EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
The 23andMe Personal Genome Service (PGS) Genetic Health Risk Test for Hereditary Thrombophilia, Alpha-1 Antitrypsin Deficiency, Alzheimer's Disease, Parkinson's Disease, Gaucher Disease Type 1, Factor XI Deficiency, Celiac Disease, G6PD Deficiency, Hereditary Hemochromatosis and Early-Onset Primary Dystonia

DECISION SUMMARY

Correction Date: November 2, 2017
This Decision Summary contains corrections to the May 2, 2017 Revised Decision Summary.
The Decision Summary originally issued April 6, 2017.

A. DEN Number:

DEN160026

B. Purpose for Submission:

De Novo request for the 23andMe Personal Genome Service (PGS) Genetic Health Risk Test for Hereditary Thrombophilia, Alpha-1 Antitrypsin Deficiency, Alzheimer's Disease, Parkinson's Disease, Gaucher Disease Type 1, Factor XI Deficiency, Celiac Disease, G6PD Deficiency, Hereditary Hemochromatosis and Early-Onset Primary Dystonia

C. Measurands:

Genomic DNA obtained from a human saliva sample

D. Type of Test:

The 23andMe PGS Genetic Health Risk Report for Hereditary Thrombophilia determines and interprets if a person has variants associated with a higher risk of developing harmful blood clots. This report is based on a qualitative genetic test for single nucleotide polymorphism detection of Factor V Leiden variant in the F5 gene (rs6025) and Prothrombin G20210A variant in the F2 gene (rs1799963/i3002432) by using the 23andMe Personal Genome Service.

The 23andMe PGS Genetic Health Risk Report for Alpha-1 Antitrypsin Deficiency (AATD) determines if a person has variants associated with a higher risk of developing AATD associated lung or liver disease. This report is based on a qualitative genetic test for single nucleotide polymorphism detection of the PI*Z (rs28929474) and PI*S (rs17580) variants in the SERPINA1 gene by using the 23andMe Personal Genome Service.

The 23andMe PGS Genetic Health Risk Report for Late-Onset Alzheimer's Disease determines if a person has variants associated with a higher risk of late-onset Alzheimer's disease. This report is based on a qualitative genetic test for single nucleotide polymorphism

detection of ε4 **(**rs429358) variant in the APOE gene by using the 23andMe Personal Genome Service.

The 23andMe PGS Genetic Health Risk Report for Parkinson's Disease determines if a person has variants associated with a higher risk of Parkinson's Disease. This report is based on a qualitative genetic test for single nucleotide polymorphism detection of G2019S (rs34637584) variant in the LRRK2 gene and N370S (rs76763715) variant in the GBA gene by using the 23andMe Personal Genome Service.

The 23andMe PGS Genetic Health Risk Report for Gaucher Disease Type 1 determines if a person has variants associated with a higher risk of Gaucher Disease. This report is based on a qualitative genetic test for single nucleotide polymorphism detection of the N370S (rs76763715) variant, 84GG (rs387906315) variant and V394L (rs80356769) variant in the GBA gene by using the 23andMe Personal Genome Service.

The 23andMe PGS Genetic Health Risk Report for Factor XI Deficiency determines if a person has variants associated with a higher risk of developing Factor XI Deficiency. This report is based on a qualitative genetic test for single nucleotide polymorphism detection of the F283L (rs121965064), E117X (rs121965063), and IVS14+1G>A (rs373297713) variants in the FXI gene by using the 23andMe Personal Genome Service.

The 23andMe PGS Genetic Health Risk Report for Celiac disease determines if a person has variants associated with a higher risk of developing Celiac disease. This report is based on a qualitative genetic test for single nucleotide polymorphism detection of the HLA-DQ2.5 haplotype (rs2187668) in the HLA-DQA1 gene by using the 23andMe Personal Genome Service.

The 23andMe PGS Genetic Health Risk Report for Glucose-6-Phosphate-Dehydrogenase Deficiency determines if a person has variants associated with a higher risk of developing G6PD Deficiency and a higher risk for episodes of anemia. This report is based on a qualitative genetic test for single nucleotide polymorphism detection of the Val68Met (rs1050828) variant in the G6PD gene by using the 23andMe Personal Genome Service.

The 23andMe PGS Genetic Health Risk Report for Hereditary Hemochromatosis determines if a person has variants associated with hereditary hemochromatosis and a higher risk of developing iron overload. This report is based on a qualitative genetic test for the C282Y (rs1800562) and H63D (rs1799945) variants in the HFE gene by using the 23andMe Personal Genome Service.

The 23andMe PGS Genetic Health Risk Report for Early-Onset Primary Dystonia determines if a person has variants associated with a higher risk of developing Early-Onset Primary Dystonia. This report is based on a qualitative genetic test for 3 base pair nucleotide deletion (rs724159981) called the deltaE302/303 variant in the DYT1 gene by using the 23andMe Personal Genome Service.

E. Applicant:

23andMe, Inc.

F. Proprietary and Established Names:

23andMe Personal Genome Service

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.5950

2. Classification:

   Class II

3. Product code(s):

   PTA

4. Panel:

   Immunology

H. Indications for use:

1. Indications for use:

   The 23andMe Personal Genome Service (PGS) Test uses qualitative genotyping to detect the following clinically relevant variants in genomic DNA isolated from human saliva collected from individuals ≥18 years with the Oragene Dx model OGD-500.001 for the purpose of reporting and interpreting Genetic Health Risks (GHR):

   The 23andMe PGS Genetic Health Risk Report for Hereditary Thrombophilia is indicated for reporting of the Factor V Leiden variant in the F5 gene, and the Prothrombin G20210A variant in the F2 gene. This report describes if a person has variants associated with a higher risk of developing harmful blood clots, but it does not describe a person's overall risk of developing harmful blood clots. This test is most relevant for people of European descent.

   The 23andMe PGS Genetic Health Risk Report for Alpha-1 Antitrypsin Deficiency is indicated for reporting of the PI*Z and PI*S variants in the SERPINA1 gene. This report describes if a person has variants associated with AAT deficiency and a higher risk for lung or liver disease, but it does not describe a person's overall risk of developing lung or liver disease. This test is most relevant for people of European descent.

The 23andMe PGS Genetic Health Risk Report for Late-onset Alzheimer's Disease is indicated for reporting of the ε4 variant in the APOE gene. The report describes if a person's genetic result is associated with an increased risk of developing Late-onset Alzheimer's Disease, but it does not describe a person's overall risk of developing Alzheimer's Disease. The ε4 variant included in this report is found and has been studied in many ethnicities. Detailed risk estimates have been studied the most in people of European descent.

The 23andMe PGS Genetic Health Risk Report for Parkinson's Disease is indicated for reporting of the G2019S variant in the LRRK2 gene and the N370S variant in the GBA gene. The report describes if a person's genetic result is associated with an increased risk of developing Parkinson's disease, but it does not describe a person's overall risk of developing Parkinson's disease. The test is most relevant for people of European, Ashkenazi Jewish, and North African Berber descent.

The 23andMe PGS Genetic Health Risk Report for Gaucher Disease Type 1 is indicated for reporting of the N370S, 84GG, and V394L variants in the GBA gene. This report describes if a person has variants associated with an increased risk for developing symptoms of Gaucher Disease Type 1, but it does not describe a person's overall risk of developing Gaucher Disease Type 1. This test is most relevant for people of Ashkenazi Jewish descent.

The 23andMe PGS Genetic Health Risk Report for Factor XI Deficiency is indicated for reporting of the variants F283L, E117X, IVS14+1G>A in the F11 gene. This report describes if a person has a variant associated with Factor XI deficiency and the potential for a higher risk of excessive bleeding following trauma or surgery, but it does not describe a person's overall risk for excessive bleeding. This test is most relevant for people of Ashkenazi Jewish descent.

The 23andMe PGS Genetic Health Risk Report for Celiac Disease is indicated for reporting of a variant associated with the HLA-DQ2.5 haplotype. The report describes if a person has a haplotype associated with an increased risk of developing celiac disease, but it does not describe a person's overall risk for developing celiac disease. This report is most relevant for people of European descent.

The 23andMe PGS Genetic Health Risk Report for Glucose-6-Phosphate-Dehydrogenase Deficiency is indicated for reporting of the Val68Met variant in the G6PD gene. This report describes if a person has a variant associated with G6PD deficiency and a higher risk for episodes of anemia, but it does not describe a person's overall risk of developing anemia. This test is most relevant for people of African descent.

The 23andMe PGS Genetic Health Risk Report for Hereditary Hemochromatosis is indicated for reporting of the C282Y and H63D variants in the HFE gene. This report describes if a person has variants associated with hereditary hemochromatosis and a higher risk for iron overload, but it does not describe a person's overall risk of developing iron overload. This report is most relevant for people of European descent.

The 23andMe PGS Genetic Health Risk Report for Early-Onset Primary Dystonia (DYT1/TOR1A-Related) is indicated for reporting of the deltaE302/303 variant in the DYT1 gene. This report describes if a person has variants associated with a higher risk for early-onset primary dystonia, but it does not describe a person's overall risk of developing dystonia. This report is most relevant for people of Ashkenazi Jewish descent.

2. Special conditions for use statements:
   a. For over-the-counter (OTC) use.
   b. This test is not a substitute for visits to a healthcare provider. It is recommended that you consult with a healthcare provider if you have any questions or concerns about your results.
   c. The 23andMe PGS Genetic Health Risk Tests for Hereditary Thrombophilia, Alpha-1 Antitrypsin Deficiency, Alzheimer's disease, Parkinson's disease, Gaucher Disease, Factor XI Deficiency, Celiac disease, and Glucose-6-Phosphate-Dehydrogenase Deficiency, Early-Onset Primary Dystonia and Hereditary Hemochromatosis do not detect all genetic variants associated with the aforementioned diseases. The absence of a variant tested does not rule out the presence of other genetic variants that may be disease-related.
   d. The test is intended for users ≥ 18 years old.
   e. The test does not diagnose any specific health conditions. Results should not be used to make medical decisions.
   f. The laboratory may not be able to process a user's sample. The probability that the laboratory cannot process a sample can be up to 7.6%.
   g. A user's race, ethnicity, age, and sex may affect how the genetic test results are interpreted.
   h. Subject to meeting the limitations contained in the special controls under regulation 21 CFR 866.5950.

3. Special instrument requirements:

   The 23andMe PGS Genetic Health Risk Tests for Hereditary Thrombophilia, Alpha-1 Antitrypsin Deficiency, Alzheimer's disease, Parkinson's disease, Gaucher Disease Type I, Factor XI Deficiency, Celiac Disease, and Glucose-6-Phosphate-Dehydrogenase Deficiency, Early-Onset Primary Dystonia and Hereditary Hemochromatosis are to be performed using the Tecan Evo and Illumina iScan instruments.

   GenomeStudio is a modular software application that is used to view and analyze genotypic data obtained from the iScan. Coregen software conducts a variety of control checks on the file, resulting in a final genotype profile for each sample. These data are used to generate test reports on a user's genotype and associated risk of disease.

I. Device Description:

The 23andMe PGS is a currently-marketed, non-invasive genetic information service that combines qualitative genotyping data covering genetic ancestry, traits, and certain heritable health conditions from a single multiplex assay with descriptive information derived from peer reviewed, published genetic research studies. It is a direct-to-consumer, over-the-counter, non-diagnostic, DNA genetic testing service intended to provide information and tools for individual users.

A user's saliva is self-collected using the Oragene·Dx device manufactured by DNA Genotek, Inc. (previously cleared under K141410), which consists of a sealable collection tube containing a stabilizing buffer solution. Once the sample is collected, it is shipped to one of two Clinical Laboratory Improvement Amendments (CLIA)-certified laboratories for processing.

DNA is isolated from the saliva and tested in a multiplex assay using a customized genotyping chip and instrumentation manufactured by Illumina. The multiplex assay simultaneously tests for more than 500,000 variants, including those for the indications proposed herein.

The raw data is generated using Illumina GenomeStudio software, and then sent to 23andMe (the Manufacturer). The data are analyzed using the Manufacturer's proprietary Coregen software, and a genotype is determined for each tested variant. The results for certain of these variants are used to generate personalized reports for users that provide information about the diseases associated with the detected variant.

Personalized reports are generated for each user that provide results of the testing performed. These reports tell the user which variant(s) has/have been detected in their sample and provide information on the risk of disease associated with the variant(s). If no variant was detected, that information is also provided. The personalized reports are designed to present scientific concepts to users in an easy-to-understand format. The reports provide scientifically valid information about the risks associated with the presence of a particular variant. The reports are designed to help users understand the meaning of their results and any appropriate actions that may be taken based on their results.

J. Substantial Equivalence Information:

1. Predicate device name:

   No predicate device exists.

2. Predicate 510(k) number:

   Not applicable.

3. Comparison with predicate:

   Not applicable.

K. Standard/Guidance Document Referenced:

Not applicable.

L. Test Principle:

The PGS is indicated to be performed using the BeadChip v4 assay (Illumina Infinium HumanOmniExpress-24 format chip), which covers more than 500,000 genetic markers. The BeadChip consists of silicon wafers etched to form wells loaded with silica beads, on which oligonucleotide capture probes are immobilized. DNA from saliva is fragmented and captured on a bead array by hybridization to immobilized variant-specific primers, followed by extension with hapten-labeled nucleotides. The primers hybridize adjacent to the variants and are extended with a single nucleotide corresponding to the variant allele. The incorporated hapten-modified nucleotides are detected by adding fluorescently labeled antibodies in several steps to amplify the signals. The Tecan Evo and Illumina iScan instruments are used for extraction and processing of the DNA, and the BeadChip for scanning and quantification of the results. The genotype content is separated, analyzed, and then integrated into pre-defined report templates specific for each condition associated with each genotype. Genotypes are determined using the GenomeStudio and Coregen software packages.

M. Performance Characteristics:

Saliva samples were collected using the Oragene·Dx saliva collection device (OGD-500.001). The samples were tested on the Illumina Infinium BeadChip. Results were analyzed using the Illumina iScan System and GenomeStudio and Coregen software.

1. Analytical performance:
   The results of all the analytical performance studies met the pre-determined acceptance criteria.

a. Precision/Reproducibility

Reproducibility for Variants Associated with Hereditary Thrombophilia

Reproducibility studies were conducted for the two genes associated with hereditary thrombophilia: Factor V Leiden and Prothrombin G20210A. The reproducibility studies were designed to determine the imprecision due to assay run, lot, instrument, operator, day, and site. Three Factor V Leiden common (wild type) DNA samples, two Factor V heterozygous DNA samples, one Factor V Leiden homozygous rare DNA sample, three Prothrombin G20210A common (wild type) DNA samples, one Prothrombin G20210A heterozygous DNA sample, and one Prothrombin G20210A homozygous rare DNA sample were included in the precision /reproducibility studies. Genotypes of these cell line samples were confirmed through bidirectional Sanger sequencing. These samples were genotyped by the PGS Test in a blinded fashion.

Samples were genotyped by the PGS Test using multiple lots of reagents, three operator teams, and nine different combinations of two instrument types (Tecan ([three instruments] and iScan [3 instruments]), over the course of five days, at two independent laboratory sites. This precision study yielded 100% correct genotype calls for all samples with a valid call across multiple days, operator teams, instruments, and reagent lots at both laboratory sites. Information regarding samples that failed quality control (FQC) was also evaluated.

Factor V Leiden

The combined data of the reproducibility study is presented in the table below:

Genotype	Number of Replicates (including FQCs)	Number of Correct Calls	Number of Incorrect Calls	Number of FQCs	Percentage of FQCs
CC (homozygous common)	1080	1061	0	19	1.76%
CT (heterozygous)	540	533	0	7	1.30%
TT (homozygous rare)	270	258	0	12	4.44%
Total	1890	1852	0	38	2.01%

Genotyping results produced 98.0 % (1852/1890) replicates that were called correctly and 2.01% (38/1890) replicates that did not pass quality control (QC) acceptance criteria. Samples with FQC on the first run are re-tested per laboratory standard operating procedures (SOPs); an anticipated rate of samples with two FQCs based on the reproducibility study data of cell line samples is 0.04% (0.020 x 0.020).

