



Donor 6322

Genetic Testing Summary

Fairfax Cryobank recommends reviewing this genetic testing summary with your healthcare provider to determine suitability.

Last Updated: 04/21/21

Donor Reported Ancestry: English, Scottish, Irish, Polish

Jewish Ancestry: No

Genetic Test*	Result	Comments/Donor's Residual Risk**
Chromosome analysis (karyotype)	Normal male karyotype	No evidence of clinically significant chromosome abnormalities
Hemoglobin evaluation	Normal hemoglobin fractionation and MCV/MCH results	Reduced risk to be a carrier for sickle cell anemia, beta thalassemia, alpha thalassemia trait (aa/-- and a-/a-) and other hemoglobinopathies
Cystic Fibrosis (CF) carrier screening	Negative by gene sequencing in the CFTR gene	1/440
Spinal Muscular Atrophy (SMA) carrier screening	Negative for deletions of exon 7 in the SMN1 gene	1/894
Expanded Genetic Disease Carrier Screening Panel attached- 283 diseases by gene sequencing	<p>Carrier: Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (SACS)</p> <p>Carrier: Leber Congenital Amaurosis 10 and Other CEP290-Related Ciliopathies (CEP290)</p> <p>Negative for other genes sequenced.</p>	Partner testing recommended before using this donor.

*No single test can screen for all genetic disorders. A negative screening result significantly reduces, but cannot eliminate, the risk for these conditions in a pregnancy.

**Donor residual risk is the chance the donor is still a carrier after testing negative.



Patient Information

Name: Donor 6322
 Date of Birth: [REDACTED]
 Sema4 ID: [REDACTED]
 Client ID: [REDACTED]
 Indication: Carrier Testing

Specimen Information

Specimen Type: Blood
 Date Collected: 07/30/2020
 Date Received: 07/31/2020
 Final Report: 08/13/2020

Referring Provider

[REDACTED]
 Fairfax Cryobank, Inc.
 [REDACTED]
 [REDACTED]

Expanded Carrier Screen (283) Minus TSE

Number of genes tested: 283

SUMMARY OF RESULTS AND RECOMMENDATIONS

⊕ Positive	⊖ Negative
<p>Carrier of Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (AR) Associated gene(s): <i>SACS</i> Variant(s) Detected: c.8393C>A, p.P2798Q, Likely Pathogenic, Heterozygous (one copy)</p> <p>Carrier of Leber Congenital Amaurosis 10 and Other CEP290-Related Ciliopathies (AR) Associated gene(s): <i>CEP290</i> Variant(s) Detected: c.5668G>T, p.G1890X, Pathogenic, Heterozygous (one copy)</p>	<p>Negative for all other genes tested To view a full list of genes and diseases tested please see Table 1 in this report</p>

AR=Autosomal recessive; XL=X-linked

Recommendations

- Testing the partner for the above positive disorder(s) and genetic counseling are recommended.
- Please note that for female carriers of X-linked diseases, follow-up testing of a male partner is not indicated.
- CGG repeat analysis of *FMR1* for fragile X syndrome is not performed on males as repeat expansion of premutation alleles is not expected in the male germline.
- Individuals of Asian, African, Hispanic and Mediterranean ancestry should also be screened for hemoglobinopathies by CBC and hemoglobin electrophoresis.
- Consideration of residual risk by ethnicity after a negative carrier screen is recommended for the other diseases on the panel, especially in the case of a positive family history for a specific disorder.

Interpretation of positive results

Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (AR)

Results and Interpretation

A heterozygous (one copy) likely pathogenic missense variant, c.8393C>A, p.P2798Q, was detected in the *SACS* gene (NM_014363.5). When this variant is present in trans with a pathogenic variant, it is considered to be causative for autosomal recessive spastic ataxia of Charlevoix-Saguenay. Therefore, this individual is expected to be at least a carrier for autosomal recessive spastic ataxia of Charlevoix-Saguenay. Heterozygous carriers are not expected to exhibit symptoms of this disease.

What is Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay?

Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS) is an autosomal recessive disorder that is caused by pathogenic variants in the gene *SACS*, and has the highest prevalence among the French Canadian population. The clinical presentation includes progressive muscle damage and lack of muscle coordination, peripheral weakness and numbness caused by nerve damage (neuropathy), and muscle spasms (spasticity) in the lower limbs. For individuals of Quebec ancestry, the onset of symptoms usually begins between age 12-18 months with difficulty and unsteadiness while walking. Non-Quebec individuals with ARSACS often do not experience symptoms until later in childhood or adulthood. Other symptoms include involuntary eye movements, loss of sensation due to the progressive nerve damage, deformities of the fingers and feet, difficulties with speech, and yellow streaks in the retina. The cognitive skills of those affected with ARSACS tend to be in the lower range of normal, and while the disease is progressive, most individuals are capable of coping with daily living tasks. No genotype-phenotype correlation is currently known.

Leber Congenital Amaurosis 10 and Other CEP290-Related Ciliopathies (AR)

Results and Interpretation

A heterozygous (one copy) pathogenic premature stop codon, c.5668G>T, p.G1890X, was detected in the *CEP290* gene (NM_025114.3). When this variant is present in trans with a pathogenic variant, it is considered to be causative for a *CEP290*-related ciliopathy. Therefore, this individual is expected to be at least a carrier for a *CEP290*-related ciliopathy. Heterozygous carriers are not expected to exhibit symptoms of this disease.

What is Leber Congenital Amaurosis 10 and Other CEP290-Related Ciliopathies?

CEP290-related ciliopathies include four different overlapping disorders, known as Leber congenital amaurosis, Bardet-Biedl syndrome, Joubert syndrome and Meckel syndrome. All diseases are inherited in an autosomal recessive manner and can be caused by pathogenic variants in the gene *CEP290*. Leber congenital amaurosis manifests with vision loss at birth or in early infancy. Patients have profound loss of vision at an early age, and some have been reported to have intellectual disability. Bardet-Biedl syndrome is characterized by obesity, intellectual disability, kidney disease, and loss of vision beginning with loss of night vision and progression to tunnel vision and blindness. Clinical features of Joubert syndrome include intellectual disability, brain malformations, ocular problems including uncontrollable eye movements and loss of vision, and kidney cysts leading to end-stage renal disease. Meckel syndrome often manifests before birth and is characterized by occipital encephalocele, brain malformations, facial dysmorphism, renal agenesis, and extra digits. Life expectancy varies according to the phenotype; it is not reduced in patients with Leber congenital amaurosis, but death in infancy is expected in patients with Meckel syndrome. Currently, it is not possible to predict which phenotype a patient will have based on the variants inherited.

