Fairfax Cryobank
The Trusted Choice for Donor Sperm

# Donor 6277

## Genetic Testing Summary

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

Last Updated: 3/6/21

Donor Reported Ancestry: French, German 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: Carbamoylphosphate Synthetase I Deficiency (CPS1)<br><br>Carrier: Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency (CYP21A2)-Non-classic variant<br><br>Carrier: Glycine Encephalopathy (GLDC)<br><br>Negative for other genes sequenced | Partner testing recommended before using this donor. |

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

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

**Patient Information**
Name: Donor 6277
Date of Birth: [redacted]
Sema4 ID: [redacted]
Client ID: [redacted]
Indication: Carrier Testing

**Specimen Information**
Specimen Type: Blood
Date Collected: 08/17/2020
Date Received: 08/18/2020
Final Report: 09/02/2020

**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 Carbamoylphosphate Synthetase I Deficiency (AR)**<br>Associated gene(s): *CPS1*<br>Variant(s) Detected: c.1837-8A>G, Likely Pathogenic, Heterozygous (one copy)<br><br>**Carrier of Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency (AR)**<br>Associated gene(s): *CYP21A2*<br>Variant(s) Detected: c.841G>T, p.V281L, Pathogenic, Heterozygous (one copy)<br><br>**Carrier of Glycine Encephalopathy (*GLDC*-Related) (AR)**<br>Associated gene(s): *GLDC*<br>Variant(s) Detected: c.2316-1G>A, Likely Pathogenic, Heterozygous (one copy) | **Negative for all other genes tested**<br>To view a full list of genes and diseases tested please see Table 1 in this report |

*AR=Autosomal recessive; XL=X-linked*

## Recommendations

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

# Interpretation of positive results

## Carbamoylphosphate Synthetase I Deficiency (AR)

### Results and Interpretation

A heterozygous (one copy) likely pathogenic intronic variant, c.1837-8A>G, was detected in the *CPS1* gene (NM_001875.4). When this variant is present in trans with a pathogenic variant, it is considered to be causative for carbamoylphosphate synthetase I deficiency. Therefore, this individual is expected to be at least a carrier for carbamoylphosphate synthetase I deficiency. Heterozygous carriers are not expected to exhibit symptoms of this disease.

### What is Carbamoylphosphate Synthetase I Deficiency?

Carbamoylphosphate synthetase I deficiency is an autosomal recessive disorder caused by pathogenic variants in the gene *CPS1* . It is a pan-ethnic disease with higher prevalence among Caucasians. Affected individuals develop high levels of ammonia in the bloodstream, which may be fatal or may result in permanent brain damage. Symptoms will present within the first few days of life and include sleepiness, poorly regulated breathing or body temperature, unwillingness to feed, vomiting, unusual body movements, and seizures. Developmental delay and intellectual disability are also associated with this disorder. Those affected with carbamoylphosphate synthetase I deficiency will always be at risk for hyperammonemia and will require constant surveillance. Some individuals will develop a later-onset form of the disease, but are still at risk for life-threatening bouts of hyperammonemia. The severity of the disease cannot be predicted based on the inherited variants.

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

### Results and Interpretation

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

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

**Inheritance:** Autosomal Recessive

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

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

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders resulting from deficiency in the enzymes involved in cortisol biosynthesis. The majority (95%) of CAH cases are due to 21-hydroxylase deficiency (21-OHD CAH), which is caused by homozygous or compound heterozygous pathogenic variants in the gene *CYP21A2*. Approximately 20% of mutant alleles have deletions of 30 kb that have been generated by unequal meiotic crossing-over between the two genes. Another 75% of mutant alleles are due to gene conversion events, where an inactivating mutation from the *CYP21A1P* pseudogene is introduced into one copy of the *CYP21A2* gene, thus making the gene non-functional. Three different forms of 21-OHD CAH have been reported: a classic salt wasting form, a classic simple virilizing form, and a non-classic form.

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

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

### Glycine Encephalopathy (*GLDC*-Related) (AR)

**Results and Interpretation**

A heterozygous (one copy) likely pathogenic splice site variant, c.2316-1G>A, was detected in the *GLDC* gene (NM_000170.2). When this variant is present in trans with a pathogenic variant, it is considered to be causative for glycine encephalopathy (*GLDC*-related). Therefore, this individual is expected to be at least a carrier for glycine encephalopathy (*GLDC*-related). Heterozygous carriers are not expected to exhibit symptoms of this disease.

