



## Donor 6745

### Genetic Testing Summary

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

Last Updated: 06/27/22

Donor Reported Ancestry: Scottish, Danish, Swedish, German, English, Irish, Dutch, Italian, Austrian

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 and gene sequencing in the SMN1 gene	1/1107
Expanded Genetic Disease Carrier Screening Panel attached- 283 diseases by gene sequencing	<p>Carrier: Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency (CYP21A2)</p> <p>Negative for other genes sequenced</p>	Partner testing recommended before using this donor.

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

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

#### Patient Information

Name: Donor 6745  
Date of Birth: [REDACTED]  
Sema4 ID: [REDACTED]  
Client ID: [REDACTED]  
Indication: Carrier Screening

#### Specimen Information

Specimen Type: Blood  
Date Collected: 09/20/2021  
Date Received: 09/22/2021  
Final Report: 10/06/2021

#### Referring Provider

[REDACTED]  
Fairfax Cryobank, Inc.

## Expanded Carrier Screen Minus TSE (283 genes) with Personalized Residual Risk

### SUMMARY OF RESULTS AND RECOMMENDATIONS

⊕ Positive	⊖ Negative
<p><b>Carrier of Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency (AR)</b></p> <p>Associated gene(s): <i>CYP21A2</i></p> <p>Variant(s) Detected: c.1357C&gt;T, p.P453S, Pathogenic, Heterozygous (one copy)</p>	<p><b>Negative for all other genes tested</b></p> <p>To view a full list of genes and diseases tested please see Table 1 in this report</p>

AR=Autosomal recessive; XL=X-linked

### Recommendations

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

## Interpretation of positive results

### 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.1357C>T, p.P453S, Pathogenic, Heterozygous (one copy)  
**Genes analyzed:** *CYP21A2* (NM\_000500.6)

**Inheritance:** Autosomal Recessive

A heterozygous (one copy) pathogenic missense variant, c.1357C>T, p.P453S, was detected in the *CYP21A2* gene (NM\_000500.6). Please note that this variant is typically causative for the non-classic form of congenital adrenal hyperplasia (PMID: 29450859). Variants associated with the non-classic form usually cause non-classic congenital adrenal hyperplasia when found in trans with a pathogenic allele, regardless of whether the second variant is associated with classic or non-classic disease (PMID: 29450859). Therefore, this individual is expected to be at least a carrier for non-classic congenital adrenal hyperplasia. Heterozygous carriers are not expected to exhibit symptoms of this disease.

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

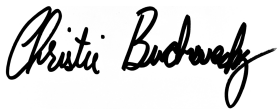
Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders resulting from deficiency in the enzymes involved in cortisol biosynthesis. The majority (95%) of CAH cases are due to 21-hydroxylase deficiency (21-OHD CAH), which is caused by homozygous or compound heterozygous pathogenic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

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

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

## Test description

This patient was tested for a panel of diseases using a combination of sequencing, targeted genotyping and copy number analysis. Please note that negative results reduce but do not eliminate the possibility that this individual is a carrier for one or more of the disorders tested. Please see Table 1 for a list of genes and diseases tested with the patient's personalized residual risk. If personalized residual risk is not provided, please see the complete residual risk table at [go.sema4.com/residualrisk](https://go.sema4.com/residualrisk). Only variants determined to be pathogenic or likely pathogenic are reported in this carrier screening test.



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Laboratory Medical Consultant: George A. Diaz, M.D., Ph.D

## Genes and diseases tested

The personalized residual risks listed below are specific to this individual. The complete residual risk table is available at [go.sema4.com/residualrisk](https://go.sema4.com/residualrisk)

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

Disease	Gene	Inheritance Pattern	Status	Detailed Summary
<b>Positive</b>				
Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency	CYP21A2	AR	Carrier	CYP21A2 copy number: 2 No pathogenic copy number variants detected CYP21A2 sequencing: c.1357C>T, p.P453S Pathogenic, Heterozygous (one copy)
<b>Negative</b>				
3-Beta-Hydroxysteroid Dehydrogenase Type II Deficiency	HSD3B2	AR	Reduced Risk	Personalized Residual Risk: 1 in 3,300
3-Methylcrotonyl-CoA Carboxylase Deficiency (MCCC1-Related)	MCCC1	AR	Reduced Risk	Personalized Residual Risk: 1 in 3,400
3-Methylcrotonyl-CoA Carboxylase Deficiency (MCCC2-Related)	MCCC2	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,200
3-Methylglutaconic Aciduria, Type III	OPA3	AR	Reduced Risk	Personalized Residual Risk: 1 in 50,000
3-Phosphoglycerate Dehydrogenase Deficiency	PHGDH	AR	Reduced Risk	Personalized Residual Risk: 1 in 63,000
6-Pyruvoyl-Tetrahydropterin Synthase Deficiency	PTS	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,800
Abetalipoproteinemia	MTTP	AR	Reduced Risk	Personalized Residual Risk: 1 in 3,200
Achromatopsia (CNGB3-related)	CNGB3	AR	Reduced Risk	Personalized Residual Risk: 1 in 8,600
Acrodermatitis Enteropathica	SLC39A4	AR	Reduced Risk	Personalized Residual Risk: 1 in 12,000
Acute Infantile Liver Failure	TRMU	AR	Reduced Risk	Personalized Residual Risk: 1 in 9,400
Acyl-CoA Oxidase I Deficiency	ACOX1	AR	Reduced Risk	Personalized Residual Risk: 1 in 39,000
Adenosine Deaminase Deficiency	ADA	AR	Reduced Risk	Personalized Residual Risk: 1 in 5,100
Adrenoleukodystrophy, X-Linked	ABCD1	XL	Reduced Risk	Personalized Residual Risk: 1 in 19,000
Aicardi-Goutieres Syndrome (SAMHD1-Related)	SAMHD1	AR	Reduced Risk	Personalized Residual Risk: 1 in 10,000
Alpha-Mannosidosis	MAN2B1	AR	Reduced Risk	Personalized Residual Risk: 1 in 6,200
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 Personalized Residual Risk: 1 in 10,000
Alpha-Thalassemia Intellectual Disability Syndrome	ATRX	XL	Reduced Risk	Personalized Residual Risk: 1 in 48,000
Alport Syndrome (COL4A3-Related)	COL4A3	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,800
Alport Syndrome (COL4A4-Related)	COL4A4	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,800
Alport Syndrome (COL4A5-Related)	COL4A5	XL	Reduced Risk	Personalized Residual Risk: 1 in 150,000
Alstrom Syndrome	ALMS1	AR	Reduced Risk	Personalized Residual Risk: 1 in 3,800
Andermann Syndrome	SLC12A6	AR	Reduced Risk	Personalized Residual Risk: 1 in 151,000
Argininosuccinic Aciduria	ASL	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,200
Aromatase Deficiency	CYP19A1	AR	Reduced Risk	Personalized Residual Risk: 1 in 5,400
Arthrogryposis, Mental Retardation, and Seizures	SLC35A3	AR	Reduced Risk	Personalized Residual Risk: 1 in 454,000
Asparagine Synthetase Deficiency	ASNS	AR	Reduced Risk	Personalized Residual Risk: 1 in 202,000
Aspartylglycosaminuria	AGA	AR	Reduced Risk	Personalized Residual Risk: 1 in 13,000
Ataxia With Isolated Vitamin E Deficiency	TTPA	AR	Reduced Risk	Personalized Residual Risk: 1 in 61,000
Ataxia-Telangiectasia	ATM	AR	Reduced Risk	

