Fairfax Cryobank
The Trusted Choice for Donor Sperm

Donor 5787

Genetic Testing Summary

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

Last Updated: 12/18/19

Donor Reported Ancestry: African (Benin) 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	Low MCV, MCH (see below)	Likely carrier for alpha thalassemia and/or beta globin hemoglobinopathy (confirmed below)
Cystic Fibrosis (CF) carrier screening	Negative by gene sequencing in the CFTR gene	1/630
Spinal Muscular Atrophy (SMA) carrier screening	Negative for deletions of exon 7 in the SMN1 gene	1/4300
Expanded Genetic Disease Carrier Screening Panel attached- 283 diseases by gene sequencing	Carrier: 6-Pyruvoyl-Tetrahydropterin Synthetase Deficiency (PTS) Carrier: Alpha Thalassemia (HBA1/HBA2) -a/-a Carrier: Beta-Globin-Related Hemoglobinopathies (HBB) HbC trait Carrier: Congenital Adrenal Hyperplasia due to 21 Hydroxylase Deficiency (CYP21A2) Non-classic variant Negative for other genes sequenced.	Partner testing is 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 5787
Date of Birth: [redacted]
Sema4 ID: [redacted]
Client ID [redacted]
Indication: Carrier Testing

Specimen Information
Specimen Type: Blood
Date Collected: 07/03/2019
Date Received: 07/05/2019
Final Report: 07/18/2019

Referring Provider
[redacted]
Fairfax Cryobank, Inc.
[redacted]

Expanded Carrier Screen (283)

Number of genes tested: 283

SUMMARY OF RESULTS AND RECOMMENDATIONS

⊕ Positive	⊖ Negative
Carrier of 6-Pyruvoyl-Tetrahydropterin Synthase Deficiency (AR) Associated gene(s): *PTS* Variant(s) Detected: c.169_171delGTG, p.V57del, Pathogenic, Heterozygous (one copy) **Carrier of Alpha-Thalassemia (AR)** Associated gene(s): *HBA1/HBA2* Variant(s) Detected: Two copies of the alpha 3.7 deletion **Carrier of Beta-Globin-Related Hemoglobinopathies (AR)** Associated gene(s): *HBB* Variant(s) Detected: c.19G>A, p.E7K (HbC), Pathogenic, Heterozygous (one copy) **Carrier of Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency (AR)** Associated gene(s): *CYP21A2* Variant(s) Detected: c.841G>T, p.V281L, Pathogenic,Heterozygous (one copy)	**Negative for all other genes tested** To view a full list of genes and diseases tested please see Table 1 in this report

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

6-Pyruvoyl-Tetrahydropterin Synthase Deficiency (AR)

Results and Interpretation

A heterozygous (one copy) pathogenic inframe deletion, c.169_171delGTG, p.V57del, was detected in the *PTS* gene (NM_000317.2). When this variant is present in trans with a pathogenic variant, it is considered to be causative for 6-pyruvoyl-tetrahydropterin synthase deficiency. Therefore, this individual is expected to be at least a carrier for 6-pyruvoyl-tetrahydropterin synthase deficiency. Heterozygous carriers are not expected to exhibit symptoms of this disease.

What is 6-Pyruvoyl-Tetrahydropterin Synthase Deficiency?

6-pyruvoyl-tetrahydropterin synthase deficiency is an autosomal recessive disorder caused by pathogenic variants in the *PTS* gene. It has been reported in patients of different ethnicities, although it has an increased prevalence in individuals of Asian ancestry. Clinical features are typically noted at birth but can present in infancy. Patients with the severe form of the disease typically develop an excess of phenylalanine in the blood (hyperphenylalaninemia). Symptoms include psychomotor retardation, abnormal muscle tone, convulsions, fatigue, irritability, hyperthermia, hypersalivation and difficulty swallowing. The mild form of 6-pyruvoyl-tetrahydropterin synthase deficiency does not include neurological symptoms and is associated with mild to moderate transient hyperphenylalaninemia. Life expectancy is variable and no genotype-phenotype correlation has been reported.

Alpha-Thalassemia (AR)

Results and Interpretation

HBA1 Copy Number: 2
HBA2 Copy Number: 0
Two copies of the alpha 3.7 deletion detected
HBA1/HBA2 Sequencing: Negative

Gene(s) analyzed: *HBA1* (NM_000558.4) and *HBA2* (NM_000517.4)

Inheritance: Autosomal Recessive

This patient carries a homozygous alpha 3.7 deletion, resulting in the loss of two copies of the alpha-globin gene and is therefore a carrier of the alpha-thalassemia trait (-a/-a). No pathogenic or likely pathogenic variants were identified by sequence analysis.

Typically, individuals have four functional alpha-globin genes: 2 copies of *HBA1* and 2 copies of *HBA2*, whose expression is regulated by a cis-acting regulatory element HS-40. Alpha-thalassemia carriers have three (silent carrier) or two (carrier of the alpha-thalassemia trait) functional alpha-globin genes with or without a mild phenotype.

