Fairfax Cryobank
The Trusted Choice for Donor Sperm

Donor 4461

Genetic Testing Summary

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

Last Updated: 08/03/2020

Donor Reported Ancestry: Polish, Lithuanian, Puerto Rican Jewish Ancestry: Yes

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 for 99 mutations in the CFTR gene	1/300
Spinal Muscular Atrophy (SMA) carrier screening	Negative for deletions of exon 7 in the SMN1 gene	1/370
Panel of 17 genes by genotyping-attached	Negative for mutations tested	
Special Testing		
21-Hydroxylase Deficient Classical Congenital Adrenal Hypoplasia	Negative for 1 mutation in the CYP21A2	1/86
Retinitis Pigmentosa 59 (DHDDS)	Negative by gene sequencing in the DHDDS gene	1/9900
Non-Syndromic Hearing Loss (GJB2)	Negative by gene sequencing in the GJB2 gene	1/210
Gaucher Disease (GBA)	Negative by gene sequencing in the GBA gene	1/280

*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.

Counsyl

Results Recipient	Male	Female
Fairfax Cryobank [redacted]	Name: 4461 4461 DOB: [redacted] Ethnicity: Ashkenazi Jewish Sample Type: EDTA Blood Date of Collection: 06/03/2013 Date Received: 06/05/2013 Barcode: [redacted] Indication: Egg or Sperm Donor	Not tested

Report Date: 06/07/2013

Counsyl Test Results Summary (Egg or Sperm Donor)

The Counsyl test (**Fairfax Cryobank Jewish Panel**) uses copy number analysis and targeted genotyping as described in the methods section on page 2 to determine carrier status associated with **17 diseases**. Please refer to page 3 for a complete list of diseases and genes included in this panel.

4461 4461

4461 4461's DNA test shows that he is not a carrier of any disease-causing mutation tested.

Partner

The reproductive risk presented is based on a hypothetical pairing with a partner of the same ethnic group.

Reproductive Risk Summary

No increased reproductive risks to highlight. Please refer to the following pages for detailed information about the results.

Clinical Notes

- If necessary, patients can discuss residual risks with their physician or a genetic counselor. To schedule a complimentary appointment to speak with a genetic counselor about these results, please visit counsyl.com/counseling/.

ENTERED NP 06.11.13

Counsyl

Male

Name: 4461 4461
DOB: [redacted]

Female

Not tested

Methods and Limitations

4461 4461: targeted genotyping and copy number analysis.

Targeted genotyping: Targeted DNA mutation analysis is used to simultaneously determine the genotype of 179 variants associated with 16 diseases. The test is not validated for detection of homozygous mutations, and although rare, asymptomatic individuals affected by the disease may not be genotyped accurately.

Copy number analysis: Targeted copy number analysis is used to determine the copy number of exon 7 of the SMN1 gene relative to other genes. Other mutations may interfere with this analysis. Some individuals with two copies of SMN1 are carriers with two SMN1 genes on one chromosome and a SMN1 deletion on the other chromosome. In addition, a small percentage of SMA cases are caused by nondeletion mutations in the SMN1 gene. Thus, a test result of two SMN1 copies significantly reduces the risk of being a carrier; however, there is still a residual risk of being a carrier and subsequently a small risk of future affected offspring for individuals with two or more SMN1 gene copies. Some SMA cases arise as the result of de novo mutation events which will not be detected by carrier testing.

Limitations: In an unknown number of cases, nearby genetic variants may interfere with mutation detection. Other possible sources of diagnostic error include sample mix-up, trace contamination, bone marrow transplantation, blood transfusions and technical errors. The Counsyl test does not fully address all inherited forms of intellectual disability, birth defects and genetic disease. A family history of any of these conditions may warrant additional evaluation. Furthermore, not all mutations will be identified in the genes analyzed and additional testing may be beneficial for some patients. For example, individuals of African, Southeast Asian, and Mediterranean ancestry are at increased risk for being carriers for hemoglobinopathies, which can be identified by CBC and hemoglobin electrophoresis or HPLC (*ACOG Practice Bulletin No. 78. Obstet Gynecol 2007;109:229-37*) and additional Tay-Sachs disease testing can be performed using a biochemical assay (*Gross et al. Genet Med 2008:10(1):54-56*).