					Personalized Residual Risk: 1 in 1,300
Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay	SACS	AR	Reduced Risk		Personalized Residual Risk: 1 in 2,600
Bardet-Biedl Syndrome (BBS10-Related)	BBS10	AR	Reduced Risk		Personalized Residual Risk: 1 in 2,700
Bardet-Biedl Syndrome (BBS12-Related)	BBS12	AR	Reduced Risk		Personalized Residual Risk: 1 in 9,900
Bardet-Biedl Syndrome (BBS1-Related)	BBS1	AR	Reduced Risk		Personalized Residual Risk: 1 in 6,400
Bardet-Biedl Syndrome (BBS2-Related)	BBS2	AR	Reduced Risk		Personalized Residual Risk: 1 in 1,200
Bare Lymphocyte Syndrome, Type II	CIITA	AR	Reduced Risk		Personalized Residual Risk: 1 in 35,000
Bartter Syndrome, Type 4A	BSND	AR	Reduced Risk		Personalized Residual Risk: 1 in 91,000
Bernard-Soulier Syndrome, Type A1	GP1BA	AR	Reduced Risk		Personalized Residual Risk: 1 in 42,000
Bernard-Soulier Syndrome, Type C	GP9	AR	Reduced Risk		Personalized Residual Risk: 1 in 3,300
Beta-Globin-Related Hemoglobinopathies	HBB	AR	Reduced Risk		Personalized Residual Risk (Beta-Globin-Related Hemoglobinopathies): 1 in 2,000 Personalized Residual Risk (Beta-Globin-Related Hemoglobinopathies: HbS Variant): 1 in 790,000 Personalized Residual Risk (Beta-Globin-Related Hemoglobinopathies: HbC Variant): 1 in 2,107,000
Beta-Ketothiolase Deficiency	ACAT1	AR	Reduced Risk		Personalized Residual Risk: 1 in 5,400
Bilateral Frontoparietal Polymicrogyria	GPR56	AR	Reduced Risk		Personalized Residual Risk: 1 in 203,000
Biotinidase Deficiency	BTB	AR	Reduced Risk		Personalized Residual Risk: 1 in 500
Bloom Syndrome	BLM	AR	Reduced Risk		Personalized Residual Risk: 1 in 7,400
Canavan Disease	ASPA	AR	Reduced Risk		Personalized Residual Risk: 1 in 4,000
Carbamoylphosphate Synthetase I Deficiency	CPS1	AR	Reduced Risk		Personalized Residual Risk: 1 in 1,100
Carnitine Palmitoyltransferase IA Deficiency	CPT1A	AR	Reduced Risk		Personalized Residual Risk: 1 in 24,000
Carnitine Palmitoyltransferase II Deficiency	CPT2	AR	Reduced Risk		Personalized Residual Risk: 1 in 670
Carpenter Syndrome	RAB23	AR	Reduced Risk		Personalized Residual Risk: 1 in 21,000
Cartilage-Hair Hypoplasia	RMRP	AR	Reduced Risk		Personalized Residual Risk: 1 in 960
Cerebral Creatine Deficiency Syndrome 1	SLC6A8	XL	Reduced Risk		Personalized Residual Risk: 1 in 208,000
Cerebral Creatine Deficiency Syndrome 2	GAMT	AR	Reduced Risk		Personalized Residual Risk: 1 in 2,100
Cerebrotendinous Xanthomatosis	CYP27A1	AR	Reduced Risk		Personalized Residual Risk: 1 in 3,900
Charcot-Marie-Tooth Disease, Type 4D	NDRG1	AR	Reduced Risk		Personalized Residual Risk: 1 in 730,000
Charcot-Marie-Tooth Disease, Type 5 / Arts Syndrome	PRPS1	XL	Reduced Risk		Personalized Residual Risk: 1 in 114,000
Charcot-Marie-Tooth Disease, X-Linked	GJB1	XL	Reduced Risk		Personalized Residual Risk: 1 in 11,000
Choreoacanthocytosis	VPS13A	AR	Reduced Risk		Personalized Residual Risk: 1 in 13,000
Choroideremia	CHM	XL	Reduced Risk		Personalized Residual Risk: 1 in 125,000
Chronic Granulomatous Disease (CYBA-Related)	CYBA	AR	Reduced Risk		Personalized Residual Risk: 1 in 5,000
Chronic Granulomatous Disease (CYBB-Related)	CYBB	XL	Reduced Risk		Personalized Residual Risk: 1 in 294,000
Citrin Deficiency	SLC25A13	AR	Reduced Risk		Personalized Residual Risk: 1 in 12,000
Citrullinemia, Type 1	ASS1	AR	Reduced Risk		Personalized Residual Risk: 1 in 2,500
Cohen Syndrome	VPS13B	AR	Reduced Risk		Personalized Residual Risk: 1 in 6,400
Combined Malonic and Methylmalonic Aciduria	ACSF3	AR	Reduced Risk		Personalized Residual Risk: 1 in 2,400
Combined Oxidative Phosphorylation Deficiency 1	GFM1	AR	Reduced Risk		Personalized Residual Risk: 1 in 13,000
Combined Oxidative Phosphorylation Deficiency 3	TSFM	AR	Reduced Risk		Personalized Residual Risk: 1 in 27,000
Combined Pituitary Hormone Deficiency 2	PROP1	AR	Reduced Risk		Personalized Residual Risk: 1 in 2,800
Combined Pituitary Hormone Deficiency 3	LHX3	AR	Reduced Risk		Personalized Residual Risk: 1 in 140,000
Combined SAP Deficiency	PSAP	AR	Reduced Risk		Personalized Residual Risk: 1 in 44,000
Congenital Adrenal Hyperplasia due to 17-Alpha-Hydroxylase Deficiency	CYP17A1	AR	Reduced Risk		Personalized Residual Risk: 1 in 1,800
Congenital Amegakaryocytic Thrombocytopenia	MPL	AR	Reduced Risk		Personalized Residual Risk: 1 in 3,100