Test description

This patient was tested for a panel of diseases using a combination of sequencing, targeted genotyping and copy number analysis. Please note that negative results reduce but do not eliminate the possibility that this individual is a carrier for one or more of the disorders tested. Please see Table 1 for a list of genes and diseases tested, and go.sema4.com/residualrisk for specific detection rates and residual risk by ethnicity. With individuals of mixed ethnicity, it is recommended to use the highest residual risk estimate. Only variants determined to be pathogenic or likely pathogenic are reported in this carrier screening test.

Christie Buchovecky, PhD, Assistant Director, Reproductive Genomics
 Laboratory Medical Consultant: George A. Diaz, M.D., Ph.D.

Genes and diseases tested

For specific detection rates and residual risk by ethnicity, please visit go.sema4.com/residualrisk

Table 1: List of genes and diseases tested with detailed results

Disease	Gene	Inheritance Pattern	Status	Detailed Summary
⊕ Positive				
Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay	<i>SACS</i>	AR	Carrier	c.8393C>A, p.P2798Q, Likely Pathogenic, Heterozygous (one copy)
Leber Congenital Amaurosis 10 and Other CEP290-Related Ciliopathies	<i>CEP290</i>	AR	Carrier	c.5668G>T, p.G1890X, Pathogenic, Heterozygous (one copy)
⊖ Negative				
3-Beta-Hydroxysteroid Dehydrogenase Type II Deficiency	<i>HSD3B2</i>	AR	Reduced Risk	
3-Methylcrotonyl-CoA Carboxylase Deficiency (MCCC1-Related)	<i>MCCC1</i>	AR	Reduced Risk	
3-Methylcrotonyl-CoA Carboxylase Deficiency (MCCC2-Related)	<i>MCCC2</i>	AR	Reduced Risk	
3-Methylglutaconic Aciduria, Type III	<i>OPA3</i>	AR	Reduced Risk	
3-Phosphoglycerate Dehydrogenase Deficiency	<i>PHGDH</i>	AR	Reduced Risk	
6-Pyruvoyl-Tetrahydropterin Synthase Deficiency	<i>PTS</i>	AR	Reduced Risk	
Abetalipoproteinemia	<i>MTTP</i>	AR	Reduced Risk	
Achromatopsia (CNGB3-related)	<i>CNGB3</i>	AR	Reduced Risk	
Acrodermatitis Enteropathica	<i>SLC39A4</i>	AR	Reduced Risk	
Acute Infantile Liver Failure	<i>TRMU</i>	AR	Reduced Risk	
Acyl-CoA Oxidase I Deficiency	<i>ACOX1</i>	AR	Reduced Risk	
Adenosine Deaminase Deficiency	<i>ADA</i>	AR	Reduced Risk	
Adrenoleukodystrophy, X-Linked	<i>ABCD1</i>	XL	Reduced Risk	
Aicardi-Goutieres Syndrome (SAMHD1-Related)	<i>SAMHD1</i>	AR	Reduced Risk	
Alpha-Mannosidosis	<i>MAN2B1</i>	AR	Reduced Risk	
Alpha-Thalassemia	<i>HBA1/HBA2</i>	AR	Reduced Risk	<i>HBA1</i> Copy Number: 2 <i>HBA2</i> Copy Number: 2 No pathogenic copy number variants detected <i>HBA1/HBA2</i> Sequencing: Negative

Alpha-Thalassemia Mental Retardation Syndrome	<i>ATRX</i>	XL	Reduced Risk
Alport Syndrome (<i>COL4A3</i> -Related)	<i>COL4A3</i>	AR	Reduced Risk
Alport Syndrome (<i>COL4A4</i> -Related)	<i>COL4A4</i>	AR	Reduced Risk
Alport Syndrome (<i>COL4A5</i> -Related)	<i>COL4A5</i>	XL	Reduced Risk
Alstrom Syndrome	<i>ALMS1</i>	AR	Reduced Risk
Andermann Syndrome	<i>SLC12A6</i>	AR	Reduced Risk
Argininosuccinic Aciduria	<i>ASL</i>	AR	Reduced Risk
Aromatase Deficiency	<i>CYP19A1</i>	AR	Reduced Risk
Arthrogryposis, Mental Retardation, and Seizures	<i>SLC35A3</i>	AR	Reduced Risk
Asparagine Synthetase Deficiency	<i>ASNS</i>	AR	Reduced Risk
Aspartylglycosaminuria	<i>AGA</i>	AR	Reduced Risk
Ataxia With Isolated Vitamin E Deficiency	<i>TTPA</i>	AR	Reduced Risk
Ataxia-Telangiectasia	<i>ATM</i>	AR	Reduced Risk
Bardet-Biedl Syndrome (<i>BBS10</i> -Related)	<i>BBS10</i>	AR	Reduced Risk
Bardet-Biedl Syndrome (<i>BBS12</i> -Related)	<i>BBS12</i>	AR	Reduced Risk
Bardet-Biedl Syndrome (<i>BBS1</i> -Related)	<i>BBS1</i>	AR	Reduced Risk
Bardet-Biedl Syndrome (<i>BBS2</i> -Related)	<i>BBS2</i>	AR	Reduced Risk
Bare Lymphocyte Syndrome, Type II	<i>CIITA</i>	AR	Reduced Risk
Bartter Syndrome, Type 4A	<i>BSND</i>	AR	Reduced Risk
Bernard-Soulier Syndrome, Type A1	<i>GP1BA</i>	AR	Reduced Risk
Bernard-Soulier Syndrome, Type C	<i>GP9</i>	AR	Reduced Risk
Beta-Globin-Related Hemoglobinopathies	<i>HBB</i>	AR	Reduced Risk
Beta-Ketothiolase Deficiency	<i>ACAT1</i>	AR	Reduced Risk
Bilateral Frontoparietal Polymicrogyria	<i>GPR56</i>	AR	Reduced Risk
Biotinidase Deficiency	<i>BTD</i>	AR	Reduced Risk
Bloom Syndrome	<i>BLM</i>	AR	Reduced Risk
Canavan Disease	<i>ASPA</i>	AR	Reduced Risk
Carbamoylphosphate Synthetase I Deficiency	<i>CPS1</i>	AR	Reduced Risk
Carnitine Palmitoyltransferase IA Deficiency	<i>CPT1A</i>	AR	Reduced Risk
Carnitine Palmitoyltransferase II Deficiency	<i>CPT2</i>	AR	Reduced Risk
Carpenter Syndrome	<i>RAB23</i>	AR	Reduced Risk
Cartilage-Hair Hypoplasia	<i>RMRP</i>	AR	Reduced Risk
Cerebral Creatine Deficiency Syndrome 1	<i>SLC6A8</i>	XL	Reduced Risk
Cerebral Creatine Deficiency Syndrome 2	<i>GAMT</i>	AR	Reduced Risk
Cerebrotendinous Xanthomatosis	<i>CYP27A1</i>	AR	Reduced Risk
Charcot-Marie-Tooth Disease, Type 4D	<i>NDRG1</i>	AR	Reduced Risk

