



Donor 6177

Genetic Testing Summary

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

Last Updated: 07/01/24

Donor Reported Ancestry: 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: Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency (CYP21A2)- Non-classic</p> <p>Carrier: Gaucher Disease (GBA)</p> <p>Carrier: Krabbe Disease (GALC)</p> <p>Carrier: Nemaline Myopathy 2 (NEB)</p> <p>Negative for other genes sequenced.</p>	<p>Partner testing recommended before using this donor.</p> <p>Carriers of mutations in the GBA gene are at 5.5 to 7-fold increased risk above the general population risk to develop Parkinson disease. The incidence of Parkinson's Disease in the general population for those over 65 is 0.2%. Being a carrier for GBA increases the risk to about 1.4%. The overwhelming majority of patients who carry GBA mutations do not develop Parkinson disease.</p>
Special Testing		
Genes: CIB2, RNASEH2B	Negative by gene sequencing	

*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 6177
 Date of Birth: [REDACTED]
 Sema4 ID: [REDACTED]
 Client ID: [REDACTED]
 Indication: Carrier Testing

Specimen Information

Specimen Type: Blood
 Date Collected: 01/26/2021
 Date Received: 01/27/2021
 Final Report: 02/10/2021

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 Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency (AR) Associated gene(s): <i>CYP21A2</i> Variant(s) Detected: c.841G>T, p.V281L, Pathogenic, Heterozygous (one copy)</p> <p>Carrier of Gaucher Disease (AR) Associated gene(s): <i>GBA</i> Variant(s) Detected: c.1297G>T, p.V433L, Pathogenic, Heterozygous (one copy)</p> <p>Carrier of Krabbe Disease (AR) Associated gene(s): <i>GALC</i> Variant(s) Detected: c.334A>G, p.T112A, Pathogenic, Heterozygous (one copy)</p> <p>Carrier of Nemaline Myopathy 2 (AR) Associated gene(s): <i>NEB</i> Variant(s) Detected: c.23847+2T>C, Likely 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. Please note that residual risks for X-linked diseases (including full repeat expansions for Fragile X syndrome) may not be accurate for males and the actual residual risk is likely to be lower.

Interpretation of positive results

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)

Genes 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.

Gaucher Disease (AR)

Results and Interpretation

A heterozygous (one copy) pathogenic missense variant, c.1297G>T, p.V433L, was detected in the *GBA* gene (NM_001005741.2). When this variant is present in trans with a pathogenic variant, it is considered to be causative for Gaucher disease. Therefore, this individual is expected to be at least a carrier for Gaucher disease. Heterozygous carriers are not expected to exhibit symptoms of this disease, but have an increased risk of developing Parkinson's disease. This risk is approximately five times higher than the general population in heterozygous carriers and 10-20 times higher than the general population in homozygous carriers (PMID: 31010158).

What is Gaucher Disease?

Gaucher disease is an autosomal recessive disease caused by pathogenic variants in the gene *GBA*. While it is found in populations worldwide, it is most prevalent in individuals of Ashkenazi Jewish descent. Gaucher disease has variable clinical features and can be divided into the following subtypes.

- Type 1 is characterized by bone disease and the lack of neurological involvement. The bone disease can vary in severity from asymptomatic to destruction of bone tissue and painful "bone crises". Patients often have anemia and abnormal blood cell counts and may have lung disease. Some patients may be asymptomatic.
- Type 2 is a severe form that begins in infancy and usually results in death by the age of 2 years. It is characterized by severe neurologic deterioration, seizures, anemia, poor feeding and failure to thrive.
- The perinatal-lethal form is a more severe subtype of type 2, where accumulation of fluid in the fetus results in death in utero, or in the first several days of life. Some patients do not have the excess fluid, but die within three months.
- Type 3 is characterized by neurologic deterioration, as with type 2, but onset may be anywhere from childhood to adulthood, and progresses more slowly. Patients develop seizures and declining intelligence. Patients also experience the bone disease and anemia seen in type 1.
- The cardiovascular form is a subtype of type 3 that is characterized by calcification of the heart valves during adolescence. Patients may also have problems controlling their eye movements. The cardiac manifestations are usually fatal.