Congenital Disorder of Glycosylation, Type Ia	PMM2	AR	Reduced Risk	Personalized Residual Risk: 1 in 540
Congenital Disorder of Glycosylation, Type Ib	MPI	AR	Reduced Risk	Personalized Residual Risk: 1 in 5,600
Congenital Disorder of Glycosylation, Type Ic	ALG6	AR	Reduced Risk	Personalized Residual Risk: 1 in 4,100
Congenital Insensitivity to Pain with Anhidrosis	NTRK1	AR	Reduced Risk	Personalized Residual Risk: 1 in 5,700
Congenital Myasthenic Syndrome (CHRNA3-Related)	CHRNA3	AR	Reduced Risk	Personalized Residual Risk: 1 in 4,100
Congenital Myasthenic Syndrome (RAPSN-Related)	RAPSN	AR	Reduced Risk	Personalized Residual Risk: 1 in 2,900
Congenital Neutropenia (HAX1-Related)	HAX1	AR	Reduced Risk	Personalized Residual Risk: 1 in 82,000
Congenital Neutropenia (VPS45-Related)	VPS45	AR	Reduced Risk	Personalized Residual Risk: 1 in 163,000
Corneal Dystrophy and Perceptive Deafness	SLC4A11	AR	Reduced Risk	Personalized Residual Risk: 1 in 4,600
Corticosterone Methyltransferase Deficiency	CYP11B2	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,500
Cystic Fibrosis	CFTR	AR	Reduced Risk	Personalized Residual Risk: 1 in 440
Cystinosis	CTNS	AR	Reduced Risk	Personalized Residual Risk: 1 in 7,700
D-Bifunctional Protein Deficiency	HSD17B4	AR	Reduced Risk	Personalized Residual Risk: 1 in 5,000
Deafness, Autosomal Recessive 77	LOXHD1	AR	Reduced Risk	Personalized Residual Risk: 1 in 6,700
Duchenne Muscular Dystrophy / Becker Muscular Dystrophy	DMD	XL	Reduced Risk	Personalized Residual Risk: 1 in 10,000
Dyskeratosis Congenita (RTKL1-Related)	RTKL1	AR	Reduced Risk	Personalized Residual Risk: 1 in 9,800
Dystrophic Epidermolysis Bullosa	COL7A1	AR	Reduced Risk	Personalized Residual Risk: 1 in 900
Ehlers-Danlos Syndrome, Type VIIC	ADAMTS2	AR	Reduced Risk	Personalized Residual Risk: 1 in 243,000
Ellis-van Creveld Syndrome (EVC-Related)	EVC	AR	Reduced Risk	Personalized Residual Risk: 1 in 4,200
Emery-Dreifuss Myopathy 1	EMD	XL	Reduced Risk	Personalized Residual Risk: 1 in 833,000
Enhanced S-Cone Syndrome	NR2E3	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,600
Ethylmalonic Encephalopathy	ETHE1	AR	Reduced Risk	Personalized Residual Risk: 1 in 3,400
Fabry Disease	GLA	XL	Reduced Risk	Personalized Residual Risk: 1 in 7,700
Factor IX Deficiency	F9	XL	Reduced Risk	Personalized Residual Risk: 1 in 5,100
Factor XI Deficiency	F11	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,500
Familial Autosomal Recessive Hypercholesterolemia	LDLRAP1	AR	Reduced Risk	Personalized Residual Risk: 1 in 136,000
Familial Dysautonomia	IKBKAP	AR	Reduced Risk	Personalized Residual Risk: 1 in 51,000
Familial Hypercholesterolemia	LDLR	AR	Reduced Risk	Personalized Residual Risk: 1 in 280
Familial Hyperinsulinism (ABCC8-Related)	ABCC8	AR	Reduced Risk	Personalized Residual Risk: 1 in 450
Familial Hyperinsulinism (KCNJ11-Related)	KCNJ11	AR	Reduced Risk	Personalized Residual Risk: 1 in 5,300
Familial Mediterranean Fever	MEFV	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,200
Fanconi Anemia, Group A	FANCA	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,100
Fanconi Anemia, Group C	FANCC	AR	Reduced Risk	Personalized Residual Risk: 1 in 12,000
Fanconi Anemia, Group G	FANCG	AR	Reduced Risk	Personalized Residual Risk: 1 in 28,000
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. Personalized Residual Risk: 1 in 19,000
Fumarate Deficiency	FH	AR	Reduced Risk	Personalized Residual Risk: 1 in 2,500
GRACILE Syndrome and Other BCS1L-Related Disorders	BCS1L	AR	Reduced Risk	Personalized Residual Risk: 1 in 3,900
Galactokinase Deficiency	GALK1	AR	Reduced Risk	Personalized Residual Risk: 1 in 2,700
Galactosemia	GALT	AR	Reduced Risk	Personalized Residual Risk: 1 in 3,200
Gaucher Disease	GBA	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,300
Gitelman Syndrome	SLC12A3	AR	Reduced Risk	Personalized Residual Risk: 1 in 290
Glutaric Acidemia, Type I	GCDH	AR	Reduced Risk	Personalized Residual Risk: 1 in 2,700
Glutaric Acidemia, Type IIa	ETFA	AR	Reduced Risk	Personalized Residual Risk: 1 in 4,700
Glutaric Acidemia, Type IIc	ETFDH	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,700