Charcot-Marie-Tooth Disease, Type 5 / Arts Syndrome	<i>PRPS1</i>	XL	Reduced Risk	
Charcot-Marie-Tooth Disease, X-Linked	<i>GJB1</i>	XL	Reduced Risk	
Choreoacanthocytosis	<i>VPS13A</i>	AR	Reduced Risk	
Choroideremia	<i>CHM</i>	XL	Reduced Risk	
Chronic Granulomatous Disease (CYBA-Related)	<i>CYBA</i>	AR	Reduced Risk	
Chronic Granulomatous Disease (CYBB-Related)	<i>CYBB</i>	XL	Reduced Risk	
Citrin Deficiency	<i>SLC25A13</i>	AR	Reduced Risk	
Citrullinemia, Type 1	<i>ASS1</i>	AR	Reduced Risk	
Cohen Syndrome	<i>VPS13B</i>	AR	Reduced Risk	
Combined Malonic and Methylmalonic Aciduria	<i>ACSF3</i>	AR	Reduced Risk	
Combined Oxidative Phosphorylation Deficiency 1	<i>GFM1</i>	AR	Reduced Risk	
Combined Oxidative Phosphorylation Deficiency 3	<i>TSFM</i>	AR	Reduced Risk	
Combined Pituitary Hormone Deficiency 2	<i>PROP1</i>	AR	Reduced Risk	
Combined Pituitary Hormone Deficiency 3	<i>LHX3</i>	AR	Reduced Risk	
Combined SAP Deficiency	<i>PSAP</i>	AR	Reduced Risk	
Congenital Adrenal Hyperplasia due to 17-Alpha-Hydroxylase Deficiency	<i>CYP17A1</i>	AR	Reduced Risk	
Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency	<i>CYP21A2</i>	AR	Reduced Risk	<i>CYP21A2</i> copy number: 2 <i>CYP21A2</i> sequencing: Negative
Congenital Amegakaryocytic Thrombocytopenia	<i>MPL</i>	AR	Reduced Risk	
Congenital Disorder of Glycosylation, Type Ia	<i>PMM2</i>	AR	Reduced Risk	
Congenital Disorder of Glycosylation, Type Ib	<i>MPI</i>	AR	Reduced Risk	
Congenital Disorder of Glycosylation, Type Ic	<i>ALG6</i>	AR	Reduced Risk	
Congenital Insensitivity to Pain with Anhidrosis	<i>NTRK1</i>	AR	Reduced Risk	
Congenital Myasthenic Syndrome (CHRNE-Related)	<i>CHRNE</i>	AR	Reduced Risk	
Congenital Myasthenic Syndrome (RAPSN-Related)	<i>RAPSN</i>	AR	Reduced Risk	
Congenital Neutropenia (HAX1-Related)	<i>HAX1</i>	AR	Reduced Risk	
Congenital Neutropenia (VPS45-Related)	<i>VPS45</i>	AR	Reduced Risk	
Corneal Dystrophy and Perceptive Deafness	<i>SLC4A11</i>	AR	Reduced Risk	
Corticosterone Methyloxidase Deficiency	<i>CYP11B2</i>	AR	Reduced Risk	
Cystic Fibrosis	<i>CFTR</i>	AR	Reduced Risk	
Cystinosis	<i>CTNS</i>	AR	Reduced Risk	
D-Bifunctional Protein Deficiency	<i>HSD17B4</i>	AR	Reduced Risk	
Deafness, Autosomal Recessive 77	<i>LOXHD1</i>	AR	Reduced Risk	
Duchenne Muscular Dystrophy / Becker Muscular Dystrophy	<i>DMD</i>	XL	Reduced Risk	
Dyskeratosis Congenita (RTEL1-Related)	<i>RTEL1</i>	AR	Reduced Risk	

Dystrophic Epidermolysis Bullosa	COL7A1	AR	Reduced Risk	
Ehlers-Danlos Syndrome, Type VIIC	ADAMTS2	AR	Reduced Risk	
Ellis-van Creveld Syndrome (EVC-Related)	EVC	AR	Reduced Risk	
Emery-Dreifuss Myopathy 1	EMD	XL	Reduced Risk	
Enhanced S-Cone Syndrome	NR2E3	AR	Reduced Risk	
Ethylmalonic Encephalopathy	ETHE1	AR	Reduced Risk	
Fabry Disease	GLA	XL	Reduced Risk	
Factor IX Deficiency	F9	XL	Reduced Risk	
Factor XI Deficiency	F11	AR	Reduced Risk	
Familial Autosomal Recessive Hypercholesterolemia	LDLRAP1	AR	Reduced Risk	
Familial Dysautonomia	IKBKAP	AR	Reduced Risk	
Familial Hypercholesterolemia	LDLR	AR	Reduced Risk	
Familial Hyperinsulinism (ABCC8-Related)	ABCC8	AR	Reduced Risk	
Familial Hyperinsulinism (KCNJ11-Related)	KCNJ11	AR	Reduced Risk	
Familial Mediterranean Fever	MEFV	AR	Reduced Risk	
Fanconi Anemia, Group A	FANCA	AR	Reduced Risk	
Fanconi Anemia, Group C	FANCC	AR	Reduced Risk	
Fanconi Anemia, Group G	FANCG	AR	Reduced Risk	
Fragile X Syndrome	FMR1	XL	Reduced Risk	<p>FMR1 CGG repeat sizes: Not Performed</p> <p>FMR1 Sequencing: Negative</p> <p>Fragile X CGG triplet repeat expansion testing was not performed at this time, as the patient has either been previously tested or is a male.</p>
Fumarase Deficiency	FH	AR	Reduced Risk	
GRACILE Syndrome and Other BCS1L-Related Disorders	BCS1L	AR	Reduced Risk	
Galactokinase Deficiency	GALK1	AR	Reduced Risk	
Galactosemia	GALT	AR	Reduced Risk	
Gaucher Disease	GBA	AR	Reduced Risk	
Gitelman Syndrome	SLC12A3	AR	Reduced Risk	
Glutaric Acidemia, Type I	GCDH	AR	Reduced Risk	
Glutaric Acidemia, Type IIa	ETFAL	AR	Reduced Risk	
Glutaric Acidemia, Type IIc	ETFDH	AR	Reduced Risk	
Glycine Encephalopathy (AMT-Related)	AMT	AR	Reduced Risk	
Glycine Encephalopathy (GLDC-Related)	GLDC	AR	Reduced Risk	
Glycogen Storage Disease, Type II	GAA	AR	Reduced Risk	
Glycogen Storage Disease, Type III	AGL	AR	Reduced Risk	
Glycogen Storage Disease, Type IV / Adult Polyglucosan Body Disease	GBE1	AR	Reduced Risk	