This test was developed and its performance characteristics determined by Counsyl, Inc. It has not been cleared or approved by the US Food and Drug Administration (FDA). The FDA does not require this test to go through premarket review. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical testing. These results are adjunctive to the ordering physician's workup. Literature citations validating reported variants are available upon request. CLIA Number: **#05D1102604**.

Lab Director:

Hyunseok Kang

H. Peter Kang, MD

Counsyl

Male

Name: 4461 4461
DOB: [redacted]

Female

Not tested

Diseases Tested

ABCC8-Related Hyperinsulinism - **Gene:** ABCC8. **Variants (3):** F1388del, V187D, 3992-9G>A. **Detection rate**: Ashkenazi Jewish 90%.

Bloom Syndrome - **Gene:** BLM. **Variant (1):** 2281del6ins7. **Detection rate**: Ashkenazi Jewish 99%.

Canavan Disease - **Gene:** ASPA. **Variants (4):** E285A, Y231X, A305E, IVS2-2A>G. **Detection rate**: Ashkenazi Jewish 98%.

Cystic Fibrosis - **Gene:** CFTR. **Variants (99):** G85E, R117H, R334W, R347P, A455E, G542X, G551D, R553X, R560T, R1162X, W1282X, N1303K, F508del, I507del, 2184delA, 3659delC, 621+1G>T, 711+1G>T, 1717-1G>A, 1898+1G>A, 2789+5G>A, 3120+1G>A, 3849+10kbC>T, E60X, R75X, E92X, Y122X, G178R, R347H, Q493X, V520F, S549N, P574H, M1101K, D1152H, 2143delT, 394delTT, 444delA, 1078delT, 3876delA, 3905insT, 1812-1G>A, 3272-26A>G, 2183AA>G, S549R(A>C), R117C, L206W, G330X, T338I, R352Q, S364P, G480C, C524X, S549R(T>G), Q552X, A559T, G622D, R709X, K710X, R764X, Q890X, R1066C, W1089X, Y1092X, R1158X, S1196X, W1204X(c.3611G>A), Q1238X, S1251N, S1255X, 3199del6, 574delA, 663delT, 935delA, 936delTA, 1677delTA, 1949del84, 2043delG, 2055del9>A, 2108delA, 3171delC, 3667del4, 3791delC, 1288insTA, 2184insA, 2307insA, 2869insG, 296+12T>C, 405+1G>A, 405+3A>C, 406-1G>A, 711+5G>A, 712-1G>T, 1898+1G>T, 1898+5G>T, 3120G>A, 457TAT>G, 3849+4A>G, Q359K/T360K. **Detection rate**: Ashkenazi Jewish 97%.

Familial Dysautonomia - **Gene:** IKBKAP. **Variants (2):** IVS20+6T>C, R696P. **Detection rate**: Ashkenazi Jewish >99%.

Fanconi Anemia Type C - **Gene:** FANCC. **Variants (3):** IVS4+4A>T, 322delG, R548X. **Detection rate**: Ashkenazi Jewish 99%.

Gaucher Disease - **Gene:** GBA. **Variants (10):** N370S, L444P, 84GG, IVS2+1G>A, V394L, R496H, D409H, D409V, R463C, R463H. **Detection rate**: Ashkenazi Jewish 95%.

Glycogen Storage Disease Type Ia - **Gene:** G6PC. **Variants (7):** R83C, Q347X, Q27fsdelC, 459insTA, R83H, G188R, Q242X. **Detection rate**: Ashkenazi Jewish 99%.

Hb Beta Chain-Related Hemoglobinopathy (Including Beta Thalassemia and Sickle Cell Disease) - **Gene:** HBB. **Variants (28):** Hb S, K17X, Q39X, Phe41fs, Ser9fs, IVS-II-654, IVS-II-745, IVS-II-850, IVS-I-6, IVS-I-110, IVS-I-5, IVS-I-1(G>A), -88C>T, -28A>G, -29A>G, Lys8fs, Phe71fs, IVS-II-849(A>C), IVS-II-849(A>G), Gly24 T>A, -87C>G, Hb C, W15X, Gly16fs, Glu6fs, Hb E, Hb D-Punjab, Hb O-Arab. **Detection rate**: Ashkenazi Jewish 83%.

Hexosaminidase A Deficiency (Including Tay-Sachs Disease) - **Gene:** HEXA. **Variants (9):** 1278insTATC, IVS12+1G>C, G269S, IVS9+1G>A, R178H, IVS7+1G>A, 7.6kb del, G250D, R170W. **Detection rate**: Ashkenazi Jewish 92%.