**What is Glycine Encephalopathy (*GLDC*-Related)?**

Glycine encephalopathy (*GLDC*-related) is an autosomal recessive disease caused by pathogenic variants in the gene *GLDC* . This disease may affect people of any ethnicity, but is more common in Arab people from Israel. Symptoms may begin soon after birth or later in infancy. Babies who are symptomatic soon after birth experience lethargy, muscle weakness, and muscle jerks. These early symptoms resolve but patients have intellectual disability and seizures throughout life. Individuals whose symptoms start later in infancy experience muscle weakness, seizures, and developmental delay. Long-term symptoms include severe to mild intellectual disability and seizures. Rarely, an individual affected with a milder form of the disease can present later in life with less severe symptoms. Life expectancy is variable, depending on the severity of symptoms, and can range from infancy to adulthood. It is not currently possible to predict the severity of disease based on the inherited variants.

# 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.

**Rebekah Zimmerman, Ph.D., FACMG, Laboratory Director**
Laboratory Medical Consultant: George A. Diaz, M.D., Ph.D.

# Genes and diseases tested

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

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

| Disease | Gene | Inheritance Pattern | Status | Detailed Summary |
|---|---|---|---|---|
| ⊕ **Positive** | | | | |
| **Carbamoylphosphate Synthetase I Deficiency** | *CPS1* | AR | Carrier | c.1837-8A>G, Likely Pathogenic, Heterozygous (one copy) |
| **Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency** | *CYP21A2* | AR | Carrier | *CYP21A2* copy number: 2<br>No pathogenic copy number variants detected<br>*CYP21A2* sequencing: c.841G>T, p.V281L, Pathogenic, Heterozygous (one copy) |
| **Glycine Encephalopathy (*GLDC*-Related)** | *GLDC* | AR | Carrier | c.2316-1G>A, 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<br>*HBA2* Copy Number: 2<br>No pathogenic copy number variants detected<br>*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 | |
| 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 | |

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|---|---|---|---|---|
| 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<br>*FMR1* Sequencing: Negative<br>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 | |