Glycogen Storage Disease, Type Ia	<i>G6PC</i>	AR	Reduced Risk
Glycogen Storage Disease, Type Ib	<i>SLC37A4</i>	AR	Reduced Risk
Glycogen Storage Disease, Type V	<i>PYGM</i>	AR	Reduced Risk
Glycogen Storage Disease, Type VII	<i>PFKM</i>	AR	Reduced Risk
HMG-CoA Lyase Deficiency	<i>HMGCL</i>	AR	Reduced Risk
Hemochromatosis, Type 2A	<i>HFE2</i>	AR	Reduced Risk
Hemochromatosis, Type 3	<i>TFR2</i>	AR	Reduced Risk
Hereditary Fructose Intolerance	<i>ALDOB</i>	AR	Reduced Risk
Hereditary Spastic Paraparesis 49	<i>TECPR2</i>	AR	Reduced Risk
Hermansky-Pudlak Syndrome, Type 1	<i>HPS1</i>	AR	Reduced Risk
Hermansky-Pudlak Syndrome, Type 3	<i>HPS3</i>	AR	Reduced Risk
Holocarboxylase Synthetase Deficiency	<i>HLCS</i>	AR	Reduced Risk
Homocystinuria (CBS-Related)	<i>CBS</i>	AR	Reduced Risk
Homocystinuria due to <i>MTHFR</i> Deficiency	<i>MTHFR</i>	AR	Reduced Risk
Homocystinuria, cblE Type	<i>MTRR</i>	AR	Reduced Risk
Hydrolethalus Syndrome	<i>HYLS1</i>	AR	Reduced Risk
Hyperomithinemia-Hyperammonemia-Homocitrullinuria Syndrome	<i>SLC25A15</i>	AR	Reduced Risk
Hypohidrotic Ectodermal Dysplasia 1	<i>EDA</i>	XL	Reduced Risk
Hypophosphatasia	<i>ALPL</i>	AR	Reduced Risk
Inclusion Body Myopathy 2	<i>GNE</i>	AR	Reduced Risk
Infantile Cerebral and Cerebellar Atrophy	<i>MED17</i>	AR	Reduced Risk
Isovaleric Acidemia	<i>IVD</i>	AR	Reduced Risk
Joubert Syndrome 2	<i>TMEM216</i>	AR	Reduced Risk
Joubert Syndrome 7 / Meckel Syndrome 5 / COACH Syndrome	<i>RPGRIP1L</i>	AR	Reduced Risk
Junctional Epidermolysis Bullosa (<i>LAMA3</i> -Related)	<i>LAMA3</i>	AR	Reduced Risk
Junctional Epidermolysis Bullosa (<i>LAMB3</i> -Related)	<i>LAMB3</i>	AR	Reduced Risk
Junctional Epidermolysis Bullosa (<i>LAMC2</i> -Related)	<i>LAMC2</i>	AR	Reduced Risk
Krabbe Disease	<i>GALC</i>	AR	Reduced Risk
Lamellar Ichthyosis, Type 1	<i>TGM1</i>	AR	Reduced Risk
Leber Congenital Amaurosis 13	<i>RDH12</i>	AR	Reduced Risk
Leber Congenital Amaurosis 2 / Retinitis Pigmentosa 20	<i>RPE65</i>	AR	Reduced Risk
Leber Congenital Amaurosis 5	<i>LCA5</i>	AR	Reduced Risk
Leber Congenital Amaurosis 8 / Retinitis Pigmentosa 12 / Pigmented Paravenous Chorioretinal Atrophy	<i>CRB1</i>	AR	Reduced Risk
Leigh Syndrome, French-Canadian Type	<i>LRPPRC</i>	AR	Reduced Risk

Lethal Congenital Contracture Syndrome 1 / Lethal Arthrogryposis with Anterior Horn Cell Disease	<i>GLE1</i>	AR	Reduced Risk
Leukoencephalopathy with Vanishing White Matter	<i>EIF2B5</i>	AR	Reduced Risk
Limb-Girdle Muscular Dystrophy, Type 2A	<i>CAPN3</i>	AR	Reduced Risk
Limb-Girdle Muscular Dystrophy, Type 2B	<i>DYSF</i>	AR	Reduced Risk
Limb-Girdle Muscular Dystrophy, Type 2C	<i>SGCG</i>	AR	Reduced Risk
Limb-Girdle Muscular Dystrophy, Type 2D	<i>SGCA</i>	AR	Reduced Risk
Limb-Girdle Muscular Dystrophy, Type 2E	<i>SGCB</i>	AR	Reduced Risk
Limb-Girdle Muscular Dystrophy, Type 2I	<i>FKRP</i>	AR	Reduced Risk
Lipoamide Dehydrogenase Deficiency	<i>DLD</i>	AR	Reduced Risk
Lipoid Adrenal Hyperplasia	<i>STAR</i>	AR	Reduced Risk
Lipoprotein Lipase Deficiency	<i>LPL</i>	AR	Reduced Risk
Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency	<i>HADHA</i>	AR	Reduced Risk
Lysinuric Protein Intolerance	<i>SLC7A7</i>	AR	Reduced Risk
Maple Syrup Urine Disease, Type 1a	<i>BCKDHA</i>	AR	Reduced Risk
Maple Syrup Urine Disease, Type 1b	<i>BCKDHB</i>	AR	Reduced Risk
Meckel 1 / Bardet-Biedl Syndrome 13	<i>MKS1</i>	AR	Reduced Risk
Medium Chain Acyl-CoA Dehydrogenase Deficiency	<i>ACADM</i>	AR	Reduced Risk
Megalencephalic Leukoencephalopathy with Subcortical Cysts	<i>MLC1</i>	AR	Reduced Risk
Menkes Disease	<i>ATP7A</i>	XL	Reduced Risk
Metachromatic Leukodystrophy	<i>ARSA</i>	AR	Reduced Risk
Methylmalonic Acidemia (MMAA-Related)	<i>MMAA</i>	AR	Reduced Risk
Methylmalonic Acidemia (MMAB-Related)	<i>MMAB</i>	AR	Reduced Risk
Methylmalonic Acidemia (MUT-Related)	<i>MUT</i>	AR	Reduced Risk
Methylmalonic Aciduria and Homocystinuria, Cobalamin C Type	<i>MMACHC</i>	AR	Reduced Risk
Methylmalonic Aciduria and Homocystinuria, Cobalamin D Type	<i>MMADHC</i>	AR	Reduced Risk
Microphthalmia / Anophthalmia	<i>VSX2</i>	AR	Reduced Risk
Mitochondrial Complex I Deficiency (ACAD9-Related)	<i>ACAD9</i>	AR	Reduced Risk
Mitochondrial Complex I Deficiency (NDUFAF5-Related)	<i>NDUFAF5</i>	AR	Reduced Risk
Mitochondrial Complex I Deficiency (NDUFS6-Related)	<i>NDUFS6</i>	AR	Reduced Risk
Mitochondrial DNA Depletion Syndrome 6 / Navajo Neurohepatopathy	<i>MPV17</i>	AR	Reduced Risk
Mitochondrial Myopathy and Sideroblastic Anemia 1	<i>PUS1</i>	AR	Reduced Risk
Mucopolipidosis II / IIIA	<i>GNPTAB</i>	AR	Reduced Risk