Some pathogenic variants are associated with a specific type of Gaucher disease. However, there is significant variability in the phenotypes, even between identical twins. Therefore, it is not always possible to predict the severity of disease based on genotype.

Krabbe Disease (AR)

Results and Interpretation

A heterozygous (one copy) pathogenic missense variant, c.334A>G, p.T112A, was detected in the *GALC* gene (NM_000153.3). Please note that this is a mild variant and is not expected to result in a disease phenotype when homozygous or when found in trans with the p.Y319C mild variant, unless present as part of a complex allele. If found in trans with a severe pathogenic variant, the individual is expected to develop Krabbe disease. When this variant is present in trans with a pathogenic variant, it is considered to be causative for Krabbe disease. Therefore, this individual is expected to be at least a carrier for Krabbe disease. Heterozygous carriers are not expected to exhibit symptoms of this disease.

What is Krabbe Disease?

Krabbe disease is an autosomal recessive disorder caused by pathogenic variants in the gene *GALC*. While it has been identified in patients worldwide, it is more prevalent in specific groups of Druze and Muslim Arabs in Israel. The classical form of the disease has an onset in infancy. After several months of normal development, infants become irritable and develop spasticity and rigidity. Psychomotor and mental regression proceeds rapidly, and the infant becomes blind and non-responsive within several weeks or months. The average life span is 13 months. Approximately 15% of patients have a later-onset form of the disease, in which the severity is highly variable. Onset can occur anywhere between the age of 1 year and middle age, and deterioration proceeds more slowly. Specific variants have been determined to cause the infantile or late-onset forms of the disease, and therefore the phenotype may be predicted for most genotypes.

Nemaline Myopathy 2 (AR)

Results and Interpretation

A heterozygous (one copy) likely pathogenic splice site variant, c.23847+2T>C, was detected in the *NEB* gene (NM_001271208.1). When this variant is present in trans with a pathogenic variant, it is considered to be causative for nemaline myopathy 2. Therefore, this individual is expected to be at least a carrier for nemaline myopathy 2. Heterozygous carriers are not expected to exhibit symptoms of this disease.

What is Nemaline Myopathy 2?

Nemaline myopathy 2 is an autosomal recessive neuromuscular disorder that is caused by pathogenic variants in the gene *NEB*. While it is found in different ethnicities around the world, it is more prevalent in individuals of Ashkenazi Jewish or Finnish descent due to the presence of founder mutations. This disorder is characterized by muscle weakness and the presence of rod-shaped structures (nemaline bodies or rods) in affected muscle fibers. The typical, and most common, form is characterized by the infantile onset of a slowly progressive or non-progressive weakness of facial, bulbar, and respiratory muscles and neck flexors. Initial weakness is primarily proximal, with later distal involvement. Patients usually have difficulty walking and cannot run, but many remain ambulatory into adulthood. Life expectancy may not be reduced. In the most severe form, which is less common, decreased fetal movements are noticed before birth, and patients will not achieve the ability to sit or walk. These patients also have joint contractures and difficulty breathing, and usually do not survive infancy. It is not currently possible to predict the severity of the disease based on the genotype.

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.

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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				
Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency	<i>CYP21A2</i>	AR	Carrier	<i>CYP21A2</i> copy number: 2 No pathogenic copy number variants detected <i>CYP21A2</i> sequencing: c.841G>T, p.V281L, Pathogenic, Heterozygous (one copy)
Gaucher Disease	<i>GBA</i>	AR	Carrier	c.1297G>T, p.V433L, Pathogenic, Heterozygous (one copy)
Krabbe Disease	<i>GALC</i>	AR	Carrier	c.334A>G, p.T112A, Pathogenic, Heterozygous (one copy)