Glycine Encephalopathy (AMT-Related)	AMT	AR	Reduced Risk	Personalized Residual Risk: 1 in 5,700
Glycine Encephalopathy (GLDC-Related)	GLDC	AR	Reduced Risk	Personalized Residual Risk: 1 in 760
Glycogen Storage Disease, Type II	GAA	AR	Reduced Risk	Personalized Residual Risk: 1 in 520
Glycogen Storage Disease, Type III	AGL	AR	Reduced Risk	Personalized Residual Risk: 1 in 5,600
Glycogen Storage Disease, Type IV / Adult Polyglucosan Body Disease	GBE1	AR	Reduced Risk	Personalized Residual Risk: 1 in 2,400
Glycogen Storage Disease, Type Ia	G6PC	AR	Reduced Risk	Personalized Residual Risk: 1 in 5,300
Glycogen Storage Disease, Type Ib	SLC37A4	AR	Reduced Risk	Personalized Residual Risk: 1 in 7,300
Glycogen Storage Disease, Type V	PYGM	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,200
Glycogen Storage Disease, Type VII	PFKM	AR	Reduced Risk	Personalized Residual Risk: 1 in 4,300
HMG-CoA Lyase Deficiency	HMGCL	AR	Reduced Risk	Personalized Residual Risk: 1 in 2,700
Hemochromatosis, Type 2A	HFE2	AR	Reduced Risk	Personalized Residual Risk: 1 in 12,000
Hemochromatosis, Type 3	TFR2	AR	Reduced Risk	Personalized Residual Risk: 1 in 11,000
Hereditary Fructose Intolerance	ALDOB	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,900
Hereditary Spastic Paraparesis 49	TECPR2	AR	Reduced Risk	Personalized Residual Risk: 1 in 116,000
Hermansky-Pudlak Syndrome, Type 1	HPS1	AR	Reduced Risk	Personalized Residual Risk: 1 in 3,500
Hermansky-Pudlak Syndrome, Type 3	HPS3	AR	Reduced Risk	Personalized Residual Risk: 1 in 49,000
Holocarboxylase Synthetase Deficiency	HLCS	AR	Reduced Risk	Personalized Residual Risk: 1 in 5,500
Homocystinuria (CBS-Related)	CBS	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,400
Homocystinuria due to MTHFR Deficiency	MTHFR	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,300
Homocystinuria, cblE Type	MTRR	AR	Reduced Risk	Personalized Residual Risk: 1 in 9,600
Hydroletharus Syndrome	HYLS1	AR	Reduced Risk	Personalized Residual Risk: 1 in 52,000
Hyperornithinemia-Hyperammonemia-Homocitrullinuria Syndrome	SLC25A15	AR	Reduced Risk	Personalized Residual Risk: 1 in 5,700
Hypohidrotic Ectodermal Dysplasia 1	EDA	XL	Reduced Risk	Personalized Residual Risk: 1 in 22,000
Hypophosphatasia	ALPL	AR	Reduced Risk	Personalized Residual Risk: 1 in 790
Inclusion Body Myopathy 2	GNB	AR	Reduced Risk	Personalized Residual Risk: 1 in 2,000
Infantile Cerebral and Cerebellar Atrophy	MED17	AR	Reduced Risk	Personalized Residual Risk: 1 in 129,000
Isovaleric Acidemia	IVD	AR	Reduced Risk	Personalized Residual Risk: 1 in 2,000
Joubert Syndrome 2	TMEM216	AR	Reduced Risk	Personalized Residual Risk: 1 in 152,000
Joubert Syndrome 7 / Meckel Syndrome 5 / COACH Syndrome	RPGRIP1L	AR	Reduced Risk	Personalized Residual Risk: 1 in 32,000
Junctional Epidermolysis Bullosa (LAMA3-Related)	LAMA3	AR	Reduced Risk	Personalized Residual Risk: 1 in 21,000
Junctional Epidermolysis Bullosa (LAMB3-Related)	LAMB3	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,900
Junctional Epidermolysis Bullosa (LAMC2-Related)	LAMC2	AR	Reduced Risk	Personalized Residual Risk: 1 in 77,000
Krabbe Disease	GALC	AR	Reduced Risk	Personalized Residual Risk: 1 in 860
Lamellar Ichthyosis, Type 1	TGM1	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,500
Leber Congenital Amaurosis 10 and Other CEP290-Related Ciliopathies	CEP290	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,100
Leber Congenital Amaurosis 13	RDH12	AR	Reduced Risk	Personalized Residual Risk: 1 in 5,500
Leber Congenital Amaurosis 2 / Retinitis Pigmentosa 20	RPE65	AR	Reduced Risk	Personalized Residual Risk: 1 in 2,500
Leber Congenital Amaurosis 5	LCA5	AR	Reduced Risk	Personalized Residual Risk: 1 in 14,000
Leber Congenital Amaurosis 8 / Retinitis Pigmentosa 12 / Pigmented Paravenous Chorioretinal Atrophy	CRB1	AR	Reduced Risk	Personalized Residual Risk: 1 in 990
Leigh Syndrome, French-Canadian Type	LRPPRC	AR	Reduced Risk	Personalized Residual Risk: 1 in 32,000
Lethal Congenital Contracture Syndrome 1 / Lethal Arthrogyposis with Anterior Horn Cell Disease	GLE1	AR	Reduced Risk	Personalized Residual Risk: 1 in 10,000
Leukoencephalopathy with Vanishing White Matter	EIF2B5	AR	Reduced Risk	Personalized Residual Risk: 1 in 2,300
Limb-Girdle Muscular Dystrophy, Type 2A	CAPN3	AR	Reduced Risk	Personalized Residual Risk: 1 in 960



