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Patient	Sample	Referring Doctor
Patient Name: 5587 Donor **Date of Birth:** 5/[redacted]1997 **Reference #:** TSBCA-S4DONOR5587 **Indication:** Carrier Testing **Test Type:** Cystic Fibrosis Carrier Screen	**Specimen Type:** Saliva **Lab #:** 18103341CF **Date Collected:** 11/27/2018 **Date Received:** 11/28/2018 **Final Report:** 12/12/2018	**Lorraine Bonner, M.D.** **The Sperm Bank of California** **2115 Milvia Street** **Suite 201** **Berkeley, CA 94704** Fax: **510-841-0332**

RESULTS

Clare MS CGC 12/13/18

NEGATIVE for cystic fibrosis
No pathogenic or likely pathogenic variants detected in *CFTR*

Gene analyzed: *CFTR* (NM_000492.3)
Inheritance: Autosomal Recessive

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

Interpretation
Next generation sequencing and targeted genotyping of the *CFTR* gene were performed. No pathogenic or likely pathogenic *CFTR* sequence or copy number variants were identified. These negative results reduce but do not eliminate the possibility that this individual is a carrier. See Table of Residual Risks Based on Ethnicity. With individuals of mixed ethnicity, it is recommended to use the highest residual risk estimate.

Table of Residual Risk Based On Ethnicity

Ethnicity	Carrier Frequency	Detection Rate	Residual Risk
African	1 in 58	91%	1 in 630
Ashkenazi Jewish	1 in 24	98%	1 in 1,200
East Asian	1 in 277	80%	1 in 1,400
Finnish	1 in 75	93%	1 in 1,100
Caucasian	1 in 23	95%	1 in 440
Latino	1 in 40	96%	1 in 1,000
South Asian	1 in 73	91%	1 in 800
Worldwide	1 in 33	94%	1 in 500

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Test Methods and Comments

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

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.

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Multiplex Ligation-Dependent Probe Amplification (MLPA) (Analytical Detection Rate >99%)
MLPA® for cystic fibrosis (*CFTR*) will only be performed if indicated for confirmation of detected CNVs. If performed, the copy numbers of all 27 *CFTR* exons were analyzed. 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%.

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.

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.

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

SELECTED REFERENCES

Carrier Screening
Grody W et al. ACMG position statement on prenatal/preconception expanded carrier screening. *Genet Med.* 2013 15:482-3.
Variant Classification:
Richards S et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015 May;17(5):405-24

Additional disease-specific references available upon request.

This case has been reviewed and electronically signed by Ruth Kornreich, Ph.D., FACMG, Laboratory Director

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

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Patient	Sample	Referring Doctor
Patient Name: 5587 Donor **Date of Birth:** 5/[redacted]/1997 **Reference #:** TSBCA-S4DONOR5587 **Indication:** Carrier Testing **Test Type:** Spinal Muscular Atrophy Carrier Screen (includes enhanced analysis)	**Specimen Type:** Saliva **Lab #:** 18103341SM **Date Collected:** 11/27/2018 **Date Received:** 11/28/2018 **Final Report:** 12/12/2018	**Lorraine Bonner, M.D.** **The Sperm Bank of California** **2115 Milvia Street** **Suite 201** **Berkeley, CA 94704** Fax: **510-841-0332**

RESULTS

NEGATIVE for spinal muscular atrophy
SMN1 Copy Number: 2
SMN2 Copy Number: 0
c.*3+80T>G: Negative

Clinical MS CGC 12/13/18

Negative copy number result
Decreased risk of being an *SMN1* silent (2+0) carrier (see *SMA Table*)

Genes analyzed: *SMN1* (NM_000344.3) and *SMN2* (NM_017411.3)
Inheritance: Autosomal Recessive

Recommendations
Consideration of residual risk by ethnicity after a negative carrier screen is recommended, especially in the case of a positive family history for spinal muscular atrophy.

Interpretation
This patient is negative for loss of *SMN1* copy number. Complete loss of *SMN1* is causative in spinal muscular atrophy (SMA). Two copies of *SMN1* were detected in this individual, which significantly reduces the risk of being an SMA carrier. Parallel testing to assess the presence of an *SMN1* duplication allele was also performed to detect a single nucleotide polymorphism (SNP), c.*3+80T>G, in intron 7 of the *SMN1* gene. This individual was found to be negative for this change and is therefore, at a decreased risk of being a silent (2+0) carrier, see *SMA Table* for residual risk estimates based on ethnicity.

SMA Table: Carrier detection and residual risk estimates before and after testing for c.*3+80T>G

Ethnicity	Carrier Frequency	Detection rate	Residual risk after negative result*	Detection rate with *SMN1* c.*3+80T>G	Residual risk c.*3+80T>G negative	Residual risk c.*3+80T>G positive
African American	1 in 85	71%	1 in 160	91%	1 in 455	1 in 49
Ashkenazi Jewish	1 in 76	90%	1 in 672	93%	1 in 978	1 in 10
East Asian	1 in 53	94%	1 in 864	95%	1 in 901	1 in 12
Caucasian	1 in 48	95%	1 in 803	95%	1 in 894	1 in 23
Latino	1 in 63	91%	1 in 609	94%	1 in 930	1 in 47
South Asian	1 in 103	87%	1 in 637	87%	1 in 637	1 in 608
Sephardic Jewish	1 in 34	96%	1 in 696	97%	1 in 884	1 in 12

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*Residual risk with two copies *SMN1* detected using dosage sensitive methods. The presence of three or more copies of *SMN1* reduces the risk of being an *SMN1* carrier between 5 - 10 fold, depending on ethnicity.
FOR INDIVIDUALS WITH MIXED ETHNICITY, USE HIGHEST RESIDUAL RISK ESTIMATE
^ Parental follow-up will be requested for confirmation

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Patient:	5587 Donor	DOB:	5/[redacted]/1997	Lab #:	18103341SM

Test Methods and Comments

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

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 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.

Residual Risk Calculations
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, which have been calculated from internal data. 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.

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

Selected References

Carrier Screening
Grody W et al. ACMG position statement on prenatal/preconception expanded carrier screening. *Genet Med.* 2013 15:482-3.
Spinal Muscular Atrophy:
Luo M et al. An Ashkenazi Jewish SMN1 haplotype specific to duplication alleles improves pan-ethnic carrier screening for spinal muscular atrophy. *Genet Med.* 2014 16:149-56.
Variant Classification:
Richards S et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015 May;17(5):405-24

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Patient:	5587 Donor	DOB:	5/●/1997	Lab #:	18103341SM

Additional disease-specific references available upon request.

This case has been reviewed and electronically signed by Ruth Kornreich, Ph.D., FACMG, Laboratory Director

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