



Donor 5107

Genetic Testing Summary

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

Last Updated: 05/17/21

Donor Reported Ancestry: Dutch, 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 genotyping of 130 mutations- in the CFTR gene	1/476
Spinal Muscular Atrophy (SMA) carrier screening	Negative for deletions of exon 7 in the SMN1 gene	1/632
Standard testing attached- 22 diseases by genotyping	Negative for mutations tested	
Special Testing		
Limb-Girdle Muscular Dystrophy Type 2A	Negative by gene sequencing in the CAPN3 gene	1/720
Usher Syndrome Type 1B	Negative by gene sequencing in the MYO7A gene	1/780
Bloom Syndrome	Negative by gene sequencing in the BLM gene	1/7400

Galactosemia	Negative by gene sequencing in the GALT gene	1/1600
Familial Mediterranean Fever	Negative by gene sequencing in the MEFV gene	1/1200
Achromatopsia (CNGB3)	Negative by gene sequencing in the CNGB3 gene	1/8600
Multiple Sulfatase Deficiency (SUMF1)	Negative by gene sequencing in the SUMF1 gene	1/2500
Wilson Disease (ATP7B)	Negative by gene sequencing in the ATP7B gene	1/350

*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.

Ordering Practice:

Practice Code: 926
Fairfax Cryobank
3015 Williams Drive, #110, Fairfax, VA,
22031, US
Physician: Suzanne Seitz
Report Generated: 2015-09-08
Report Updated: 2015-09-09

5107 [REDACTED]

DOB: [REDACTED]
Gender: Male
Ethnicity: European
Procedure ID: 28909
Kit Barcode: [REDACTED]
Method: Genotyping
Specimen: Blood, #30577
Specimen Collection: 2015-08-28
Specimen Received: 2015-08-31
Specimen Analyzed: 2015-09-08

Partner Not Tested

SUMMARY OF RESULTS

NO MUTATIONS IDENTIFIED


5107 [REDACTED] was not identified to carry any of the mutations tested.

All mutations analyzed were not detected, reducing but not eliminating your chance to be a carrier for the associated genetic diseases. A list of all the diseases and mutations you were screened for is included later in this report. The test does not screen for every possible genetic disease.

For disease information, please visit www.recombine.com/diseases. To speak with a Genetic Counselor, call [855.OUR.GENES](tel:855.OUR.GENES).

♂ Male

Panel: Fairfax Cryobank Panel , Diseases Tested: 21, Mutations Tested: 382, Genes Tested: 22, Null Calls: 0

Assay performed by 
Reprogenetics

CLIA ID: 31D1054821

Lab Technician Bo Chu

Reviewed by Pere Colls, PhD, HCLD, Lab Director

Methods and Limitations

Genotyping: Genotyping is performed using the Illumina Infinium Custom HD Genotyping assay to identify mutations in >200 genes. The assay is not validated for homozygous mutations, and it is possible that individuals affected with disease may not be accurately genotyped.

Spinal Muscular Atrophy: Spinal Muscular Atrophy is tested for via an Identity-by-State shared haplotype comparison algorithm. Detection is limited to haplotypes within our library of known carriers of the most common mutation (deletion of Exon 7).

































Limitations: In some cases, genetic variations other than that which is being assayed may interfere with mutation detection, resulting in false-negative or false-positive results. Additional sources of error include, but are not limited to: sample contamination, sample mix-up, bone marrow transplantation, blood transfusions, and technical errors.

The test does not test for all forms of genetic disease, birth defects, and intellectual disability. All results should be interpreted in the context of family history; additional evaluation may be indicated based on a history of these conditions. Additional testing may be necessary to determine mutation phase in individuals identified to carry more than one mutation in the same gene. All mutations included within the genes assayed may not be detected, and additional testing may be appropriate for some individuals.

Diseases & Mutations Assayed

● High Impact ● Treatment Benefits ● X-Linked ● Moderate Impact

H	T	X	M	Disease	#	Mutations
●	●	●	●	Alpha Thalassemia	10	♂ Genotyping SEA deletion, 11.1kb deletion, c.207C>A (p.N69K), c.223G>C (p.D75G), c.2T>C (p.M1T), c.207C>G (p.N69K), c.340_351delCTCCCGCCGAG (p.L114_E117del), c.377T>C (p.L126P), c.427T>C (p.X143Qext32), c.*+94A>G
●	●	●	●	Beta Thalassemia	83	♂ Genotyping c.17_18delCT, c.20delA (p.E7Gfs), c.217insA (p.S73Kfs), c.223+702_444+342del620insAAGTAGA, c.230delC, c.25_26delAA, c.315+1G>A, c.315+2T>C, c.316-197C>T, c.316-146T>G, c.315+745C>G, c.316-1G>A, c.316-1G>C, c.316-2A>G, c.316-3C>A, c.316-3C>G, c.4delG (p.V2Cfs), c.51delC (p.K18Rfs), c.93-21G>A, c.92+1G>A, c.92+5G>A, c.92+5G>C, c.92+5G>T, c.92+6T>C, c.93-1G>A, c.93-1G>T, c.-50A>C, c.a-78g, c.a-79g, c.a-81g, c.A52T (p.K18X), c.c-137g, c.c-138t, c.c-151t, c.C118T (p.Q40X), c.G169C (p.G57R), c.G295A (p.V99M), c.G34A (p.V12I), c.G415C (p.A139P), c.G47A (p.W16X), c.G48A (p.W16X), c.t-80a, c.T2C (p.M1T), c.T75A (p.G25G), c.444+111A>G, c.g-29a, c.68_74delAAGTTGG, c.G92C (p.R31T), c.27_28insG, c.92+1G>T, c.92+1G>C, c.93-15T>G, c.93-1G>C, c.112delT, c.G113A (p.W38X), c.G114A (p.W38X), c.126delC, c.444+113A>G, c.250delG, c.225delC, c.383_385delAGG (p.Q128_A129delQAinsP), c.321_322insG (p.N109fs), c.316-1G>T, c.316-2A>C, c.316-106C>T, c.287_288insA (p.L97fs), c.271G>T (p.E91X), c.203_204delTG (p.V68Afs), c.154delC (p.P52fs), c.135delC (p.F46fs), c.92+2T>A, c.92+2T>C, c.90C>T (p.G30G), c.59A>G (p.N20S), c.46delT (p.W16Gfs), c.45_46insG (p.L16fs), c.36delT (p.T13fs), c.2T>G (p.M1R), c.1A>G (p.M1V), c.c-137t, c.c-136g, c.c-142t, c.c-140t
●	●	●	●	Bloom Syndrome	24	♂ Genotyping c.2207_2212delATCTGAinsTAGATTC (p.Y736Lfs), c.2407insT, c.557_559delCAA (p.S186X), c.1284G>A (p.W428X), c.1701G>A (p.W567X), c.1933C>T (p.Q645X), c.C2528T (p.T843I), c.C2695T (p.R899X), c.G3107T (p.C1036F), c.2923delC (p.Q975K), c.3558+1G>T, c.3875-2A>G, c.2074+2T>A, c.2343_2344dupGA (p.781EfsX), c.380delC (p.127Tfs), c.3564delC (p.1188Dfs), c.4008delG (p.1336Rfs), c.C947G (p.S316X), c.2193+1_2193+9del9, c.C1642T (p.Q548X), c.3143delA (p.1048NfsX), c.356_357delTA (p.Cys120Hisfs), c.4076+1delG, c.C3281A (p.S1094X)
●	●	●	●	Canavan Disease	8	♂ Genotyping c.433-2A>G, c.A854C (p.E285A), c.C693A (p.Y231X), c.C914A (p.A305E), c.A71G (p.E24G), c.C654A (p.C218X), c.T2C (p.M1T), c.G79A (p.G27R)