What is Alpha-Thalassemia?

Alpha-thalassemia is an autosomal recessive condition that affects the red blood cells. It can affect people of any ethnicity, but is more common in people who can trace their ancestry to Southeast Asia, India, equatorial Africa, the Mediterranean, or the Arabian Peninsula. There are two major forms of alpha-thalassemia:

- Hemoglobin Bart syndrome is caused by a loss of all 4 alpha-globin genes (--/--). It is very severe, and fetuses are either stillborn or die shortly after birth.
- Alpha-thalassemia (also called HbH disease) is caused by a loss of 3 alpha-globin genes (-a/--). This disease results in anemia, an enlarged spleen, and mild jaundice. Most individuals are mildly disabled by this condition. Some people with more severe disease require frequent blood transfusions.

The type of disease as well as the severity of symptoms can be predicted based on the genetic variants detected. Carriers may have mild anemia.

Beta-Globin-Related Hemoglobinopathies (AR)

Results and Interpretation

A heterozygous (one copy) pathogenic missense variant, c.19G>A, p.E7K (HbC), was detected in the *HBB* gene (NM_000518.4). This variant is considered to be pathogenic and when present in trans with a pathogenic variant causative for HbC disease or another beta-globin related hemoglobinopathy. Therefore, this individual is expected to be at least a carrier for HbC disease. Carriers are not expected to exhibit symptoms.

What is Beta-Globin-Related Hemoglobinopathies?

Pathogenic variants in the beta-globin gene (*HBB*) cause a variety of autosomal recessive diseases of aberrant hemoglobin, the protein that carries oxygen in the blood. The most frequent hemoglobinopathies are beta-thalassemia, sickle cell disease and HbC disease.

- In individuals with beta-thalassemia, hemoglobin is not properly synthesized and results in small red blood cells that are inefficient at carrying oxygen. Individuals with severe beta-thalassemia require life-long blood transfusions and chelation therapy to remove the extra iron that results from the blood transfusions. Individuals with milder forms of beta-thalassemia may not require transfusions. Although current treatments can extend the life expectancy into adulthood, patients usually do not survive beyond their 30s as a result of cardiac complications of iron overload. Individuals carrying one pathogenic allele causing beta-thalassemia in addition to 5 or more copies of HBA may develop a thalassemia intermedia phenotype with a variable clinical presentation, and may require recurrent transfusions.
- Sickle cell disease is caused by the inheritance of two copies of Hemoglobin S (HbS), encoded by a specific *HBB* variant. Symptoms typically first present in infancy or childhood and include chronic anemia, pain and/or swelling in the hands and feet, episodes of severe pain, and infections. The clinical presentation is highly variable between affected individuals. The life expectancy for individuals with sickle cell disease is in the 40s but may be increasing. HbS can also cause related diseases if it is inherited along with a different type of variant in *HBB*
- HbC disease is caused by the inheritance of two copies of Hemoglobin C (HbC), encoded by a specific *HBB* variant. HbC disease causes mild anemia in some patients, but the majority of affected individuals do not have any symptoms and have a normal life expectancy. HbC can also cause disease if it is inherited with another type of abnormal hemoglobin, the most common being HbS. The inheritance of one copy each of HbS and HbC result in SC disease, which may cause chronic anemia, pain and/or swelling in the hands and feet, episodes of severe pain, infections, and retinal disease. The life expectancy for individuals with SC disease is in the 60s.

The type of disease that will develop can be predicted based on the variants inherited. Variants causing beta-thalassemia are prevalent in Mediterranean and South-East Asian populations, whereas HbS is most common in people of African, Mediterranean, Middle Eastern, and Indian ancestry. HbC is most common in people of African descent.

Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency (AR)

Results and Interpretation

CYP21A2 copy number: 2
No pathogenic copy number variants detected
CYP21A2 sequencing: c.841G>T, p.V281L, Pathogenic,Heterozygous (one copy)

Gene(s) analyzed: *CYP21A2* (NM_000500.6)

Inheritance: Autosomal Recessive

A heterozygous (one copy) pathogenic missense variant, c.841G>T, p.V281L, was detected in the *CYP21A2* gene (NM_000500.6). Please note that this variant is typically causative for the non-classic form of congenital adrenal hyperplasia (PMID: 29450859). Variants associated with the non-classic form usually cause non-classic congenital adrenal hyperplasia when found in trans with a pathogenic allele, regardless of whether the second variant is associated with classic or non-classic disease (PMID: 29450859). Therefore, this individual is expected to be at least a carrier for non-classic congenital adrenal hyperplasia. Heterozygous carriers are not expected to exhibit symptoms of this disease.

What is congenital adrenal hyperplasia (due to 21-hydroxylase deficiency)?