Mucopolidosis III Gamma	<i>GNPTG</i>	AR	Reduced Risk
Mucopolidosis IV	<i>MCOLN1</i>	AR	Reduced Risk
Mucopolysaccharidosis Type I	<i>IDUA</i>	AR	Reduced Risk
Mucopolysaccharidosis Type II	<i>IDS</i>	XL	Reduced Risk
Mucopolysaccharidosis Type IIIA	<i>SGSH</i>	AR	Reduced Risk
Mucopolysaccharidosis Type IIIB	<i>NAGLU</i>	AR	Reduced Risk
Mucopolysaccharidosis Type IIIC	<i>HGSNAT</i>	AR	Reduced Risk
Mucopolysaccharidosis Type IIID	<i>GNS</i>	AR	Reduced Risk
Mucopolysaccharidosis Type IVb / GM1 Gangliosidosis	<i>GLB1</i>	AR	Reduced Risk
Mucopolysaccharidosis type IX	<i>HYAL1</i>	AR	Reduced Risk
Mucopolysaccharidosis type VI	<i>ARSB</i>	AR	Reduced Risk
Multiple Sulfatase Deficiency	<i>SUMF1</i>	AR	Reduced Risk
Muscle-Eye-Brain Disease and Other <i>POMGNT1</i> -Related Congenital Muscular Dystrophy-Dystroglycanopathies	<i>POMGNT1</i>	AR	Reduced Risk
Myoneurogastrointestinal Encephalopathy	<i>TYMP</i>	AR	Reduced Risk
Myotubular Myopathy 1	<i>MTM1</i>	XL	Reduced Risk
N-Acetylglutamate Synthase Deficiency	<i>NAGS</i>	AR	Reduced Risk
Nemaline Myopathy 2	<i>NEB</i>	AR	Reduced Risk
Nephrogenic Diabetes Insipidus, Type II	<i>AQP2</i>	AR	Reduced Risk
Nephrotic Syndrome (<i>NPHS1</i> -Related) / Congenital Finnish Nephrosis	<i>NPHS1</i>	AR	Reduced Risk
Nephrotic Syndrome (<i>NPHS2</i> -Related) / Steroid-Resistant Nephrotic Syndrome	<i>NPHS2</i>	AR	Reduced Risk
Neuronal Ceroid-Lipofuscinosis (<i>CLN3</i> -Related)	<i>CLN3</i>	AR	Reduced Risk
Neuronal Ceroid-Lipofuscinosis (<i>CLN5</i> -Related)	<i>CLN5</i>	AR	Reduced Risk
Neuronal Ceroid-Lipofuscinosis (<i>CLN6</i> -Related)	<i>CLN6</i>	AR	Reduced Risk
Neuronal Ceroid-Lipofuscinosis (<i>CLN8</i> -Related)	<i>CLN8</i>	AR	Reduced Risk
Neuronal Ceroid-Lipofuscinosis (<i>MFSD8</i> -Related)	<i>MFSD8</i>	AR	Reduced Risk
Neuronal Ceroid-Lipofuscinosis (<i>PPT1</i> -Related)	<i>PPT1</i>	AR	Reduced Risk
Neuronal Ceroid-Lipofuscinosis (<i>TPP1</i> -Related)	<i>TPP1</i>	AR	Reduced Risk
Niemann-Pick Disease (<i>SMPD1</i> -Related)	<i>SMPD1</i>	AR	Reduced Risk
Niemann-Pick Disease, Type C (<i>NPC1</i> -Related)	<i>NPC1</i>	AR	Reduced Risk
Niemann-Pick Disease, Type C (<i>NPC2</i> -Related)	<i>NPC2</i>	AR	Reduced Risk
Nijmegen Breakage Syndrome	<i>NBN</i>	AR	Reduced Risk
Non-Syndromic Hearing Loss (<i>GJB2</i> -Related)	<i>GJB2</i>	AR	Reduced Risk
Odonto-Onycho-Dermal Dysplasia / Schopf-Schulz-Passarge Syndrome	<i>WNT10A</i>	AR	Reduced Risk
Omenn Syndrome (<i>RAG2</i> -Related)	<i>RAG2</i>	AR	Reduced Risk