H	T	X	M	Disease	#	Mutations
				Cystic Fibrosis	130	<p>♂ Genotyping c.1029delC, 1153_1154insAT, c.1519_1521delATC (p.507delI), c.1521_1523delCTT (p.508delF), c.1545_1546delTA (p.Y515Xfs), c.1585-1G>A, c.164+12T>C, c.1680-886A>G, c.1680-1G>A, c.1766+1G>A, c.1766+1G>T, c.1766+5G>T, c.1818delB4, c.1911delG, c.1923delCTCAAACTinsA, c.1973delGAAATTCATCTinsAGAAA, c.2052delA (p.K684fs), c.2052insA (p.Q685fs), c.2051_2052delAAinsG (p.K684SfsX38), c.2174insA, c.261delTT, c.2657+5G>A, c.273+1G>A, c.273+3A>C, c.274-1G>A, c.2988+1G>A, c.3039delC, c.3140-26A>G, c.325delTATinsG, c.3527delC, c.3535delACCA, c.3691delT, c.3717+12191C>T, c.3744delA, c.3773_3774insT (p.L1258fs), c.442delA, c.489+1G>T, c.531delT, c.579+1G>T, c.579+5G>A (IVS4+5G>A), c.803delA (p.N268fs), c.805_806delAT (p.I269fs), c.933_935delCTT (p.311delF), c.A1645C (p.S549R), c.A2128T (p.K710X), c.C1000T (p.R334W), c.C1013T (p.T338I), c.C1364A (p.A455E), c.C1477T (p.Q493X), c.C1572A (p.C524X), c.C1654T (p.Q552X), c.C1657T (p.R553X), c.C1721A (p.P574H), c.C2125T (p.R709X), c.C223T (p.R75X), c.C2668T (p.Q890X), c.C3196T (p.R1066C), c.C3276G (p.Y1092X), c.C3472T (p.R1158X), c.C3484T (p.R1162X), c.C349T (p.R117C), c.C3587G (p.S1196X), c.C3712T (p.Q1238X), c.C3764A (p.S1255X), c.C3909G (p.N1303K), c.G1040A (p.R347H), c.G1040C (p.R347P), c.G1438T (p.G480C), c.G1624T (p.G542X), c.G1646A (p.S549N), c.G1646T (p.S549I), c.G1652A (p.G551D), c.G1675A (p.A559T), c.G1679C (p.R560T), c.G178T (p.E60X), c.G1865A (p.G622D), c.G254A (p.G85E), c.G271A (p.G91R), c.G274T (p.E92X), c.G3209A (p.R1070Q), c.G3266A (p.W1089X), c.G3454C (p.D1152H), c.G350A (p.R117H), c.G3611A (p.W1204X), c.G3752A (p.S1251N), c.G3846A (p.W1282X), c.G3848T (p.R1283M), c.G532A (p.G178R), c.G988T (p.G330X), c.T1090C (p.S364P), c.T3302A (p.M1101K), c.T617G (p.L206W), c.C14T (p.P5L), c.G19T (p.E7X), c.G171A (p.W57X), c.313delA (p.I105fs), c.G328C (p.D110H), c.580-1G>T, c.G1055A (p.R352Q), c.C1075A (p.Q359K), c.C1079A (p.T360K), c.T1647G (p.S549R), c.1976delA (p.N659fs), c.C2290T (p.R764X), c.2737_2738insG (p.Y913X), c.3067_3072delATAGTG (p.I1023_V1024delT), c.3536_3539delCCAA (p.T1179fs), c.3659delC (p.T1220fs), c.G3808A (p.D1270N), c.G4056C (p.Q1352H), c.C4364G (p.S1455X), c.C4003T (p.L1335F), c.G2538A (p.W846X), c.C200T (p.P67L), c.C4426T (p.Q1476X), c.1116+1G>A, c.1986_1989delAACT (p.T663R), c.2089_2090insA (p.R697Kfs), c.2215delG (p.V739Y), c.T263G (p.L196X), c.3022delG (p.V1008S), c.3908dupA (p.N1303Kfs), c.C658T (p.Q220X), c.C868T (p.Q290X), c.1526delG (p.G509fs), c.2908+1085-3367+260del7201, c.C11A (p.S4X), c.A3700G (p.I1234V), c.A416T (p.H139I), c.T366A (p.Y122X)</p>
				Familial Dysautonomia	4	<p>♂ Genotyping c.2204+6T>C, c.C2741T (p.P914L), c.G2087C (p.R696P), c.C2128T (p.Q710X)</p>
				Familial Hyperinsulinism: Type 1: ABCC8 Related	10	<p>♂ Genotyping c.3989-9G>A, c.4159_4161delTTC (p.1387delF), c.C4258T (p.R1420C), c.C4477T (p.R1493W), c.G2147T (p.G716V), c.G4055C (p.R1352P), c.T560A (p.V187D), c.4516G>A (p.E1506K), c.C2506T (p.Q836X), c.579+2T>A</p>
				Fanconi Anemia: Type C	8	<p>♂ Genotyping c.456+4A>T, c.67delG, c.C37T (p.Q13X), c.C553T (p.R185X), c.T1661C (p.L554P), c.C1642T (p.R548X), c.G66A (p.W22X), c.G65A (p.W22X)</p>
				Gaucher Disease	6	<p>♂ Genotyping c.84_85insG, c.A1226G (p.N409S), c.A1343T (p.D448V), c.C1504T (p.R502C), c.G1297T (p.V433L), c.G1604A (p.R535H)</p>
				Glycogen Storage Disease: Type IA	13	<p>♂ Genotyping c.376_377insTA, c.79delC, c.979_981delTTC (p.327delF), c.C1039T (p.Q347X), c.C247T (p.R83C), c.C724T (p.Q242X), c.G248A (p.R83H), c.G562C (p.G188R), c.G648T, c.G809T (p.G270V), c.A113T (p.D38V), c.975delG (p.L326fs), c.724delC</p>
				Joubert Syndrome	1	<p>♂ Genotyping c.G35T (p.R12L)</p>
				Maple Syrup Urine Disease: Type 1B	6	<p>♂ Genotyping c.G1114T (p.E372X), c.G548C (p.R183P), c.G832A (p.G278S), c.C970T (p.R324X), c.G487T (p.E163X), c.C853T (p.R285X)</p>