Results for Factor V Leiden stratified by site and genotype (all valid calls were 100% correct at both sites):

Site 1					
Genotype	**Number of Replicates (including FQCs)**	**Number of Correct Calls**	**Number of Incorrect Calls**	**Number of FQCs**	**Percentage of FQCs**
CC (homozygous common)	540	537	0	3	0.56%
CT (heterozygous)	270	268	0	2	0.74%
TT (homozygous rare)	135	135	0	0	0.00%

Total	945	940	0	5	0.53%

Site 2					
Genotype	**Number of Replicates (including FQCs)**	**Number of Correct Calls**	**Number of Incorrect Calls**	**Number of FQCs**	**Percentage of FQCs**
CC (homozygous common)	540	524	0	16	2.96%
CT (heterozygous)	270	265	0	5	1.85%
TT (homozygous rare)	135	123	0	12	8.89%
Total	945	912	0	33	3.49%

Prothrombin G20210A

The combined data of the reproducibility study of Prothrombin G20210A is presented in the table below:

Genotype	**Number of Replicates (including FQCs)**	**Number of Correct Calls**	**Number of Incorrect Calls**	**Number of FQCs**	**Percentage of FQCs**
GG (homozygous common)	1080	1060	0	20	1.85%
AG (heterozygous)	270	262	0	8	2.96%
AA (homozygous rare)	270	268	0	2	0.74%
Total	1620	1590	0	30	1.85%

Genotyping results produced 98.1 % (1590/1620) replicates that were called correctly and 1.85% (30/1620) replicates that did not pass QC acceptance criteria. Samples with FQC on the first run are re-tested per laboratory SOPs; therefore, an anticipated rate of samples with two FQCs based on the reproducibility study data of cell line samples is 0.03% (0.0185 x 0.0185).

Results for Prothrombin G2010A stratified by site and genotype (all valid calls were 100% correct at both sites):

Site 1					
Genotype	**Number of Replicates (including FQCs)**	**Number of Correct Calls**	**Number of Incorrect Calls**	**Number of FQCs**	**Percentage of FQCs**
GG (homozygous common)	540	539	0	1	0.19%
AG (heterozygous)	135	132	0	3	2.22%
AA (homozygous rare)	135	134	0	1	0.74%
Total	810	805	0	5	0.62%

Site 2					
Genotype	**Number of Replicates (including FQCs)**	**Number of Correct Calls**	**Number of Incorrect Calls**	**Number of FQCs**	**Percentage of FQCs**
GG (homozygous common)	540	521	0	19	1.48%
AG (heterozygous)	135	130	0	5	3.7
AA (homozygous rare)	135	134	0	1	0.74%
Total	810	785	0	25	3.09%

Reproducibility for Variants Associated with Alpha-1 Antitrypsin Deficiency

Reproducibility studies were conducted for the two variants associated with Alpha-1 Antitrypsin Deficiency: PI*Z and PI*S in the SERPINA1 gene. Two PI*Z SERPINA1 homozygous common DNA samples, one heterozygous PI*Z SERPINA1 DNA sample, one PI*Z SERPINA1 homozygous rare DNA sample, two PI*S SERPINA1 homozygous common DNA samples, one heterozygous PI*S SERPINA1 DNA sample, and one PI*S SERPINA1 homozygous rare DNA sample were included in the reproducibility study. The study design and data analysis for Alpha-1 Antitrypsin Deficiency was identical to the Hereditary Thrombophilia reproducibility study reported above.

PI*Z SERPINA1

The combined data of the reproducibility study of PI*Z SERPINA1 is presented in the table below

Genotypee	Number of Replicates (including FQCs)	Number of Correct Calls	Number of Incorrect Calls	Number of FQCs	Percentage of FQCs
CC (homozygous common)	810	797	0	13	1.60%
CT (heterozygous)	270	261	0	9	3.33%
TT (homozygous rare)	270	270	0	0	0%
Total	1350	1328	0	22	1.63%

Genotyping results produced 98.4 % (1328/1350) replicates that were called correctly and 1.63% (22/1350) replicates that did not pass QC acceptance criteria. Samples with FQC on the first run are re-tested per laboratory SOPs; therefore, an anticipated rate of samples with two FQCs based on the reproducibility study data of cell line samples is 0.03% (0.0163 x 0.0163).

Results for P1*Z SERPINA1 stratified by site and genotype (all valid calls were 100% correct at both sites):

Site 1					
Genotype	**Number of Replicates (including FQCs)**	**Number of Correct Calls**	**Number of Incorrect Calls**	**Number of FQCs**	**Percentage of FQCs**
CC (homozygous common)	405	404	0	1	0.25%
CT (heterozygous)	135	131	0	4	2.97%
TT (homozygous rare)	135	135	0	0	0.00%
Total	675	670	0	5	0.74%

Site 2					
Genotpye	**Number of Replicates (including FQCs)**	**Number of Correct Calls**	**Number of Incorrect Calls**	**Number of FQCs**	**Percentage of FQCs**
CC (homozygous common)	405	393	0	12	2.96%
CT (heterozygous)	135	130	0	5	3.70%
TT (homozygous rare)	135	135	0	0	0.00%

Site 2					
Genotpye	**Number of Replicates (including FQCs)**	**Number of Correct Calls**	**Number of Incorrect Calls**	**Number of FQCs**	**Percentage of FQCs**
Total	675	658	0	17	2.52%

PI*S SERPINA1

The combined data of the reproducibility study of PI*S SERPINA1 is presented in the table below.

Genotype	Number of Replicates (including FQCs)	Number of Correct Calls	Number of Incorrect Calls	Number of FQCs	Percentage of FQCs
TT (homozygous common)	810	797	0	13	1.60%
AT (heterozygous)	270	269	0	1	0.37%
AA (homozygous rare)	225	216	0	9	4.00%
Total	1305	1282	0	23	1.76%

Genotyping results produced 98.2 % (1282/1305) replicates that were called correctly and 1.76% (23/1305) replicates that did not pass QC acceptance criteria. Samples with FQC on the first run are re-tested per laboratory SOPs; therefore, an anticipated rate of samples with two FQCs based on the reproducibility study data of cell line samples is 0.03% (0.0176 x 0.0176).

Results for P1*S SERPINA1 stratified by site and genotype (all valid calls were 100% correct at both sites):

Site 1					
Genotype	**Number of Replicates (including FQCs)**	**Number of Correct Calls**	**Number of Incorrect Calls**	**Number of FQCs**	**Percentage of FQCs**
TT (homozygous common) 2 samples	405	404	0	1	0.25%
AT (heterozygous) 1 sample	135	135	0	0	0.00%
AA (homozygous rare) 1 sample	135	133	0	2	1.48%
Total	675	672	0	3	0.44%

Site 2

Genotpye	Number of Replicates (including FQCs)	Number of Correct Calls	Number of Incorrect Calls	Number of FQCs	Percentage of FQCs
TT (homozygous common) 2 samples	405	393	0	12	2.96%
AT (heterozygous) 1 sample	135	134	0	1	0.74%
AA (homozygous rare) 1 sample	90	83	0	7	7.78%
Total	630	610	0	20	3.17%

Reproducibility for the Variant Associated with Late-onset Alzheimer's Disease

Reproducibility studies were conducted for the APOE ε4 variant associated with Late-onset Alzheimer's Disease. Three APOE ε4 homozygous common DNA samples, two heterozygous APOE ε4 DNA samples, and one APOE ε4 homozygous rare DNA sample were included in the reproducibility study. These DNA samples were genotyped by the PGS Test in a blinded fashion, using three lots of reagents, three operator teams per day, and nine different combinations of two instrument types (Tecan [3 instruments] and iScan [3 instruments]), over the course of three days, at each of two independent laboratory sites.

The combined data of the reproducibility study of APOE ε4 is presented in the table below.

Genoptye	Number of Replicates (including FQCs)	Number of Correct Calls	Number of Incorrect Calls	Number of FQCs	Percentage of FQCs
TT (homozygous common)	486	477	0	9	1.89%
CT (heterozygous)	324	318	0	6	1.89%
CC (homozygous rare)	162	162	0	0	0.00%
Total	972	957	0	15	1.54%

Genotyping results produced 98.5 % (957/972) replicates that were called correctly and 1.54% (15/972) replicates that did not pass QC acceptance criteria. Samples with FQC on the first run are re-tested per laboratory SOPs; therefore, an anticipated rate of samples with two FQCs based on the precision study data of cell line samples is 0.02% (0.0154x0.0154).

Results for APOE ε4 stratified by site and genotype (all valid calls were 100% correct at both sites):

Site 1					
Genotype	**Number of Replicates (including FQCs)**	**Number of Correct Calls**	**Number of Incorrect Calls**	**Number of FQCs**	**Percentage of FQCs**
TT (homozygous common)	243	242	0	1	0.41%
CT (heterozygous)	162	162	0	0	0.00%
CC (homozygous rare)	81	81	0	0	0.00%
Total	486	485	0	1	0.21%

Site 2					
Sample	**Number of Replicates (including FQCs)**	**Number of Correct Calls**	**Number of Incorrect Calls**	**Number of FQCs**	**Percentage of FQCs**
TT (homozygous common)	243	235	0	8	3.40%
CT (heterozygous)	162	156	0	6	3.85%
CC (homozygous rare)	81	81	0	0	0.00%
Total	486	472	0	14	2.97%

Reproducibility for Variants Associated with Parkinson's Disease:

Reproducibility studies were conducted for two variants associated with Parkinson's Disease: G2019S in the LRRK2 gene and N370S in the GBA gene. Two G2019S LRRK2 homozygous common DNA samples, one heterozygous G2019S LRRK2 DNA sample, one homozygous rare G2019S LRRK2 DNA sample, two N370S GBA homozygous common DNA samples, three heterozygous N370S GBA DNA samples, and one homozygous rare N370S GBA DNA samples were included in the reproducibility study. The study design and data analysis for Parkinson's Disease was identical to the Hereditary Thrombophilia precision study reported above.

G2019S LRRK2

The combined data of the reproducibility study of G2019S LRRK2 is presented in the table below:

Genotype	Number of Replicates (including FQCs)	Number of Correct Calls	Number of Incorrect Calls	Number of FQCs	Percentage of FQCs
GG (homozygous common)	324	315	0	9	2.77%
AG (heterozygous)	162	161	0	1	0.62%
AA (homozygous rare)	162	162	0	0	0.00%
Total	648	638	0	10	1.54%

Genotyping results produced 98.5 % (638/648) replicates that were called correctly and 1.54% (10/648) replicates that did not pass QC acceptance criteria. Samples with FQC on the first run are re-tested per laboratory SOPs; therefore, an anticipated rate of samples with two FQCs based on the precision study data of cell line samples is 0.02% (0.0154 x 0.0154).

Results for G2109S LRRK2 stratified by site and genotype (all valid calls were 100% correct at both sites):

Site 1					
Genotype	**Number of Replicates (including FQCs)**	**Number of Correct Calls**	**Number of Incorrect Calls**	**Number of FQCs**	**Percentage of FQCs**
GG (homozygous common)	162	161	0	1	0.62%
AG (heterozygous)	81	81	0	0	0.00
AA (homozygous rare)	81	81	0	0	0.00
Total	324	323	0	1	0.31%

Site 2					
Genotype	**Number of Replicates (including FQCs)**	**Number of Correct Calls**	**Number of Incorrect Calls**	**Number of FQCs**	**Percentage of FQCs**
GG (homozygous common)	162	154	0	8	3.09%
AG (heterozygous)	81	80	0	1	1.23%
AA (homozygous rare)	81	81	0	0	0.00%
Total	324	315	0	9	2.78%

N370S GBA

The combined data of the reproducibility study of N370S GBA is presented in the table below:

Genotype	Number of Replicates (including FQCs)	Number of Correct Calls	Number of Incorrect Calls	Number of FQCs	Percentage of FQCs
TT (homozygous common)	324	314	0	10	3.09%
CT (heterozygous)	486	466	0	20	4.12%
CC (homozygous rare)	162	155	0	7	4.32%
Total	972	935	0	37	3.81%

Genotyping results produced 96.2 % (935/972) replicates that were called correctly and 3.81% (37/972) replicates that did not pass QC acceptance criteria. Samples with FQC on the first run are re-tested per laboratory SOPs; therefore, an anticipated rate of samples with two FQCs based on the reproducibility study data of cell line samples is 0.15% (0.0381 x 0.0381).

Results for N370S GBA stratified by site and genotype (all valid calls were 100% correct at both sites):

Site 1					
Genotype	**Number of Replicates (including FQCs)**	**Number of Correct Calls**	**Number of Incorrect Calls**	**Number of FQCs**	**Percentage of FQCs**
TT (homozygous common) 2 samples	162	158	0	4	1.23%
CT (heterozygous) 3 samples	243	232	0	11	4.53%
CC (homozygous rare) 1 sample	81	77	0	4	4.94%
Total	486	467	0	19	3.91%

Site 2					
Genotype	**Number of Replicates (including FQCs)**	**Number of Correct Calls**	**Number of Incorrect Calls**	**Number of FQCs**	**Percentage of FQCs**
TT (homozygous common)	162	156	0	6	3.70%

Site 2					
Genotype	**Number of Replicates (including FQCs)**	**Number of Correct Calls**	**Number of Incorrect Calls**	**Number of FQCs**	**Percentage of FQCs**
CT (heterozygous)	243	234	0	9	3.70%
CC (homozygous rare)	81	78	0	3	3.70%
Total	486	468	0	18	3.70%

Reproducibility for the Variant Associated with Gaucher Disease Type I

Reproducibility studies were conducted for the N370S variant in the GBA gene associated with Gaucher Disease Type I. Two N370S GBA homozygous common DNA samples, three heterozygous N370S GBA DNA samples, and one homozygous rare N370S GBA DNA sample were included in the reproducibility study. The study design and data analysis for Gaucher Disease was identical to the Alzheimer's Disease study reported above.

N370S GBA

The combined data of the reproducibility study of N370S GBA is presented in the table below:

Genotypee	**Number of Replicates (including FQCs)**	**Number of Correct Calls**	**Number of Incorrect Calls**	**Number of FQCs**	**Percentage of FQCs**
TT (homozygous common)	525	510	0	15	2.86%
CT (heterozygous)	784	768	0	26	3.32%
CC (homozygous rare)	262	254	0	8	3.05%
Total	1571	1532	0	49	3.12%

Genotyping results produced 97.5 % (1532/1571) replicates that were called correctly and 3.12% (49/1571) replicates that did not pass QC acceptance criteria. Samples with FQC on the first run are re-tested per laboratory SOPs; therefore, an anticipated rate of samples with two FQCs based on the reproducibility study data of cell line samples is 0.10% (0.0312 x 0.0312).

Results for N370S GBA stratified by site and genotype (all valid calls were 100% correct at both sites):

Site 1

Genotype	Number of Replicates (including FQCs)	Number of Correct Calls	Number of Incorrect Calls	Number of FQCs	Percentage of FQCs
TT (homozygous common)	264	258	0	6	2.27%
CT (heterozygous)	394	383	0	11	2.79%
CC (homozygous rare	131	127	0	4	3.05%
Total	789	768	0	21	2.66%

Site 2					
Genotype	**Number of Replicates (including FQCs)**	**Number of Correct Calls**	**Number of Incorrect Calls**	**Number of FQCs**	**Percentage of FQCs**
TT (homozygous common)	261	252	0	9	3.45%
CT (heterozygous)	390	385	0	15	3.85%
CC (homozygous rare)	131	127	0	4	3.05%
Total	782	754	0	28	3.58%

The Manufacturer affirmed that reproducibility studies were completed for the variants described in the table below. The study design, acceptance criteria, and results for the reproducibility were the same for each of the variants listed in the table and the same as the studies that were completed for the variants for Hereditary Thrombophilia, Alpha-1 Antitrypsin Deficiency, Alzheimer's Disease, Parkinson's Disease, and Gaucher Disease Type 1 (N370S variant). Each variant was tested with wild-type, heterozygous and homozygous samples, except where indicated.

Disease	Gene	Variant	Precision Completed
Gaucher Disease Type 1	GBA	84GG$^{\Psi}$	Yes
Gaucher Disease Type 1	GBA	V394L$^{\Psi}$	Yes
Factor XI Deficiency	Factor XI	F283L	Yes
Factor XI Deficiency	Factor XI	E117X	Yes
Factor XI Deficiency	Factor XI	IVS14+1G>A$^{\Psi}$	Yes
Celiac Disease	HLA-DQA1	HLA-DQA1	Yes
Glucose-6-Phosphate Dehydrogenase Deficiency (G6PD)	G6PD	Val68Met*	Yes
Hereditary Hemochromatosis	HFE	C282Y	Yes
Hereditary Hemochromatosis	HFE	H63D	Yes

Disease	Gene	Variant	Precision Completed
Early-Onset Primary Dystonia	DYT1	deltaE302/303[Ψ]	Yes

*Hemizygous genotype not included
[Ψ]Homozygous genotype samples were not tested therefore homozygous test results will not be reported to user.

b. *Linearity/assay Reportable Range:*

Not applicable.

c. *Traceability, Stability, Expected Values (controls, calibrators, or methods):*

The PGS requires two types of controls: the sample processing control and the reproducibility control. The control material is genotyped on the 23andMe BeadChip according to routine SOPs at the contracted laboratory sites. Each new lot of the reproducibility control is tested by comparison with reference BeadChip genotype results.

