTS-3; BRGDA 1; *SDHAF2* : Hereditary paragangliomas and pheochromocytomas ; *SDHB* : Hereditary paragangliomas and pheochromocytomas ;*SDHC* : Hereditary paragangliomas and pheochromocytomas ; *SDHD* : Hereditary paragangliomas and pheochromocytomas ;

SERPINC1: Thrombophilia due to antithrombin III deficiency; *SGCD*: Cardiomyopathy, dilated; *SMAD3*: Loeys-Dietz syndrome 1C ; *SMAD4* : Juvenile polyposis syndrome; *STK11* : Peutz-Jeghers syndrome ; *TGFB2*: Loeys-Dietz syndrome, type 4; *TGFB3*: Arrhythmogenic right ventricular dysplasia 1; *TGFBR1* : Loeys-Dietz syndrome, type 1A and type 2A; *TGFBR2* : Hereditary nonpolyposis colorectal cancer/Lynch syndrome / Loeys-Dietz syndrome ; *TMEM127*: Susceptibility to pheochromocytoma; *TMEM43*: Arrhythmogenic right ventricular dysplasia 5; *TNNI3*: Cardiomyopathy, dilated; Cardiomyopathy, familial hypertrophic; *TNNT2*: Cardiomyopathy, dilated; Cardiomyopathy, familial hypertrophic; *TP53* : Li-Fraumeni syndrome ; *TPM1*: Cardiomyopathy, dilated; Cardiomyopathy, familial hypertrophic; *TSC1*: Tuberous sclerosis complex; *TSC2*: Tuberous sclerosis complex; *VHL* : von Hippel-Lindau syndrome

AUTOSOMAL RECESSIVE *(Homozygotes/compound heterozygotes)*

ATP7B: Wilson disease ; *BCHE*: Pseudocholinesterase deficiency (homozygotes for null alleles only); *BLM*: Bloom syndrome; *CASQ2*: Ventricular tachycardia, catecholaminergic polymorphic; *CFTR*: Cystic fibrosis; *CPT2*: CPT deficiency, hepatic, type II; *F5*: Factor V deficiency; *GAA*: Glycogen storage disease II; *HAMP*: Hemochromatosis, type 2B; *HFE*: Hemochromatosis *C282Y only (mild/moderate genetic risk gene); *HFE2*: Hemochromatosis, type 2A; *IDUA*: Mucopolysaccharidosis; *DLRAP1*: Hypercholesterolemia, familial; *PAH*: Phenylketonuria; *PCBD1*: Hyperphenylalaninemia, BH4-deficient, D; *PTS*: Hyperphenylalaninemia, BH4-deficient, A; *QDPR*: Hyperphenylalaninemia, BH4-deficient, C; *SERPINA1*: Emphysema due to AAT deficiency; *SLC25A13*:Citrullinemia, adult-onset type II; *SLC37A* : Glycogen storage disease Ib; Glycogen storage disease Ic; *SLC7A9*: Cystinuria; *SLC3A1*: Cystinuria

X-LINKED

DMD: Becker muscular dystrophy; Cardiomyopathy, dilated; Duchenne muscular dystrophy; *EMD*: Emery- Dreifuss muscular dystrophy 1; *GLA*: Fabry disease; *OTC*: Ornithine transcarbamylase deficiency