H	T	X	M	Disease	#	Mutations
●	●	○	○	Maple Syrup Urine Disease: Type 3	8	♂ Genotyping c.104_105insA, c.G685T (p.G229C), c.A214G (p.K72E), c.A1081G (p.M361V), c.G1123A (p.E375K), c.T1178C (p.I393T), c.C1463T (p.P488L), c.A1483G (p.R495G)
●	○	○	○	Mucopolidosis: Type IV	4	♂ Genotyping c.406-2A>G, c.G1084T (p.D362Y), c.C304T (p.R102X), c.244delC (p.L82fsX)
●	○	○	○	Nemaline Myopathy: NEB Related	1	♂ Genotyping c.7434_7536del2502bp
●	○	○	○	Niemann-Pick Disease: Type A	6	♂ Genotyping c.996delC, c.G1493T (p.R498L), c.T911C (p.L304P), c.C1267T (p.H423Y), c.G1734C (p.K578N), c.1493G>A (p.R498H)
●	○	○	○	Spinal Muscular Atrophy: SMN1 Linked	19	♂ Genotyping DEL EXON 7, c.22_23insA, c.43C>T (p.Q15X), c.91_92insT, c.305G>A (p.W102X), c.400G>A (p.E134K), c.439_443delGAAGT, c.558delA, c.585_586insT, c.683T>A (p.L228X), c.734C>T (p.P245L), c.768_778dupTGCTGATGCTT, c.815A>G (p.Y272C), c.821C>T (p.T274I), c.823G>A (p.G275S), c.834+2T>G, c.835-18_835-12delCCTTTAT, c.835G>T, c.836G>T
●	○	○	○	Tay-Sachs Disease	30	♂ Genotyping c.1073+1G>A, c.1277_1278insTATC, c.1421+1G>C, c.805+1G>A, c.C532T (p.R178C), c.G533A (p.R178H), c.G805A (p.G269S), c.C1510T (p.R504C), c.G1496A (p.R499H), c.G509A (p.R170Q), c.A1003T (p.I335F), c.910_912delTTC (p.305delF), c.G749A (p.G250D), c.T632C (p.F211S), c.C629T (p.S210F), c.613delC, c.A611G (p.H204R), c.G598A (p.V200M), c.A590C (p.K197T), c.571-1G>T, c.C540G (p.Y180X), c.T538C (p.Y180H), c.G533T (p.R178L), c.C508T (p.R170W), c.C409T (p.R137X), c.T380G (p.L127R), c.346+1G>C, c.T116G (p.L39R), c.G78A (p.W26X), c.A1G (p.M1V)
●	○	○	○	Usher Syndrome: Type 1F	6	♂ Genotyping c.C733T (p.R245X), c.2067C>A (p.Y684X), c.C7T (p.R3X), c.C1942T (p.R648X), c.2800C>T (p.R934X), c.4272delA (p.L1425fs)
●	○	○	○	Usher Syndrome: Type 3	4	♂ Genotyping c.T144G (p.N48K), c.T359A (p.M120K), c.300T>G (p.Y176X), c.C634T (p.Q212X)
●	○	○	○	Walker-Warburg Syndrome	1	♂ Genotyping c.1167insA (p.F390fs)

Patient	Sample	Referring Doctor
Patient Name: Donor 5107 Date of Birth: [REDACTED] Reference #: FFAXCB-S45107 Indication: Carrier Testing Test Type: Custom Carrier Screen (ECS)	Specimen Type: Purified DNA Lab #: [REDACTED] Date Collected: 4/11/2019 Date Received: 4/13/2019 Final Report: 4/26/2019	Fairfax Cryobank, Inc. [REDACTED] [REDACTED] [REDACTED] [REDACTED]

Results

Negative: No clinically significant variant(s) detected

Gene(s) analyzed: *MYO7A* and *CAPN3*

Recommendations:

Consideration of residual risk by ethnicity after a negative carrier screen is recommended, especially in the case of a positive family history for a specific disorder.