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders resulting from deficiency in the enzymes involved in cortisol biosynthesis. The majority (95%) of CAH cases are due to 21-hydroxylase deficiency (21-OHD CAH), which is caused by homozygous or compound heterozygous pathogenic variants in the gene *CYP21A2*. Approximately 20% of mutant alleles have deletions of 30 kb that have been generated by unequal meiotic crossing-over between the two genes. Another 75% of mutant alleles are due to gene conversion events,

where an inactivating mutation from the *CYP21A1P* pseudogene is introduced into one copy of the *CYP21A2* gene, thus making the gene non-functional. Three different forms of 21-OHD CAH have been reported: a classic salt wasting form, a classic simple virilizing form, and a non-classic form.

- The classic salt wasting form results from a nonfunctional enzyme and is the most severe. The phenotype includes prenatal onset of virilization and inadequate adrenal aldosterone secretion that can result in fatal salt-wasting crises.
- The classic simple virilizing form results from low levels of functional enzyme and involves prenatal virilization but no salt-wasting.
- The non-classic form, which results from a mild enzyme deficiency, occurs postnatally and involves phenotypes associated with hyperandrogenism, such as hirsutism, delayed menarche, and infertility.

Treatment for the classic forms of the disorder include glucocorticoid and mineralocorticoid replacement therapy, as well as the possibility of feminizing genitoplasty, while patients with the non-classic form usually do not require treatment. The life expectancy for this disorder can be normal with treatment, however the occurrence of salt-wasting crises can be fatal.

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.

Anastasia Larmore, Ph.D., Assistant Laboratory Director
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**				
6-Pyruvoyl-Tetrahydropterin Synthase Deficiency	*PTS*	AR	Carrier	c.169_171delGTG, p.V57del, Pathogenic, Heterozygous (one copy)
Alpha-Thalassemia	*HBA1/HBA2*	AR	Carrier	*HBA1* Copy Number: 2 *HBA2* Copy Number: 0 Two copies of the alpha 3.7 deletion detected *HBA1/HBA2* Sequencing: Negative
Beta-Globin-Related Hemoglobinopathies	*HBB*	AR	Carrier	c.19G>A, p.E7K (HbC), Pathogenic, Heterozygous (one copy)
Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency	*CYP21A2*	AR	Carrier	*CYP21A2* copy number: 2 No pathogenic copy number variants detected *CYP21A2* sequencing: c.841G>T, p.V281L, Pathogenic,Heterozygous (one copy)

Negative			
3-Beta-Hydroxysteroid Dehydrogenase Type II Deficiency	*HSD3B2*	AR	Reduced Risk
3-Methylcrotonyl-CoA Carboxylase Deficiency (*MCCC1*-Related)	*MCCC1*	AR	Reduced Risk
3-Methylcrotonyl-CoA Carboxylase Deficiency (*MCCC2*-Related)	*MCCC2*	AR	Reduced Risk
3-Methylglutaconic Aciduria, Type III	*OPA3*	AR	Reduced Risk
3-Phosphoglycerate Dehydrogenase Deficiency	*PHGDH*	AR	Reduced Risk
Abetalipoproteinemia	*MTTP*	AR	Reduced Risk
Achromatopsia	*CNGB3*	AR	Reduced Risk
Acrodermatitis Enteropathica	*SLC39A4*	AR	Reduced Risk
Acute Infantile Liver Failure	*TRMU*	AR	Reduced Risk
Acyl-CoA Oxidase I Deficiency	*ACOX1*	AR	Reduced Risk
Adenosine Deaminase Deficiency	*ADA*	AR	Reduced Risk
Adrenoleukodystrophy, X-Linked	*ABCD1*	XL	Reduced Risk
Aicardi-Goutières Syndrome (*SAMHD1*-Related)	*SAMHD1*	AR	Reduced Risk
Alpha-Mannosidosis	*MAN2B1*	AR	Reduced Risk
Alpha-Thalassemia Mental Retardation Syndrome	*ATRX*	XL	Reduced Risk
Alport Syndrome (*COL4A3*-Related)	*COL4A3*	AR	Reduced Risk
Alport Syndrome (*COL4A4*-Related)	*COL4A4*	AR	Reduced Risk
Alport Syndrome (*COL4A5*-Related)	*COL4A5*	XL	Reduced Risk
Alstrom Syndrome	*ALMS1*	AR	Reduced Risk
Andermann Syndrome	*SLC12A6*	AR	Reduced Risk
Argininosuccinic Aciduria	*ASL*	AR	Reduced Risk
Aromatase Deficiency	*CYP19A1*	AR	Reduced Risk
Arthrogryposis, Mental Retardation, and Seizures	*SLC35A3*	AR	Reduced Risk
Asparagine Synthetase Deficiency	*ASNS*	AR	Reduced Risk
Aspartylglycosaminuria	*AGA*	AR	Reduced Risk
Ataxia With Isolated Vitamin E Deficiency	*TTPA*	AR	Reduced Risk
Ataxia-Telangiectasia	*ATM*	AR	Reduced Risk
Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay	*SACS*	AR	Reduced Risk
Bardet-Biedl Syndrome (*BBS10*-Related)	*BBS10*	AR	Reduced Risk
Bardet-Biedl Syndrome (*BBS12*-Related)	*BBS12*	AR	Reduced Risk
Bardet-Biedl Syndrome (*BBS1*-Related)	*BBS1*	AR	Reduced Risk
Bardet-Biedl Syndrome (*BBS2*-Related)	*BBS2*	AR	Reduced Risk