The sample processing control is run on every sample genotyping plate and the reproducibility control is run approximately once per week. Historical data from all such runs were analyzed for one lot of the sample processing control spanning three months and one lot of the reproducibility control spanning one year.

Stability protocols and acceptance criteria were reviewed and acceptable. The information provided demonstrates that the sample processing control is stable for up to three months and the reproducibility control is stable for up to 12 months.

d. *Detection Limit:*

<u>Detection limit study for variants in the Factor V Leiden, Prothrombin G20210A, SERPINA1, APOE, LRRK2, and GBA variants</u>

The Limit of Detection (LoD) study was performed to determine the lowest concentration of DNA that is necessary for successful assignment of the correct variant using the 23andMe PGS Test. The Manufacturer presented the results of the LoD study for the following genes and variants: Factor V Leiden F5 and Prothrombin G20210A F2 variants for Hereditary Thrombophilia; PI*Z and PI*S in SERPINA1 for Alpha-1 Antitrypsin Deficiency; ε4 variant in the APOE gene for Alzheimer's disease; and the G2019S variant in the LRRK2 gene and the N370S variant in the GBA gene for Parkinson's and Gaucher disease. Each sample was diluted to three different DNA concentrations and genotyped by the PGS Test in a blinded fashion using three lots of reagents. To confirm the genotype call, each sample was also sequenced by bidirectional sequencing. Genotype calls from the PGS Test were compared with sequenced genotypes to determine the rates of correct genotype calls at each DNA concentration. The LoD was defined as the lowest DNA concentration at which at least 95% of samples yielded the correct call.

The LoD study yielded 100% correct genotype calls for all samples and all reagent lots tested at sample DNA concentrations of 5, 15, and 50 ng/µL. Therefore, the study passed the LoD acceptance criteria at a sample DNA concentration of 5 ng/µL. This LoD study shows that the PGS Test is valid for the variants tested using samples with a DNA concentration between 5 ng/µL to 50 ng/µL. The Manufacturer has claimed a LoD of 15 ng/uL.

The Manufacturer affirmed that detection limit studies were completed for the variants described in the table below. The study design, acceptance criteria, and results for the detection limit studies were the same for each of the variants listed in the table and the same as the studies that were completed for the variants for Hereditary Thrombophilia, AATD, Alzheimer's Disease, Parkinson's Disease, and Gaucher Disease, reported above. Each variant was tested with a wild-type, heterozygous, and homozygous sample, except where indicated.

Disease	Gene	Variant	LoD Completed
Gaucher Disease Type 1	GBA	84GG$^{\Psi}$	Yes
Gaucher Disease Type 1	GBA	V394L$^{\Psi}$	Yes
Factor XI Deficiency	Factor XI	F283L	Yes
Factor XI Deficiency	Factor XI	E117X	Yes
Factor XI Deficiency	Factor XI	IVS14+1G>A$^{\Psi}$	Yes
Celiac Disease	HLA-DQA1	HLA-DQA1	Yes
Glucose-6-Phosphate Dehydrogenase Deficiency (G6PD)	G6PD	Val68Met*	Yes
Hereditary Hemochromatosis	HFE	C282Y	Yes
Hereditary Hemochromatosis	HFE	H63D	Yes
Early-Onset Primary Dystonia	DYT1	deltaE302/303$^{\Psi}$	Yes

*Hemizygous genotype not included
$^{\Psi}$Homozygous genotype samples were not tested therefore homozygous test results will not be reported to user.

e. *Interfering Substances*

Endogenous and Exogenous substances

A series of studies were conducted to assess the effects of endogenous substances, exogenous substances, microbial substances, and smoking on the 23andMe PGS Test. The results of the Endogenous Interference Study can be found in the Decision Summary for DEN140044.

The PGS Test requires the use of an FDA cleared collection device (K141410). The cleared device includes instructions for use instructing the user to not eat, drink, smoke, or chew gum for 30 minutes prior to collecting their saliva, thus minimizing the presence of interferents in the sample. The Instructions for Use (IFU) was tested

for user comprehension and a paper version of the IFU is included in every collection kit. Should an interfering substance be present after DNA has been extracted and an insufficient concentration or quality of DNA is available, then the sample is managed per standard operating procedures that are pre-determined by the Manufacturer.

Interfering Mutations
Analyses were performed to identify potentially interfering variants within the 50 nucleotide probe-binding region of the variant being detected. If samples for the potentially interfering mutations were not available to test the impact on detection of the corresponding clinically relevant variant, the Manufacturer added a statement in the device labeling stating that the impact of that potentially interfering mutation on the performance of the device has not been evaluated. The results follow for each claimed variant.

Interfering Mutations for Hereditary Thrombophilia:
Two potentially interfering mutations for Factor V Leiden and one potentially interfering mutation for Prothrombin G20210A were identified. For Factor V Leiden the potential interfering mutations include rs760488939 and rs763859650. For Prothrombin G20210A the potential interfering mutation is rs112016113. Interference due to these mutations was not tested.

Interfering Mutations for Alph-1 Antitrypsin Deficiency:
Nine potentially interfering mutations were identified for PI*Z SERPINA1and six potentially interfering mutation in PI*S SERPINA1. The potentially interfering mutations include: rs148362959, rs533419579, rs551595739, rs201774333, rs143370956, rs1131139, rs200945035, rs373630097 and rs9630 for PI*Z variant and rs538675821, rs550592374, rs141095970, rs149537225, rs1049800 and rs2230075 for PI*S variant. Interference due to these mutations was not tested.

Interfering Mutations for Alzheimer's disease:
Three potentially interfering mutations were identified for APOEε4. The potential interfering mutations include rs11542041, rs573658040, and rs543363163. Interference due to these mutations was not tested.

Interfering Mutations for Parkinson’s Disease:
Two potentially interfering mutations each for LRRK2 and GBA variants were identified. For the G2019S variant for LRRK2 the potential interfering mutations include rs150219613 and rs183394865. For the N370S variant for GBA the potential interfering mutations include rs187143994 and rs111417507. Interference due to these mutations was not tested.

Interfering Mutations for Gaucher Disease:
Seven potentially interfering mutations were identified for GBA. The potentially interfering mutations include: rs104886460, rs143187997 and rs150466109 for the 84GG variant; rs14917112 and rs201499639 for the V394L variant; and rs187143994 and rs111417507 for the N370S variant. Interference due to these mutations was not tested.

Interfering Mutations for Factor XI Deficiency:
Eight potentially interfering mutations were identified for Factor XI. The potential interfering mutations include rs200218867, rs549554738, rs569456903, and rs147592940 for the F283L variant and rs200593979, rs5973, rs199657604 and rs34807019 for the E117X variant. Interference due to these mutations was not tested.

Interfering Mutations for Celiac Disease:
Six potentially interfering mutations were identified for HLA-DQA1. The potential interfering mutations include rs373744062, rs34481484, rs535725525, rs116178934, rs118073417, and rs9272482. Interference due to these mutations was not tested.

Interfering Mutations for Glucose-6-Phosphate Dehydrogenase Deficiency:
One potentially interfering mutation was identified for Val68Met. The potential interfering mutation is rs138687036. Interference due to this mutation was not tested.

Interfering Mutations for Hereditary Hemochromatosis:
Seven potentially interfering mutations were identified for Hemochromatosis. The potentially interfering mutations include rs140080192 and rs143175221 for the C282Y variant and rs28934889, rs147297176, rs147426902, rs556335391 and rs62625342 for the H63D variant. Interference due to these mutations was not tested.

Interfering Mutations for Early Onset Primary Dystonia:
One potentially interfering mutation was identified for ΔE302/303. The potential interfering mutation is rs188191403. Interference due to this mutation was not tested.

f. Assay Cut-off:

Not applicable.

g. Specimen Stability at 2–8° C

Saliva samples for testing are collected with the Oragene·Dx collection device. See K141410 for sample stability information.

h. Shipping Stability

Saliva samples are shipped for testing in the Oragene·Dx collection device. See K141410 for sample shipping stability information.

2. Comparison Studies:

a. Comparison with Sanger Bidirectional Sequencing:

Comparison for Hereditary Thrombophilia, Alpha-1 Antitrypsin Deficiency, Alzheimer's Disease, Parkinson's Disease, Gaucher Disease:

Accuracy was evaluated through calculation of agreement of the genetic variant determinations between the 23andMe PGS Test and results from Sanger bidirectional sequencing (comparator). All PGS genotyping was performed at an independent laboratory site. Saliva samples were selected from the 23andMe customer biobank based on their predetermined genotype. All chosen samples were then genotyped using Sanger bidirectional sequencing. Genotyping results were compared between the PGS test and bidirectional sequencing to calculate percent agreement (PA) with the sequencing results considered to be "truth." The comparison study for each report is provided below. The accuracy data generated for each test report met the pre-defined acceptance criteria outlined in the special controls.

Comparison for Hereditary Thrombophilia:

The goal of the comparison studies was to evaluate genotype assignment between the 23andMe PGS Test and the gold standard, Sanger bidirectional sequencing, for the Factor V Leiden variant of the F5 gene and the Prothrombin G20210A variant of the F2 gene.

Factor V Leiden variant of the F5 gene				
		Sanger Bidirectional Sequencing		
	Genotype	CC	CT	TT
23andMe PGS Test	CC (homozygous common)	68	0	0
	CT (heterozygous)	0	68	0
	TT (homozygous rare)	0	0	67
	'no calls' or 'invalid"	0	0	0
Total		68	68	67

Percent Agreement (PA) of PGS test results with comparator results of saliva samples:
PA (CC|CC) = 100% (68/68) with 95% CI: 94.7% to 100%;
PA (CT|CT) = 100% (68/68) with 95% CI: 94.7% to 100%;
PA (TT|TT) =100% (67/67) with 95% CI: 94.6% to 100%;
Percent of no calls or invalid is 0.0% (0/203) with 95% CI: 0.0% to 1.9%.

The minor variant frequency for Factor V Leiden in individuals of European descent reported in published literature is 3%–15%; technical (analytical) positive predictive values for 23andMe PGS test results of CT and TT are ≥ 99.5% and ≥ 99.1% correspondingly.

Prothrombin G20210A variant of the F2 gene				
		Sanger Bidirectional Sequencing		
	Genotype	GG	AG	AA
23andMe PGS Test	GG (homozygous common)	68	0	0
	AG (heterozygous)	0	67	0
	AA (homozygous rare)	0	0	66
	'no calls' or 'invalid"	0	0	0

Total	68	67	66

Percent Agreement (PA) of PGS test results with comparator results of saliva samples:
PA (GG|GG) = 100% (68/68) with 95% CI: 94.7% to 100%;
PA (AG|AG) = 100% (67/67) with 95% CI: 94.6% to 100%;
PA (AA|AA) =100% (66/66) with 95% CI: 94.5% to 100%;
Percent of no calls or invalid is 0.0% (0/201) with 95% CI: 0.0% to 1.9%.

The minor variant frequency for Prothrombin G20210A in individuals of European descent reported in published literature is 1%–3%; technical (analytical) positive predictive values for 23andMe PGS test results of AG and AA are ≥ 98.6% and ≥ 97.2% correspondingly.

Comparison for Alpha-1 Antitrypsin Deficiency

The goal of the comparison studies was to evaluate genotype assignment between the 23andMe PGS Test and a gold standard, Sanger bidirectional sequencing for the PI*S and PI*Z variants of the SERPINA1 gene. The study design and acceptance criteria were identical to the study for Hereditary Thrombophilia.

PI*Z variant of the SERPINA1 gene				
		Sanger Bidirectional Sequencing		
	Genotype	CC	CT	TT
23andMe PGS Test	CC (homozygous common)	70	0	0
	CT (heterozygous)	0	68	0
	TT (homozygous rare)	0	0	69
	'no calls' or 'invalid"	0	0	0
Total		70	68	69

Percent Agreement (PA) of PGS test results with comparator results of saliva samples:
PA (CC|CC) = 100% (70/70) with 95% CI: 94.8% to 100%;
PA (CT|CT) = 100% (68/68) with 95% CI: 94.7% to 100%;
PA (TT|TT) =100% (69/69) with 95% CI: 94.7% to 100%;
Percent of no calls or invalid is 0.0% (0/207) with 95% CI: 0.0% to 1.8%.

Minor variant frequency for PI*Z SERPINA1 in individuals of European descent in the 23andMe database was 3.63%; technical (analytical) positive predictive values for 23andMe PGS test result of TT and CT are 99.3% and 99.6% correspondingly.

PI*S variant of the SERPINA1 gene				
		Sanger Bidirectional Sequencing		
	Genotype	TT	AT	AA
23andMe PGS Test	TT (homozygous common)	65	0	0
	AT (heterozygous)	0	68	0
	AA (homozygous rare)	0	0	69

	'no calls' or 'invalid"	0	0	0
Total		65	68	69

Percent Agreement (PA) of device results with comparator results of saliva samples:
PA (TT|TT) = 100% (65/65) with 95% CI: 94.4% to 100%
PA (AT|AT) = 100% (68/68) with 95% CI: 94.7% to 100%
PA (AA|AA) =100% (69/69) with 95% CI: 94.7% to 100%
Percent of no calls or invalid is 0.0% (0/202) with 95% CI: 0.0% to 1.9%

Minor variant frequency for PI*S SERPINA1 in individuals of European descent in the 23andMe database was 7.97%; technical (analytical) positive predictive values for 23andMe PGS test result of TT and CT are 99.7% and 99.8% correspondingly.

Comparison for Late-onset Alzheimer's Disease:

The goal of the comparison study was to evaluate genotype assignment between the 23andMe PGS Test and a gold standard, Sanger bidirectional sequencing for the ε4 variant of the APOE gene. The study design and acceptance criteria were identical to Hereditary Thrombophilia.

ε4 variant of the APOE gene				
		Sanger Bidirectional Sequencing		
	Genotype	TT	CT	CC
23andMe PGS Test	TT (homozygous common)	316	0	0
	CT (heterozygous)	0	126	0
	CC (homozygous rare)	0	1	101
	'no calls' or 'invalid"	4	2	5
Total		320	129	106

Percent Agreement (PA) of device results with comparator results of saliva samples:
PA (TT|TT) = 100% (316/316) with 95% CI: 98.8% to 100%
PA (CT|CT) = 99.2% (126/127) with 95% CI: 95.7% to 99.9%
PA (CC|CC) =100% (101/101) with 95% CI: 96.3% to 100%
Percent of no calls or invalid is 2.0% (11/555) with 95% CI: 1.1% to 3.4%

APOE ε4 genotype frequencies in individuals of European descent reported in published literature are 1.8% for CC genotype and 23.9% for CT genotype; technical (analytical) positive predictive values for 23andMe PGS test results of CC and CT are 90.5% and 99.9% correspondingly.

Comparison for Parkinson's Disease
The goal of the comparison studies was to evaluate genotype assignment between the 23andMe PGS Test and a gold standard, Sanger bidirectional sequencing for the G2019S variant of the LRRK2 gene and the N370S variant of the GBA gene. The

study design and acceptance criteria were identical to the study for Hereditary Thrombophilia.

G2019S variant of the LRRK2 gene				
	Genotype	Sanger Bidirectional Sequencing		
		GG	AG	AA
23andMe PGS Test	GG (homozygous common)	25	0	0
	AG (heterozygous)	0	51	0
	AA (homozygous rare)	0	0	4
	'no calls' or 'invalid"	0	0	0
Total		25	51	4

Percent Agreement (PA) of device results with comparator results of saliva samples:
PA (GG|GG) = 100% (25/25) with 95% CI: 86.7% to 100%
PA (AG|AG) = 100% (51/51) with 95% CI: 93% to 100%
PA (AA|AA) =100% (4/4) with 95% CI: 51.0% to 100%
Percent of no calls or invalid is 0.0% (0/80) with 95% CI: 0.0% to 4.6%

Minor variant frequency for G2019S LRRK2 in Ashkenazi Jewish descent reported in published literature is 1.7%; technical (analytical) positive predictive values for 23andMe PGS test results of AG and AA are 99.2% and 98.4% correspondingly.

Minor variant frequency for G2019S LRRK2 in North African Berber descent reported in published literature is 1.5%; technical (analytical) positive predictive values for 23andMe PGS test results of AG and AA are 99.1% and 98.2% correspondingly.