Interpretation:

Screening for the presence of pathogenic variants in the *MYO7A* and *CAPN3* genes which are associated with Usher syndrome, type 1B and limb-girdle muscular dystrophy, type 2A, respectively, was performed by next generation sequencing and possibly genotyping for select variants on DNA extracted from this patient's sample. No clinically significant variants were detected during this analysis.

Please note that negative results reduce but do not eliminate the possibility that this individual is a carrier for the disorder(s) tested. Please see table of residual risks 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.

Comments:

This carrier screening test masks likely benign variants and variants of uncertain significance (VUS) if there are any. Only known pathogenic variants or likely pathogenic variants which are expected to result in deleterious effects on protein function are reported. If reporting of likely benign variants and VUS is desired in this patient, please contact the laboratory (tel. 212-241-2537) to request an amended report.

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.

Patient: Donor 5107

DOB: [REDACTED]

Lab #: [REDACTED]

Table of Residual Risks by Ethnicity

Please note: This table displays residual risks after a negative result for each of the genes and corresponding disorders. **If a patient is reported to be a carrier of a disease, their residual risk is 1 and this table does not apply for that disease.**

Disease (Inheritance)	Gene	Ethnicity	Carrier Frequency	Detection Rate	Residual Risk	Analytical Detection Rate
Limb-Girdle Muscular Dystrophy, Type 2A (AR) NM_000070.2	CAPN3	African	1 in 111	64%	1 in 310	99%
		Ashkenazi Jewish	1 in 563	99%	1 in 56,200	
		East Asian	1 in 104	78%	1 in 470	
		Finnish	1 in 411	73%	1 in 1,600	
		Caucasian	1 in 103	86%	1 in 720	
		Latino	1 in 144	91%	1 in 1,700	
		South Asian	1 in 223	80%	1 in 1,100	
		Worldwide	1 in 127	84%	1 in 770	
		Amish	N/A	99%	N/A	
Usher Syndrome, Type IB (AR) NM_000260.3	MYO7A	African	1 in 174	79%	1 in 820	99%
		Ashkenazi Jewish	1 in 345	69%	1 in 1,100	
		East Asian	1 in 119	31%	1 in 170	
		Finnish	1 in 285	80%	1 in 1,400	
		Caucasian	1 in 129	84%	1 in 780	
		Latino	1 in 300	79%	1 in 1,400	
		South Asian	1 in 61	93%	1 in 810	
		Worldwide	1 in 119	82%	1 in 650	

AR: Autosomal Recessive

N/A: Not Available

This case has been reviewed and electronically signed by Anastasia Larmore, PhD, Assistant Director

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

Patient: Donor 5107

DOB: [REDACTED]

Lab #: [REDACTED]

Test Methods and Comments

Genomic DNA isolated from this patient was analyzed by one or more of the following methodologies, as applicable:

Fragile X CGG Repeat Analysis (Analytical Detection Rate >99%)

PCR amplification using Asuragen, Inc. AmplideX® *FMR1* PCR reagents followed by capillary electrophoresis for allele sizing was performed. Samples positive for *FMR1* CGG repeats in the premutation and full mutation size range were further analyzed by Southern blot analysis to assess the size and methylation status of the *FMR1* CGG repeat.

Genotyping (Analytical Detection Rate >99%)

Multiplex PCR amplification and allele specific primer extension analyses using the MassARRAY® System were used to identify variants that are complex in nature or are present in low copy repeats. Rare sequence variants may interfere with assay performance.

Multiplex Ligation-Dependent Probe Amplification (MLPA) (Analytical Detection Rate >99%)

MLPA® probe sets and reagents from MRC-Holland were used for copy number analysis of specific targets versus known control samples. False positive or negative results may occur due to rare sequence variants in target regions detected by MLPA probes. Analytical sensitivity and specificity of the MLPA method are both 99%.

For alpha thalassemia, the copy numbers of the *HBA1* and *HBA2* genes were analyzed. Alpha-globin gene deletions, triplications, and the Constant Spring (CS) mutation are assessed. This test is expected to detect approximately 90% of all alpha-thalassemia mutations, varying by ethnicity. Carriers of alpha-thalassemia with three or more *HBA* copies on one chromosome, and one or no copies on the other chromosome, may not be detected. With the exception of triplications, other benign alpha-globin gene polymorphisms will not be reported. Analyses of *HBA1* and *HBA2* are performed in association with long-range PCR of the coding regions followed by short-read sequencing.

For Duchenne muscular dystrophy, the copy numbers of all *DMD* exons were analyzed. Potentially pathogenic single exon deletions and duplications are confirmed by a second method. Analysis of *DMD* is performed in association with sequencing of the coding regions.

For congenital adrenal hyperplasia, the copy number of the *CYP21A2* gene was analyzed. This analysis can detect large deletions due to unequal meiotic crossing-over between *CYP21A2* and the pseudogene *CYP21A1P*. These 30-kb deletions make up approximately 20% of *CYP21A2* pathogenic alleles. This test may also identify certain point mutations in *CYP21A2* caused by gene conversion events between *CYP21A2* and *CYP21A1P*. Some carriers may not be identified by dosage sensitive methods as this testing cannot detect individuals with two copies (duplication) of the *CYP21A2* gene on one chromosome and loss of *CYP21A2* (deletion) on the other chromosome. Analysis of *CYP21A2* is performed in association with long-range PCR of the coding regions followed by short-read sequencing.

For spinal muscular atrophy (SMA), the copy numbers of the *SMN1* and *SMN2* genes were analyzed. The individual dosage of exons 7 and 8 as well as the combined dosage of exons 1, 4, 6 and 8 of *SMN1* and *SMN2* were assessed. Copy number gains and losses can be detected with this assay. Depending on ethnicity, 6 - 29 % of carriers will not be identified by dosage sensitive methods as this testing cannot detect individuals with two copies (duplication) of the *SMN1* gene on one chromosome and loss of *SMN1* (deletion) on the other chromosome (silent 2+0 carrier) or individuals that carry an intragenic mutation in *SMN1*. Please also note that 2% of individuals with SMA have an *SMN1* mutation that occurred *de novo*. Typically in these cases, only one parent is an SMA carrier.