Bare Lymphocyte Syndrome, Type II	*CIITA*	AR	Reduced Risk
Bartter Syndrome, Type 4A	*BSND*	AR	Reduced Risk
Bernard-Soulier Syndrome, Type A1	*GP1BA*	AR	Reduced Risk
Bernard-Soulier Syndrome, Type C	*GP9*	AR	Reduced Risk
Beta-Ketothiolase Deficiency	*ACAT1*	AR	Reduced Risk
Bilateral Frontoparietal Polymicrogyria	*GPR56*	AR	Reduced Risk
Biotinidase Deficiency	*BTD*	AR	Reduced Risk
Bloom Syndrome	*BLM*	AR	Reduced Risk
Canavan Disease	*ASPA*	AR	Reduced Risk
Carbamoylphosphate Synthetase I Deficiency	*CPS1*	AR	Reduced Risk
Carnitine Palmitoyltransferase IA Deficiency	*CPT1A*	AR	Reduced Risk
Carnitine Palmitoyltransferase II Deficiency	*CPT2*	AR	Reduced Risk
Carpenter Syndrome	*RAB23*	AR	Reduced Risk
Cartilage-Hair Hypoplasia	*RMRP*	AR	Reduced Risk
Cerebral Creatine Deficiency Syndrome 1	*SLC6A8*	XL	Reduced Risk
Cerebral Creatine Deficiency Syndrome 2	*GAMT*	AR	Reduced Risk
Cerebrotendinous Xanthomatosis	*CYP27A1*	AR	Reduced Risk
Charcot-Marie-Tooth Disease, Type 4D	*NDRG1*	AR	Reduced Risk
Charcot-Marie-Tooth Disease, Type 5 / Arts Syndrome	*PRPS1*	XL	Reduced Risk
Charcot-Marie-Tooth Disease, X-Linked	*GJB1*	XL	Reduced Risk
Choreoacanthocytosis	*VPS13A*	AR	Reduced Risk
Choroideremia	*CHM*	XL	Reduced Risk
Chronic Granulomatous Disease (*CYBA*-Related)	*CYBA*	AR	Reduced Risk
Chronic Granulomatous Disease (*CYBB*-Related)	*CYBB*	XL	Reduced Risk
Citrin Deficiency	*SLC25A13*	AR	Reduced Risk
Citrullinemia, Type 1	*ASS1*	AR	Reduced Risk
Cohen Syndrome	*VPS13B*	AR	Reduced Risk
Combined Malonic and Methylmalonic Aciduria	*ACSF3*	AR	Reduced Risk
Combined Oxidative Phosphorylation Deficiency 1	*GFM1*	AR	Reduced Risk
Combined Oxidative Phosphorylation Deficiency 3	*TSFM*	AR	Reduced Risk
Combined Pituitary Hormone Deficiency 2	*PROP1*	AR	Reduced Risk
Combined Pituitary Hormone Deficiency 3	*LHX3*	AR	Reduced Risk
Combined SAP Deficiency	*PSAP*	AR	Reduced Risk
Congenital Adrenal Hyperplasia due to 17-Alpha-Hydroxylase Deficiency	*CYP17A1*	AR	Reduced Risk
Congenital Amegakaryocytic Thrombocytopenia	*MPL*	AR	Reduced Risk