Omenn Syndrome / Severe Combined Immunodeficiency, Athabaskan-Type	<i>DCLRE1C</i>	AR	Reduced Risk
Ornithine Aminotransferase Deficiency	<i>OAT</i>	AR	Reduced Risk
Ornithine Transcarbamylase Deficiency	<i>OTC</i>	XL	Reduced Risk
Osteopetrosis 1	<i>TCIRG1</i>	AR	Reduced Risk
Pendred Syndrome	<i>SLC26A4</i>	AR	Reduced Risk
Phenylalanine Hydroxylase Deficiency	<i>PAH</i>	AR	Reduced Risk
Polycystic Kidney Disease, Autosomal Recessive	<i>PKHD1</i>	AR	Reduced Risk
Polyglandular Autoimmune Syndrome, Type 1	<i>AIRE</i>	AR	Reduced Risk
Pontocerebellar Hypoplasia, Type 1A	<i>VRK1</i>	AR	Reduced Risk
Pontocerebellar Hypoplasia, Type 6	<i>RARS2</i>	AR	Reduced Risk
Primary Carnitine Deficiency	<i>SLC22A5</i>	AR	Reduced Risk
Primary Ciliary Dyskinesia (<i>DNAH5</i> -Related)	<i>DNAH5</i>	AR	Reduced Risk
Primary Ciliary Dyskinesia (<i>DNAI1</i> -Related)	<i>DNAI1</i>	AR	Reduced Risk
Primary Ciliary Dyskinesia (<i>DNAI2</i> -Related)	<i>DNAI2</i>	AR	Reduced Risk
Primary Hyperoxaluria, Type 1	<i>AGXT</i>	AR	Reduced Risk
Primary Hyperoxaluria, Type 2	<i>GRHPR</i>	AR	Reduced Risk
Primary Hyperoxaluria, Type 3	<i>HOGA1</i>	AR	Reduced Risk
Progressive Cerebello-Cerebral Atrophy	<i>SEPSECS</i>	AR	Reduced Risk
Progressive Familial Intrahepatic Cholestasis, Type 2	<i>ABCB11</i>	AR	Reduced Risk
Propionic Acidemia (<i>PCCA</i> -Related)	<i>PCCA</i>	AR	Reduced Risk
Propionic Acidemia (<i>PCCB</i> -Related)	<i>PCCB</i>	AR	Reduced Risk
Pycnodysostosis	<i>CTSK</i>	AR	Reduced Risk
Pyruvate Dehydrogenase E1-Alpha Deficiency	<i>PDHA1</i>	XL	Reduced Risk
Pyruvate Dehydrogenase E1-Beta Deficiency	<i>PDHB</i>	AR	Reduced Risk
Renal Tubular Acidosis and Deafness	<i>ATP6V1B1</i>	AR	Reduced Risk
Retinitis Pigmentosa 25	<i>EYS</i>	AR	Reduced Risk
Retinitis Pigmentosa 26	<i>CERKL</i>	AR	Reduced Risk
Retinitis Pigmentosa 28	<i>FAM161A</i>	AR	Reduced Risk
Retinitis Pigmentosa 59	<i>DHDDS</i>	AR	Reduced Risk
Rhizomelic Chondrodysplasia Punctata, Type 1	<i>PEX7</i>	AR	Reduced Risk
Rhizomelic Chondrodysplasia Punctata, Type 3	<i>AGPS</i>	AR	Reduced Risk
Roberts Syndrome	<i>ESCO2</i>	AR	Reduced Risk
Salla Disease	<i>SLC17A5</i>	AR	Reduced Risk
Sandhoff Disease	<i>HEXB</i>	AR	Reduced Risk
Schimke Immunoosseous Dysplasia	<i>SMARCAL1</i>	AR	Reduced Risk

Segawa Syndrome	<i>TH</i>	AR	Reduced Risk	
Sjogren-Larsson Syndrome	<i>ALDH3A2</i>	AR	Reduced Risk	
Smith-Lemli-Opitz Syndrome	<i>DHCR7</i>	AR	Reduced Risk	
Spinal Muscular Atrophy	<i>SMN1</i>	AR	Reduced Risk	<i>SMN1</i> copy number: 2 <i>SMN2</i> copy number: 1 c.*3+80T>G: Negative
Spondylothoracic Dysostosis	<i>MESP2</i>	AR	Reduced Risk	
Steel Syndrome	<i>COL27A1</i>	AR	Reduced Risk	
Stuve-Wiedemann Syndrome	<i>LIFR</i>	AR	Reduced Risk	
Sulfate Transporter-Related Osteochondrodysplasia	<i>SLC26A2</i>	AR	Reduced Risk	
Tay-Sachs Disease	<i>HEXA</i>	AR	Reduced Risk	
Tyrosinemia, Type I	<i>FAH</i>	AR	Reduced Risk	
Usher Syndrome, Type IB	<i>MYO7A</i>	AR	Reduced Risk	
Usher Syndrome, Type IC	<i>USH1C</i>	AR	Reduced Risk	
Usher Syndrome, Type ID	<i>CDH23</i>	AR	Reduced Risk	
Usher Syndrome, Type IF	<i>PCDH15</i>	AR	Reduced Risk	
Usher Syndrome, Type IIA	<i>USH2A</i>	AR	Reduced Risk	
Usher Syndrome, Type III	<i>CLRN1</i>	AR	Reduced Risk	
Very Long Chain Acyl-CoA Dehydrogenase Deficiency	<i>ACADVL</i>	AR	Reduced Risk	
Walker-Warburg Syndrome and Other <i>FKTN</i> -Related Dystrophies	<i>FKTN</i>	AR	Reduced Risk	
Wilson Disease	<i>ATP7B</i>	AR	Reduced Risk	
Wolman Disease / Cholesteryl Ester Storage Disease	<i>LIPA</i>	AR	Reduced Risk	
X-Linked Juvenile Retinoschisis	<i>RS1</i>	XL	Reduced Risk	
X-Linked Severe Combined Immunodeficiency	<i>IL2RG</i>	XL	Reduced Risk	
Zellweger Syndrome Spectrum (<i>PEX10</i> -Related)	<i>PEX10</i>	AR	Reduced Risk	
Zellweger Syndrome Spectrum (<i>PEX1</i> -Related)	<i>PEX1</i>	AR	Reduced Risk	
Zellweger Syndrome Spectrum (<i>PEX2</i> -Related)	<i>PEX2</i>	AR	Reduced Risk	
Zellweger Syndrome Spectrum (<i>PEX6</i> -Related)	<i>PEX6</i>	AR	Reduced Risk	

AR=Autosomal recessive; XL=X-linked

Test methods and comments

Genomic DNA isolated from this patient was analyzed by one or more of the following methodologies, as applicable:

Fragile X CGG Repeat Analysis (Analytical Detection Rate >99%)

PCR amplification using Asuragen, Inc. AmpliDeX[®] *FMR1* PCR reagents followed by capillary electrophoresis for allele sizing was performed. Samples positive for *FMR1* CGG repeats in the premutation and full mutation size range were further analyzed by Southern blot analysis to assess the size and methylation status of the *FMR1* CGG repeat.

Genotyping (Analytical Detection Rate >99%)

Multiplex PCR amplification and allele specific primer extension analyses using the MassARRAY® System were used to identify certain recurrent variants that are complex in nature or are present in low copy repeats. Rare sequence variants may interfere with assay performance.

Multiplex Ligation-Dependent Probe Amplification (MLPA) (Analytical Detection Rate >99%)

MLPA® probe sets and reagents from MRC-Holland were used for copy number analysis of specific targets versus known control samples. False positive or negative results may occur due to rare sequence variants in target regions detected by MLPA probes. Analytical sensitivity and specificity of the MLPA method are both 99%.

For alpha thalassemia, the copy numbers of the *HBA1* and *HBA2* genes were analyzed. Alpha-globin gene deletions, triplications, and the Constant Spring (CS) mutation are assessed. This test is expected to detect approximately 90% of all alpha-thalassemia mutations, varying by ethnicity. Carriers of alpha-thalassemia with three or more *HBA* copies on one chromosome, and one or no copies on the other chromosome, may not be detected. With the exception of triplications, other benign alpha-globin gene polymorphisms will not be reported. Analyses of *HBA1* and *HBA2* are performed in association with long-range PCR of the coding regions followed by short-read sequencing.