The presence of the c.*3+80T>G (chr5:70,247,901T>G) variant allele in an individual with Ashkenazi Jewish or Asian ancestry is typically indicative of a duplication of *SMN1*. When present in an Ashkenazi Jewish or Asian individual with two copies of *SMN1*, c.*3+80T>G is likely indicative of a silent (2+0) carrier. In individuals with two copies of *SMN1* with African American, Hispanic or Caucasian ancestry, the presence or absence of c.*3+80T>G significantly increases or decreases, respectively, the likelihood of being a silent 2+0 silent carrier.

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

MLPA for Gaucher disease (*GBA*), cystic fibrosis (*CFTR*), and non-syndromic hearing loss (*GJB2/GJB6*) will only be performed if indicated for confirmation of detected CNVs. If *GBA* analysis was performed, the copy numbers of exons 1, 3, 4, and 6 - 10 of the *GBA* gene (of 11 exons total) were analyzed. If *CFTR* analysis was performed, the copy numbers of all 27 *CFTR* exons were analyzed. If *GJB2/GJB6* analysis was performed, the copy number of the two *GJB2* exons were analyzed, as well as the presence or absence of the two upstream deletions of the *GJB2* regulatory region, del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854).

Patient: Donor 5107

DOB: [REDACTED]

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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™QXT technology was used with a custom capture library to target the exonic regions and intron/exon splice junctions of the relevant genes, as well as a number of UTR, intronic or promoter regions that contain previously reported mutations. Samples were pooled and sequenced on the Illumina HiSeq 2500 platform in the Rapid Run mode or the Illumina NovaSeq platform in the Xp workflow, using 100 bp paired-end reads. The sequencing data was analyzed using a custom bioinformatics algorithm designed and validated in house.

The coding exons and splice junctions of the known protein-coding RefSeq genes were assessed for the average depth of coverage (minimum of 20X) and data quality threshold values. Most exons not meeting a minimum of >20X read depth across the exon are further analyzed by Sanger sequencing. Please note that several genomic regions present difficulties in mapping or obtaining read depth >20X. The exons contained within these regions are noted within Table 1 (as "Exceptions") and will not be reflexed to Sanger sequencing if the mapping quality or coverage is poor. Any variants identified during testing in these regions are confirmed by a second method and reported if determined to be pathogenic or likely pathogenic. However, as there is a possibility of false negative results within these regions, detection rates and residual risks for these genes have been calculated with the presumption that variants in these exons will not be detected, unless included in the MassARRAY® genotyping platform.

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

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

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

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

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

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

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

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

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DOB: [REDACTED]

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determine the phase (cis/trans configuration) of the *CYP21A2* alleles identified. Family studies may be required in certain scenarios where phasing is required to determine the carrier status.

Residual Risk Calculations

Carrier frequencies and detection rates for each ethnicity were calculated through the combination of internal curations of >28,000 variants and genomic frequency data from >138,000 individuals across seven ethnic groups in the gnomAD database. Additional variants in HGMD and novel deleterious variants were also incorporated into the calculation. Residual risk values are calculated using a Bayesian analysis combining the *a priori* risk of being a pathogenic mutation carrier (carrier frequency) and the detection rate. They are provided only as a guide for assessing approximate risk given a negative result, and values will vary based on the exact ethnic background of an individual. This report does not represent medical advice but should be interpreted by a genetic counselor, medical geneticist or physician skilled in genetic result interpretation and the relevant medical literature.

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

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

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

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

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

SELECTED REFERENCES

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Ashkenazi Jewish Disorders:

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

Duchenne Muscular Dystrophy:

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

Variant Classification:

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Additional disease-specific references available upon request.

Patient: Donor 5107

DOB: [REDACTED]

Lab #: [REDACTED]

Patient	Sample	Referring Doctor
Patient Name: Donor 5107 Date of Birth: [REDACTED] Reference #: FFAXCB-S45107 Indication: Carrier Testing Test Type: Unmask Additional Gene(s) V1E	Specimen Type: Blood Lab #: [REDACTED] Date Collected: 4/11/2019 Date Received: 5/31/2019 Final Report: 6/13/2019	Fairfax Cryobank, Inc. [REDACTED] [REDACTED] [REDACTED] [REDACTED]

RESULTS

Negative: No clinically significant variant(s) detected

Gene(s) analyzed: *BLM*, *GALT*, and *MEFV*

Recommendations:

Consideration of residual risk by ethnicity after a negative carrier screen is recommended, especially in the case of a positive family history for a specific disorder.

Interpretation:

Screening for the presence of pathogenic variants in the *BLM*, *GALT*, and *MEFV* genes which are associated with Bloom syndrome, galactosemia, and familial Mediterranean fever, respectively, was performed by next generation sequencing and possibly genotyping for select variants on DNA extracted from this patient's sample. No clinically significant variants were detected during this analysis.

Please note that negative results reduce but do not eliminate the possibility that this individual is a carrier for the disorder(s) tested. Please see table of residual risks 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.

Comments:

This carrier screening test masks likely benign variants and variants of uncertain significance (VUS) if there are any. Only known pathogenic variants or likely pathogenic variants which are expected to result in deleterious effects on protein function are reported. If reporting of likely benign variants and VUS is desired in this patient, please contact the laboratory (tel. 212-241-2537) to request an amended report.