G2019S LRRK2 heterozygous frequency in European descent observed in the 23andMe database was 0.09%; technical (analytical) positive predictive values for 23andMe PGS test results of AG and AA are 75.9% and 57.6% correspondingly.

N370S variant of the GBA gene				
	Genotype	Sanger Bidirectional Sequencing		
		TT	CT	CC
23andMe PGS Test	TT (homozygous common)	25	0	0
	CT (heterozygous)	0	60	0
	CC (homozygous rare)	0	0	26
	'no calls' or 'invalid"	0	0	0
Total		25	60	26

Percent Agreement (PA) of device results with comparator results of saliva samples:
PA (TT|TT) = 100% (25/25) with 95% CI: 86.7% to 100 %
PA (CT|CT) = 100% (60/60) with 95% CI: 94% to 100%
PA (CC|CC) =100% (26/26) with 95% CI: 87.1% to 100%
Percent of no calls or invalid is 0.0% (0/111) with 95% CI: 0.0 % to 3.3 %

Minor variant frequency for N370S GBA in Ashkenazi Jewish descent reported in published literature is 5-6%; technical (analytical) positive predictive values for 23andMe PGS test results of AG and AA are ≥ 99.7% and ≥ 99.5% correspondingly. Minor variant frequency for N370S GBA in European descent reported in published literature is 0.5%; technical (analytical) positive predictive values for 23andMe PGS test results of AG and AA are 97.2% and 94.6% correspondingly.

Comparison for Gaucher Disease Type 1:

The goal of the comparison study was to evaluate genotype assignment between the 23andMe PGS Test and a gold standard, Sanger bidirectional DNA sequencing for the N370S variant of the GBA gene. The study design and acceptance criteria were identical to the study for Hereditary Thrombophilia.

N370S variant of the GBA gene				
		Sanger Bidirectional Sequencing		
	Genotype	TT	CT	CC
23andMe PGS Test	TT (homozygous common)	25	0	0
	CT (heterozygous)	0	60	0
	CC (homozygous rare)	0	0	26
	'no calls' or 'invalid"	0	0	0
Total		25	60	26

Percent Agreement (PA) of device results with comparator results of saliva samples:
PA (TT|TT) = 100% (25/25) with 95% CI: 86.7% to 100 %
PA (CT|CT) = 100% (60/60) with 95% CI: 94% to 100%
PA (CC|CC) =100% (26/26) with 95% CI: 87.1% to 100%
Percent of no calls or invalid is 0.0% (0/111) with 95% CI: 0.0% to 3.3 %
Minor variant frequency for N370S GBA in Ashkenazi Jewish descent observed in the 23andMe database is 6.52%; technical (analytical) positive predictive values for 23andMe PGS test results of CT and CC are 99.8% and 99.6% correspondingly.

Comparison studies for Gaucher Disease Type 1, Factor XI Deficiency, Celiac Disease and Glucose-6-Phosphate-Dehydrogenase Deficiency:

The Manufacturer affirmed that comparison studies were completed for the variants described in the table below. The study design, acceptance criteria, and results for comparison studies were the same for each of the variants listed in the table below and the same as the studies that were completed for the variants for Hereditary Thrombophilia, AATD, Alzheimer's Disease, Parkinson's Disease and Gaucher Disease, above. Each variant was tested with wild-type, heterozygous, and homozygous samples, except where indicated.

Disease	**Gene**	**Variant**	**Comparison Study**

			Completed
Gaucher Disease Type 1	GBA	84GG$^{\Psi}$	Yes
Gaucher Disease Type 1	GBA	V394L$^{\Psi}$	Yes
Factor XI Deficiency	Factor XI	F283L	Yes
Factor XI Deficiency	Factor XI	E117X	Yes
Factor XI Deficiency	Factor XI	IVS14+1G>A$^{\Psi}$	Yes
Celiac Disease	HLA-DQA1	HLA-DQA1	Yes
Glucose-6-Phosphate Dehydrogenase Deficiency (G6PD)	G6PD	Val68Met*	Yes
Hereditary Hemochromatosis	HFE	C282Y	Yes
Hereditary Hemochromatosis	HFE	H63D	Yes
Early-Onset Primary Dystonia	DYT1	deltaE302/303$^{\Psi}$	Yes

*Hemizygous genotype not included
$^{\Psi}$Homozygous genotype samples were not tested therefore homozygous test results will not be reported to user.

b. Matrix Comparison:
Not applicable. This test is for use with human saliva samples only.

3. Clinical Studies:

a. Disease Description and Clinical Summary

Clinical performance for this test was assessed using published data and studies to demonstrate user comprehension of the labeling and test results. The 23andMe PGS Genetic Health Risk Test meets the special controls for clinical performance.

Hereditary Thrombophilia

The 23andMe PGS Genetic Health Risk Test for Hereditary Thrombophilia is indicated for the detection of the Factor V Leiden variant (rs6025, c.1601G>A) in the F5 gene and the Prothrombin G20210A variant (rs1799963/i3002432, c.*97G>A) in the F2 gene. These variants are associated with higher risk for developing harmful blood clots, or venous thromboembolism (VTE; includes deep vein thrombosis and pulmonary embolism) (Simone, 2013)[1]. Common signs and symptoms of VTE include chest pain, difficulty breathing (in the case of pulmonary embolism), and pain, tenderness, swelling, or redness in one or both legs (in the case of deep vein thrombosis) (Dupras, 2013)[2].

[1] Simone B et al. (2013) "Risk of venous thromboembolism associated with single and combined effects of Factor V Leiden, Prothrombin 20210A and Methylenetetrahydrofolate reductase C677T: a meta-analysis involving over 11,000 cases and 21,000 controls." Eur J Epidemiol. 28(8):621–47.
[2] Dupras D et al. (Updated January 2013) "Institute for Clinical Systems Improvement. Venous Thromboembolism Diagnosis and Treatment." http://bit.ly/VTE0113.

Hereditary thrombophilia is a genetic condition that results in the increased tendency to develop blood clots due to a defect in the coagulation system. The risk of developing VTE, the most common type of blood clot, increases with age and other risk factors (Anderson, 2003)[3]. This condition may also be associated with recurrent late pregnancy loss (Kujovich, 2011)[4]. Hereditary thrombophilia does not usually warrant prophylactic medical management, but anticoagulants are sometimes used in specific situations such as surgery or pregnancy (Kujovich, 2011).

Approximately 1 in 1,000 individuals of European descent in the general population develop VTE each year (Raskob, 2014)[5]; and lifetime risk of developing VTE for individuals from birth to 80 years is 80 in 1,000 individuals. In people with one copy of the Factor V Leiden variant, it is estimated that about 2 out of 1,000 develop VTE each year, and in people with two copies it is estimated that about 15 out of 1,000 develop VTE each year (Heit, 2005[6]; Juul, 2004[7]; Kujovich, 2011). Overall, at least 1 in 20 people of European descent have a variant linked to hereditary thrombophilia (Kujovich, 2011; Roberts, 2009[8]).

The 23andMe PGS Genetic Health Risk Report for Hereditary Thrombophilia specifically presents the test results for these variants. The report describes whether a person has these variants, and provides information about the health risks associated with these variants. It does not provide a definitive determination of a person's overall risk for developing VTE. Many additional factors, including non-genetic factors and genetic variants not covered by the test, also affect whether a person develops VTE (Anderson, 2003). This test does not detect all genetic variants related to hereditary thrombophilia or any other variants in the F5 and F2 genes.

Clinical Summary for Hereditary Thrombophilia

To support test results, a meta-analysis study done by Simone et al. was used to calculate likelihood ratios (LR, an estimate of how the test result affects the chances of a condition). A lower bound of 95% confidence interval for LR greater than 1 indicates that the test result is associated with the disease. The results of this analysis are provided in the table below with a summary of the relevant numbers for each clinical scenario; post-test lifetime risk R is calculated for average lifetime risk of π=8% as $R/(1-R)=LR\cdot\pi/(1-\pi)$.

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[3] Anderson FA and Spencer FA. (2003) "Risk factors for venous thromboembolism." Circulation.107(23 Suppl 1):I9–16.
[4] Kujovich JL. (2011) "Factor V Leiden thrombophilia." Genet Med. 13(1):1–16.
[5] Raskob GE et al. (2014) "Thrombosis: a major contributor to global disease burden." Arterioscler Thromb Vasc Biol. 34(11):2363–71.
[6] Heit JA et al. (2005) "The incidence of venous thromboembolism among Factor V Leiden carriers: a community-based cohort study." J Thromb Haemost. 3(2):305–11.
[7] Juul K et al. (2004) "Factor V Leiden and the risk for venous thromboembolism in the adult Danish population." Ann Intern Med. 140(5):330–7.
[8] Roberts et al. (2009) "Venous thromboembolism and ethnicity." Br J Haematol. 146(4):369–83.

Test Results	References	Study Summary	LR	95% CI for LR	Post-test risk for π=8%
Zero F5 or F2 variants	Simone, 2013	For the joint risk estimates across F2 and F5 genes, the study included meta-analysis of 4,343 Caucasian cases with VTE and 3,725 controls. (Overall analysis comprised of 11,239 cases and 21,521 controls across 31 studies.)	0.99	0.98 to 0.99	Average Risk
"Heterozygous F5" One copy	Simone, 2013	For the Factor V Leiden risk estimates, the study included meta-analysis of 9,081 Caucasian cases with VTE and 17,513 controls. (Overall analysis comprised of 11,239 cases and 21,521 controls across 31 studies.)	2.69	2.51 to 2.89	19.0%
"Homozygous F5" Two Copies	Simone, 2013	For the Factor V Leiden risk estimates, the study included meta-analysis of 9,081 Caucasian cases with VTE and 17,513 controls. (Overall analysis comprised of 11,239 cases and 21,521 controls across 31 studies.)	9.32	6.01 to 14.46	44.8%
"F2 AG Heterozygote" One copy	Simone, 2013	For the PT20210A variant risk estimates, the study included meta-analysis of 9,134 Caucasian cases with VTE and 17,606 controls. (Overall analysis comprised of 11,239 cases and 21,521 controls across 31 studies.)	2.34	2.15 to 2.57	16.9%
"F2 AA Homozygote" Two Copies	Simone, 2013	For the PT20210A variant risk estimates, the study included meta-analysis of 9,134 Caucasian cases with VTE and 17,606 controls. (Overall analysis comprised of 11,239 cases and 21,521 controls across 31 studies.)	13.49	4.73 to 38.45	54.0%
At Least one F2 copy + At least one F5 copy	Simone, 2013	For the joint risk estimates across F2 and F5 genes, the study included meta-analysis of 4,343 Caucasian cases with VTE and 3,725 controls. (Overall analysis comprised of 11,239 cases and 21,521 controls across 31 studies.)	1.32*	1.06 to 1.66	10.3%

*The risk estimate for one copy of each variant is not as high as for people with two copies of one variant. But because a patient with both genotypes (F5 and F2) are managed similarly in a clinical setting, the test report provided to the user will have the same interpretation as the test report for both genotypes.

Alpha-1 Antitrypsin Deficiency

The 23andMe PGS Genetic Health Risk Test for Alpha-1 Antitrypsin Deficiency is indicated for the detection of the PI*Z variant (rs28929474, c.1096G>A) and the PI*S

variant (rs17580, c.863A>T) in the SERPINA1 gene. The PI*Z variant is associated with lower serum levels of the alpha-1 antitrypsin (AAT) protein, which increases the risk of both emphysema and liver disease (American Thoracic Society and European Respiratory Society, 2003)[9]. The PI*S variant is associated with slightly lower serum levels of the AAT protein. Although the PI*S variant is not associated with clinical symptoms on its own, individuals who have both the PI*Z and PI*S variants do have an increased risk of emphysema (American Thoracic Society and European Respiratory Society, 2003). It is estimated that 98% of individuals with diseases related to AATD have harbor the PI*Z variant (Blanco, 2017[10]). Typical signs and symptoms of AAT deficiency include shortness of breath and wheezing, chronic cough, recurrent lung infections, lung disease including emphysema, and liver disease including cirrhosis (American Thoracic Society and European Respiratory Society, 2003; Stoller, 2006[11]).

The variants associated with AATD lead to amino acid substitutions in the Alpha-1 Antitrypsin (AAT) protein that decrease serum concentrations. In individuals with the PI*Z variant, variant AAT protein accumulates in the liver. Low levels of serum AAT lead to increased neutrophil elastase enzyme activity in the lung, causing tissue damage and lung disease. Accumulation of variant AAT protein in the liver is the most widely accepted explanation for the cause of lung disease in individuals with the PI*Z variant (American Thoracic Society and European Respiratory Society, 2003).

AAT deficiency is a genetic condition, and the risk of developing symptoms is influenced by factors such as smoking, family history, and occupational exposure to mineral dust, airborne chemicals, and other pollutants (American Thoracic Society and European Respiratory Society, 2003; Senn, 2005[12]). There is no cure for AAT deficiency, and treatment focuses on managing the symptoms of emphysema and/or liver disease (Stoller, 2006). People with this condition are encouraged to avoid smoking (Stoller, 2006).

AAT deficiency is most common in people of Northern European descent (American Thoracic Society and European Respiratory Society, 2003; de Serres and Blanco, 2012[13]). In the United States, 1 in 3000 to 1 in 5000 people has this condition (American Thoracic Society and European Respiratory Society, 2003). Individuals who have one or two copies of the PI*S variant are likely not at increased risk for either emphysema or liver disease (Stoller, 2006). Individuals who have one copy of the PI*Z variant may have

[9] American Thoracic Society; European Respiratory Society (2003) "American Thoracic Society/European Respiratory Society statement: standards for the diagnosis and management of individuals with alpha-1 antitrypsin deficiency." Am J Respir Crit Care Med. 168(7):818–900.
[10] Blanco I et. al. (2017) “A lpha-1 antitrypsin Pi*Z gene frequency and Pi*ZZ genotype numbers worldwide: an update.” International Journal of COPD 2017:12 561–569
[11] Stoller JK et al. (2006) "Alpha-1 Antitrypsin Deficiency." In: Pagon RA et al., editors. GeneReviews.
[12] Senn O et al. (2005) "alpha1-Antitrypsin deficiency and lung disease: risk modification by occupational and environmental inhalants." Eur Respir J. 26(5):909–17.
[13] de Serres FJ and Blanco I (2012). Prevalence of α1-antitrypsin deficiency alleles PI*S and PI*Z worldwide and effective screening for each of the five phenotypic classes PI*MS, PI*MZ, PI*SS, PI*SZ, and PI*ZZ: a comprehensive review. Ther Adv Respir Dis. 6(5):277–95.

a higher risk of developing emphysema if they smoke (Molloy, 2014)[14], but evidence is mixed regarding whether these individuals have an increased risk of developing liver disease (American Thoracic Society and European Respiratory Society, 2003). Individuals who have two copies of the PI*Z variant have a >80% chance of developing emphysema during their lifetime (Stoller, 2006), and a 30–40% chance of developing liver disease after the age of 50 (American Thoracic Society and European Respiratory Society, 2003). Individuals who have one copy of the PI*S variant and one copy of the PI*Z variant and who smoke have a 20–50% chance of developing emphysema during their lifetime (Stoller, 2006), but there is not enough evidence to determine whether these individuals have an increased risk of developing liver disease.

Clinical Summary for Alpha-1 Antitrypsin Deficiency

Deficiency in Alpha-1 Antitrypsin (AAT), a serum protease inhibitor, has been demonstrated to increase the risk of developing emphysema and liver disease. AAT is encoded by the SERPINA1 gene. There are > 50 known alleles of this gene, but the most common are the PI*M, PI*S, and PI*Z alleles (Blanco, 2017). The PI*Z allele is the most common allele associated with AAT deficiency, and individuals who are homozygous for this variant have significantly lower concentrations of serum AAT. Other genotypes including PI*MZ and PI*SZ are associated with AATD, but factors including smoking or exposure to other hazardous respiratory environments contribute to the unmasking of AATD and progression of disease in individuals with these genotypes.

Test Result	References	Study Summary
PI*MS	Stoller, 2006	**Lung disease**: Individuals with this genotype are not likely at increased risk of developing emphysema.
		Liver disease: Individuals with this genotype are not likely at increased risk of cirrhosis.
PI*SS	American Thoracic Society and European Respiratory Society, 2003; Molloy, 2014	**Lung disease**: Individuals with this genotype are not likely at increased risk of developing emphysema
		Liver disease: Individuals with this genotype are not likely at increased risk of cirrhosis
PI*MZ	American Thoracic Society and European Respiratory Society, 2003; Molloy, 2014	**Lung disease**: Individuals with this genotype who are non-smokers are not likely at increased risk of developing emphysema. Smokers with this genotype have a slightly increased risk of developing emphysema. Additional clinical studies are needed to estimate the risk of lung disease in individuals with this genotype.