For Duchenne muscular dystrophy, the copy numbers of all *DMD* exons were analyzed. Potentially pathogenic single exon deletions and duplications are confirmed by a second method. Analysis of *DMD* is performed in association with sequencing of the coding regions.

For congenital adrenal hyperplasia, the copy number of the *CYP21A2* gene was analyzed. This analysis can detect large deletions typically due to unequal meiotic crossing-over between *CYP21A2* and the pseudogene *CYP21A1P*. Classic 30-kb deletions make up approximately 20% of *CYP21A2* pathogenic alleles. This test may also identify certain point mutations in *CYP21A2* caused by gene conversion events between *CYP21A2* and *CYP21A1P*. Some carriers may not be identified by dosage sensitive methods as this testing cannot detect individuals with two copies (duplication) of the *CYP21A2* gene on one chromosome and loss of *CYP21A2* (deletion) on the other chromosome. Analysis of *CYP21A2* is performed in association with long-range PCR of the coding regions followed by short-read sequencing.

For spinal muscular atrophy (SMA), the copy numbers of the *SMN1* and *SMN2* genes were analyzed. The individual dosage of exons 7 and 8 as well as the combined dosage of exons 1, 4, 6 and 8 of *SMN1* and *SMN2* were assessed. Copy number gains and losses can be detected with this assay. Depending on ethnicity, 6 - 29 % of carriers will not be identified by dosage sensitive methods as this testing cannot detect individuals with two copies (duplication) of the *SMN1* gene on one chromosome and loss of *SMN1* (deletion) on the other chromosome (silent 2+0 carrier) or individuals that carry an intragenic mutation in *SMN1*. Please also note that 2% of individuals diagnosed with SMA have a causative *SMN1* variant that occurred *de novo*, and therefore cannot be picked up by carrier screening in the parents. Analysis of *SMN1* is performed in association with short-read sequencing of exons 2a-7, followed by confirmation using long-range PCR (described below).

The presence of the c.*3+80T>G (chr5:70,247,901T>G) variant allele in an individual with Ashkenazi Jewish or Asian ancestry is typically indicative of a duplication of *SMN1*. When present in an Ashkenazi Jewish or Asian individual with two copies of *SMN1*, c.*3+80T>G is likely indicative of a silent (2+0) carrier. In individuals with two copies of *SMN1* with African American, Hispanic or Caucasian ancestry, the presence or absence of c.*3+80T>G significantly increases or decreases, respectively, the likelihood of being a silent 2+0 silent carrier.

MLPA for Gaucher disease (*GBA*), cystic fibrosis (*CFTR*), and non-syndromic hearing loss (*GJB2/GJB6*) will only be performed if indicated for confirmation of detected CNVs. If *GBA* analysis was performed, the copy numbers of exons 1, 3, 4, and 6 - 10 of the *GBA* gene (of 11 exons total) were analyzed. If *CFTR* analysis was performed, the copy numbers of all 27 *CFTR* exons were analyzed. If *GJB2/GJB6* analysis was performed, the copy number of the two *GJB2* exons were analyzed, as well as the presence or absence of the two upstream deletions of the *GJB2* regulatory region, del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854).

Next Generation Sequencing (NGS) (Analytical Detection Rate >95%)

NGS was performed on a panel of genes for the purpose of identifying pathogenic or likely pathogenic variants.

Agilent SureSelect™XT Low Input technology was used with a custom capture library to target the exonic regions and intron/exon splice junctions of the relevant genes, as well as a number of UTR, intronic or promoter regions that contain previously reported mutations. Libraries

were pooled and sequenced on the Illumina NovaSeq 9000 platform, using paired-end 100 bp reads. The sequencing data was analyzed using a custom bioinformatics algorithm designed and validated in house.

The coding exons and splice junctions of the known protein-coding RefSeq genes were assessed for the average depth of coverage (minimum of 20X) and data quality threshold values. Most exons not meeting a minimum of >20X read depth across the exon are further analyzed by Sanger sequencing. Please note that several genomic regions present difficulties in mapping or obtaining read depth >20X. These regions, which are described below, will not be reflexed to Sanger sequencing if the mapping quality or coverage is poor. Any variants identified during testing in these regions are confirmed by a second method and reported if determined to be pathogenic or likely pathogenic. However, as there is a possibility of false negative results within these regions, detection rates and residual risks for these genes have been calculated with the presumption that variants in these exons will not be detected, unless included in the MassARRAY® genotyping platform.

Exceptions: *ABCD1* (NM_000033.3) exons 8 and 9; *ADA* (NM_000022.2) exon 1; *ADAMTS2* (NM_014244.4) exon 1; *AGPS* (NM_003659.3) chr2:178,257,512 - 178,257,649 (partial exon 1); *ALMS1* (NM_015120.4) chr2:73,612,990 - 73,613,041 (partial exon 1); *CEP290* (NM_025114.3) exon 5, exon 7, chr12:88,519,017 - 88,519,039 (partial exon 13), chr12:88,514,049 - 88,514,058 (partial exon 15), chr12:88,502,837 - 88,502,841 (partial exon 23), chr12:88,481,551 - 88,481,589 (partial exon 32), chr12:88,471,605 - 88,471,700 (partial exon 40); *CFTR* (NM_000492.3) exon 10; *COL4A4* (NM_000092.4) chr2:227,942,604 - 227,942,619 (partial exon 25); *CYP11B2* (NM_000498.3) exons 3 - 7; *DNAI2* (NM_023036.4) chr17:72,308,136 - 72,308,147 (partial exon 12); *EVC* (NM_153717.2) exon 1; *FH* (NM_000143.3) exon 1; *GAMT* (NM_000156.5) exon 1; *GLDC* (NM_000170.2) exon 1; *GNPTAB* (NM_024312.4) chr17:4,837,000 - 4,837,400 (partial exon 2); *GNPTG* (NM_032520.4) exon 1; *HGSNAT* (NM_152419.2) exon 1; *IDS* (NM_000202.6) exon 3; *LIFR* (NM_002310.5) exon 19; *NEB* (NM_001271208.1) exons 82 - 105; *NPC1* (NM_000271.4) chr18:21,123,519 - 21,123,538 (partial exon 14); *PUS1* (NM_025215.5) ; chr12:132,414,446 - 132,414,532 (partial exon 2); *RPGRIP1L* (NM_015272.2) exon 23; *SGSH* (NM_000199.3) chr17:78,194,022 - 78,194,072 (partial exon 1); *SLC6A8* (NM_005629.3) exons 3 and 4.