Congenital Disorder of Glycosylation, Type Ia	*PMM2*	AR	Reduced Risk
Congenital Disorder of Glycosylation, Type Ib	*MPI*	AR	Reduced Risk
Congenital Disorder of Glycosylation, Type Ic	*ALG6*	AR	Reduced Risk
Congenital Insensitivity to Pain with Anhidrosis	*NTRK1*	AR	Reduced Risk
Congenital Myasthenic Syndrome (*CHRNE*-Related)	*CHRNE*	AR	Reduced Risk
Congenital Myasthenic Syndrome (*RAPSN*-Related)	*RAPSN*	AR	Reduced Risk
Congenital Neutropenia (*HAX1*-Related)	*HAX1*	AR	Reduced Risk
Congenital Neutropenia (*VPS45*-Related)	*VPS45*	AR	Reduced Risk
Corneal Dystrophy and Perceptive Deafness	*SLC4A11*	AR	Reduced Risk
Corticosterone Methyloxidase Deficiency	*CYP11B2*	AR	Reduced Risk
Cystic Fibrosis	*CFTR*	AR	Reduced Risk
Cystinosis	*CTNS*	AR	Reduced Risk
D-Bifunctional Protein Deficiency	*HSD17B4*	AR	Reduced Risk
Deafness, Autosomal Recessive 77	*LOXHD1*	AR	Reduced Risk
Duchenne Muscular Dystrophy / Becker Muscular Dystrophy	*DMD*	XL	Reduced Risk
Dyskeratosis Congenita (*RTEL1*-Related)	*RTEL1*	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	*FMR1* CGG repeat sizes: Not Performed *FMR1* Sequencing: Negative 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.
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	*ETFA*	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	*G6PC*	AR	Reduced Risk	
Glycogen Storage Disease, Type Ib	*SLC37A4*	AR	Reduced Risk	
Glycogen Storage Disease, Type V	*PYGM*	AR	Reduced Risk	
Glycogen Storage Disease, Type VII	*PFKM*	AR	Reduced Risk	
HMG-CoA Lyase Deficiency	*HMGCL*	AR	Reduced Risk	
Hemochromatosis, Type 2A	*HFE2*	AR	Reduced Risk	
Hemochromatosis, Type 3	*TFR2*	AR	Reduced Risk	
Hereditary Fructose Intolerance	*ALDOB*	AR	Reduced Risk	
Hereditary Spastic Paraparesis 49	*TECPR2*	AR	Reduced Risk	
Hermansky-Pudlak Syndrome, Type 1	*HPS1*	AR	Reduced Risk	
Hermansky-Pudlak Syndrome, Type 3	*HPS3*	AR	Reduced Risk	
Holocarboxylase Synthetase Deficiency	*HLCS*	AR	Reduced Risk	
Homocystinuria (*CBS*-Related)	*CBS*	AR	Reduced Risk	
Homocystinuria due to *MTHFR* Deficiency	*MTHFR*	AR	Reduced Risk	
Homocystinuria, cblE Type	*MTRR*	AR	Reduced Risk	
Hydrolethalus Syndrome	*HYLS1*	AR	Reduced Risk	
Hyperornithinemia-Hyperammonemia-Homocitrullinuria Syndrome	*SLC25A15*	AR	Reduced Risk	

Hypohidrotic Ectodermal Dysplasia 1	*EDA*	XL	Reduced Risk
Hypophosphatasia	*ALPL*	AR	Reduced Risk
Inclusion Body Myopathy 2	*GNE*	AR	Reduced Risk
Infantile Cerebral and Cerebellar Atrophy	*MED17*	AR	Reduced Risk
Isovaleric Acidemia	*IVD*	AR	Reduced Risk
Joubert Syndrome 2	*TMEM216*	AR	Reduced Risk
Joubert Syndrome 7 / Meckel Syndrome 5 / COACH Syndrome	*RPGRIP1L*	AR	Reduced Risk
Junctional Epidermolysis Bullosa (*LAMA3*-Related)	*LAMA3*	AR	Reduced Risk
Junctional Epidermolysis Bullosa (*LAMB3*-Related)	*LAMB3*	AR	Reduced Risk
Junctional Epidermolysis Bullosa (*LAMC2*-Related)	*LAMC2*	AR	Reduced Risk
Krabbe Disease	*GALC*	AR	Reduced Risk
Lamellar Ichthyosis, Type 1	*TGM1*	AR	Reduced Risk
Leber Congenital Amaurosis 10 and Other CEP290-Related Ciliopathies	*CEP290*	AR	Reduced Risk
Leber Congenital Amaurosis 13	*RDH12*	AR	Reduced Risk
Leber Congenital Amaurosis 2 / Retinitis Pigmentosa 20	*RPE65*	AR	Reduced Risk
Leber Congenital Amaurosis 5	*LCA5*	AR	Reduced Risk
Leber Congenital Amaurosis 8 / Retinitis Pigmentosa 12 / Pigmented Paravenous Chorioretinal Atrophy	*CRB1*	AR	Reduced Risk
Leigh Syndrome, French-Canadian Type	*LRPPRC*	AR	Reduced Risk
Lethal Congenital Contracture Syndrome 1 / Lethal Arthrogryposis with Anterior Horn Cell Disease	*GLE1*	AR	Reduced Risk
Leukoencephalopathy with Vanishing White Matter	*EIF2B5*	AR	Reduced Risk
Limb-Girdle Muscular Dystrophy, Type 2A	*CAPN3*	AR	Reduced Risk
Limb-Girdle Muscular Dystrophy, Type 2B	*DYSF*	AR	Reduced Risk
Limb-Girdle Muscular Dystrophy, Type 2C	*SGCG*	AR	Reduced Risk
Limb-Girdle Muscular Dystrophy, Type 2D	*SGCA*	AR	Reduced Risk
Limb-Girdle Muscular Dystrophy, Type 2E	*SGCB*	AR	Reduced Risk
Limb-Girdle Muscular Dystrophy, Type 2I	*FKRP*	AR	Reduced Risk
Lipoamide Dehydrogenase Deficiency	*DLD*	AR	Reduced Risk
Lipoid Adrenal Hyperplasia	*STAR*	AR	Reduced Risk
Lipoprotein Lipase Deficiency	*LPL*	AR	Reduced Risk
Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency	*HADHA*	AR	Reduced Risk
Lysinuric Protein Intolerance	*SLC7A7*	AR	Reduced Risk
Maple Syrup Urine Disease, Type 1a	*BCKDHA*	AR	Reduced Risk
Maple Syrup Urine Disease, Type 1b	*BCKDHB*	AR	Reduced Risk