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|---|---|---|---|
| GRACILE Syndrome and Other *BCS1L*-Related Disorders | *BCS1L* | AR | Reduced Risk |
| Galactokinase Deficiency | *GALK1* | AR | Reduced Risk |
| Galactosemia | *GALT* | AR | Reduced Risk |
| Gaucher Disease | *GBA* | AR | Reduced Risk |
| Gitelman Syndrome | *SLC12A3* | AR | Reduced Risk |
| Glutaric Acidemia, Type I | *GCDH* | AR | Reduced Risk |
| Glutaric Acidemia, Type IIa | *ETFA* | AR | Reduced Risk |
| Glutaric Acidemia, Type IIc | *ETFDH* | AR | Reduced Risk |
| Glycine Encephalopathy (*AMT*-Related) | *AMT* | AR | Reduced Risk |
| Glycogen Storage Disease, Type II | *GAA* | AR | Reduced Risk |
| Glycogen Storage Disease, Type III | *AGL* | AR | Reduced Risk |
| Glycogen Storage Disease, Type IV / Adult Polyglucosan Body Disease | *GBE1* | AR | Reduced Risk |
| Glycogen Storage Disease, Type Ia | *G6PC* | AR | Reduced Risk |
| Glycogen Storage Disease, Type Ib | *SLC37A4* | AR | Reduced Risk |
| Glycogen Storage Disease, Type V | *PYGM* | AR | Reduced Risk |
| Glycogen Storage Disease, Type VII | *PFKM* | AR | Reduced Risk |
| HMG-CoA Lyase Deficiency | *HMGCL* | AR | Reduced Risk |
| Hemochromatosis, Type 2A | *HFE2* | AR | Reduced Risk |
| Hemochromatosis, Type 3 | *TFR2* | AR | Reduced Risk |
| Hereditary Fructose Intolerance | *ALDOB* | AR | Reduced Risk |
| Hereditary Spastic Paraparesis 49 | *TECPR2* | AR | Reduced Risk |
| Hermansky-Pudlak Syndrome, Type 1 | *HPS1* | AR | Reduced Risk |
| Hermansky-Pudlak Syndrome, Type 3 | *HPS3* | AR | Reduced Risk |
| Holocarboxylase Synthetase Deficiency | *HLCS* | AR | Reduced Risk |
| Homocystinuria (*CBS*-Related) | *CBS* | AR | Reduced Risk |
| Homocystinuria due to *MTHFR* Deficiency | *MTHFR* | AR | Reduced Risk |
| Homocystinuria, cblE Type | *MTRR* | AR | Reduced Risk |
| Hydrolethalus Syndrome | *HYLS1* | AR | Reduced Risk |
| Hyperornithinemia-Hyperammonemia-Homocitrullinuria Syndrome | *SLC25A15* | AR | Reduced Risk |
| Hypohidrotic Ectodermal Dysplasia 1 | *EDA* | XL | Reduced Risk |
| Hypophosphatasia | *ALPL* | AR | Reduced Risk |
| Inclusion Body Myopathy 2 | *GNE* | AR | Reduced Risk |
| Infantile Cerebral and Cerebellar Atrophy | *MED17* | AR | Reduced Risk |
| Isovaleric Acidemia | *IVD* | AR | Reduced Risk |
| Joubert Syndrome 2 | *TMEM216* | AR | Reduced Risk |
| Joubert Syndrome 7 / Meckel Syndrome 5 / COACH Syndrome | *RPGRIP1L* | AR | Reduced Risk |
| Junctional Epidermolysis Bullosa (*LAMA3*-Related) | *LAMA3* | AR | Reduced Risk |
| Junctional Epidermolysis Bullosa (*LAMB3*-Related) | *LAMB3* | AR | Reduced Risk |
| Junctional Epidermolysis Bullosa (*LAMC2*-Related) | *LAMC2* | AR | Reduced Risk |
| Krabbe Disease | *GALC* | AR | Reduced Risk |
| Lamellar Ichthyosis, Type 1 | *TGM1* | AR | Reduced Risk |
| Leber Congenital Amaurosis 10 and Other CEP290-Related Ciliopathies | *CEP290* | AR | Reduced Risk |
| Leber Congenital Amaurosis 13 | *RDH12* | AR | Reduced Risk |
| Leber Congenital Amaurosis 2 / Retinitis Pigmentosa 20 | *RPE65* | AR | Reduced Risk |
| Leber Congenital Amaurosis 5 | *LCA5* | AR | Reduced Risk |
| Leber Congenital Amaurosis 8 / Retinitis Pigmentosa 12 / Pigmented Paravenous Chorioretinal Atrophy | *CRB1* | AR | Reduced Risk |
| Leigh Syndrome, French-Canadian Type | *LRPPRC* | AR | Reduced Risk |
| Lethal Congenital Contracture Syndrome 1 / Lethal Arthrogryposis with Anterior Horn Cell Disease | *GLE1* | AR | Reduced Risk |
| Leukoencephalopathy with Vanishing White Matter | *EIF2B5* | AR | Reduced Risk |
| Limb-Girdle Muscular Dystrophy, Type 2A | *CAPN3* | AR | Reduced Risk |
| Limb-Girdle Muscular Dystrophy, Type 2B | *DYSF* | AR | Reduced Risk |
| Limb-Girdle Muscular Dystrophy, Type 2C | *SGCG* | AR | Reduced Risk |
| Limb-Girdle Muscular Dystrophy, Type 2D | *SGCA* | AR | Reduced Risk |