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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Table of Residual Risks by Ethnicity

Please note: This table displays residual risks after a negative result for each of the genes and corresponding disorders. **If a patient is reported to be a carrier of a disease, their residual risk is 1 and this table does not apply for that disease.**

Disease (Inheritance)	Gene	Ethnicity	Carrier Frequency	Detection Rate	Residual Risk	Analytical Detection Rate
Bloom Syndrome (AR) NM_000057.2	<i>BLM</i>	African	1 in 532	99%	1 in 53,100	99%
		Ashkenazi Jewish	1 in 117	99%	1 in 11,700	
		East Asian	1 in 337	99%	1 in 33,600	
		Finnish	1 in 712	99%	1 in 71,100	
		Caucasian	1 in 358	95%	1 in 7,400	
		Latino	1 in 495	99%	1 in 49,400	
		South Asian	1 in 636	95%	1 in 12,500	
		Worldwide	1 in 357	97%	1 in 11,800	
Familial Mediterranean Fever (AR) NM_000243.2	<i>MEFV</i> †	African	1 in 230	74%	1 in 870	99%
		Ashkenazi Jewish	1 in 8	99%	1 in 720	
		East Asian	1 in 141	96%	1 in 3,400	
		Finnish	1 in 29	99%	1 in 2,800	
		Caucasian	1 in 40	97%	1 in 1,200	
		Latino	1 in 74	95%	1 in 1,500	
		South Asian	1 in 56	95%	1 in 1,000	
		Worldwide	1 in 40	97%	1 in 1,200	
		Sepharic Jewish	1 in 14	99%	1 in 1,300	
		Armenian	1 in 5	99%	1 in 400	
Galactosemia (AR) NM_000155.3	<i>GALT</i>	African	1 in 87	86%	1 in 610	99%
		Ashkenazi Jewish	1 in 181	96%	1 in 4,100	
		East Asian	1 in 208	40%	1 in 350	
		Finnish	1 in 4085	68%	1 in 12,600	
		Caucasian	1 in 123	92%	1 in 1,600	
		Latino	1 in 219	93%	1 in 3,000	
		South Asian	1 in 342	81%	1 in 1,800	
		Worldwide	1 in 156	85%	1 in 1,000	
		Irish Travellers	1 in 11	99%	1 in 1,000	

† Carrier frequencies include milder and reduced penetrance forms of the disease; therefore, carrier frequencies may appear higher than reported in the literature.

AR: Autosomal Recessive

This case has been reviewed and electronically signed by Anastasia Larmore, PhD, Assistant Director

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

Patient: Donor 5107

DOB: [REDACTED]

Lab #: [REDACTED]

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. AmpliX[®] *FMR1* PCR reagents followed by capillary electrophoresis for allele sizing was performed. Samples positive for *FMR1* CGG repeats in the premutation and full mutation size range were further analyzed by Southern blot analysis to assess the size and methylation status of the *FMR1* CGG repeat.

Genotyping (Analytical Detection Rate >99%)

Multiplex PCR amplification and allele specific primer extension analyses using the MassARRAY[®] System were used to identify variants that are complex in nature or are present in low copy repeats. Rare sequence variants may interfere with assay performance.

Multiplex Ligation-Dependent Probe Amplification (MLPA) (Analytical Detection Rate >99%)

MLPA[®] probe sets and reagents from MRC-Holland were used for copy number analysis of specific targets versus known control samples. False positive or negative results may occur due to rare sequence variants in target regions detected by MLPA probes. Analytical sensitivity and specificity of the MLPA method are both 99%.

For alpha thalassemia, the copy numbers of the *HBA1* and *HBA2* genes were analyzed. Alpha-globin gene deletions, triplications, and the Constant Spring (CS) mutation are assessed. This test is expected to detect approximately 90% of all alpha-thalassemia mutations, varying by ethnicity. Carriers of alpha-thalassemia with three or more *HBA* copies on one chromosome, and one or no copies on the other chromosome, may not be detected. With the exception of triplications, other benign alpha-globin gene polymorphisms will not be reported. Analyses of *HBA1* and *HBA2* are performed in association with long-range PCR of the coding regions followed by short-read sequencing.

For Duchenne muscular dystrophy, the copy numbers of all *DMD* exons were analyzed. Potentially pathogenic single exon deletions and duplications are confirmed by a second method. Analysis of *DMD* is performed in association with sequencing of the coding regions.

For congenital adrenal hyperplasia, the copy number of the *CYP21A2* gene was analyzed. This analysis can detect large deletions due to unequal meiotic crossing-over between *CYP21A2* and the pseudogene *CYP21A1P*. These 30-kb deletions make up approximately 20% of *CYP21A2* pathogenic alleles. This test may also identify certain point mutations in *CYP21A2* caused by gene conversion events between *CYP21A2* and *CYP21A1P*. Some carriers may not be identified by dosage sensitive methods as this testing cannot detect individuals with two copies (duplication) of the *CYP21A2* gene on one chromosome and loss of *CYP21A2* (deletion) on the other chromosome. Analysis of *CYP21A2* is performed in association with long-range PCR of the coding regions followed by short-read sequencing.

For spinal muscular atrophy (SMA), the copy numbers of the *SMN1* and *SMN2* genes were analyzed. The individual dosage of exons 7 and 8 as well as the combined dosage of exons 1, 4, 6 and 8 of *SMN1* and *SMN2* were assessed. Copy number gains and losses can be detected with this assay. Depending on ethnicity, 6 - 29 % of carriers will not be identified by dosage sensitive methods as this testing cannot detect individuals with two copies (duplication) of the *SMN1* gene on one chromosome and loss of *SMN1* (deletion) on the other chromosome (silent 2+0 carrier) or individuals that carry an intragenic mutation in *SMN1*. Please also note that 2% of individuals with SMA have an *SMN1* mutation that occurred *de novo*. Typically in these cases, only one parent is an SMA carrier.

The presence of the c.*3+80T>G (chr5:70,247,901T>G) variant allele in an individual with Ashkenazi Jewish or Asian ancestry is typically indicative of a duplication of *SMN1*. When present in an Ashkenazi Jewish or Asian individual with two copies of *SMN1*, c.*3+80T>G is likely indicative of a silent (2+0) carrier. In individuals with two copies of *SMN1* with African American, Hispanic or Caucasian ancestry, the presence or absence of c.*3+80T>G significantly increases or decreases, respectively, the likelihood of being a silent 2+0 silent carrier.

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

MLPA for Gaucher disease (*GBA*), cystic fibrosis (*CFTR*), and non-syndromic hearing loss (*GJB2/GJB6*) will only be performed if indicated for confirmation of detected CNVs. If *GBA* analysis was performed, the copy numbers of exons 1, 3, 4, and 6 - 10 of the *GBA* gene (of 11 exons total) were analyzed. If *CFTR* analysis was performed, the copy numbers of all 27 *CFTR* exons were analyzed. If *GJB2/GJB6* analysis was performed, the copy number of the two *GJB2* exons were analyzed, as well as the presence or absence of the two upstream deletions of the *GJB2* regulatory region, del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854).