[14] Molloy K et al. (2014) "Clarification of the risk of chronic obstructive pulmonary disease in alpha1-antitrypsin deficiency PiMZ heterozygotes." Am J Respir Crit Care Med. 189(4):419–27.

Test Result	References	Study Summary
		Liver disease: Only a few studies have looked at risk for liver disease in individuals with this genotype, and findings have been inconclusive. More clinical studies are needed to determine if there is any additional risk of liver disease in individuals with this genotype.
PI*SZ	American Thoracic Society and European Respiratory Society, 2003; Stoller, 2006	**Lung disease**: Individuals with this genotype who are non- smokers are not likely at increased risk of developing emphysema. However, clinical studies estimate that 20–50% of smokers with this genotype will develop signs and symptoms of emphysema during their lifetime.
		Liver disease: There are not enough clinical studies of individuals with this genotype to determine whether they are at increased risk for liver disease including cirrhosis.
PI*ZZ	American Thoracic Society and European Respiratory Society, 2003; Stoller, 2006	**Lung disease**: Individuals with this genotype have an increased risk of developing emphysema. Clinical studies have demonstrated that greater than 80% of individuals with this genotype will develop signs and symptoms of emphysema during their lifetime.
		Liver disease: Individuals with this genotype have an increased risk of developing liver disease. Clinical studies have demonstrated that that individuals with this genotype have a 30–40% chance of developing cirrhosis after the age of 50

Late-onset Alzheimer's disease

The 23andMe PGS Genetic Health Risk Report for Late-onset Alzheimer's Disease is indicated for detection of the ε4 variant (rs429358, c.388T>C) in the APOE gene (Reitz, 2013)[15]. The gene for APOE, the major cholesterol transporter, has common variants ε2, ε3, and ε4. The ε4 variant, rs429358, is associated with higher risk of developing Alzheimer's Disease (Alzheimer's Association, 2016[16]; Reitz, 2013) at or after the age of 65 years. Typical signs and symptoms of Alzheimer's Disease include memory loss that worsens with time, mood and personality changes, difficulty planning or solving problems, confusion with time or place, and trouble completing activities of daily living (Alzheimer's Association, 2016). There is currently no cure for Alzheimer's Disease, but certain medications may be used to delay or ease symptoms (Alzheimer's Association, 2016).

[15] Reitz C et al. (2013) "Variants in the ATP-binding cassette transporter (ABCA7), apolipoprotein E ϵ4,and the risk of late-onset Alzheimer disease in African Americans." JAMA. 309(14):1483–92.
[16] Alzheimer's Association. (2016) "2016 Alzheimer's disease facts and figures." Alzheimers Dement. 12(4):459–509.

Approximately 40–65% of Alzheimer's patients have one or two copies of the ε4 variant, but not all people who have one or two copies of the ε4 variant will develop Alzheimer's Disease (Alzheimer's Association, 2016). The risk of developing Alzheimer's Disease is also influenced by factors such as age, sex, family history, heart health, diet, intellectual activity, exercise, ethnicity, and other genes (Altmann, 2014[17]; Alzheimer's Association, 2016; Beecham, 2014[18]; Cass, 2017[19]; Cupples, 2004[20]; Mayeda, 2016[21]; Morris, 2015[22]; Naj, 2014[23]; Reitz, 2011[24]; Sando, 2008[25]; Satizabal, 2016[26]; Tang, 1998[27]). Alzheimer's Disease affects people of all ethnicities, but elderly African Americans and Hispanics are more likely to develop this condition than people of other ethnicities (Alzheimer's Association, 2016; Tang, 1998). Approximately 1 in 9 people over the age of 65 has Alzheimer's Disease (Alzheimer's Association, 2016). In the general population, <1% of people are expected to develop Alzheimer's Disease by age 65, 3% of people are expected to develop Alzheimer's Disease by age 75, and 11-14% of people are expected to develop Alzheimer's Disease by age 85 (Genin, 2011). Among people of European descent with no copies of ε4, <1% of people are expected to develop Alzheimer's Disease by age 65, 1–2% of people are expected to develop Alzheimer's disease by age 75, and 5–10% of people are expected to develop Alzheimer's Disease by age 85 (Genin, 2011). Among people of European descent with one copy of ε4, <1 to 1% of people are expected to develop Alzheimer's Disease by age 65, 4–7% of people are expected to develop Alzheimer's Disease by age 75, and 20–30% of people are expected to develop Alzheimer's Disease by age 85 (Genin, 2011). Among people of European descent with two copies of ε4, 2–4% of people are expected to develop Alzheimer's disease by age 65, 28% of people are expected to develop Alzheimer's Disease by age 75, and 51–60% of people are expected to develop Alzheimer's Disease by age 85 (Genin, 2011).

[17] Altmann A et al. (2014) "Sex modifies the APOE-related risk of developing Alzheimer disease." Ann Neurol. 75(4):563–73.
[18] Beecham GW et al. (2014) "Genome-wide association meta-analysis of neuropathologic features of Alzheimer's disease and related dementias." PLoS Genet. 10(9):e1004606.
[19] Cass SP. (2017) "Alzheimer's Disease and Exercise: A Literature Review." Curr Sports Med Rep. 16(1):19–22.
[20] Cupples LA et al. (2004) "Estimating risk curves for first-degree relatives of patients with Alzheimer's disease: the REVEAL study." Genet Med. 6(4):192–6.
[21] Mayeda ER et al. (2016) "Inequalities in dementia incidence between six racial and ethnic groups over 14 years." Alzheimers Dement. 12(3):216–24.
[22] Morris MC et al. (2015) "MIND diet associated with reduced incidence of Alzheimer's disease." Alzheimers Dement. 11(9):1007–14.
[23] Naj AC et al. (2014) "Effects of multiple genetic loci on age at onset in late-onset Alzheimer disease: a genome-wide association study." JAMA Neurol. 71(11):1394–404.
[24] Reitz C et al. (2011) "Epidemiology of Alzheimer disease." Nat Rev Neurol. 7(3):137–52.
[25] Sando SB et al. (2008) "Risk-reducing effect of education in Alzheimer's disease." Int J Geriatr Psychiatry. 23(11):1156–62.
[26] Satizabal CL et al. (2016). "Incidence of Dementia over Three Decades in the Framingham Heart Study." N Engl J Med. 374(6):523–32.)
[27] Tang MX et al. (1998) "The APOE-epsilon4 allele and the risk of Alzheimer disease among African Americans, whites, and Hispanics." JAMA. 279(10):751–5.

The 23andMe PGS Genetic Health Risk Report for Alzheimer's Disease presents the test results for only the ε4 variant. The report describes whether a person has this variant and provides information about risk associated with this variant. It does not provide a definitive determination of a person's overall risk for developing Alzheimer's Disease. Many additional factors, including non-genetic factors and genetic variants not covered by the test, also affect whether a person develops Alzheimer's Disease (Alzheimer's Association, 2016). This test does not detect all genetic variants related to Alzheimer's Disease or any other variants in the APOE gene.

Clinical Summary for Late-onset Alzheimer's Disease

The likelihood ratios presented in the table below are estimated based on published studies of variant frequencies in various populations. A lower bound of 95% confidence interval for LR greater than 1 indicates that the test result is associated with the disease.

Test Result	Genotype	Ethnicity	LR	95% CI for LR	References	Study Summary
No ε4	ε2/ε2	European	0.3	0.1-–0.5	Farrer, 1997[28]	A meta-analysis of 5930 patients who met criteria for probable or definite Alzheimer's disease and 8607 controls. Among study participants, there were 5107 Caucasian Alzheimer's patients and 6262 Caucasian controls; 235 African American Alzheimer's patients and 240 African American controls; 261 Hispanic Alzheimer's patients and 267 Hispanic controls; and 336 East Asian Alzheimer's patients and 1977 East Asian controls.
	ε2/ε3	European	0.4	0.3–0.4		
	ε3/ε3	European	0.6	0.57–0.62		
One copy of ε4 variant	ε2/ε4	European	1	0.8–1.3		
	ε3/ε4	European	1.9	1.8–2.1		
Two copies of ε4	ε4/ε4	European	8.2	6.8–10.0		
No ε4	ε2/ε2	African American	0.3	0.04–2.7	Murrell, 2006[29]	This study included 162 African American cases with Alzheimer's Disease and 318 controls.
	ε2/ε3	African American	0.5	0.3–0.9		
	ε3/ε3	African American	0.7	0.6–0.9		
One copy of ε4	ε2/ε4	African American	0.9	0.4–2.3		
	ε3/ε4	African American	1.6	1.2–2.1		

[28] Farrer LA et al. (1997) "Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium." JAMA. 278(16):1349–56.
[29] Murrell JR et al. (2006) "Association of apolipoprotein E genotype and Alzheimer disease in African Americans." Arch Neurol. 63(3):431–4.

Test Result	Genotype	Ethnicity	LR	95% CI for LR	References	Study Summary
Two copies of ε4	ε4/ε4	African American	3.8	1.9–7.6		
No ε4	ε2/ε2	South Asian	0.1	(0.02–0.9)	Agarwal, 2014[30]	A meta-analysis of 417 South Asian cases with Alzheimer's Disease and 651 controls.
	ε2/ε3	South Asian	0.5	(0.3–0.8)		
	ε3/ε3	South Asian	0.6	(0.6–0.7)		
One copy of ε4	ε2/ε4	South Asian	4.1	(2.0–8.3)		
	ε3/ε4	South Asian	3.2	(2.5–4.1)		
Two copies of ε4	ε4/ε4	South Asian	5.7	(2.3–14.0)		
No ε4	ε2/ε2	East Asian	1.1	(0.5–2.3)	Liu, 2014[31]	A large study that included 1,576 Chinese cases with Alzheimer's Disease and 1,741 controls.
	ε2/ε3	East Asian	0.7	(0.5–0.8)		
	ε3/ε3	East Asian	0.7	(0.6–0.7)		
One copy of ε4	ε2/ε4	East Asian	1.9	(1.2–3.0)		
	ε3/ε4	East Asian	2.5	(2.2–2.9)		
Two copies of ε4	ε4/ε4	East Asian	25.6	(10.5–62.6)		

Parkinson's Disease

The 23andMe PGS Genetic Health Risk Test for Parkinson's Disease is indicated for the detection of the G2019S variant (rs34637584, c.6055G>A) in the LRRK2 gene and the N370S variant (rs76763715, c.1226A>G) in the GBA gene. These variants are associated with higher risk for developing Parkinson's Disease (Clark, 2006[32]; Sidransky, 2009[33]). Typical signs and symptoms of Parkinson's disease include slow movement, tremor, muscle stiffness, problems with balance, and memory loss in some cases (Farlow, 2004)[34]. There is currently no cure for Parkinson's Disease, but certain medications may be used to delay or ease symptoms (Farlow, 2004).

[30] Agarwal R et al. (2014) "Association of apolipoprotein E genetic variation in Alzheimer's disease in Indian population: a meta-analysis." Am J Alzheimers Dis Other Demen. 29(7):575–82.
[31] Liu M et al. (2014) "Apolipoprotein E gene polymorphism and Alzheimer's disease in Chinese population: a meta-analysis." Sci Rep. 4:4383.
[32] Clark LN et al. (2006) "Frequency of LRRK2 mutations in early- and late-onset Parkinson disease." Neurology. 67(10):1786–91
[33] Sidransky E et al. (2009) "Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease." N Engl J Med. 361(17):1651–61.
[34] Farlow J et al. (2004) "Parkinson Disease Overview." In: Pagon RA et al., editors. GeneReviews.

Most cases of Parkinson's disease are sporadic and approximately 10% of all Parkinson's cases are familial (Klein and Westenberger, 2012)[35]. The same genetic variants can cause both sporadic and familial Parkinson's disease. 1–2% of all cases of Parkinson's Disease and 5–7% of familial cases are linked to the G2019S variant in the LRRK2 gene (Farlow, 2004). 8–14% of all cases of Parkinson's Disease are linked to variants in the GBA gene (Klein and Westenberger, 2012), and approximately half of these are linked to the N370S variant (Sidransky, 2009). The risk of developing Parkinson's Disease is also influenced by factors such as age, family history, sex, and exposure to certain chemicals (Farlow, 2004; Kenborg, 2015[36]; Noyce, 2012[37]; Wooten, 2004[38]).

Parkinson's disease affects people of all ethnicities, and approximately 1 in 100 people over the age of 55 has Parkinson's Disease (Farlow, 2004). Approximately 25% of individuals of European or Ashkenazi Jewish descent who have one copy of the G2019S variant will develop Parkinson's Disease during their lifetime (Clark, 2006; Marder, 2015[39]). Roughly 6% of individuals of Ashkenazi Jewish descent who have one copy of the N370S variant will develop Parkinson's Disease during their lifetime, as will 9% of individuals of Ashkenazi Jewish descent who have two copies of the N370S variant (Alcalay, 2014)[40].

The 23andMe PGS Genetic Health Risk Report for Parkinson's Disease detects the G2019S variant (rs34637584, c.6055G>A) in the LRRK2 gene and the N370S variant (rs76763715, c.1226A>G) in the GBA gene. The report describes whether a person has these variants, and provides information about risk of developing Parkinson's Disease associated with these variants. It does not provide a definitive determination of a person's overall risk for developing Parkinson's Disease. Many additional factors affect whether a person develops Parkinson's Disease, including non-genetic factors and genetic variants not covered by the test (Farlow, 2004; Noyce, 2012). This test does not detect all genetic variants related to Parkinson's Disease or any other variants in the LRRK2 and GBA genes.

Clinical Summary for Parkinson 's Disease

The following table summarizes the likelihood ratios estimated based on published studies of the frequencies of the claimed Parkinson's disease variants in the various

[35] Klein C and Westenberger A. (2012) "Genetics of Parkinson's disease." Cold Spring Harb Perspect Med. 2(1):a008888.
[36] Kenborg L et al. (2015) "Lifestyle, family history, and risk of idiopathic Parkinson disease: a large Danish case-control study." Am J Epidemiol. 181(10):808–16.
[37] Noyce AJ et al. (2012) "Meta-analysis of early nonmotor features and risk factors for Parkinson disease." Ann Neurol. 72(6):893–901.
[38] Wooten GF et al. (2004) "Are men at greater risk for Parkinson's disease than women?" J Neurol Neurosurg Psychiatry. 75(4):637–9.
[39] Marder K et al. (2015) "Age-specific penetrance of LRRK2 G2019S in the Michael J. Fox Ashkenazi Jewish LRRK2 Consortium." Neurology. 85(1):89–95.
[40] Alcalay RN et al. (2014) "Comparison of Parkinson risk in Ashkenazi Jewish patients with Gaucher disease and GBA heterozygotes." JAMA Neurol. 71(6):752–7.

populations. A lower bound of 95% confidence interval for LR greater than 1 indicates that the test result is associated with the disease.

Test result	Ethnicity	LR	95% CI for LR	References	Study Summary
No G2019S variants detected	Ashkenazi Jewish	0.87	0.83 – 0.91	Orr-Urtreger,2007[41]	A study of 344 Ashkenazi Jewish patients with Parkinson's Disease and 252 Ashkenazi Jewish age and sex-matched controls. 51 cases were heterozygous for G2019S, compared to 5 controls. A secondary analysis was run to correct for the potential masking effect of genes associated with early-onset Parkinson's Disease, which included 254 Ashkenazi Jewish late-onset Parkinson's Disease patients (>50 years old) and 318 Ashkenazi Jewish controls (also >50 years old). 35 cases were heterozygous for G2019S, compared to 8 controls.
One or two copies of G2019S detected *	Ashkenazi Jewish	6.23	3.77– 10.3)		
No N370S variants	Ashkenazi Jewish	0.93	0.91 – 0.96	Gan-Or, 2015[42]	A meta-analysis of 11,453 patients with Parkinson's Disease and 14,565 controls. Among study participants with 0 copies of N370S, there were 869 Ashkenazi Jewish individuals with Parkinson's Disease and 3,580 controls. Among study participants with 1 or 2 copies of N370S, there were 131 Ashkenazi Jewish individuals with Parkinson's Disease and 225 controls. The number of cases and number of controls for each genotype were used to calculate likelihood ratios for people of Ashkenazi Jewish descent.
One or two copies of N370S detected *	Ashkenazi Jewish	2.08	1.69 – 2.56		
No G2019S variants detected	European	Not available	Not available	Not available	Not available
One or two copies of G2019S detected *	European	Not available	Not available		
No N370S variants	European	0.97	0.96 – 0.99	Lesage, 2011[43]	A study of 1130 European Parkinson's disease patients and 391 controls. The cases included 525 families and 605 patients with sporadic Parkinson's disease. 35 cases and 2 controls carried the N370S variant
One or two copies of N370S detected *	European	6.11	1.48 – 25.28		

[41] Orr-Urtreger A et al. (2007) "The LRRK2 G2019S mutation in Ashkenazi Jews with Parkinson disease: is there a gender effect?" Neurology. 69(16):1595–602.
[42] Gan-Or, Z., Amshalom, I., Kilarski, L. L., Bar-Shira, A., Gana-Weisz, M., Mirelman, A. Orr-Urtreger, A. (2015). Differential effects of severe vs mild GBA mutations on Parkinson disease. Neurology, 84(9), 880–887.
[43] Lesage S et al. (2011) "Large-scale screening of the Gaucher's disease-related glucocerebrosidase gene in Europeans with Parkinson's disease." Hum Mol Genet. 20(1):202–10.