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|---|---|---|---|
| Limb-Girdle Muscular Dystrophy, Type 2E | *SGCB* | AR | Reduced Risk |
| Limb-Girdle Muscular Dystrophy, Type 2I | *FKRP* | AR | Reduced Risk |
| Lipoamide Dehydrogenase Deficiency | *DLD* | AR | Reduced Risk |
| Lipoid Adrenal Hyperplasia | *STAR* | AR | Reduced Risk |
| Lipoprotein Lipase Deficiency | *LPL* | AR | Reduced Risk |
| Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency | *HADHA* | AR | Reduced Risk |
| Lysinuric Protein Intolerance | *SLC7A7* | AR | Reduced Risk |
| Maple Syrup Urine Disease, Type 1a | *BCKDHA* | AR | Reduced Risk |
| Maple Syrup Urine Disease, Type 1b | *BCKDHB* | AR | Reduced Risk |
| Meckel 1 / Bardet-Biedl Syndrome 13 | *MKS1* | AR | Reduced Risk |
| Medium Chain Acyl-CoA Dehydrogenase Deficiency | *ACADM* | AR | Reduced Risk |
| Megalencephalic Leukoencephalopathy with Subcortical Cysts | *MLC1* | AR | Reduced Risk |
| Menkes Disease | *ATP7A* | XL | Reduced Risk |
| Metachromatic Leukodystrophy | *ARSA* | AR | Reduced Risk |
| Methylmalonic Acidemia (*MMAA*-Related) | *MMAA* | AR | Reduced Risk |
| Methylmalonic Acidemia (*MMAB*-Related) | *MMAB* | AR | Reduced Risk |
| Methylmalonic Acidemia (*MUT*-Related) | *MUT* | AR | Reduced Risk |
| Methylmalonic Aciduria and Homocystinuria, Cobalamin C Type | *MMACHC* | AR | Reduced Risk |
| Methylmalonic Aciduria and Homocystinuria, Cobalamin D Type | *MMADHC* | AR | Reduced Risk |
| Microphthalmia / Anophthalmia | *VSX2* | AR | Reduced Risk |
| Mitochondrial Complex I Deficiency (*ACAD9*-Related) | *ACAD9* | AR | Reduced Risk |
| Mitochondrial Complex I Deficiency (*NDUFAF5*-Related) | *NDUFAF5* | AR | Reduced Risk |
| Mitochondrial Complex I Deficiency (*NDUFS6*-Related) | *NDUFS6* | AR | Reduced Risk |
| Mitochondrial DNA Depletion Syndrome 6 / Navajo Neurohepatopathy | *MPV17* | AR | Reduced Risk |
| Mitochondrial Myopathy and Sideroblastic Anemia 1 | *PUS1* | AR | Reduced Risk |
| Mucolipidosis II / IIIA | *GNPTAB* | AR | Reduced Risk |
| Mucolipidosis III Gamma | *GNPTG* | AR | Reduced Risk |
| Mucolipidosis IV | *MCOLN1* | AR | Reduced Risk |
| Mucopolysaccharidosis Type I | *IDUA* | AR | Reduced Risk |
| Mucopolysaccharidosis Type II | *IDS* | XL | Reduced Risk |
| Mucopolysaccharidosis Type IIIA | *SGSH* | AR | Reduced Risk |
| Mucopolysaccharidosis Type IIIB | *NAGLU* | AR | Reduced Risk |
| Mucopolysaccharidosis Type IIIC | *HGSNAT* | AR | Reduced Risk |
| Mucopolysaccharidosis Type IIID | *GNS* | AR | Reduced Risk |
| Mucopolysaccharidosis Type IVb / GM1 Gangliosidosis | *GLB1* | AR | Reduced Risk |
| Mucopolysaccharidosis type IX | *HYAL1* | AR | Reduced Risk |
| Mucopolysaccharidosis type VI | *ARSB* | AR | Reduced Risk |
| Multiple Sulfatase Deficiency | *SUMF1* | AR | Reduced Risk |
| Muscle-Eye-Brain Disease and Other *POMGNT1*-Related Congenital Muscular Dystrophy-Dystroglycanopathies | *POMGNT1* | AR | Reduced Risk |
| Myoneurogastrointestinal Encephalopathy | *TYMP* | AR | Reduced Risk |
| Myotubular Myopathy 1 | *MTM1* | XL | Reduced Risk |
| N-Acetylglutamate Synthase Deficiency | *NAGS* | AR | Reduced Risk |
| Nemaline Myopathy 2 | *NEB* | AR | Reduced Risk |
| Nephrogenic Diabetes Insipidus, Type II | *AQP2* | AR | Reduced Risk |
| Nephrotic Syndrome (*NPHS1*-Related) / Congenital Finnish Nephrosis | *NPHS1* | AR | Reduced Risk |
| Nephrotic Syndrome (*NPHS2*-Related) / Steroid-Resistant Nephrotic Syndrome | *NPHS2* | AR | Reduced Risk |
| Neuronal Ceroid-Lipofuscinosis (*CLN3*-Related) | *CLN3* | AR | Reduced Risk |
| Neuronal Ceroid-Lipofuscinosis (*CLN5*-Related) | *CLN5* | AR | Reduced Risk |
| Neuronal Ceroid-Lipofuscinosis (*CLN6*-Related) | *CLN6* | AR | Reduced Risk |
| Neuronal Ceroid-Lipofuscinosis (*CLN8*-Related) | *CLN8* | AR | Reduced Risk |
| Neuronal Ceroid-Lipofuscinosis (*MFSD8*-Related) | *MFSD8* | AR | Reduced Risk |
| Neuronal Ceroid-Lipofuscinosis (*PPT1*-Related) | *PPT1* | AR | Reduced Risk |

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|---|---|---|---|---|
| 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<br>*SMN2* copy number: 2<br>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 | |

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|---|---|---|---|
| **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 8$^{th}$ "unassigned" group, which were then used to select residual risk values for each gene. For individuals belonging to multiple high-level ethnic groupings, a weighting strategy was used to select the most appropriate residual risk. For genes that had insufficient data to calculate ethnic-specific residual risk values, or for sub-ethnic groupings that fell into the "unassigned" group, a "worldwide" residual risk was used. This "worldwide" residual risk was calculated using data from all available continental-level groups.

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

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

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

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Additional disease-specific references available upon request.