Nemaline Myopathy 2	NEB	AR	Carrier	c.23847*2T>C, Likely 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	
6-Pyruvoyl-Tetrahydropterin Synthase Deficiency	PTS	AR	Reduced Risk	
Abetalipoproteinemia	MTTP	AR	Reduced Risk	
Achromatopsia (CNGB3-related)	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-Goutieres Syndrome (SAMHD1-Related)	SAMHD1	AR	Reduced Risk	
Alpha-Mannosidosis	MAN2B1	AR	Reduced Risk	
Alpha-Thalassemia	HBA1/HBA2	AR	Reduced Risk	HBA1 Copy Number: 2 HBA2 Copy Number: 2 No pathogenic copy number variants detected HBA1/HBA2 Sequencing: Negative
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-Globin-Related Hemoglobinopathies	HBB	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	<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 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	<i>COL7A1</i>	AR	Reduced Risk
Ehlers-Danlos Syndrome, Type VIIC	<i>ADAMTS2</i>	AR	Reduced Risk
Ellis-van Creveld Syndrome (EVC-Related)	<i>EVC</i>	AR	Reduced Risk
Emery-Dreifuss Myopathy 1	<i>EMD</i>	XL	Reduced Risk
Enhanced S-Cone Syndrome	<i>NR2E3</i>	AR	Reduced Risk
Ethylmalonic Encephalopathy	<i>ETHE1</i>	AR	Reduced Risk
Fabry Disease	<i>GLA</i>	XL	Reduced Risk
Factor IX Deficiency	<i>F9</i>	XL	Reduced Risk
Factor XI Deficiency	<i>F11</i>	AR	Reduced Risk
Familial Autosomal Recessive Hypercholesterolemia	<i>LDLRAP1</i>	AR	Reduced Risk
Familial Dysautonomia	<i>IKBKAP</i>	AR	Reduced Risk
Familial Hypercholesterolemia	<i>LDLR</i>	AR	Reduced Risk
Familial Hyperinsulinism (ABCC8-Related)	<i>ABCC8</i>	AR	Reduced Risk
Familial Hyperinsulinism (KCNJ11-Related)	<i>KCNJ11</i>	AR	Reduced Risk
Familial Mediterranean Fever	<i>MEFV</i>	AR	Reduced Risk
Fanconi Anemia, Group A	<i>FANCA</i>	AR	Reduced Risk
Fanconi Anemia, Group C	<i>FANCC</i>	AR	Reduced Risk
Fanconi Anemia, Group G	<i>FANCG</i>	AR	Reduced Risk

Disease	Gene	Inheritance	Risk	Notes
Fragile X Syndrome	<i>FMR1</i>	XL	Reduced Risk	<i>FMR1</i> CGG repeat sizes: Not Performed <i>FMR1</i> 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	<i>FH</i>	AR	Reduced Risk	
GRACILE Syndrome and Other <i>BCS1L</i> -Related Disorders	<i>BCS1L</i>	AR	Reduced Risk	
Galactokinase Deficiency	<i>GALK1</i>	AR	Reduced Risk	
Galactosemia	<i>GALT</i>	AR	Reduced Risk	
Gitelman Syndrome	<i>SLC12A3</i>	AR	Reduced Risk	
Glutaric Acidemia, Type I	<i>GCDH</i>	AR	Reduced Risk	
Glutaric Acidemia, Type IIa	<i>ETF A</i>	AR	Reduced Risk	
Glutaric Acidemia, Type IIc	<i>ETFDH</i>	AR	Reduced Risk	
Glycine Encephalopathy (<i>AMT</i> -Related)	<i>AMT</i>	AR	Reduced Risk	
Glycine Encephalopathy (<i>GLDC</i> -Related)	<i>GLDC</i>	AR	Reduced Risk	
Glycogen Storage Disease, Type II	<i>GAA</i>	AR	Reduced Risk	
Glycogen Storage Disease, Type III	<i>AGL</i>	AR	Reduced Risk	
Glycogen Storage Disease, Type IV / Adult Polyglucosan Body Disease	<i>GBE1</i>	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 (<i>CBS</i> -Related)	<i>CBS</i>	AR	Reduced Risk	
Homocystinuria due to <i>MTHFR</i> Deficiency	<i>MTHFR</i>	AR	Reduced Risk	
Homocystinuria, cbI Type	<i>MTRR</i>	AR	Reduced Risk	
Hydroletharus 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	
Lamellar Ichthyosis, Type 1	<i>TGM1</i>	AR	Reduced Risk	
Leber Congenital Amaurosis 10 and Other CEP290-Related Ciliopathies	<i>CEP290</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
Mucopolipidosis III Gamma	<i>GNPTG</i>	AR	Reduced Risk
Mucopolipidosis 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 POMGNT1-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
Nephrogenic Diabetes Insipidus, Type II	<i>AQP2</i>	AR	Reduced Risk
Nephrotic Syndrome (NPHS1-Related) / Congenital Finnish Nephrosis	<i>NPHS1</i>	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 Transcarbamylase 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
Spondylothoracic Dysostosis	MESP2	AR	Reduced Risk

SMN1 copy number: 2
 SMN2 copy number: 2
 c.*3+80T>G: Negative

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
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 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 SureSelectTMXT 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 SureSelect™XT 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.