Meckel 1 / Bardet-Biedl Syndrome 13	*MKS1*	AR	Reduced Risk
Medium Chain Acyl-CoA Dehydrogenase Deficiency	*ACADM*	AR	Reduced Risk
Megalencephalic Leukoencephalopathy with Subcortical Cysts	*MLC1*	AR	Reduced Risk
Menkes Disease	*ATP7A*	XL	Reduced Risk
Metachromatic Leukodystrophy	*ARSA*	AR	Reduced Risk
Methylmalonic Acidemia (*MMAA*-Related)	*MMAA*	AR	Reduced Risk
Methylmalonic Acidemia (*MMAB*-Related)	*MMAB*	AR	Reduced Risk
Methylmalonic Acidemia (*MUT*-Related)	*MUT*	AR	Reduced Risk
Methylmalonic Aciduria and Homocystinuria, Cobalamin C Type	*MMACHC*	AR	Reduced Risk
Methylmalonic Aciduria and Homocystinuria, Cobalamin D Type	*MMADHC*	AR	Reduced Risk
Microphthalmia / Anophthalmia	*VSX2*	AR	Reduced Risk
Mitochondrial Complex I Deficiency (*ACAD9*-Related)	*ACAD9*	AR	Reduced Risk
Mitochondrial Complex I Deficiency (*NDUFAF5*-Related)	*NDUFAF5*	AR	Reduced Risk
Mitochondrial Complex I Deficiency (*NDUFS6*-Related)	*NDUFS6*	AR	Reduced Risk
Mitochondrial DNA Depletion Syndrome 6 / Navajo Neurohepatopathy	*MPV17*	AR	Reduced Risk
Mitochondrial Myopathy and Sideroblastic Anemia 1	*PUS1*	AR	Reduced Risk
Mucolipidosis II / IIIA	*GNPTAB*	AR	Reduced Risk
Mucolipidosis III Gamma	*GNPTG*	AR	Reduced Risk
Mucolipidosis IV	*MCOLN1*	AR	Reduced Risk
Mucopolysaccharidosis Type I	*IDUA*	AR	Reduced Risk
Mucopolysaccharidosis Type II	*IDS*	XL	Reduced Risk
Mucopolysaccharidosis Type IIIA	*SGSH*	AR	Reduced Risk
Mucopolysaccharidosis Type IIIB	*NAGLU*	AR	Reduced Risk
Mucopolysaccharidosis Type IIIC	*HGSNAT*	AR	Reduced Risk
Mucopolysaccharidosis Type IIID	*GNS*	AR	Reduced Risk
Mucopolysaccharidosis Type IVb / GM1 Gangliosidosis	*GLB1*	AR	Reduced Risk
Mucopolysaccharidosis type IX	*HYAL1*	AR	Reduced Risk
Mucopolysaccharidosis type VI	*ARSB*	AR	Reduced Risk
Multiple Sulfatase Deficiency	*SUMF1*	AR	Reduced Risk
Muscle-Eye-Brain Disease and Other *POMGNT1*-Related Congenital Muscular Dystrophy-Dystroglycanopathies	*POMGNT1*	AR	Reduced Risk
Myoneurogastrointestinal Encephalopathy	*TYMP*	AR	Reduced Risk
Myotubular Myopathy 1	*MTM1*	XL	Reduced Risk
N-Acetylglutamate Synthase Deficiency	*NAGS*	AR	Reduced Risk