Test result	Ethnicity	LR	95% CI for LR	References	Study Summary
No G2019S variants detected	North African Berber	0.64	0.60 – 0.68	Trinh, 2014[44]	A study of Arab-Berber individuals from Tunisia; 220 unrelated participants were heterozygous for the G2019S variant, and 46 participants were relatives who had Parkinson's disease. The lifetime risk of developing Parkinson's disease was 98% at age 80 for the probands, and 91% at age 80 for the affected relatives.
One or two copies of G2019S detected *	North African Berber	12.67	7.17 – 22.33		
No N370S variants	North African Berber	Not available	Not available	Not available	Not available
One or two copies of N370S detected *	North African Berber	Not available	Not available		

* Subjects with one and two copies of these variants are combined into one group because people with two copies are extremely rare.

Gaucher Disease Type 1

The 23andMe PGS Genetic Health Risk Test for Gaucher Disease Test is intended to detect and identify three variants in the GBA gene (N370S, 84GG, and V394L) to provide information on genetic health risks for individuals with certain combinations of these variants. These variants are primarily found in individuals with type 1 Gaucher disease, although rare individuals with an 84GG or V394L variant and a second, non-N370S variant, may have type 2 or type 3 Gaucher disease (Koprivica, 2000;[45] Theophilus, 1989[46]).

Gaucher Disease is an inherited disorder caused by mutations in the GBA gene encoding an enzyme called glucocerebrosidase (Tsuji[47], 1987; Tsuji, 1988[48]). Loss of function mutations in GBA can cause a lipid called glucocerebroside to build up to harmful levels in various organs.

There are three main types of Gaucher Disease, and type 1 – which includes approximately 90% of patients – is distinct due to its lack of central nervous system

[44] Trinh J et al. (2014) "Comparative study of Parkinson's disease and leucine-rich repeat kinase 2 p.G2019S parkinsonism." Neurobiol Aging. 35(5):1125–31.
[45] Koprivica, V., Stone, D. L., Park, J. K., Callahan, M., Frisch, A., Cohen, I. J., … Sidransky, E. (2000). "Analysis and classification of 304 mutant alleles in patients with type 1 and type 3 Gaucher disease." American Journal of Human Genetics, 66(6), 1777–1786.
[46] Theophilus, B., Latham, T., Grabowski, G. A., & Smith, F. I. (1989)." Gaucher disease: molecular heterogeneity and phenotype-genotype correlations." American Journal of Human Genetics, 45(2), 212–225.
[47] Tsuji S et al. (1987). "A mutation in the human glucocerebrosidase gene in neuronopathic Gaucher's disease." N Engl J Med. 316(10):570–5.
[48] Tsuji S et al. (1988). "Genetic heterogeneity in type 1 Gaucher disease: multiple genotypes in Ashkenazic and non-Ashkenazic individuals." Proc Natl Acad Sci U S A. 85(7):2349–52

involvement (Mistry, 2015)[49]. The main clinical features of type 1 Gaucher Disease include bone abnormalities, enlarged liver and spleen, anemia, low platelet counts, and growth impairment (Grabowski, 2008)[50]; patients with type 2 and type 3 disease—which are typically much more severe— also present with early-onset neurological symptoms. Gaucher Disease is relatively common among individuals of Ashkenazi Jewish descent, affecting approximately 1 in 850 individuals, compared with 1 in 40,000 individuals in the general population (Mistry, 2015). About one out of every 18 people with Ashkenazi Jewish ancestry is a carrier for Gaucher Disease (Gross, 2008)[51].
More than 300 different disease-causing variants have been identified in the GBA gene (Grabowski, 2008). Four specific GBA mutations—N370S, 84GG, L444P, and IVS2+1G>A—are included in the standard panel of genetic disease tests available to couples of Ashkenazi Jewish ancestry who are pregnant or considering having a child and are recommended by the American College of Medical Genetics and Genomics (ACMG) (Gross, 2008). The PGS Test will include two of these four variants, (N370S and 84GG) plus a third variant (V394L). This test is unable to detect the remaining two ACMG-recommended variants. Together, the PGS Test will cover approximately 92% of the disease-causing alleles in individuals of Ashkenazi Jewish descent (Gross, 2008).

The presence of an N370S variant—in either a homozygous or compound heterozygous form—could lead to Gaucher Disease type 1. Although the disease course in compound heterozygotes is typically more severe than in N370S homozygotes, there is significant phenotypic variability among individuals with both genotypes.

Early studies suggested that more than half of N370S homozygotes may remain asymptomatic throughout adulthood (Grabowski, 1997)[52], although more recent studies suggest that these individuals are likely to have disease manifestations upon follow-up (Balwani, 2010)[53].

N370S is the most common GBA allele, accounting for 86% of alleles among Ashkenazi Jewish Gaucher Disease patients (Gross., 2008) and 30% of alleles among Gaucher Disease patients of non-Ashkenazi Jewish descent (Horowitz,1993[54]; Filocamo, 2002[55]). N370S and V394L are missense variants that result in reduced enzymatic activity (Montfort, 2004[56]; Grace, 1994[57]); 84GG is a one-nucleotide insertion that results in a truncated protein lacking enzymatic activity (Beutler, 1991)[58].

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[49] Mistry PK et al. (2015). "Understanding the natural history of Gaucher disease." Am J Hematol. 90 Suppl 1:S6–11
[50] Grabowski GA. (2008). "Phenotype, diagnosis, and treatment of Gaucher's disease." Lancet. 372(9645):1263–71.
[51] Gross SJ et al. (2008). "Carrier screening in individuals of Ashkenazi Jewish descent." Genet Med. 10(1):54–6.
[52] Grabowski GA. (1997). "Gaucher disease: gene frequencies and genotype/phenotype correlations." Genet Test. 1(1):5–12
[53] Balwani M et al. (2010). "Type 1 Gaucher disease: significant disease manifestations in "asymptomatic"homozygotes." Arch Intern Med. 170(16):1463–9
[54] Horowitz M et al. (1993). "Prevalence of nine mutations among Jewish and non-Jewish Gaucher disease patients." Am. J. Hum. Genet. 53(4):921–930.
[55] Filocamo M et al. (2002). "Analysis of the glucocerebrosidase gene and mutation profile in 144 Italian gaucher patients." Hum. Mutat. 20(3):234–5.
[56] Montfort M et al. (2004). "Functional analysis of 13 GBA mutant alleles identified in Gaucher disease patients: Pathogenic changes and "modifier" polymorphisms." Hum Mutat. 23(6):567–75.

Clinical Summary for Gaucher Disease Type 1

Individuals with two copies of the N370S variant will be told they are at risk of developing symptoms of Gaucher Disease Type 1. Numerical risk estimates for disease manifestation range from 30% (Beutler, 1993)[59] to nearly 100% (Mistry, 2015), depending on whether symptoms are self-reported or detected by clinical, laboratory, or imaging tests. Because of this variability, numerical risk estimates will not be provided in the report.

Test Result	Ancestry	Number of patients	Phenotype	References
N370S/N370S	Ashkenazi Jewish	54	Mild Gaucher Disease	Beutler, 1992[60]
N370S/N370S	Jewish and non-Jewish European	83	Type 1 Gaucher Disease	Horowitz, 1993
N370S/N370S	Spanish	26	Type 1 Gaucher Disease	Alfonso, 2007[61]
N370S/N370S	Italian	13	Type 1 Gaucher Disease	Filocamo, 2002
N370S/84GG	Ashkenazi Jewish	25	More severe symptoms than N370S homozygotes, on average	Beutler, 1992
N370S/84GG	Jewish	35	Type 1 Gaucher Disease	Horowitz, 1993
N370S/84GG	Spanish	4	Type 1 Gaucher Disease	Alfonso, 2007
N370S/V394L	Ashkenazi Jewish	3	Not specified	Beutler, 1992
N370S/V394L	Primarily Jewish	18	Relatively mild Gaucher Disease	Elstein, 2005[62]

Factor XI Deficiency

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[57] Grace, M. E., Desnick, R. J., & Pastores, G. M. (1997).” Identification and expression of acid beta-glucosidase mutations causing severe type 1 and neurologic type 2 Gaucher disease in non-Jewish patients” Journal of Clinical Investigation, 99(10), 2530–2537.

[58] Beutler, E., Gelbart, T., Kuhl, W., Sorge, J., & West, C. (1991).” Identification of the second common Jewish Gaucher disease mutation makes possible population-based screening for the heterozygous state.” Proceedings of the National Academy of Sciences of the United States of America, 88(23), 10544–10547.

[59] Beutler E et al. (1993). "Gaucher disease: gene frequencies in the Ashkenazi Jewish population." Am J Hum Genet. 52(1):85–8.

[60] Beutler E et al. (1992). "Mutations in Jewish patients with Gaucher disease." Blood. 79(7):1662–6.

[61] Alfonso P et al (2007). "Mutation analysis and genotype/phenotype relationships of Gaucher disease patients in Spain." J Hum Genet. 52(5):391–6.

[62] Elstein D, Guedalia J, Doniger GM, et al. (2005).” Computerized cognitive testing in patients with type I Gaucher disease: effects of enzyme replacement and substrate reduction.” Genet Med. 7(2):124–30.

The 23andMe PGS Genetic Health Risk Report for Factor XI deficiency (also known as Hemophilia C or Rosenthal syndrome) is indicated for reporting of the variants F283L, E117X, and IVS14+1G>A in the *F11* gene (MIM number 264900). This test is intended to determine if a person has one or a combination of these variants, which are associated with the potential for a higher risk of excessive bleeding following trauma or surgery involving tissues with high fibrinolytic activity (for example, the urogenital tract and oral cavity; (Asakai., 1991[63]; Gomez and Bolton-Maggs, 2008[64]). Individuals with Factor XI deficiency do not have increased risk for spontaneous bleeding and Factor XI deficiency is considered a mild to moderate bleeding disorder (Gomez and Bolton-Maggs, 2008). The three variants reported by this test are most common in individuals of Ashkenazi Jewish descent, making this test most relevant for this population.

Currently the diagnosis for severe Factor XI deficiency is demonstration of a prolonged aPTT (activated partial thromboplastin time) test result, and normal prothrombin time (PT) and thrombin time (TT). Factor XI assays to measure factor activity can be used for confirmation of a deficiency. Current treatment is only necessary for patients who are undergoing surgery or dental extraction, with treatment consisting of fresh frozen plasma administration.

Inherited Factor XI deficiency is rare in the general population, with an estimated prevalence of 1/1,000,000 people (OrphaNet, ORPHA:329). Certain ethnic groups have significantly increased prevalence of inherited Factor XI deficiency, with the most significant prevalence attributed to the Ashkenazi Jewish population, with one study indicating ~8% of subjects were heterozygous for a variant associated with Factor XI deficiency (and thus at least partial Factor XI deficiency; Shpilberg, 1995[65]). Factor XI deficiency is thus considered one of the most common inherited disorders among Ashkenazi Jews.

Factor XI deficiency is described as an autosomal recessive disorder with variable penetrance (Gomez and Bolton-Maggs, 2008). Although patients heterozygous for variants associated with Factor XI deficiency have lower bleeding risk compared to homozygous or compound heterozygous patients, studies indicate that 20–50% of individuals heterozygous for variants associated with the deficiency bleed excessively (Bolton-Maggs, 1996[66]). There is evidence that lower levels of other coagulation factors likely influence the tendency to bleed (Gomez and Bolton-Maggs, 2008).

Over 200 variants of the F11 gene are associated with Factor XI deficiency (Duga and Salomon, 2013[67]), the 23andMe PGS for Factor XI deficiency detects two of the most common variants associated with severe Factor XI deficiency in the Ashkenazi Jewish

[63] Asakai et al., (1991). "Factor XI deficiency in Ashkenazi Jews in Israel." NEJM 325(3): 153–158.
[64] Gomez and Bolton-Maggs, 2008. "Factor XI deficiency." Haemophilia 14(6):1183–1189.
[65] Shpilberg et al., (1995). "One of two common mutations causing Factor XI deficiency in Ashkenazi Jews (Type II) is also prevalent in Iraqi Jews, who represent the ancient gene pool of Jews." Blood 85(2):429–432.
[66] Bolton-Maggs, (2008). "Factor XI deficiency and its management," in Schulman, S (ed.), Treatment of Hemophilia." Quebec: The World Federation of Hemophilia, Volume 14:1–10.
[67] Duga and Salomon, (2013). "Congenital factor XI deficiency: an update." Semin Thromb Hemost 39(6): 621–631.

population: E117X (Glu117Stop, Type II mutation) and F283L (Phe283Leu, or Type III mutation). These two variants were found to be associated with the majority of 414 unrelated Israeli Jewish patients with severe Factor XI deficiency (Peretz, 2013[68]) and found with similar frequencies in another study involving 49 unrelated Ashkenazi Jewish patients with Factor XI deficiency (Asakai, 1991). This indicates that genotypes associated with the majority of Factor XI deficiency in the Ashkenazi Jewish population are E117X/E117X homozygotes, F283L/F283L homozygotes, or E117X/F283L compound heterozygotes. The F283L variant has thus far only been associated with the Ashkenazi Jewish population, whereas the E117X variant is found in other ethnic groups (Peretz, 1997[69]). The third variant – IVS14+1G>A (Type I mutation) – is also found with some, albeit significantly lower, frequency (~1% in the study of 414 Israeli Jewish patients with severe Factor XI deficiency; Peretz, 2013).

Molecular genetic evaluation indicates that the E117X mutation is a nonsense mutation that results in premature termination of the polypeptide, likely resulting in a truncated or unstable Factor XI protein (Asakai, 1989[70]). The F283L point mutation is a substitution of the amino acid phenylalanine for leucine and may result in a destabilized Factor XI protein (Asakai, 1989; Asakai, 1991). The IVS14+1G>A is a splice site mutation that likely leads to destabilized mRNA or, if the mRNA is translated, a truncated or unstable Factor XI protein (Asakai, 1989). There appears to be correlation between the level of Factor XI coagulant activity and genotype. The order of least to most Factor XI activity associated with a given genotype is: E117X/E117X homozygotes < E117X/F283L compound heterozygotes < F283L/F283L homozygotes (Asakai, 1991).

Although genotype may correlate to Factor XI activity, it has also been found that the level of Factor XI activity does not predict the clinical bleeding severity for patients with Factor XI deficiency (Peyvandi, 2012[71]). Studies indicate that asymptomatic patients can be found to have the same level of Factor XI activity as individuals who have had a severe bleeding event (Gomez and Bolton-Maggs, 2008).

The 23andMe PGS for Factor XI deficiency is only indicated for Factor XI deficiency caused by the F283L, E117X, and IVS14+1G>A variants. Because the liver is the site of synthesis of Factor XI, acquired Factor XI deficiency can be associated with liver failure, or, in rare instances, after liver transplantation from a Factor XI-deficient donor (Yankol, 2015[72]). Factor XI deficiency can also be caused by inhibition of Factor XI by allo-

[68] Peretz et al., (2013). "Type I mutation in the F11 gene is a third ancestral mutation which causes factor XI deficiency in Ashkenazi Jews." J Thromb Haemost 11(4): 724–730.
[69] Peretz et al., (1997). "The two common mutations causing factor XI deficiency in Jews stem from distinct founders: one of ancient Middle Eastern origin and another of more recent European origin." Blood 90(7): 2654–2659.
[70] Asakai et al., (1989). "Factor XI (plasma thromboplastin antecedent) deficiency in Ashkenazi Jews is a bleeding disorder that can result from three types of point mutations." PNAS 86(20): 7667–7671.
[71] Peyvandi et al., (2012). "Coagulation factor activity and clinical bleeding severity in rare bleeding disorders: results from the European Network of Rare Bleeding Disorders." J Thromb Haemost 10(4): 615–621.
[72] Yankol et al., (2015). "Acquired Factor XI deficiency: a rare complication after liver transplantation." Transplant Proc 47(1): 179–181.

antibodies or auto-antibodies, as is seen after plasma replacement therapy (Salomon, 2006[73]), or in patients with autoimmune diseases (as is seen rarely in patients with systemic lupus erythematosus; Bortoli, 2009)[74], respectively.