Limb-Girdle Muscular Dystrophy, Type 2B	DYSF	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,100
Limb-Girdle Muscular Dystrophy, Type 2C	SGCG	AR	Reduced Risk	Personalized Residual Risk: 1 in 4,900
Limb-Girdle Muscular Dystrophy, Type 2D	SGCA	AR	Reduced Risk	Personalized Residual Risk: 1 in 3,500
Limb-Girdle Muscular Dystrophy, Type 2E	SGCB	AR	Reduced Risk	Personalized Residual Risk: 1 in 31,000
Limb-Girdle Muscular Dystrophy, Type 2I	FKRP	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,400
Lipoamide Dehydrogenase Deficiency	DLD	AR	Reduced Risk	Personalized Residual Risk: 1 in 14,000
Lipoid Adrenal Hyperplasia	STAR	AR	Reduced Risk	Personalized Residual Risk: 1 in 3,600
Lipoprotein Lipase Deficiency	LPL	AR	Reduced Risk	Personalized Residual Risk: 1 in 2,400
Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency	HADHA	AR	Reduced Risk	Personalized Residual Risk: 1 in 5,900
Lysinuric Protein Intolerance	SLC7A7	AR	Reduced Risk	Personalized Residual Risk: 1 in 3,000
Maple Syrup Urine Disease, Type 1a	BCKDHA	AR	Reduced Risk	Personalized Residual Risk: 1 in 5,100
Maple Syrup Urine Disease, Type 1b	BCKDHB	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,100
Meckel Syndrome 1 / Bardet-Biedl Syndrome 13	MKS1	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,700
Medium Chain Acyl-CoA Dehydrogenase Deficiency	ACADM	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,800
Megalencephalic Leukoencephalopathy with Subcortical Cysts	MLC1	AR	Reduced Risk	Personalized Residual Risk: 1 in 4,300
Menkes Disease	ATP7A	XL	Reduced Risk	Personalized Residual Risk: 1 in 172,000
Metachromatic Leukodystrophy	ARSA	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,000
Methylmalonic Acidemia (MMAA-Related)	MMAA	AR	Reduced Risk	Personalized Residual Risk: 1 in 15,000
Methylmalonic Acidemia (MMAB-Related)	MMAB	AR	Reduced Risk	Personalized Residual Risk: 1 in 12,000
Methylmalonic Acidemia (MUT-Related)	MUT	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,300
Methylmalonic Aciduria and Homocystinuria, Cobalamin C Type	MMACHC	AR	Reduced Risk	Personalized Residual Risk: 1 in 6,800
Methylmalonic Aciduria and Homocystinuria, Cobalamin D Type	MMADHC	AR	Reduced Risk	Personalized Residual Risk: 1 in 219,000
Microphthalmia / Anophthalmia	VSX2	AR	Reduced Risk	Personalized Residual Risk: 1 in 40,000
Mitochondrial Complex I Deficiency (ACAD9-Related)	ACAD9	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,800
Mitochondrial Complex I Deficiency (NDUFAF5-Related)	NDUFAF5	AR	Reduced Risk	Personalized Residual Risk: 1 in 98,000
Mitochondrial Complex I Deficiency (NDUFS6-Related)	NDUFS6	AR	Reduced Risk	Personalized Residual Risk: 1 in 353,000
Mitochondrial DNA Depletion Syndrome 6 / Navajo Neurohepatopathy	MPV17	AR	Reduced Risk	Personalized Residual Risk: 1 in 4,400
Mitochondrial Myopathy and Sideroblastic Anemia 1	PUS1	AR	Reduced Risk	Personalized Residual Risk: 1 in 449,000
Mucopolipidosis II / IIIA	GNPTAB	AR	Reduced Risk	Personalized Residual Risk: 1 in 2,100
Mucopolipidosis III Gamma	GNPTG	AR	Reduced Risk	Personalized Residual Risk: 1 in 68,000
Mucopolipidosis IV	MCOLN1	AR	Reduced Risk	Personalized Residual Risk: 1 in 9,400
Mucopolysaccharidosis Type I	IDUA	AR	Reduced Risk	Personalized Residual Risk: 1 in 3,300
Mucopolysaccharidosis Type II	IDS	XL	Reduced Risk	Personalized Residual Risk: 1 in 76,000
Mucopolysaccharidosis Type IIIA	SGSH	AR	Reduced Risk	Personalized Residual Risk: 1 in 2,700
Mucopolysaccharidosis Type IIIB	NAGLU	AR	Reduced Risk	Personalized Residual Risk: 1 in 950
Mucopolysaccharidosis Type IIIC	HGSNAT	AR	Reduced Risk	Personalized Residual Risk: 1 in 3,200
Mucopolysaccharidosis Type IIID	GNS	AR	Reduced Risk	Personalized Residual Risk: 1 in 137,000
Mucopolysaccharidosis Type IVb / GM1 Gangliosidosis	GLB1	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,700
Mucopolysaccharidosis type IX	HYAL1	AR	Reduced Risk	Personalized Residual Risk: 1 in 149,000
Mucopolysaccharidosis type VI	ARSB	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,300
Multiple Sulfatase Deficiency	SUMF1	AR	Reduced Risk	Personalized Residual Risk: 1 in 69,000
Muscle-Eye-Brain Disease and Other POMGNT1-Related Congenital Muscular Dystrophy-Dystroglycanopathies	POMGNT1	AR	Reduced Risk	Personalized Residual Risk: 1 in 4,200
Myoneurogastrointestinal Encephalopathy	TYMP	AR	Reduced Risk	Personalized Residual Risk: 1 in 2,100
Myotubular Myopathy 1	MTM1	XL	Reduced Risk	