Figure S1

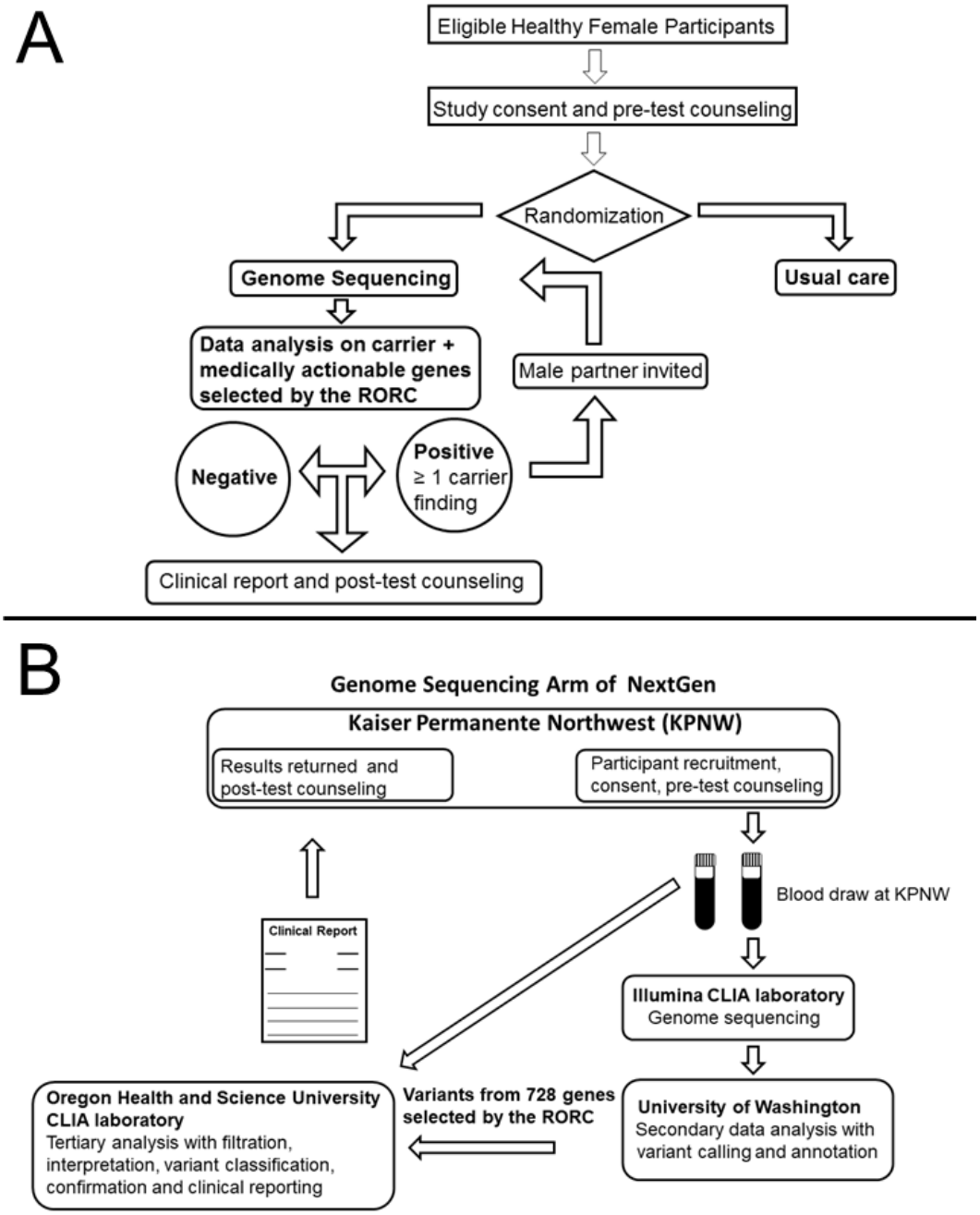

Figure S1: NextGen Study Design

A) A schematic representation of the sequential model for carrier screening in the NextGen study design. Participant surveys were conducted at the beginning of the study, upon receiving results, as well as a final survey at the end of the study for both arms, the Usual Care arm and the GS arm. B) Collaborators in the NextGen study. Further details are in the Methods section in the main text. RORC – Return of Results Committee

Table S1

Variant type	Percentage (%) (n= 195)
missense	48
nonsense	19
frameshift	18
Splice-error	11
Multi-exon deletion	2
Synonymous/splice-error	1
Whole gene deletion	0.5
In-frame deletion	0.5

Table S1: Frequency of the various types of variants reported in the study.
The percentage is based on 195 'distinct' variants (any repeated variant was not included) and includes both known and novel variants. The distribution of variant type is dependent on the pathogenic mechanism of disease for the genes-disorder categories as well as the sensitivity and specificity of the sequencing platform.