This test will detect variants within the exons and the intron-exon boundaries of the target regions. Variants outside these regions may not be detected, including, but not limited to, UTRs, promoters, and deep intronic areas, or regions that fall into the Exceptions mentioned above. This technology may not detect all small insertion/deletions and is not diagnostic for repeat expansions and structural genomic variation. In addition, a mutation(s) in a gene not included on the panel could be present in this patient.

Variant interpretation and classification was performed based on the American College of Medical Genetics Standards and Guidelines for the Interpretation of Sequence Variants (Richards et al, 2015). All potentially pathogenic variants may be confirmed by either a specific genotyping assay or Sanger sequencing, if indicated. Any benign variants, likely benign variants or variants of uncertain significance identified during this analysis will not be reported.

Next Generation Sequencing for SMN1

Exonic regions and intron/exon splice junctions of *SMN1* and *SMN2* were captured, sequenced, and analyzed as described above. Any variants located within exons 2a-7 and classified as pathogenic or likely pathogenic were confirmed to be in either *SMN1* or *SMN2* using gene-specific long-range PCR analysis followed by Sanger sequencing. Variants located in exon 1 cannot be accurately assigned to either *SMN1* or *SMN2* using our current methodology, and so these variants are considered to be of uncertain significance and are not reported.

Copy Number Variant Analysis (Analytical Detection Rate >95%)

Large duplications and deletions were called from the relative read depths on an exon-by-exon basis using a custom exome hidden Markov model (XHMM) algorithm. Deletions or duplications determined to be pathogenic or likely pathogenic were confirmed by either a custom arrayCGH platform, quantitative PCR, or MLPA (depending on CNV size and gene content). While this algorithm is designed to pick up deletions and duplications of 2 or more exons in length, potentially pathogenic single-exon CNVs will be confirmed and reported, if detected.

Exon Array (Confirmation method) (Accuracy >99%)

The customized oligonucleotide microarray (Oxford Gene Technology) is a highly-targeted exon-focused array capable of detecting medically relevant microdeletions and microduplications at a much higher resolution than traditional aCGH methods. Each array matrix has approximately 180,000 60-mer oligonucleotide probes that cover the entire genome. This platform is designed based on human genome NCBI Build 37 (hg19) and the CGH probes are enriched to target the exonic regions of the genes in this panel.

Quantitative PCR (Confirmation method) (Accuracy >99%)

The relative quantification PCR is utilized on a Roche Universal Library Probe (UPL) system, which relates the PCR signal of the target region in one group to another. To test for genomic imbalances, both sample DNA and reference DNA is amplified with primer/probe sets that specific

to the target region and a control region with known genomic copy number. Relative genomic copy numbers are calculated based on the standard $\Delta\Delta C_t$ formula.

Long-Range PCR (Analytical Detection Rate >99%)

Long-range PCR was performed to generate locus-specific amplicons for *CYP21A2*, *HBA1* and *HBA2* and *GBA*. The PCR products were then prepared for short-read NGS sequencing and sequenced. Sequenced reads were mapped back to the original genomic locus and run through the bioinformatics pipeline. If indicated, copy number from MLPA was correlated with the sequencing output to analyze the results. For *CYP21A2*, a certain percentage of healthy individuals carry a duplication of the *CYP21A2* gene, which has no clinical consequences. In cases where two copies of a gene are located on the same chromosome in tandem, only the second copy will be amplified and assessed for potentially pathogenic variants, due to size limitations of the PCR reaction. However, because these alleles contain at least two copies of the *CYP21A2* gene in tandem, it is expected that this patient has at least one functional gene in the tandem allele and this patient is therefore less likely to be a carrier. When an individual carries both a duplication allele and a pathogenic variant, or multiple pathogenic variants, the current analysis may not be able to determine the phase (cis/trans configuration) of the *CYP21A2* alleles identified. Family studies may be required in certain scenarios where phasing is required to determine the carrier status.

Residual Risk Calculations

Carrier frequencies and detection rates for each ethnicity were calculated through the combination of internal curations of >30,000 variants and genomic frequency data from >138,000 individuals across seven ethnic groups in the gnomAD database. Additional variants in HGMD and novel deleterious variants were also incorporated into the calculation. Residual risk values are calculated using a Bayesian analysis combining the *a priori* risk of being a pathogenic mutation carrier (carrier frequency) and the detection rate. They are provided only as a guide for assessing approximate risk given a negative result, and values will vary based on the exact ethnic background of an individual. This report does not represent medical advice but should be interpreted by a genetic counselor, medical geneticist or physician skilled in genetic result interpretation and the relevant medical literature.

Personalized Residual Risk Calculations

Agilent SureSelectTMXT Low-Input technology was utilized in order to create whole-genome libraries for each patient sample. Libraries were then pooled and sequenced on the Illumina NovaSeq platform. Each sequencing lane was multiplexed to achieve 0.4-2x genome coverage, using paired-end 100 bp reads. The sequencing data underwent ancestral analysis using a customized, licensed bioinformatics algorithm that was validated in house. Identified sub-ethnic groupings were binned into one of 7 continental-level groups (African, East Asian, South Asian, Non-Finnish European, Finnish, Native American, and Ashkenazi Jewish) or, for those ethnicities that matched poorly to the continental-level groups, an 8th "unassigned" group, which were then used to select residual risk values for each gene. For individuals belonging to multiple high-level ethnic groupings, a weighting strategy was used to select the most appropriate residual risk. For genes that had insufficient data to calculate ethnic-specific residual risk values, or for sub-ethnic groupings that fell into the "unassigned" group, a "worldwide" residual risk was used. This "worldwide" residual risk was calculated using data from all available continental-level groups.

Sanger Sequencing (Confirmation method) (Accuracy >99%)

Sanger sequencing, as indicated, was performed using BigDye Terminator chemistry with the ABI 3730 DNA analyzer with target specific amplicons. It also may be used to supplement specific guaranteed target regions that fail NGS sequencing due to poor quality or low depth of coverage (<20 reads) or as a confirmatory method for NGS positive results. False negative results may occur if rare variants interfere with amplification or annealing.

Please note these tests were developed and their performance characteristics were determined by Mount Sinai Genomics, Inc. They have not been cleared or approved by the FDA. These analyses generally provide highly accurate information regarding the patient's carrier or affected status. Despite this high level of accuracy, it should be kept in mind that there are many potential sources of diagnostic error, including misidentification of samples, polymorphisms, or other rare genetic variants that interfere with analysis. Families should understand that rare diagnostic errors may occur for these reasons.

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Additional disease-specific references available upon request.