SELECTED REFERENCES

Carrier Screening

Grody W et al. ACMG position statement on prenatal/preconception expanded carrier screening. *Genet Med.* 2013 15:482-3.

Fragile X syndrome:

Chen L et al. An information-rich CGG repeat primed PCR that detects the full range of Fragile X expanded alleles and minimizes the need for Southern blot analysis. *J Mol Diag* 2010 12:589-600.

Spinal Muscular Atrophy:

Luo M et al. An Ashkenazi Jewish SMN1 haplotype specific to duplication alleles improves pan-ethnic carrier screening for spinal muscular atrophy. *Genet Med.* 2014 16:149-56.

Ashkenazi Jewish Disorders:

Scott SA et al. Experience with carrier screening and prenatal diagnosis for sixteen Ashkenazi Jewish Genetic Diseases. *Hum. Mutat.* 2010 31:1-11.

Duchenne Muscular Dystrophy:

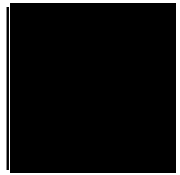
Flanigan KM et al. Mutational spectrum of DMD mutations in dystrophinopathy patients: application of modern diagnostic techniques to a large cohort. *Hum Mutat.* 2009 30:1657-66.

Variant Classification:

Richards S et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015 May;17(5):405-24

Additional disease-specific references available upon request.





Patient Information:

6177, Donor

DOB: [REDACTED]

Sex: M

MR#: 6177

Patient#: [REDACTED]

Accession:

[REDACTED]

Test#: [REDACTED]

Order#: [REDACTED]

Ext Test#: [REDACTED]

Ext Order#: [REDACTED]

Specimen Type: DNA

Collected: Not provided

Received Date: Jan 25,2024

Authorized Date: Jan 28,2024

Physician:

Seitz, Suzanne

ATTN: Seitz, Suzanne

Fairfax Cryobank

3015 Williams Drive

Fairfax, VA 22031

Phone:

Fax:

Laboratory:

Fulgent Therapeutics, LLC

CAP#: 8042697

CLIA#: 05D2043189

Laboratory Director:

Dr. Hanlin (Harry) Gao

Report Date: Feb 03,2024

Final Report

TEST PERFORMED

CIB2 Single Gene

(1 Gene Panel: *CIB2*; gene sequencing with deletion and duplication analysis)

RESULTS:

No clinically significant sequence or copy-number variants were identified in the submitted specimen.

A negative result does not rule out the possibility of a genetic predisposition nor does it rule out any pathogenic mutations of the sort not queried by this test or in areas not reliably assessed by this test.

INTERPRETATION:

Notes and Recommendations:

- As requested, this report only includes variants classified as Pathogenic, Likely Pathogenic, or Risk Allele at the time of analysis. If detected, this report does not include variants classified as of uncertain significance.
- Gene specific notes and limitations may be present. See below.
- These results should be interpreted in the context of this individual's clinical findings, biochemical profile, and family history.
- Genetic counseling is recommended. Available genetic counselors and additional resources can be found at the National Society of Genetic Counselors (NSGC; <https://www.nsgc.org>)
- Guide to Interpreting Genomic Reports: A Genomics Toolkit (CSER Consortium; February 2017) (<https://www.genome.gov/For-Health-Professionals/Provider-Genomics-Education-Resources#hep>)

GENES TESTED:

CIB2 Single Gene

1 genes tested (100.00% at >20x).

CIB2

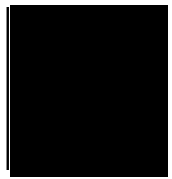
Gene Specific Notes and Limitations

No gene specific limitations apply to the genes on the tested panel.