					Personalized Residual Risk: 1 in 192,000
N-Acetylglutamate Synthase Deficiency	NAGS	AR	Reduced Risk		Personalized Residual Risk: 1 in 3,200
Nemaline Myopathy 2	NEB	AR	Reduced Risk		Personalized Residual Risk: 1 in 2,400
Nephrogenic Diabetes Insipidus, Type II	AQP2	AR	Reduced Risk		Personalized Residual Risk: 1 in 3,400
Nephrotic Syndrome (NPHS1-Related) / Congenital Finnish Nephrosis	NPHS1	AR	Reduced Risk		Personalized Residual Risk: 1 in 920
Nephrotic Syndrome (NPHS2-Related) / Steroid-Resistant Nephrotic Syndrome	NPHS2	AR	Reduced Risk		Personalized Residual Risk: 1 in 780
Neuronal Ceroid-Lipofuscinosis (CLN3-Related)	CLN3	AR	Reduced Risk		Personalized Residual Risk: 1 in 9,200
Neuronal Ceroid-Lipofuscinosis (CLN5-Related)	CLN5	AR	Reduced Risk		Personalized Residual Risk: 1 in 4,300
Neuronal Ceroid-Lipofuscinosis (CLN6-Related)	CLN6	AR	Reduced Risk		Personalized Residual Risk: 1 in 8,600
Neuronal Ceroid-Lipofuscinosis (CLN8-Related)	CLN8	AR	Reduced Risk		Personalized Residual Risk: 1 in 3,100
Neuronal Ceroid-Lipofuscinosis (MFSD8-Related)	MFSD8	AR	Reduced Risk		Personalized Residual Risk: 1 in 6,200
Neuronal Ceroid-Lipofuscinosis (PPT1-Related)	PPT1	AR	Reduced Risk		Personalized Residual Risk: 1 in 7,500
Neuronal Ceroid-Lipofuscinosis (TPP1-Related)	TPP1	AR	Reduced Risk		Personalized Residual Risk: 1 in 6,300
Niemann-Pick Disease (SMPD1-Related)	SMPD1	AR	Reduced Risk		Personalized Residual Risk: 1 in 1,800
Niemann-Pick Disease, Type C (NPC1-Related)	NPC1	AR	Reduced Risk		Personalized Residual Risk: 1 in 690
Niemann-Pick Disease, Type C (NPC2-Related)	NPC2	AR	Reduced Risk		Personalized Residual Risk: 1 in 6,600
Nijmegen Breakage Syndrome	NBN	AR	Reduced Risk		Personalized Residual Risk: 1 in 14,000
Non-Syndromic Hearing Loss (GJB2-Related)	GJB2	AR	Reduced Risk		Personalized Residual Risk: 1 in 600
Odonto-Onycho-Dermal Dysplasia / Schopf-Schulz-Passarge Syndrome	WNT10A	AR	Reduced Risk		Personalized Residual Risk: 1 in 1,900
Omenn Syndrome (RAG2-Related)	RAG2	AR	Reduced Risk		Personalized Residual Risk: 1 in 17,000
Omenn Syndrome / Severe Combined Immunodeficiency, Athabaskan-Type	DCLRE1C	AR	Reduced Risk		Personalized Residual Risk: 1 in 5,500
Ornithine Aminotransferase Deficiency	OAT	AR	Reduced Risk		Personalized Residual Risk: 1 in 6,400
Ornithine Transcarbamylase Deficiency	OTC	XL	Reduced Risk		Personalized Residual Risk: 1 in 103,000
Osteopetrosis 1	TCIRG1	AR	Reduced Risk		Personalized Residual Risk: 1 in 4,700
Pendred Syndrome	SLC26A4	AR	Reduced Risk		Personalized Residual Risk: 1 in 390
Phenylalanine Hydroxylase Deficiency	PAH	AR	Reduced Risk		Personalized Residual Risk: 1 in 340
Polycystic Kidney Disease, Autosomal Recessive	PKHD1	AR	Reduced Risk		Personalized Residual Risk: 1 in 450
Polyglandular Autoimmune Syndrome, Type 1	AIRE	AR	Reduced Risk		Personalized Residual Risk: 1 in 5,300
Pontocerebellar Hypoplasia, Type 1A	VRK1	AR	Reduced Risk		Personalized Residual Risk: 1 in 25,000
Pontocerebellar Hypoplasia, Type 6	RARS2	AR	Reduced Risk		Personalized Residual Risk: 1 in 8,600
Primary Carnitine Deficiency	SLC22A5	AR	Reduced Risk		Personalized Residual Risk: 1 in 1,500
Primary Ciliary Dyskinesia (DNAH5-Related)	DNAH5	AR	Reduced Risk		Personalized Residual Risk: 1 in 1,500
Primary Ciliary Dyskinesia (DNAI1-Related)	DNAI1	AR	Reduced Risk		Personalized Residual Risk: 1 in 5,000
Primary Ciliary Dyskinesia (DNAI2-Related)	DNAI2	AR	Reduced Risk		Personalized Residual Risk: 1 in 76,000
Primary Hyperoxaluria, Type 1	AGXT	AR	Reduced Risk		Personalized Residual Risk: 1 in 1,900
Primary Hyperoxaluria, Type 2	GRHPR	AR	Reduced Risk		Personalized Residual Risk: 1 in 11,000
Primary Hyperoxaluria, Type 3	HOGA1	AR	Reduced Risk		Personalized Residual Risk: 1 in 2,400
Progressive Cerebello-Cerebral Atrophy	SEPSECS	AR	Reduced Risk		Personalized Residual Risk: 1 in 6,400
Progressive Familial Intrahepatic Cholestasis, Type 2	ABCB11	AR	Reduced Risk		Personalized Residual Risk: 1 in 950
Propionic Acidemia (PCCA-Related)	PCCA	AR	Reduced Risk		Personalized Residual Risk: 1 in 2,600
Propionic Acidemia (PCCB-Related)	PCCB	AR	Reduced Risk		Personalized Residual Risk: 1 in 12,000
Pycnodysostosis	CTSK	AR	Reduced Risk		Personalized Residual Risk: 1 in 5,100
Pyruvate Dehydrogenase E1-Alpha Deficiency	PDHA1	XL	Reduced Risk		Personalized Residual Risk: 1 in 139,000
Pyruvate Dehydrogenase E1-Beta Deficiency	PDHB	AR	Reduced Risk		Personalized Residual Risk: 1 in 15,000
Renal Tubular Acidosis and Deafness	ATP6V1B1	AR	Reduced Risk		Personalized Residual Risk: 1 in 6,600
Retinitis Pigmentosa 25	EYS	AR	Reduced Risk		Personalized Residual Risk: 1 in 1,800