Lipoamide Dehydrogenase Deficiency - **Gene:** DLD. **Variants (2):** 105insA, G229C. **Detection rate**: Ashkenazi Jewish >99%.

Maple Syrup Urine Disease Type 1B - **Gene:** BCKDHB. **Variants (3):** R183P, G278S, E372X. **Detection rate**: Ashkenazi Jewish 99%.

Mucolipidosis IV - **Gene:** MCOLN1. **Variants (2):** 511_6944del, IVS3-2A>G. **Detection rate**: Ashkenazi Jewish 96%.

Niemann-Pick Disease, SMPD1-Associated - **Gene:** SMPD1. **Variants (4):** fsP330, L302P, R496L, p.R608del. **Detection rate**: Ashkenazi Jewish 97%.

Spinal Muscular Atrophy (copy number analysis only) - **Gene:** SMN1. **Variant (1):** SMN1 copy number. **Detection rate**: Ashkenazi Jewish 91%.

Usher Syndrome Type 1F - **Gene:** PCDH15. **Variant (1):** R245X. **Detection rate**: Ashkenazi Jewish 75%.

Usher Syndrome Type 3 - **Gene:** CLRN1. **Variant (1):** N48K. **Detection rate**: Ashkenazi Jewish 98%.

Counsyl

Male

Name: 4461 4461
DOB: [redacted]

Female

Not tested

Risk Calculations

Below are the risk calculations for all diseases tested. Since negative results do not completely rule out the possibility of being a carrier, the **residual risk** represents the patient's post-test likelihood of being a carrier and the **reproductive risk** represents the likelihood the patient's future children could inherit each disease. These risks are inherent to all carrier screening tests, may vary by ethnicity, are predicated on a negative family history and are present even after a negative test result. Inaccurate reporting of ethnicity may cause errors in risk calculation.

Disease	4461 4461 Residual Risk	Reproductive Risk
ABCC8-Related Hyperinsulinism	1 in 670	1 in 180,000
Bloom Syndrome	1 in 11,000	< 1 in 1,000,000
Canavan Disease	1 in 2,700	1 in 590,000
Cystic Fibrosis	1 in 900	1 in 98,000
Familial Dysautonomia	1 in 6,100	1 in 750,000
Fanconi Anemia Type C	1 in 8,900	< 1 in 1,000,000
Gaucher Disease	1 in 310	1 in 19,000
Glycogen Storage Disease Type Ia	1 in 7,000	< 1 in 1,000,000
Hb Beta Chain-Related Hemoglobinopathy (Including Beta Thalassemia and Sickle Cell Disease)	1 in 930	1 in 590,000
Hexosaminidase A Deficiency (Including Tay-Sachs Disease)	1 in 370	1 in 44,000
Lipoamide Dehydrogenase Deficiency	1 in 93,000	< 1 in 1,000,000
Maple Syrup Urine Disease Type 1B	1 in 9,600	< 1 in 1,000,000
Mucolipidosis IV	1 in 2,700	< 1 in 1,000,000
Niemann-Pick Disease, SMPD1-Associated	1 in 3,300	< 1 in 1,000,000
Spinal Muscular Atrophy	SMN1: 2 copies 1 in 370	1 in 60,000
Usher Syndrome Type 1F	1 in 400	1 in 160,000
Usher Syndrome Type 3	1 in 6,000	< 1 in 1,000,000

Recombine

CarrierMap™

Ordering Practice:

Practice Code: [redacted]
Fairfax Cryobank
3015 Williams Drive, #110, Fairfax, VA, 22031, US
[redacted]
Report Generated: 2017-12-20
Report Updated: 2017-12-22

Donor 4461

DOB [redacted]
Gender: Male
Ethnicity: Other and Latin American
Procedure ID: 109489
Kit Barcode: [redacted]
Specimen: Sperm, #111399
Specimen Collection: 2017-07-24
Specimen Received: 2017-12-11
Specimen Analyzed: 2017-12-18

Partner Not Tested

TEST INFORMATION

Test: CarrierMap^{GEN} (Genotyping)
Panel: Custom Panel
Diseases Tested: 1
Genes Tested: 1
Mutations Tested: 1

***Amended Report:**

Please note the sample collection date has been corrected.

SUMMARY OF RESULTS: NO MUTATIONS IDENTIFIED

Donor 4461 was not identified to carry any of the mutation(s) tested.