METHODS:

Patient: 6177, Donor; Sex: M;
DOB: [REDACTED] MR#: 6177

Accession#: [REDACTED]; FD Patient#: [REDACTED]
DocID: [REDACTED]; PAGE 1 of 3



Genomic DNA was isolated from the submitted specimen indicated above (if cellular material was submitted). DNA was barcoded, and enriched for the coding exons of targeted genes using hybrid capture technology. Prepared DNA libraries were then sequenced using a Next Generation Sequencing technology. Following alignment to the human genome reference sequence (assembly GRCh37), variants were detected in regions of at least 10x coverage. For this specimen, 100.00% and 100.00% of coding regions and splicing junctions of genes listed had been sequenced with coverage of at least 10x and 20x, respectively, by NGS or by Sanger sequencing. The remaining regions did not have 10x coverage, and were not evaluated. Variants were interpreted manually using locus specific databases, literature searches, and other molecular biological principles. To minimize false positive results, any variants that do not meet internal quality standards are confirmed by Sanger sequencing. Variants classified as pathogenic, likely pathogenic, or risk allele which are located in the coding regions and nearby intronic regions (+/- 20bp) of the genes listed above are reported. Variants outside these intervals may be reported but are typically not guaranteed. When a single pathogenic or likely pathogenic variant is identified in a clinically relevant gene with autosomal recessive inheritance, the laboratory will attempt to ensure 100% coverage of coding sequences either through NGS or Sanger sequencing technologies ("fill-in"). All genes listed were evaluated for large deletions and/or duplications. However, single exon deletions or duplications will not be detected in this assay, nor will copy number alterations in regions of genes with significant pseudogenes. Putative deletions or duplications identified by NGS are confirmed by an orthogonal method (qPCR or MLPA), unless exceeding an internally specified and validated quality score, beyond which deletions and duplications are considered real without further confirmation. New York patients: diagnostic findings are confirmed by Sanger, MLPA, or qPCR; exception SNV variants in genes for which confirmation of NGS results has been performed ≥ 10 times may not be confirmed if identified with high quality by NGS. Bioinformatics: The Fulgent Germline v2019.2 pipeline was used to analyze this specimen.

LIMITATIONS:

These test results and variant interpretation are based on the proper identification of the submitted specimen, accuracy of any stated familial relationships, and use of the correct human reference sequences at the queried loci. In very rare instances, errors may result due to mix-up or co-mingling of specimens. Positive results do not imply that there are no other contributors, genetic or otherwise, to this individual's phenotype, and negative results do not rule out a genetic cause for the indication for testing. Official gene names change over time. Fulgent uses the most up to date gene names based on HUGO Gene Nomenclature Committee (<https://www.genenames.org>) recommendations. If the gene name on report does not match that of ordered gene, please contact the laboratory and details can be provided. Result interpretation is based on the available clinical and family history information for this individual, collected published information, and Alamut annotation available at the time of reporting. This assay is designed and validated for detection of germline variants only. It is not designed or validated for the detection of low-level mosaicism or somatic mutations. This assay will not detect certain types of genomic aberrations such as translocations, inversions, or repeat expansions (eg. trinucleotide or hexanucleotide repeat expansion). DNA alterations in regulatory regions or deep intronic regions (greater than 20bp from an exon) may not be detected by this test. Unless otherwise indicated, no additional assays have been performed to evaluate genetic changes in this specimen. There are technical limitations on the ability of DNA sequencing to detect small insertions and deletions. Our laboratory uses a sensitive detection algorithm for copy number variants, however these types of alterations are not detected as reliably as single nucleotide variants. Rarely, due to systematic chemical, computational, or human error, DNA variants may be missed. Although next generation sequencing technologies and our bioinformatics analysis significantly reduce the confounding contribution of pseudogene sequences or other highly-homologous sequences, sometimes these may still interfere with the technical ability of the assay to identify pathogenic alterations in both sequencing and deletion/duplication analyses. Deletion/duplication analysis can identify alterations of genomic regions which are two or more contiguous exons in size; single exon deletions or duplications may occasionally be identified, but are not routinely detected by this test. When novel DNA duplications are identified, it is not possible to discern the genomic location or orientation of the duplicated segment, hence the effect of the duplication cannot be predicted. Where deletions are detected, it is not always possible to determine whether the predicted product will remain in-frame or not. Unless otherwise indicated, deletion/duplication analysis has not been performed in regions that have been sequenced by Sanger.