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NGS was performed on a panel of genes for the purpose of identifying pathogenic or likely pathogenic variants.

Agilent SureSelect™QXT technology was used with a custom capture library to target the exonic regions and intron/exon splice junctions of the relevant genes, as well as a number of UTR, intronic or promoter regions that contain previously reported mutations. Samples were pooled and sequenced on the Illumina HiSeq 2500 platform in the Rapid Run mode or the Illumina NovaSeq platform in the Xp workflow, using 100 bp paired-end reads. The sequencing data was analyzed using a custom bioinformatics algorithm designed and validated in house.

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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.

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Patient: Donor 5107	DOB: [REDACTED]	Lab #: [REDACTED]
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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.

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

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Additional disease-specific references available upon request.

Patient: Donor 5107

DOB: [REDACTED]

Lab #: [REDACTED]

Patient Information

Name: Donor 5107

Date of Birth: [REDACTED]

Sema4 ID: [REDACTED]

Client ID [REDACTED]

Indication: Carrier Testing

Specimen Information

Specimen Type: Purified DNA

Date Collected: [REDACTED]

Date Received: [REDACTED]

Final Report: 04/05/2021

Referring Provider

Fairfax Cryobank, Inc.

Unmask Additional Gene(s) V1E

Number of genes tested: 3

SUMMARY OF RESULTS AND RECOMMENDATIONS

 **Negative****Negative for all genes tested: *ATP7B*, *CNGB3*, and *SUMF1***To view a full list of genes and diseases tested
please see Table 1 in this report*AR=Autosomal recessive; XL=X-linked***Recommendations**

- 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.

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](https://www.sema4.com/residualrisk) for specific detection rates and residual risk by ethnicity. With individuals of mixed ethnicity, it is recommended to use the highest residual risk estimate. Only variants determined to be pathogenic or likely pathogenic are reported in this carrier screening test.

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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
⊖ Negative				
Achromatopsia (CNGB3-related)	CNGB3	AR	Reduced Risk (see table below)	
Multiple Sulfatase Deficiency	SUMF1	AR	Reduced Risk (see table below)	
Wilson Disease	ATP7B	AR	Reduced Risk (see table below)	

AR=Autosomal recessive; XL=X-linked

Table 2: Residual Risk by ethnicity for negative results

Disease (Inheritance)	Gene	Ethnicity	Carrier Frequency	Detection Rate	Residual Risk	Analytical Detection Rate
Achromatopsia (CNGB3-related) (AR) NM_000253.3	CNGB3	African	1 in 50	98%	1 in 2,300	99%
		Ashkenazi Jewish	1 in 97	99%	1 in 9,600	
		East Asian	1 in 208	99%	1 in 20,700	
		Finnish	1 in 163	99%	1 in 16,200	
		European (Non-Finnish)	1 in 87	99%	1 in 8,600	
		Native American	1 in 90	99%	1 in 8,900	
		South Asian	1 in 18	99%	1 in 1,700	
		Worldwide	1 in 61	99%	1 in 4,300	
Multiple Sulfatase Deficiency (AR) NM_182760.3	SUMF1	African	1 in 406	99%	1 in 40,500	99%
		Ashkenazi Jewish	1 in 298	99%	1 in 29,700	
		East Asian	1 in 1437	33%	1 in 2,200	
		European (Non-Finnish)	1 in 696	73%	1 in 2,500	
		Native American	1 in 1525	99%	1 in 152,000	
		South Asian	1 in 834	94%	1 in 13,100	
Wilson Disease (AR) NM_000053.3	ATP7B	Worldwide	1 in 588	65%	1 in 1,700	99%
		African	1 in 146	73%	1 in 540	
		Ashkenazi Jewish	1 in 39	97%	1 in 1,500	
		East Asian	1 in 32	78%	1 in 150	
		Finnish	1 in 114	90%	1 in 1,100	
		European (Non-Finnish)	1 in 63	82%	1 in 350	
		Native American	1 in 63	74%	1 in 240	
		South Asian	1 in 78	60%	1 in 200	
		Worldwide	1 in 65	81%	1 in 330	
		Canary Islands	1 in 25	88%	1 in 200	
		Sardinian	1 in 42	99%	1 in 4,100	
		Sephardic Jewish - North African, Iraqi, Yemenite, Iranian and Bukharian	1 in 65	99%	1 in 6,100	

* Carrier detection by HEXA enzyme analysis has a detection rate of approximately 98% (Applies to *HEXA* gene testing only).

† Carrier frequencies include milder and reduced penetrance forms of the disease. Therefore, carrier frequencies may appear higher than reported in the literature (Applies to *BTD*, *Fg*, *GJB2*, *GJB1*, *GLA*, and *MEFV* gene testing only).

‡ Please note that *GJB2* testing includes testing for the two upstream deletions, del(GJB6-D13S1830) and del(GJB6-D13S1854) (PMID:11807148 and 15994881) (Applies to *GJB2* gene testing only).

AR: Autosomal recessive; N/A: Not available; XL: X-linked

Test methods and comments

Genomic DNA isolated from this patient was analyzed by one or more of the following methodologies, as applicable:

Fragile X CGG Repeat Analysis (Analytical Detection Rate >99%)

PCR amplification using Asuragen,

Inc. AmplideX® *FMR1* PCR reagents followed by capillary electrophoresis for allele sizing was performed. Samples positive for *FMR1* CGG repeats in the premutation and full mutation size range were further analyzed by Southern blot analysis to assess the size and methylation status of the *FMR1* CGG repeat.

Genotyping (Analytical Detection Rate >99%)

Multiplex PCR amplification and allele specific primer extension analyses using the MassARRAY® System were used to identify variants that are complex in nature or are present in low copy repeats. Rare sequence variants may interfere with assay performance.