Retinitis Pigmentosa 26	CERKL	AR	Reduced Risk	Personalized Residual Risk: 1 in 13,000
Retinitis Pigmentosa 28	FAM161A	AR	Reduced Risk	Personalized Residual Risk: 1 in 34,000
Retinitis Pigmentosa 59	DHDDS	AR	Reduced Risk	Personalized Residual Risk: 1 in 601,000
Rhizomelic Chondrodysplasia Punctata, Type 1	PEX7	AR	Reduced Risk	Personalized Residual Risk: 1 in 10,000
Rhizomelic Chondrodysplasia Punctata, Type 3	AGPS	AR	Reduced Risk	Personalized Residual Risk: 1 in 620,000
Roberts Syndrome	ESCO2	AR	Reduced Risk	Personalized Residual Risk: 1 in 139,000
Salla Disease	SLC17A5	AR	Reduced Risk	Personalized Residual Risk: 1 in 8,400
Sandhoff Disease	HEXB	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,800
Schimke Immunoosseous Dysplasia	SMARCA1	AR	Reduced Risk	Personalized Residual Risk: 1 in 3,800
Segawa Syndrome	TH	AR	Reduced Risk	Personalized Residual Risk: 1 in 6,100
Sjogren-Larsson Syndrome	ALDH3A2	AR	Reduced Risk	Personalized Residual Risk: 1 in 5,500
Smith-Lemli-Opitz Syndrome	DHCR7	AR	Reduced Risk	Personalized Residual Risk: 1 in 750
Spinal Muscular Atrophy	SMN1	AR	Reduced Risk	SMN1 copy number: 2 SMN2 copy number: 2 c.*3+80T>G: Negative SMN1 Sequencing: Negative Personalized Residual Risk: 1 in 1,107
Spondylothoracic Dysostosis	MESP2	AR	Reduced Risk	Personalized Residual Risk: 1 in 382,000
Steel Syndrome	COL27A1	AR	Reduced Risk	Personalized Residual Risk: 1 in 93,000
Stuve-Wiedemann Syndrome	LIFR	AR	Reduced Risk	Personalized Residual Risk: 1 in 6,000
Sulfate Transporter-Related Osteochondrodysplasia	SLC26A2	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,800
Tay-Sachs Disease	HEXA	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,400
Tyrosinemia, Type I	FAH	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,900
Usher Syndrome, Type IB	MYO7A	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,000
Usher Syndrome, Type IC	USH1C	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,600
Usher Syndrome, Type ID	CDH23	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,400
Usher Syndrome, Type IF	PCDH15	AR	Reduced Risk	Personalized Residual Risk: 1 in 3,800
Usher Syndrome, Type IIA	USH2A	AR	Reduced Risk	Personalized Residual Risk: 1 in 290
Usher Syndrome, Type III	CLRN1	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,300
Very Long Chain Acyl-CoA Dehydrogenase Deficiency	ACADVL	AR	Reduced Risk	Personalized Residual Risk: 1 in 920
Walker-Warburg Syndrome and Other FKTN-Related Dystrophies	FKTN	AR	Reduced Risk	Personalized Residual Risk: 1 in 4,200
Wilson Disease	ATP7B	AR	Reduced Risk	Personalized Residual Risk: 1 in 350
Wolman Disease / Cholesteryl Ester Storage Disease	LIPA	AR	Reduced Risk	Personalized Residual Risk: 1 in 3,200
X-Linked Juvenile Retinoschisis	RS1	XL	Reduced Risk	Personalized Residual Risk: 1 in 40,000
X-Linked Severe Combined Immunodeficiency	IL2RG	XL	Reduced Risk	Personalized Residual Risk: 1 in 250,000
Zellweger Syndrome Spectrum (PEX10-Related)	PEX10	AR	Reduced Risk	Personalized Residual Risk: 1 in 6,300
Zellweger Syndrome Spectrum (PEX1-Related)	PEX1	AR	Reduced Risk	Personalized Residual Risk: 1 in 2,000
Zellweger Syndrome Spectrum (PEX2-Related)	PEX2	AR	Reduced Risk	Personalized Residual Risk: 1 in 77,000
Zellweger Syndrome Spectrum (PEX6-Related)	PEX6	AR	Reduced Risk	Personalized Residual Risk: 1 in 1,600