SIGNATURE:

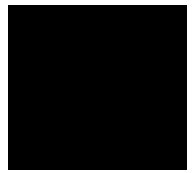


Yan Meng, Ph.D., CGMB, FACMG on 2/3/2024 12:22 PM PST
Electronically signed



DISCLAIMER:

This test was developed and its performance characteristics determined by **Fulgent Therapeutics, LLC**. It has not been cleared or approved by the FDA. The laboratory is regulated under CLIA as qualified to perform high-complexity testing. This test is used for clinical purposes. It should not be regarded as investigational or for research. Since genetic variation, as well as systematic and technical factors, can affect the accuracy of testing, the results of testing should always be interpreted in the context of clinical and familial data. For assistance with interpretation of these results, healthcare professionals may contact us directly at **(626) 350-0537** or info@fulgentgenetics.com. It is recommended that patients receive appropriate genetic counseling to explain the implications of the test result, including its residual risks, uncertainties and reproductive or medical options.



Patient Information:

6177, Donor

DOB: [REDACTED]

Sex: M

MR#: 6177

Patient#: [REDACTED]

Partner Information:

Not Tested

Physician:

Seitz, Suzanne

ATTN: Seitz, Suzanne

Fairfax Cryobank

3015 Williams Drive

Fairfax, VA 22031

Laboratory:

Fulgent Therapeutics LLC

CAP#: 8042697

CLIA#: 05D2043189

Laboratory Director:

Lawrence M. Weiss, MD

Report Date: Jun 27, 2024

Accession:

[REDACTED]

Test#: [REDACTED]

Specimen Type: DNA

Collected: Not Provided

Accession:

N/A

FINAL RESULTS



No carrier mutations identified

TEST PERFORMED

Single Gene Carrier Screening: RNASEH2B

(1 Gene Panel: *RNASEH2B*; gene sequencing with deletion and duplication analysis)

INTERPRETATION:

Notes and Recommendations:

- No carrier mutations were identified in the submitted specimen. A negative result does not rule out the possibility of a genetic predisposition nor does it rule out any pathogenic mutations in areas not assessed by this test or in regions that were covered at a level too low to reliably assess. Also, it does not rule out mutations that are of the sort not queried by this test; see Methods and Limitations for more information. A negative result reduces, but does not eliminate, the chance to be a carrier for any condition included in this screen. Please see the supplemental table for details.
- This carrier screening test does not screen for all possible genetic conditions, nor for all possible mutations in every gene tested. This report does not include variants of uncertain significance; only variants classified as pathogenic or likely pathogenic at the time of testing, and considered relevant for reproductive carrier screening, are reported. Please see the gene specific notes for details. Please note that the classification of variants can change over time.
- Patients may wish to discuss any carrier results with blood relatives, as there is an increased chance that they are also carriers. These results should be interpreted in the context of this individual's clinical findings, biochemical profile, and family history.
- Gene specific notes and limitations may be present. See below.
- Genetic counseling is recommended. Available genetic counselors and additional resources can be found at the National Society of Genetic Counselors (NSGC; <https://www.nsgc.org>)



GENES TESTED:

Custom Beacon Carrier Screening Panel - Gene

This analysis was run using the Custom Beacon Carrier Screening Panel gene list. 1 genes were tested with 100.0% of targets sequenced at >20x coverage. For more gene-specific information and assistance with residual risk calculation, see the SUPPLEMENTAL TABLE.

