Fairfax Cryobank
The Trusted Choice for Donor Sperm

Donor 6177

Genetic Testing Summary

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

Last Updated: 02/07/24

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.	Carrier: Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency (CYP21A2)- Non-classic Carrier: Gaucher Disease (GBA) Carrier: Krabbe Disease (GALC) Carrier: Nemaline Myopathy 2 (NEB) Negative for other genes sequenced.	Partner testing recommended before using this donor. 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.
Special Testing		
Gene: CIB2	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]

Expanded Carrier Screen (283) Minus TSE

Number of genes tested: 283

SUMMARY OF RESULTS AND RECOMMENDATIONS

⊕ Positive	⊖ Negative
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) **Carrier of Gaucher Disease (AR)** Associated gene(s): *GBA* Variant(s) Detected: c.1297G>T, p.V433L, Pathogenic, Heterozygous (one copy) **Carrier of Krabbe Disease (AR)** Associated gene(s): *GALC* Variant(s) Detected: c.334A>G, p.T112A, Pathogenic, Heterozygous (one copy) **Carrier of Nemaline Myopathy 2 (AR)** Associated gene(s): *NEB* Variant(s) Detected: c.23847+2T>C, Likely 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. 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 I.
- 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 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.

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

Genes and diseases tested

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

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

Disease	Gene	Inheritance Pattern	Status	Detailed Summary
⊕ **Positive**				
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)
Gaucher Disease	*GBA*	AR	Carrier	c.1297G>T, p.V433L, Pathogenic, Heterozygous (one copy)
Krabbe Disease	*GALC*	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	*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	
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	
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
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 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	*SMN1* copy number: 2 *SMN2* copy number: 2 c.*3+80T>G: Negative
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
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 SureSelect^{TM}XT Low Input technology was used with a custom capture library to target the exonic regions and intron/exon splice junctions of the relevant genes, as well as a number of UTR, intronic or promoter regions that contain previously reported mutations. Libraries were pooled and sequenced on the Illumina NovaSeq 9000 platform, using paired-end 100 bp reads. The sequencing data was analyzed using a custom bioinformatics algorithm designed and validated in house.

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

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

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

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

Next Generation Sequencing for SMN1

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

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

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

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

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

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

The relative quantification PCR is utilized on a Roche Universal Library Probe (UPL) system, which relates the PCR signal of the target region in one group to another. To test for genomic imbalances, both sample DNA and reference DNA is amplified with primer/probe sets that specific to the target region and a control region with known genomic copy number. Relative genomic copy numbers are calculated based on the standard 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 trough 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^{TM}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

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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.

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[redacted]

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

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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:

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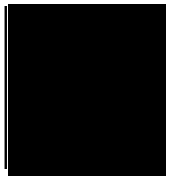

Genomic DNA was isolated from the submitted specimen indicated above (if cellular material was submitted). DNA was barcoded, and enriched for the coding exons of targeted genes using hybrid capture technology. Prepared DNA libraries were then sequenced using a Next Generation Sequencing technology. Following 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**
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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.