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 CCG Repeat Analysis (Analytical Detection Rate >99%)

PCR amplification using Asuragen, Inc. AmplideX<sup>®</sup> FMR1 PCR reagents followed by capillary electrophoresis for allele sizing was performed. Samples positive for FMR1 CCG 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 20 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.\*380T>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.\*380T>G is likely indicative of a silent (20) carrier. In individuals with two copies of *SMN1* with African American, Hispanic or Caucasian ancestry, the presence or absence of c.\*380T>G significantly increases or decreases, respectively, the likelihood of being a silent 20 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<sup>TM</sup>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<sup>®</sup> genotyping platform.

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

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

#### Next Generation Sequencing for SMN1

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

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

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

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

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

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

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

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

Long-range PCR was performed to generate locus-specific amplicons for *CYP21A2*, *HBA1* and *HBA2* and *GBA*. The PCR products were then prepared for short-read NGS sequencing and sequenced. Sequenced reads were mapped back to the original genomic locus and run through

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

### Residual Risk Calculations

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

### Personalized Residual Risk Calculations

Agilent SureSelect<sup>TM</sup>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<sup>th</sup> "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.

### Exceptions:

Gene	Transcript	Exceptions
ABC D1	NM_0000333	Exons 8 and 9
ADA	NM_000001	Exon 1

	0022.2	
ADA MTS 2	NM_01 4244.4	Exon 1
AGP S	NM_00 3659.3	chr2:178,257,512 - 178,257,649 (partial exon 1)
ALM S1	NM_01 5120.4	chr2:73,612,990 - 73,613,041 (partial exon 1)
CEP 290	NM_02 5114.3	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)
CFT R	NM_00 0492.3	Exon 10
COL 4A4	NM_00 0092.4	chr2:227,942,604 - 227,942,619 (partial exon 25)
CYP 11B2	NM_00 0498.3	Exons 3 - 7
DNA I2	NM_02 3036.4	chr17:72,308,136 - 72,308,147 (partial exon 12)
EVC	NM_15 3717.2	Exon 1
FH	NM_00 0143.3	Exon 1
GA MT	NM_00 0156.5	Exon 1
GLD C	NM_00 0170.2	Exon 1
GNP TAB	NM_02 4312.4	chr17:4,837,000 - 4,837,400 (partial exon 2)
GNP TG	NM_03 2520.4	Exon 1
HGS NAT	NM_15 2419.2	Exon 1
IDS	NM_00 0202.6	Exon 3
LIFR	NM_00 2310.5	Exon 19
NEB	NM_00 1271208.1	Exons 82 - 105
NPC 1	NM_00 0271.4	chr18:21,123,519 - 21,123,538 (partial exon 14)
PUS 1	NM_02 5215.5	chr12:132,414,446 - 132,414,532 (partial exon 2)
RPG RIP1 L	NM_01 5272.2	Exon 23

SGS H	NM_00199.3	chr17:78,194,022 - 78,194,072 (partial exon 1)
		Exons 3 and 4
		<p><b>SELECTED REFERENCES</b></p> <p><b>Carrier Screening</b> Grody W et al. ACMG position statement on prenatal/preconception expanded carrier screening. <i>Genet Med</i>. 2013 15:482-3.</p> <p><b>Fragile X syndrome:</b> 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. <i>J Mol Diag</i> 2010 12:589-600.</p> <p><b>Spinal Muscular Atrophy:</b> Luo M et al. An Ashkenazi Jewish SMN1 haplotype specific to duplication alleles improves pan-ethnic carrier screening for spinal muscular atrophy. <i>Genet Med</i>. 2014 16:149-56.</p> <p><b>Ashkenazi Jewish Disorders:</b> Scott SA et al. Experience with carrier screening and prenatal diagnosis for sixteen Ashkenazi Jewish Genetic Diseases. <i>Hum. Mutat.</i> 2010 31:1-11.</p> <p><b>Duchenne Muscular Dystrophy:</b> Flanigan KM et al. Mutational spectrum of DMD mutations in dystrophinopathy patients: application of modern diagnostic techniques to a large cohort. <i>Hum Mutat.</i> 2009 30:1657-66.</p> <p><b>Variant Classification:</b> 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. <i>Genet Med</i>. 2015 May;17(5):405-24</p> <p>Additional disease-specific references available upon request.</p>
SLC 6A8	NM_005629.3	