Nemaline Myopathy 2	*NEB*	AR	Reduced Risk
Nephrogenic Diabetes Insipidus, Type II	*AQP2*	AR	Reduced Risk
Nephrotic Syndrome (*NPHS1*-Related) / Congenital Finnish Nephrosis	*NPHS1*	AR	Reduced Risk
Nephrotic Syndrome (*NPHS2*-Related) / Steroid-Resistant Nephrotic Syndrome	*NPHS2*	AR	Reduced Risk
Neuronal Ceroid-Lipofuscinosis (*CLN3*-Related)	*CLN3*	AR	Reduced Risk
Neuronal Ceroid-Lipofuscinosis (*CLN5*-Related)	*CLN5*	AR	Reduced Risk
Neuronal Ceroid-Lipofuscinosis (*CLN6*-Related)	*CLN6*	AR	Reduced Risk
Neuronal Ceroid-Lipofuscinosis (*CLN8*-Related)	*CLN8*	AR	Reduced Risk
Neuronal Ceroid-Lipofuscinosis (*MFSD8*-Related)	*MFSD8*	AR	Reduced Risk
Neuronal Ceroid-Lipofuscinosis (*PPT1*-Related)	*PPT1*	AR	Reduced Risk
Neuronal Ceroid-Lipofuscinosis (*TPP1*-Related)	*TPP1*	AR	Reduced Risk
Niemann-Pick Disease (*SMPD1*-Related)	*SMPD1*	AR	Reduced Risk
Niemann-Pick Disease, Type C (*NPC1*-Related)	*NPC1*	AR	Reduced Risk
Niemann-Pick Disease, Type C (*NPC2*-Related)	*NPC2*	AR	Reduced Risk
Nijmegen Breakage Syndrome	*NBN*	AR	Reduced Risk
Non-Syndromic Hearing Loss (*GJB2*-Related)	*GJB2*	AR	Reduced Risk
Odonto-Onycho-Dermal Dysplasia / Schopf-Schulz-Passarge Syndrome	*WNT10A*	AR	Reduced Risk
Omenn Syndrome (*RAG2*-Related)	*RAG2*	AR	Reduced Risk
Omenn Syndrome / Severe Combined Immunodeficiency, Athabaskan-Type	*DCLRE1C*	AR	Reduced Risk
Ornithine Aminotransferase Deficiency	*OAT*	AR	Reduced Risk
Ornithine Transcarbomylase Deficiency	*OTC*	XL	Reduced Risk
Osteopetrosis 1	*TCIRG1*	AR	Reduced Risk
Pendred Syndrome	*SLC26A4*	AR	Reduced Risk
Phenylalanine Hydroxylase Deficiency	*PAH*	AR	Reduced Risk
Polycystic Kidney Disease, Autosomal Recessive	*PKHD1*	AR	Reduced Risk
Polyglandular Autoimmune Syndrome, Type 1	*AIRE*	AR	Reduced Risk
Pontocerebellar Hypoplasia, Type 1A	*VRK1*	AR	Reduced Risk
Pontocerebellar Hypoplasia, Type 6	*RARS2*	AR	Reduced Risk
Primary Carnitine Deficiency	*SLC22A5*	AR	Reduced Risk
Primary Ciliary Dyskinesia (*DNAH5*-Related)	*DNAH5*	AR	Reduced Risk
Primary Ciliary Dyskinesia (*DNAI1*-Related)	*DNAI1*	AR	Reduced Risk
Primary Ciliary Dyskinesia (*DNAI2*-Related)	*DNAI2*	AR	Reduced Risk
Primary Hyperoxaluria, Type 1	*AGXT*	AR	Reduced Risk

Primary Hyperoxaluria, Type 2	*GRHPR*	AR	Reduced Risk	
Primary Hyperoxaluria, Type 3	*HOGA1*	AR	Reduced Risk	
Progressive Cerebello-Cerebral Atrophy	*SEPSECS*	AR	Reduced Risk	
Progressive Familial Intrahepatic Cholestasis, Type 2	*ABCB11*	AR	Reduced Risk	
Propionic Acidemia (*PCCA*-Related)	*PCCA*	AR	Reduced Risk	
Propionic Acidemia (*PCCB*-Related)	*PCCB*	AR	Reduced Risk	
Pycnodysostosis	*CTSK*	AR	Reduced Risk	
Pyruvate Dehydrogenase E1-Alpha Deficiency	*PDHA1*	XL	Reduced Risk	
Pyruvate Dehydrogenase E1-Beta Deficiency	*PDHB*	AR	Reduced Risk	
Renal Tubular Acidosis and Deafness	*ATP6V1B1*	AR	Reduced Risk	
Retinitis Pigmentosa 25	*EYS*	AR	Reduced Risk	
Retinitis Pigmentosa 26	*CERKL*	AR	Reduced Risk	
Retinitis Pigmentosa 28	*FAM161A*	AR	Reduced Risk	
Retinitis Pigmentosa 59	*DHDDS*	AR	Reduced Risk	
Rhizomelic Chondrodysplasia Punctata, Type 1	*PEX7*	AR	Reduced Risk	
Rhizomelic Chondrodysplasia Punctata, Type 3	*AGPS*	AR	Reduced Risk	
Roberts Syndrome	*ESCO2*	AR	Reduced Risk	
Salla Disease	*SLC17A5*	AR	Reduced Risk	
Sandhoff Disease	*HEXB*	AR	Reduced Risk	
Schimke Immunoosseous Dysplasia	*SMARCAL1*	AR	Reduced Risk	
Segawa Syndrome	*TH*	AR	Reduced Risk	
Sjogren-Larsson Syndrome	*ALDH3A2*	AR	Reduced Risk	
Smith-Lemli-Opitz Syndrome	*DHCR7*	AR	Reduced Risk	
Spinal Muscular Atrophy	*SMN1*	AR	Reduced Risk	*SMN1* copy number: >=3 *SMN2* copy number: 1 c.*3+80T>G: Detected
Spondylothoracic Dysostosis	*MESP2*	AR	Reduced Risk	
Steel Syndrome	*COL27A1*	AR	Reduced Risk	
Stuve-Wiedemann Syndrome	*LIFR*	AR	Reduced Risk	
Sulfate Transporter-Related Osteochondrodysplasia	*SLC26A2*	AR	Reduced Risk	