Clinical Summary for Factor XI deficiency

The following table summarizes the claimed variants phenotypes based on published studies of the disease variants in the various populations.

Test result	Ancestry	Number of subjects	Phenotype	References
E117X/E117X homozygous	Ashkenazi Jewish	49 unrelated subjects	1.2±0.5% normal FXI activity; 108±18s APTT; increased frequency of injury/surgery-related bleeding	Asakai, 1991
F238L/F283L homozygous	Ashkenazi Jewish	49 unrelated subjects	9.7±3.8% normal FXI activity; 67±18s APTT; increased frequency of injury/surgery-related bleeding	Asakai, 1991
E117X/F283L compound heterozygous	Ashkenazi Jewish	49 unrelated subjects	3.3±1.6% normal FXI activity; 85±22s APTT; increased frequency of injury/surgery-related bleeding	Asakai, 1991
E117X/+ heterozygous	Ashkenazi Jewish	49 unrelated subjects	52±18% normal FXI activity; 39±7.4s APTT	Asakai, 1991
	Jewish and non-Jewish – North West England	20	45% with a bleeding history/tendency, 55% with no bleeding history/tendency	Bolton-Maggs, 1995[75]
F283L/+ heterozygous	Ashkenazi Jewish	49 unrelated subjects	67±24% normal FXI activity; 36±7.4s APTT	Asakai, 1991
IVS14+1G>A/IVS14+1G>A homozygous	Ashkenazi Jewish	13 affected families	< 1U/dL FXI activity; bleeding tendency varies though 2x more frequent than in IVS14+1G>A / + heterozygotes	Peretz, 2013
IVS14+1G>A/E117X compound heterozygous	Ashkenazi Jewish	13 affected families	bleeding tendency varies though 2x more frequent than in IVS14+1G>A/ + heterozygotes	Peretz, 2013
IVS14+1G>A/F283L compound heterozygous	Ashkenazi Jewish	13 affected families	bleeding tendency varies though 2x more frequent than in IVS14+1G>A/ + heterozygotes	Peretz, 2013
IVS14+1G>A/+ heterozygous	Ashkenazi Jewish	13 affected families	42.2 ± 8.2 U/dL FXI activity; bleeding tendency varies and bleeding less frequent than in IVS14+1G>A homozygotes or compound	Peretz, 2013

[73] Salomon et al., (2006). "Variable bleeding manifestations characterize different types of surgery in patients with severe Factor XI deficiency enabling parsimonious use of replacement therapy." Haemophilia 12(5): 490–493.
[74] Bortoli et al., (2009). "Acquired Factor XI inhibitor in systemic lupus erythematosus – case report and literature review." Semin Arthritis Rheum 39(1): 61–65.
[75] Bolton-Maggs (1995). "Definition of the bleeding tendency in factor XI-deficient kindreds: a clinical and laboratory study." Thromb Haemost 73: 194-202.

			heterozygotes	

Celiac Disease

The 23andMe PGS Genetic Health Risk Report for Celiac Disease is indicated for reporting of rs2187668, a representative or tag SNP for the HLA-DQ2.5 haplotype. This haplotype is found in the majority of patients affected with celiac disease (Dieli, 2015[76]). HLA-DQ2.5 refers to a specific major histocompatibility complex (MHC) class II molecule encoded by the HLA-DQ alleles DQA1*05 and DQB1*02. This test is intended to determine if a person has the HLA-DQ2.5 haplotype, which is associated with an increased risk of celiac disease. The 23andMe PGS for celiac disease is not indicated for reporting of the HLA-DQ8 haplotype, which is also associated with a genetic predisposition for the development of celiac disease in a minority of cases (Sollid and Lie, 2005)[77].

Celiac disease is characterized as highly variable in its expression, with a wide range of clinical symptoms and variability in the age of onset, with varying degrees of damage to the intestinal tissue (Dieli, 2005). Damage to the small intestine can be associated with clinical symptoms such as iron-deficiency anemia, diarrhea, or severe malabsorption. However, some individuals with celiac disease may be asymptomatic, even while experiencing intestinal damage (Jorres, 2007[78]; Sollid, 2002[79]). Celiac disease is currently diagnosed using histological findings from intestinal biopsies in patients consuming gluten. Serological tests of antibodies associated with the disease and HLA genotyping are also used when celiac disease is suspected (Sollid and Lie, 2005). Management of celiac disease involves removal of gluten-containing food from the diet, which results in amelioration of symptoms (Sollid and Lie, 2005).

Celiac disease is a complex inflammatory disorder in which both genetic and environmental contributions influence its expression, development, and pathology. Studies demonstrate that the disease is triggered by exposure to gluten in individuals with a genetic predisposition for the disease. The majority of the published literature on the genetics of celiac disease studied individuals of European descent. Although a polygenic disease, in which many genes are involved in determining the risk of developing celiac disease, 90% of patients with celiac disease have at least one copy of the HLA-DQ2.5 haplotype (Dieli, 2015). The HLA-DQ2.5 haplotype is present in high frequency in unaffected individuals and studies indicate that in Europe, approximately 25% of the general population tests positive for this haplotype (Sollid and Lie, 2005). Even with such a high percentage of the population positive for the HLA-DQ2.5 haplotype, it is

[76] Dieli-Crimi et al., (2015). "The genetics of celiac disease: a comprehensive review of clinical implications." J Autoimmun 64: 26–41.
[77] Sollid and Lie, (2005). "Celiac disease genetics: current concepts and practical applications." Clin Gastroenterol Hepatol 3(9): 843–851.
[78] Jores et al., (2007). "HLA-DQB1*0202 homozygosis predisposes to severe intestinal damage in celiac disease." Scand J Gastroenterol 42(1): 48–53.
[79] Sollid, (2002). "Coeliac disease: dissecting a complex inflammatory disorder." Nature Reviews 2(9): 647–655.

estimated that approximately 1% of the general population in Western countries has celiac disease (Dieli, 2015).

The HLA-DQA1 and -DQB1 genes code for the alpha and beta chains, respectively, of the HLA-DQ2 protein, which is a heterodimeric cell surface MHC class II protein. MHC class II proteins present antigens to T-cells of the immune system to initiate an immune response to foreign antigens, or to help develop immune tolerance via presentation of self-antigens. The HLA-DQ2.5 protein (DQA1*05 and DQB1*02 isoform) is thought to present gluten peptide antigens to T-cells in the intestinal mucosa, initiating an immune/inflammatory response in the intestine in response to gluten (Sollid and Lie, 2005). Activation of gluten-reactive T-cells by exposure to gluten can cause chronic inflammation of the mucosa of the small intestine in addition to atrophy of the villi lining the intestine (Sollid, 2002). A study has found that the severity of damage to the small intestine can be linked to the number of copies of the DQB1*201 allele. Greater tissue damage was found in individuals with higher DQB1*201 copy numbers, presumably due to increased presentation of activating gluten peptide to T-cells by the HLA-DQ2.5 protein, resulting in enhanced T-cell activation (Jores, 2007). Studies indicate that homozygosity for HLA-DQ2.5 may also be associated with increased risk for enteropathy-associated T-cell lymphoma (Al-Toma , 2007)[80].

The HLA-DQ2.5 haplotype is also associated with diabetes mellitus type 1, which is an autoimmune disease that results in loss of insulin expression and inability to regulate blood glucose levels (Sollid and Lie, 2005). Rates of celiac disease are found to be increased in patients with type 1 diabetes, with an observed prevalence of 2–10% (Contreas et al., 2004)[81]. Due to the high prevalence of the HLA-DQ2.5 haplotype in patients with type 1 diabetes, the use of this haplotype as an indicator of celiac disease in this patient population is less clear (Contreas et al., 2004).

Clinical Summary for Celiac Disease

The likelihood ratios for risk of celiac disease presented in the table below were calculated using a prevalence of 0.79% in the United States (Dieli, 2015) and variant frequency of 25.8% (Sollid and Lie, 2005). A lower bound of 95% confidence interval for LR greater than 1 indicates that the test result is associated with the disease.

Test Result	LR	95% CI for LR	References	Study Summary
No HLA-DQ2.5	0.30	0.29-0.31	DuBois, 2010[82]	The study included analysis of 4,533 subjects with celiac disease

[80] Al-Toma et al., (2006). "Human leukocyte antigen-DQ2 homozygosity and the development of refractory celiac disease and enteropathy-associated T-cell lymphoma." Clin Gastroenterol Hepatol 4(3): 351–319.
[81] Contreas et al., 2004. "Screening of coeliac disease in north Italian children with type 1 diabetes: limited usefulness of HLA-DQ typing." Acta Paediatr 93(5): 628-632.
[82] Dubois, P, et.al. (2010). "Multiple common variants for celiac disease influencing immune gene expression." Nature Genetics 42(4): 295–304

Test Result	LR	95% CI for LR	References	Study Summary
Haplotype				and 10,750 control subjects. All subjects were of European descent (United Kingdom, Finland, The Netherlands, and Italy).
Presence of HLA-DQ2.5 Haplotype (one or two copies)	1.87	1.85–1.89		
No HLA-DQ2.5 Haplotype	0.27	0.24-0.30	Van Heel, 2007[83]	The study included analysis of 778 subjects with celiac disease and 1,422 control subjects. All subjects were from the United Kingdom
Presence of HLA-DQ2.5 Haplotype (one or two copies)	1.90	1.83-1.95		

Glucose-6-Phosphate Dehydrogenase Deficiency

The 23andMe PGS Genetic Health Risk Test for Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency is indicated for the detection of the Val68Met variant in the G6PD gene. This variant is associated with G6PD Deficiency with higher risk for episodes of anemia (Beutler, 1994)[84] and is the most common risk variant (Val68Met) for the condition in people of African descent (Cappellini, 2008)[85]. G6PD Deficiency is a common genetic condition characterized by hemolytic anemia in response to certain factors such as infection, certain medications, or the consumption of fava beans (Cappellini, 2008 and Mason 2007[86]). Acute hemolytic anemia is caused by destruction of the older red blood cells. It appears that G6PD Deficiency is protective of severe malarial infections (Mason, 2007).

G6PD Deficiency is an X-linked disorder with hundreds of described variants (Buetler, 1994). An estimated 400 million people worldwide are affected by this enzyme deficiency, predominantly in Africa, Asia, the Middle East, and Mediterranean region (Cappellini, 2008). Rates of G6PD Deficiency range from 2.9% in the Pacific to 7.5% in Africa, though some regions can have significantly higher (>20%) or lower (0%) rates (Nkhoma, 2009)[87]. The World Health Organization has classified the different G6PD

[83] Van Heel, D., et. al. (2007). "A genome-wide association study for celiac disease identifies risk variants in the region harboring IL2 and IL21." Nature Genetics 39(7): 827–809
[84] Beutler E. 1994. G6PD deficiency. Blood 84 (11) 3613–3636
[85] Cappellini MD and Fiorelli. (2008)."Glucose -6-phosphate Dehydrogenase Deficiency." Lancet (371): 64–74
[86] Mason PJ. (2007). "G6PD deficiency: the genotype-phenotype association." Blood Rev. 21 (5): 267–83

[87] Nkhoma ET. et al.. (2009) "The Global Prevalence of Glucose-6-phosphate Dehydrogenase Deficiency: A Systematic Review and Meta- analysis." Blood Cells Mol Dis. 42 (3): 267–78

variants according to the magnitude of the enzyme deficiency and the severity of hemolysis (Beutler, 1993)[88]. The Val68Met variant is classified within the WHO class 3 variants that have been shown to have 10-60% of normal enzyme activity (Cappelini and Fiorelli, 2008).

There is no specific treatment for this enzyme deficiency, other than avoiding triggers which includes some drugs, the consumption of fava beans and infections.

The clinical performance of each of the variants detected and reported is supported by at least 3 peer-reviewed journal articles, including studies demonstrating the functional effects of the variants. Specifically, the association of the Val68Met variant to risk for G6PD Deficiency is supported by three patient studies, three functional studies, and one additional study showing the penetrance (of the G6PD enzyme deficiency) is approximately 70% in hemizygous males and approximately 50% in homozygous females (Shah, 2014)[89].

Clinical Summary for G6PD Deficiency

The following references were used to support the claimed variant for G6PD.

Output	References	Study Summary
Zero variants	Carter, 2011[90]	In a study of 2045 patients from six African counties, all G6PD deficient genotypes have the G202A (Val68Met) variant. However, the absence of this variant does not entirely exclude the possibility of G6PD deficiency. Other G6PD gene variants that could result in enzyme deficiency have been identified in Africa.
One copy (female) Heterozygous	Beutler, 1989[91] May, 2000[92] Johnson, 2009[93]	Approximately 40% reduction in enzyme activity has been reported in heterozygous females. The results will be classified as "normal" by most qualitative G6PD enzyme activity assays.

[88] Beutler E. (1993). "Study of glucose-6-phosphate dehydrogenase: history and molecular biology." AM J Hematol. 42 (1): 53–58

[89] Shah SS. et al. (2014) "Genetic Determinants of Glucose-6-phosphate Dehydrogenase Activity in Kenya." BMC Med Genet. 15: 93

[90] Carter N. et al. (2011). "Frequency of glucose-6-phosphate dehydrogenase deficiency in malaria patients from six African countries enrolled in two randomized anti-malarial clinical trials." Malar J. 10: 241

[91] Beutler E. et al (1989) "Molecular heterogeneity of glucose-6-phosphate dehydrogenase A-."Blood 74 (7): 2550–2555

[92] May J. et al. 2000."Red cell glucose-6-phosphate dehydrogenase status and pyruvate kinase activity in a Nigerian population." Trop Med Int Health. 5(2): 119–123

[93] Johnson MK. (2009) "Impact of the method of G6PD deficiency assessment on genetic association studies of malaria susceptibility." PloS One. 4(9): 7246

Output	References	Study Summary
One copy (male) Hemizygous	Beutler, 1989 May, 2000 Johnson, 2009 www.uptodate.com	Hemizygous male and homozygous females have approximately 80% reduction in enzyme activity. These individuals typically present with intermittent hemolysis with significant oxidant stress.
Two Copies Homozygous		

Hereditary Hemochromatosis

The 23andMe PGS Genetic Health Risk Report for Hereditary hemochromatosis is indicated for reporting of the variants C282Y and H63D in the *HFE* gene. Hereditary hemochromatosis (HH) is a genetic condition, specifically an autosomal recessive condition, in which the body absorbs too much iron through the gastrointestinal mucosa[94]. This leads to iron overload that can cause liver disease and other clinical manifestations. The hereditary hemochromatosis test evaluates the two most common risk variants linked to this condition, C282Y and H63D. Genetic testing is recommended for people with symptoms of HH and 1st degree relatives of confirmed HH patients[95]. Population-level screening is not recommended due to incomplete penetrance. The two variants reported by this device account for about 90% of patients with HFE-related hereditary hemochromatosis with a great majority (80–85%) due to patients with two copies of the C282Y variant and a very small number due to patients with one C282Y variant and one H63D variant. Patients with two copies of H63D variant rarely develop HFE-related hereditary hemochromatosis. Phenotypic expression only occurs in approximately 70% of people that are homozygous (i.e., have two copies) for the C282Y variant and fewer than 10% will develop severe iron overload[96]. Females have much lower clinical penetrance than men (1% vs. 25%) most likely due to menstrual blood loss and maternal iron loss during pregnancy. This test is most relevant for people of northern European origin.[97]

Early-Onset Primary Dystonia

The 23andMe PGS Genetic Health Risk Report for Early-Onset Primary Dystonia is indicated for reporting of the variant deltaE302/303 in the DYT1 gene. The DYT1 gene

[94] Seckington R, Powell L. (2000) [updated 2015 Sep 17]. *HFE*-Associated Hereditary Hemochromatosis. . GeneReviews [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2017.
[95] Bacon BR et al. (2011). Diagnosis and Management of Hemochromatosis: 2011 Practice Guidelines by the American Association for the Study of Liver Diseases. Hepatology.. 54 (1): 328-343.
[96] Allen KJ et al.(2008). Iron overload related disease in HFE hereditary hemochromatosis. N Engl J Med. 358: 221-230.
[97] Adams PC et al. (2005). Hemochromatosis and iron-overload screening in a racially diverse population. N Engl J Med. 352 (17): 1769-1778.

encodes the torsion A protein (Ozelius, 1997)[98]. This variant is a 3 base pair deletion (rs724159981) in the coding region of the DYT1 gene results in the deletion of a glutamine from the protein. The mode of inheritance is autosomal dominant and penetrance of dystonia with this variant is approximately 30% (Bressman, 1989)[99]. Signs and symptoms of early-onset primary dystonia usually appear in childhood or adolescence (Marsden, 1976)[100] although the range of age of onset is broad. This test is most relevant for people of Ashkenazi Jewish descent. Patients with dystonia with other variants in DYT1 have also been identified (Hettich, 2014)[101] and the deltaE302/303 variant has rarely been found in other dystonia patients from other ethnic groups (Klein, 1998)[102].

b. Other clinical supportive data

i. User Comprehension Study

Rationale: The Manufacturer conducted a study to assess user comprehension of the 23andMe Personal Genome Service (PGS) for Genetic Health Risk reports. The user comprehension study was performed in a sample that was demographically diverse, using quota-based sampling in a controlled laboratory-based environment. In addition to quantitative assessment of user comprehension of the test reports after viewing the educational module, the study was moderated face-to-face in order to collect observational and qualitative data on participants' overall experience with the survey.