No pathogenic mutations were identified in the genes tested, reducing but not eliminating the chance to be a carrier for the associated genetic diseases. CarrierMap assesses carrier status for genetic disease via molecular methods including targeted mutation analysis and/ or next-generation sequencing; other methodologies such as CBC and hemoglobin electrophoresis for hemoglobinopathies and enzyme analysis for Tay-Sachs disease may further refine risks for these conditions. Results should be interpreted in the context of clinical findings, family history, and/or other testing. A list of all the diseases and mutations screened for is included at the end of the report. This test does not screen for every possible genetic disease.

For additional disease information, please visit recombine.com/diseases. To speak with a Genetic Counselor, call 855.OUR.GENES.

Assay performed by Reprogenetics
CLIA ID: 31D1054821
3 Regent Street, Livingston, NJ 07039
Lab Technician: Bo Chu

Recombine CLIA # 31D2100763
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 the genes tested. The assay is not validated for homozygous mutations, and it is possible that individuals affected with disease may not be accurately genotyped.

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.

This test was developed and its performance determined by Recombine, Inc., and it has not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary.

Diseases & Mutations Assayed

21-Hydroxylase-Deficient Classical Congenital Adrenal Hyperplasia (CYP21A2):
Mutations (1): ♂ Genotyping | c.293-13C>G

Residual Risk Information

Detection rates are calculated from the primary literature and may not be available for all ethnic populations. The values listed below are for genotyping. Sequencing provides higher detection rates and lower residual risks for each disease. More precise values for sequencing may become available in the future.

Disease	Carrier Rate	Detection Rate	Residual Risk
21-Hydroxylase-Deficient Classical Congenital Adrenal Hyperplasia	♂ European: 1/62	27.65%	1/86
	♂ General: 1/62	29.34%	1/88

Patient Information
Name: Donor 4461
Date of Birth: [redacted]
Sema4 ID: [redacted]
Client ID: [redacted]
Indication: Carrier Testing

Specimen Information
Specimen Type: Purified DNA
Date Collected: 06/16/2020
Date Received: 06/19/2020
Final Report: 07/02/2020

Referring Provider
[redacted]

Custom Carrier Screen (ECS)

Number of genes tested: 3

SUMMARY OF RESULTS AND RECOMMENDATIONS

⊖ Negative

Negative for all genes tested: *DHDDS, GBA, and GJB2*
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 the genes listed above using one or more of the following methodologies: target capture and short-read sequencing, long-range PCR followed by short-read sequencing, targeted genotyping, and/or 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 view the Table of Residual Risks Based on Ethnicity at the end of this report or at **go.sema4.com/residualrisk** for gene transcripts, sequencing exceptions, specific detection rates, and residual risk estimates after a negative screening result. With individuals of mixed ethnicity, it is recommended to use the highest residual risk estimate. Only known pathogenic or likely pathogenic variants are reported. This carrier screening test does not report likely benign variants and variants of uncertain significance (VUS). If reporting of likely benign variants and VUS are desired in this patient, please contact the laboratory at 800-298-6470, option 2 to request an amended report.

Anastasia Larmore, Ph.D., Assistant Laboratory Director
Laboratory Medical Consultant: George A. Diaz, M.D., Ph.D.

Genes and diseases tested

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

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

Disease	Gene	Inheritance Pattern	Status	Detailed Summary
⊖ **Negative**				
Gaucher Disease	*GBA*	AR	Reduced Risk (see table below)	
Non-Syndromic Hearing Loss (*GJB2*-Related)	*GJB2*	AR	Reduced Risk (see table below)	
Retinitis Pigmentosa 59	*DHDDS*	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
Gaucher Disease (AR) NM_001005741.2	*GBA*	European (Non-Finnish)	1 in 164	87%	1 in 1,300	95%
		Ashkenazi Jewish	1 in 15	95%	1 in 280	
		Worldwide	1 in 158	86%	1 in 1,100	
Non-Syndromic Hearing Loss (GJB2 -Related) (AR) NM_004004.5	*GJB2*†‡	African	1 in 56	85%	1 in 360	99%
		Ashkenazi Jewish	1 in 13	94%	1 in 210	
		East Asian	1 in 5	98%	1 in 280	
		Finnish	1 in 16	99%	1 in 1,400	
		European (Non-Finnish)	1 in 18	97%	1 in 600	
		Native American	1 in 28	96%	1 in 610	
		South Asian	1 in 55	94%	1 in 970	
		Worldwide	1 in 18	97%	1 in 530	
Retinitis Pigmentosa 59 (AR) NM_024887.3	*DHDDS*	Ashkenazi Jewish	1 in 100	99%	1 in 9,900	99%
		European (Non-Finnish)	1 in 6008	99%	1 in 601,000	
		Native American	1 in 4223	99%	1 in 422,000	
		Worldwide	1 in 2009	99%	1 in 201,000	