Tay-Sachs Disease	*HEXA*	AR	Reduced Risk	Tay-Sachs disease enzyme: Non-carrier White blood cells: Non-carrier • Hex A%: 56.2% (Non-carrier : 55.0 - 72.0%; Carrier: <50%) • Total hexosaminidase activity: 1134 nmol/hr/mg Plasma: Non-carrier • Hex A%: 70.2 (Non-carrier : 58.0 - 72.0%; Carrier: <54%) • Total hexosaminidase activity: 528 nmol/hr/ml *HEXA* Sequencing: Negative
Tyrosinemia, Type I	*FAH*	AR	Reduced Risk	
Usher Syndrome, Type IB	*MYO7A*	AR	Reduced Risk	
Usher Syndrome, Type IC	*USH1C*	AR	Reduced Risk	
Usher Syndrome, Type ID	*CDH23*	AR	Reduced Risk	
Usher Syndrome, Type IF	*PCDH15*	AR	Reduced Risk	
Usher Syndrome, Type IIA	*USH2A*	AR	Reduced Risk	
Usher Syndrome, Type III	*CLRN1*	AR	Reduced Risk	
Very Long Chain Acyl-CoA Dehydrogenase Deficiency	*ACADVL*	AR	Reduced Risk	
Walker-Warburg Syndrome and Other *FKTN*-Related Dystrophies	*FKTN*	AR	Reduced Risk	
Wilson Disease	*ATP7B*	AR	Reduced Risk	
Wolman Disease / Cholesteryl Ester Storage Disease	*LIPA*	AR	Reduced Risk	
X-Linked Juvenile Retinoschisis	*RS1*	XL	Reduced Risk	
X-Linked Severe Combined Immunodeficiency	*IL2RG*	XL	Reduced Risk	
Zellweger Syndrome Spectrum (*PEX10*-Related)	*PEX10*	AR	Reduced Risk	
Zellweger Syndrome Spectrum (*PEX1*-Related)	*PEX1*	AR	Reduced Risk	
Zellweger Syndrome Spectrum (*PEX2*-Related)	*PEX2*	AR	Reduced Risk	
Zellweger Syndrome Spectrum (*PEX6*-Related)	*PEX6*	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 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 due to unequal meiotic crossing-over between *CYP21A2* and the pseudogene *CYP21A1P* . These 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 with SMA have an *SMN1* mutation that occurred *de novo* . Typically in these cases, only one parent is an SMA carrier.

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.

Pathogenic or likely pathogenic sequence variants in exon 7 may be detected during testing for the c.*3+80T>G variant allele; these will be reported if confirmed to be located in SMN1 using locus-specific Sanger primers

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^{TM}QXT 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. Samples were pooled and sequenced on the Illumina HiSeq 2500 platform in the Rapid Run mode or the Illumina NovaSeq platform in the Xp workflow, using 100 bp paired-end 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. The exons contained within these regions are noted within Table 1 (as "Exceptions") and 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.

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.

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 ΔΔCt 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 >28,000 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.

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.

Tay-Sachs Disease (TSD) Enzyme Analysis (Analytical Detection Rate ≥ 98%)

Hexosaminidase activity and Hex A% activity were measured by a standard heat-inactivation, fluorometric method using artificial 4-MU-â-N-acetyl glucosaminide (4-MUG) substrate. This assay is highly sensitive and accurate in detecting Tay-Sachs carriers and individuals affected with TSD. Normal ranges of Hex A% activity are 55.0-72.0 for white blood cells and 58.0-72.0 for plasma. It is estimated that less than 0.5% of Tay-Sachs carriers have non-carrier levels of percent Hex A activity, and therefore may not be identified by this assay. In addition, this assay may detect individuals that are carriers of or are affected with Sandhoff disease. False positive results may occur if benign variants, such as pseudodeficiency alleles, interfere with the enzymatic assay. False negative results may occur if both *HEXA* and *HEXB* pathogenic or pseudodeficiency variants are present in the same individual.

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.