Multiplex Ligation-Dependent Probe Amplification (MLPA) (Analytical Detection Rate >99%)

MLPA® probe sets and reagents from MRC-Holland were used for copy number analysis of specific targets versus known control samples. False positive or negative results may occur due to rare sequence variants in target regions detected by MLPA probes. Analytical sensitivity and specificity of the MLPA method are both 99%.

For alpha thalassemia, the copy numbers of the *HBA1* and *HBA2* genes were analyzed. Alpha-globin gene deletions, triplications, and the Constant Spring (CS) mutation are assessed. This test is expected to detect approximately 90% of all alpha-thalassemia mutations, varying by ethnicity. Carriers of alpha-thalassemia with three or more *HBA* copies on one chromosome, and one or no copies on the other chromosome, may not be detected. With the exception of triplications, other benign alpha-globin gene polymorphisms will not be reported. Analyses of *HBA1* and *HBA2* are performed in association with long-range PCR of the coding regions followed by short-read sequencing.

For Duchenne muscular dystrophy, the copy numbers of all *DMD* exons were analyzed. Potentially pathogenic single exon deletions and duplications are confirmed by a second method. Analysis of *DMD* is performed in association with sequencing of the coding regions.

For congenital adrenal hyperplasia, the copy number of the *CYP21A2* gene was analyzed. This analysis can detect large deletions due to unequal meiotic crossing-over between *CYP21A2* and the pseudogene *CYP21A1P*. These 30-kb deletions make up approximately 20% of *CYP21A2* pathogenic alleles. This test may also identify certain point mutations in *CYP21A2* caused by gene conversion events between *CYP21A2* and *CYP21A1P*. Some carriers may not be identified by dosage sensitive methods as this testing cannot detect individuals with two copies (duplication) of the *CYP21A2* gene on one chromosome and loss of *CYP21A2* (deletion) on the other chromosome. Analysis of *CYP21A2* is performed in association with long-range PCR of the coding regions followed by short-read sequencing.

For spinal muscular atrophy (SMA), the copy numbers of the *SMN1* and *SMN2* genes were analyzed. The individual dosage of exons 7 and 8 as well as the combined dosage of exons 1, 4, 6 and 8 of *SMN1* and *SMN2* were assessed. Copy number gains and losses can be detected with this assay. Depending on ethnicity, 6 - 29 % of carriers will not be identified by dosage sensitive methods as this testing cannot detect individuals with two copies (duplication) of the *SMN1* gene on one chromosome and loss of *SMN1* (deletion) on the other chromosome (silent 20 carrier) or individuals that carry an intragenic mutation in *SMN1*. Please also note that 2% of individuals with SMA have an *SMN1* mutation that occurred *de novo*. Typically in these cases, only one parent is an SMA carrier.

The presence of the c.*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 carrier.

Pathogenic or likely pathogenic sequence variants in exon 7 may be detected during testing for the c.*380T>G variant allele; these will be reported if confirmed to be located in *SMN1* using locus-specific Sanger primers.

MLPA for Gaucher disease (*GBA*), cystic fibrosis (*CFTR*), and non-syndromic hearing loss (*GJB2/GJB6*) will only be performed if indicated for confirmation of detected CNVs. If *GBA* analysis was performed, the copy numbers of exons 1, 3, 4, and 6 - 10 of the *GBA* gene (of 11 exons total) were analyzed. If *CFTR* analysis was performed, the copy numbers of all 27 *CFTR* exons were analyzed. If *GJB2/GJB6* analysis was performed, the copy number of the two *GJB2* exons were analyzed, as well as the presence or absence of the two upstream deletions of the *GJB2* regulatory region, del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854).

Next Generation Sequencing (NGS) (Analytical Detection Rate >95%)

NGS was performed on a panel of genes for the purpose of identifying pathogenic or likely pathogenic variants.

Agilent SureSelect™QXT technology was used with a custom capture library to target the exonic regions and intron/exon splice junctions of the relevant genes, as well as a number of UTR, intronic or promoter regions that contain previously reported mutations. Samples were pooled and sequenced on the Illumina HiSeq 2500 platform in the Rapid Run mode or the Illumina NovaSeq platform in the Xp workflow, using 100 bp paired-end reads. The sequencing data was analyzed using a custom bioinformatics algorithm designed and validated in house.

The coding exons and splice junctions of the known protein-coding RefSeq genes were assessed for the average depth of coverage (minimum of 20X) and data quality threshold values. Most exons not meeting a minimum of >20X read depth across the exon are further analyzed by Sanger sequencing. Please note that several genomic regions present difficulties in mapping or obtaining read depth >20X. The exons contained within these regions are noted within Table 1 (as "Exceptions") and will not be reflexed to Sanger sequencing if the mapping quality or coverage is poor. Any variants identified during testing in these regions are confirmed by a second method and reported if determined to be pathogenic or likely pathogenic. However, as there is a possibility of false negative results within these regions, detection rates and residual risks for these genes have been calculated with the presumption that variants in these exons will not be detected, unless included in the MassARRAY® genotyping platform.

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

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

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

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

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

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

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

The relative quantification PCR is utilized on a Roche Universal Library Probe (UPL) system, which relates the PCR signal of the target region in one group to another. To test for genomic imbalances, both sample DNA and reference DNA is amplified with primer/probe sets that specific to the target region and a control region with known genomic copy number. Relative genomic copy numbers are calculated based on the standard $\Delta\Delta C_t$ 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 >28,000 variants and genomic frequency data from >138,000 individuals across seven ethnic groups in the gnomAD database. Additional variants in HGMD and novel deleterious variants were also incorporated into the calculation. Residual risk values are calculated using a Bayesian analysis combining the *a priori* risk of being a pathogenic mutation carrier (carrier frequency) and the detection rate. They are provided only as a guide for assessing approximate risk given a negative result, and values will vary based on the exact ethnic background of an individual. This report does not represent medical advice but should be interpreted by a genetic counselor, medical geneticist or physician skilled in genetic result interpretation and the relevant medical literature.

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

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

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

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

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

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Additional disease-specific references available upon request.