* 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, F9, 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 followingmethodologies, as applicable:

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

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

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

The coding exons and splice junctions of the known protein-coding RefSeq genes wereassessed for the average depth of coverage (minimum of 20X) and data quality thresholdvalues. Most exons not meeting a minimum of >20X read depth across the exon are furtheranalyzed by Sanger sequencing. Please note that several genomic regions presentdifficulties in mapping or obtaining read depth >20X. The exons contained within theseregions are noted within Table 1 (as "Exceptions") and will not be reflexed to Sangersequencing if the mapping quality or coverage is poor. Any variants identified duringtesting in these regions are confirmed by a second method and reported if determined to bepathogenic or likely pathogenic. However, as there is a possibility of false negativeresults within these regions, detection rates and residual risks for these genes have beencalculated with the presumption that variants in these exons will not be detected, unlessincluded in the MassARRAY® genotyping platform.

This test will detect variants within the exons and the intron-exon boundaries of thetarget regions. Variants outside these regions may not be detected, including, but notlimited to, UTRs, promoters, and deep intronic areas, or regions that fall into theExceptions mentioned above. This technology may not detect all small insertion/deletionsand 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 ofMedical Genetics Standards and Guidelines for the Interpretation of Sequence Variants(Richards et al, 2015). All potentially pathogenic variants may be confirmed by either aspecific genotyping assay or Sanger sequencing, if indicated. Any benign variants, likelybenign variants or variants of uncertain significance identified during this analysis willnot be reported.

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

Large duplications and deletions were called from the relative read depths on anexon-by-exon basis using a custom exome hidden Markov model (XHMM) algorithm. Deletions orduplications determined to be pathogenic or likely pathogenic were confirmed by either acustom arrayCGH platform, quantitative PCR, or MLPA (depending on CNV size and gene content). While this algorithm is designed to pick updeletions and duplications of 2 or more exons in length, potentially pathogenicsingle-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-targetedexon-focused array capable of detecting medically relevant microdeletions andmicroduplications at a much higher resolution than traditional aCGH methods. Each arraymatrix has approximately 180,000 60-mer oligonucleotide probes that cover the entiregenome. This platform is designed based on human genome NCBI Build 37 (hg19) and the CGHprobes 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 testfor genomic imbalances, both sample DNA and reference DNA is amplified with primer/probesets that specific to the target region and a control region with known genomic copynumber. Relative genomic copy numbers are calculated based on the standard ΔΔCt formula.

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

Long-range PCR was performed to generate locus-specific amplicons for *CYP21A2* , *HBA1*and *HBA2*and *GBA* . The PCR products were then prepared for short-read NGS sequencing and sequenced.Sequenced reads were mapped back to the original genomic locus and run through thebioinformatics pipeline. If indicated, copy number from MLPA was correlated with thesequencing 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 locatedon the same chromosome in tandem, only the second copy will be amplified and assessed forpotentially 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 thetandem allele and this patient is therefore less likely to be a carrier. When anindividual carries both a duplication allele and a pathogenic variant, or multiplepathogenic variants, the current analysis may not be able to determine the phase(cisrans configuration) of the *CYP21A2*alleles identified. Family studies may be required in certain scenarios where phasing isrequired to determine the carrier status.

Residual Risk Calculations

Carrier frequencies and detection rates for each ethnicity were calculated through thecombination of internal curations of >28,000 variants and genomic frequency data from>138,000 individuals across seven ethnic groups in the gnomAD database. Additionalvariants in HGMD and novel deleterious variants were also incorporated into thecalculation. 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 reportdoes not represent medical advice but should be interpreted by a genetic counselor,medical geneticist or physician skilled in genetic result interpretation and the relevantmedical literature.

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

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

SELECTED REFERENCES

Carrier Screening

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

Variant Classification:

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

Additional disease-specific references available upon request.