RNASEH2B

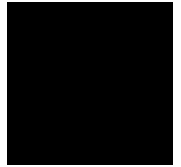
METHODS:

Genomic DNA was isolated from the submitted specimen indicated above (if cellular material was submitted). DNA was barcoded, and enriched for the coding exons of targeted genes using hybrid capture technology. Prepared DNA libraries were then sequenced using a Next Generation Sequencing technology. Following alignment to the human genome reference sequence (assembly GRCh37), variants were detected in regions of at least 10x coverage. For this specimen, 100.00% and 100.00% of coding regions and splicing junctions of genes listed had been sequenced with coverage of at least 10x and 20x, respectively, by NGS or by Sanger sequencing. The remaining regions did not have 10x coverage, and were not evaluated. Variants were interpreted manually using locus specific databases, literature searches, and other molecular biological principles. To minimize false positive results, any variants that do not meet internal quality standards are confirmed by Sanger sequencing. Variants classified as pathogenic, likely pathogenic, or risk allele which are located in the coding regions and nearby intronic regions (+/- 20bp) of the genes listed above are reported. Variants outside these intervals may be reported but are typically not guaranteed. When a single pathogenic or likely pathogenic variant is identified in a clinically relevant gene with autosomal recessive inheritance, the laboratory will attempt to ensure 100% coverage of coding sequences either through NGS or Sanger sequencing technologies ("fill-in"). All genes listed were evaluated for large deletions and/or duplications. However, single exon deletions or duplications will not be detected in this assay, nor will copy number alterations in regions of genes with significant pseudogenes. Putative deletions or duplications are analyzed using Fulgent Germline proprietary pipeline for this specimen. Bioinformatics: The Fulgent Germline v2019.2 pipeline was used to analyze this specimen.

LIMITATIONS:

General Limitations

These test results and variant interpretation are based on the proper identification of the submitted specimen, accuracy of any stated familial relationships, and use of the correct human reference sequences at the queried loci. In very rare instances, errors may result due to mix-up or co-mingling of specimens. Positive results do not imply that there are no other contributors, genetic or otherwise, to future pregnancies, and negative results do not rule out the genetic risk to a pregnancy. Official gene names change over time. Fulgent uses the most up to date gene names based on HUGO Gene Nomenclature Committee (<https://www.genenames.org>) recommendations. If the gene name on report does not match that of ordered gene, please contact the laboratory and details can be provided. Result interpretation is based on the available clinical and family history information for this individual, collected published information, and Alamut annotation available at the time of reporting. This assay is not designed or validated for the detection of low-level mosaicism or somatic mutations. This assay will not detect certain types of genomic aberrations such as translocations, inversions, or repeat expansions other than specified genes. DNA alterations in regulatory regions or deep intronic regions (greater than 20bp from an exon) may not be detected by this test. Unless otherwise indicated, no additional assays have been performed to evaluate genetic changes in this specimen. There are technical limitations on the ability of DNA sequencing to detect small insertions and deletions. Our laboratory uses a sensitive detection algorithm, however these types of alterations are not detected as reliably as single nucleotide variants. Rarely, due to systematic chemical, computational, or human error, DNA variants may be missed. Although next generation sequencing technologies and our bioinformatics analysis significantly reduce the confounding contribution of pseudogene sequences or other highly-homologous sequences, sometimes these may still interfere with the technical ability of the assay to identify pathogenic alterations in both sequencing and deletion/duplication analyses. Deletion/duplication analysis can identify alterations of genomic regions which include one whole gene (buccal swab specimens and whole blood specimens) and are two or more contiguous exons in size (whole blood specimens only); single exon deletions or duplications may occasionally be identified, but are not routinely detected by this test. When novel DNA duplications are identified, it is not possible to discern the genomic location or orientation of the duplicated segment, hence the effect of the duplication cannot be predicted. Where deletions are detected, it is not always possible to determine whether the predicted product will remain in-frame or not. Unless otherwise indicated, deletion/duplication analysis has not been performed in regions that have been sequenced by Sanger.



Gene Specific Notes and Limitations

No gene specific limitations apply to the genes on the tested panel.

SIGNATURE:



A handwritten signature in black ink, appearing to read "Yan Meng".

Yan Meng, Ph.D., CGMB, FACMG on 6/27/2024
Laboratory Director, Fulgent

DISCLAIMER:

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info@fulgentgenetics.com
www.fulgentgenetics.com



To view the supplemental table describing the carrier frequencies, detection rates, and residual risks associated with the genes on this test please visit the following link:

[Beacon Expanded Carrier Screening Supplemental Table](#)