Objectives: The user comprehension study was designed to assess comprehension of representative labeling contained in the Genetic Health Risk reports of the Hereditary Thrombophilia Test, Alpha-1 Antitrypsin Deficiency Test and G6PD Deficiency Test. This study was conducted with a demographically diverse sample to evaluate comprehension across several core comprehension concepts.

Methods: Quota-based sampling was used to recruit study participants representative of the U.S. population according to gender, age, race/ethnicity, and education level. Surveys were administered in six locations across the U.S., with a minimum of one location in each of the four census regions: Northeast, Midwest, South, and West. Approximately 120 participants were tested at each of the six locations. These participants were randomly assigned between seven study arms. Five study arms tested comprehension of test reports specific to the Hereditary Thrombophilia test reports, one arm was specific to Alpha-1 Antitrypsin Deficiency test reports, and one

[98] Ozelius, LJ et al. (1997) "The early onset torsion dystonia gene (DYT1) encodes an ATP binding protein." Nature Genetics. 17(1):40-8. PMID: 9288096
[99] Bressman, SB et al (1989) "Idiopathic dystonia among Ashkenazi Jews: evidence for autosomal dominant inheritance." Ann Neurol. 26(5):612-20.
[100] Marsden, CD et al (1976). Natural history of idiopathic torsion dystonia**.** Adv. Neurol. 14: 177-187.
[101] Hettich et al. (2014) "Biochemical and cellular analysis of human variants of the DYT1 dystonia protein, TorsinA/TOR1A." Hum Mutat. 2014 Sep;35(9):1101-13.
[102] Klein C et al. (1998). De novo mutations (GAG deletion) in the DYT1 gene in two non-Jewish patients with early-onset dystonia. Hum Mol Genet. 7(7):1133-6.

was specific to G6PD test reports. After excluding several participants according to pre-defined exclusion criteria (individuals who do not respond to the study invitation, fail to appear for their scheduled survey appointment, or do not consent to the study), 764 participants were divided amongst each the seven study arms. An investigation identified 70 participants for whom the moderators indicated they did not scroll/read through the report in order to answer the questions alongside the report. Exclusion of these 70 participants did not significantly change the demographic characteristics of the remaining 694 participants. The Manufacturer determined comprehension accuracy rates for multiple core comprehension concepts. Primary comprehension assessment addressed the following core comprehension concepts: purpose of the test, limitations of the test, ethnicity relevance, meaning of test results, the role of non-genetic risk factors, inheritance, and appropriate follow-up actions. Secondary analyses included assessment of participants' baseline knowledge of genetic testing concepts, survey completion rates, and evaluation of qualitative feedback from participants and moderators.

Data Set Characteristics: All pre-defined demographic quotas and enrollment targets were met within the expected study duration for the overall study. A total of 544 participants completed the survey task for the five Hereditary Thrombophilia test report study arms; 6 of these participants were excluded from analysis. Additionally, an investigation identified 49 participants for whom the moderators indicated that the participants did not scroll/read through the report in order to answer the questions alongside the report, therefore a total of 489 participants were included the endpoint analysis for the Hereditary Thrombophilia test report arms.

A total of 113 participants completed the survey task for the Alpha-1 Antitrypsin Deficiency test report arm; one participant was excluded from analysis. Accordingly, a total of 112 participants were included in the analysis described in the study report. An investigation identified 8 participants for whom the moderators indicated they did not scroll/read through the report in order to answer the questions alongside the report. Therefore, 104 participants were included in the endpoint analysis for the Alpha-1 Antitrypsin Deficiency test report arm.

A total of 114 participants completed the survey task for G6PD test report arm. An investigation identified 13 participants for whom moderators indicated they did not scroll/read the report, therefore a total of 101 participants were included in the endpoint analysis of the G6PD test report arm.

Results: The completion rate was 100% (764/764) for the all the subjects enrolled in the study after passing the pre-defined exclusion criteria. Final analyses of user comprehension rates for each test report listed in the table below were calculated after excluding responders that did not scroll/read through the test reports (694 participants). While the average comprehension rates per core comprehension concept ranged from 73.3% to 100 %, the overall comprehension score for all concepts across all test reports studied was greater than 90%. For the G6PD Deficiency report scenario ("1 Variant Detected"), which achieved a report-specific comprehension score of 73.3%, the Manufacturers explained that this score was due in part to the test

report describing a greater number of concepts (both personal risk and reproductive risk) than other test reports in this category. The Manufacturer will institute labeling changes in the G6PD Deficiency Report Frequently Asked Questions section as a mitigation to provide additional support to customers who may benefit from a more in-depth explanation of the information presented in the test report.

Below is a summary table of the comprehension rates by concept and the average for all representative genetic health test reports.

Comprehension Rates (%)								
	Hereditary Thrombophilia					AAT Deficiency	G6PD Deficiency	
Comprehension concept	0 Variants Detected	1 Variant Detected- Slightly Increased Risk	2 Variants Detected - Increased Risk	Result Not Determined	1 Variant Not Detected and 1 Variant Not Determined	2 Variants Detected – Not at Risk (N = 104)	1 Variant Detected - Not at Risk but Risk to Children	Overall comprehension rates (%) for all representative Genetic Health Risk reports
Test results	91.4	90.2	92.9	93.6	92.1	92.3	88.1	91.4
Purpose of test	92.4	84.3	92.9	89.4	94.4	89.4	88.1	90.1
Limitations of test	91.4	89.2	93.9	94.7	89.9	91.3	86.1	90.9
Ethnicity relevance	95.2	92.2	96.0	94.7	89.9	NA	NA	93.6
Meaning of results	100.0	91.2	94.9	96.8	97.8	94.2	73.3	92.3
Other risk factors	95.2	87.3	94.9	94.7	91.0	NA	89.1	92.0
Inheritance	NA	NA	NA	NA	NA	94.2	86.1	90.2
Appropriate follow-up	95.2	84.3	92.9	94.7	92.1	90.4	90.1	91.4
Total number of participants	N= 105	N=102	N= 99	N= 89	N = 94	N = 104	N= 101	N = 694

NA =Not Applicable

ii. Frequently Asked Questions Material

The Manufacturer has developed a Frequently Asked Questions (FAQ) section for each Genetic Health Risk Report. The FAQ section was created to provide users information to adequately understand the purpose, limitations, and the meaning of the results of the test. The FAQ section was developed using methodology consistent with the Manufacturer's labeling design, identification of primary communication messages, and label comprehension. The concepts covered in the FAQ section include: the test results, the purpose of the test, limitations of the test, relevance of race and ethnicity on test results, the meaning of the result, other risks factors that contribute to disease, appropriate follow-up procedures, how the results of the test may affect the user's family and children, and links to resources that provide additional information. Additionally, the FAQ sections provide definitions for terminology found in Genetic Health Risk Reports that is used to describe risks

associated with detected variants. The questions included in the FAQ section for each of the tests in this submission include, but are not limited to:

- What does this test do?
- What does this test not do?
- What does slightly increased risk mean?
- What does increased risk mean?
- How could my result affect my family and children?
- The report says that detailed risk estimates for the variant are best studied in people of [European, Ashkenazi Jewish, North African Berber] descent? What if I'm not of European descent?
- Where can I learn more about [disease], support groups, and other resources?
- My report says [#] variant(s) detected, what does this mean?

  Each Genetic Health Report has answers to FAQs that are specific to the variant(s) and disease being reported, where applicable. The FAQ section is included in the Genetic Health Risk report.

iii. User Opt-In page

Late-onset Alzheimer's Disease Report and Parkinson's Disease

Due to the nature of the diseases associated with these reports, as well as the current lack of effective treatments, an opt-in page was provided for Alzheimer's Disease and Parkinson's Disease test users. Prior to receiving the results of their test, users will be informed in the opt-in page that they have the choice of whether or not to receive the reports for Late-onset Alzheimer's Disease and Parkinson's Disease. Users will be directed to a page entitled "Choose your health reports" which provides the option to exclude these reports from the user's account. The report selection page includes the following statements and information to allow users to make a better-informed decision.

- The reports are about serious diseases that may not currently have an effective treatment or cure.
- Some users may be upset by learning about personal risks of developing these diseases, and risks for their family members who share DNA.
- If users tend to feel anxious or have ever been diagnosed with anxiety or depression, they may have more emotional difficulty with these reports.
- Knowing or telling others about their genetic risks could affect a user's ability to get some kinds of insurance. U.S. law (GINA) protects against genetic discrimination in health insurance and employment, but not in life, disability, or long-term care insurance. The Manufacturer states that they will not share a user's personal information with an insurance company without that user's explicit consent.
- Genetic testing for these conditions is not currently recommended by any healthcare professional organizations. In addition, the Alzheimer's Association, a

patient advocacy group, has taken the position that broad population genetic testing for Alzheimer's disease is not recommended.

- If a user does decide to view these reports, the user's reports will provide information about resources that may be helpful, including support groups, genetic counseling, and how to discuss results with family.

Results of these reports for Late-onset Alzheimer's Disease and Parkinson's Disease are locked by default, and will never be shown to users unless they have specifically chosen to receive the report at any time, including after results for other reports have been received.

4. Expected values/Reference range:

Not applicable.

N. Instrument Name:

Illumina iScan BeadChip scanner with GenomeStudio software

O. System Descriptions:

1. Modes of Operation:

Same as referenced in DEN140044

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes __X__ or No _____

Level of Concern:

Moderate

Software Description:

Same as referenced in DEN140044

Revision Level History:

A software revision history record for the 23andMe software system software was acceptable.

Unresolved Anomalies:

There are no known unresolved anomalies associated with the system software.

EMC Testing:

Not applicable.

3. Specimen Identification:

   Same as referenced in DEN140044

4. Specimen Sampling and Handling:

   Same as referenced in DEN140044

5. Calibration:

   Same as referenced in DEN140044

6. Quality Control:

   Same as referenced in DEN140044.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Refer to K141410 for saliva collection device details and study results.

Q. Proposed Labeling

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable, and the special controls for this device type.

R. Identified Risks to Health and Identified Mitigations:

The 23andMe PGS Genetic Health Risk Reports provide information derived from Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory results regarding an individual's health risk related to inherited diseases for specific genetic variants. This device is intended for the public and does not require a prescription. The risks and risk mitigations for the 23andMe PGS Genetic Health Risk tests for Hereditary Thrombophilia, Alpha-1 Antitrypsin Deficiency, Alzheimer's Disease, Parkinson's Disease, Factor XI Deficiency, Celiac Disease, G6PD Deficiency, Gaucher Disease Early-Onset Primary Dystonia and Hereditary Hemochromatosis are outlined below.

Risk of False Positive Results:

False positive results may prompt unnecessary additional testing, e.g., coagulation

workup for hereditary thrombophilia and factor XI deficiency, testing of serum AAT levels and/or pulmonary function test for AATD, enzyme activity tests for G6PD deficiency and Gaucher Disease, small bowel biopsy for celiac disease, neuroimaging for Alzheimer's disease and Parkinson's disease, additional genetic testing for early-onset primary dystonia, and iron studies and/or liver biopsy for hereditary hemochromatosis. Some of these tests are invasive and may result in adverse events. Before ordering the additional diagnostic tests, healthcare professionals routinely review the personal and family medical history and perform physical examinations. Such clinical evaluations could partially mitigate the risks associated with false positive results.

False positive results could lead to unnecessary therapies (e.g., inappropriate prophylactic therapy or dietary restriction). All medications carry some inherent risk of adverse effects. For example, individuals with hereditary thrombophilia may receive heparin prophylactically to reduce the risk of VTE. The use of heparin is associated with increased risks for post-operative bleeding and surgical re-exploration. Restrictive diet could impair an individual's quality of life, increase the risk for malnutrition and potentially weaken the immune system.

False positive results could also unnecessarily cause or enhance anxiety or depression. If the false positive results are associated with diseases that lack effective therapies or potential risk factor adjustments, the users may develop severe anxiety, depression or make inappropriate lifestyle changes.

To avoid passing the variants to their children, some users could make inappropriate reproductive choices or receive unnecessary prenatal testing, which may include amniocentesis or chorionic villus sampling. Such invasive procedures carry a risk of spontaneous abortion.

Risk of False Negative Results:

False negative results can delay the identification of genetic risk for the diseases included in these reports, potentially resulting in an inappropriate exclusion of the disease from clinical diagnosis assessment. Users may not initiate appropriate lifestyle changes, therapeutic options, or targeted surveillance.

Additional Risks:

Additional risks include the risks of erroneous result interpretation by the user, Manufacturer or the healthcare professional. The risk of result misinterpretation by a healthcare professional is low as these diseases are diagnosed based on the results of comprehensive clinical evaluation, which may include inquiry of medical and family history, clinical presentation, and physical examination, and other laboratory tests and imaging. Future clinical studies may identify additional risks not discussed in this summary or provide new data showing that the risks associated with the reported variants have changed.

Special Controls:

The special controls outlined in the Order address the risks identified above:

- Special control 1 includes a detailed description of what the 21 CFR 809.10 compliant product labeling, pre-purchase page, or test report generated by the manufacturer for users and health care professionals must include. This special control mandates that over-the-counter manufacturers of these tests must provide limiting claims for tests that are subject to this regulation to reduce inappropriate interpretation by users and health care professionals. This special control also mandates that over-the-counter manufacturers of these tests must provide information to a potential or actual test user about how to obtain access to a genetic counselor, board-certified clinical molecular geneticist, or an equivalent professional to assist in pre- and post-test counseling on the output and interpretation of the test.
- Special control 2 requires the use of a collection device that is FDA-cleared, -approved, or -classified as 510(k) exempt, with an indication for use in in vitro diagnostic use in DNA testing. The use of a FDA-compliant collection device provides assurances regarding safety, effectiveness, and quality of that component, which helps assure safety and effectiveness of the test system.
- Special control 3 includes a detailed description of information that must be provided to users in the device labeling and available on the device manufacturer's website. This special control includes an outline of technical information that should be provided for each gene or variant, an explanation of the concepts that should be explained, and a list of material that should be provided to help the user interpret their test results. This special control includes a summary of the clinical and analytical performance information that must be generated to support claims listed on the manufacturer's website. This special control provides details on what analytical testing must be performed and provides criteria for appropriate standards that must be met for performance for many of the components of analytical testing. The controls also provide information on required testing for user comprehension of test reports to limit erroneous interpretation of the tests by users. This mitigates risk by lowering the probability of inaccurate test results and by reducing inappropriate interpretation by users.
- Special control 4 outlines the indications for use that must not be included in intended use for tests that are subject to this regulation. This special control mitigates risk by limiting claims for tests under this regulation that may lead to inappropriate interpretations and influence harmful clinical actions by users.

Identified Risks to Health and Identified Mitigations

Identified Risks to Health	Identified Mitigations
Incorrect understanding of the device and test system	General controls and special controls (1), (3), and (4)

Incorrect test results (false positives, false negatives)	General controls and special controls (2) and (3)
Incorrect interpretation of test results	General controls and special controls (1), (3), and (4).

S. Benefit/Risk Analysis:

SUMMARY	
Summary of Benefits	(1) Direct user access to tests for genetic risk of diseases The PGS tests provides users with easier access to their own health data compared to traditional genetic tests. This test does not require prescriptions from healthcare professionals. The sample collection kits are mailed directly to the users. Geographic location will not restrict an individual's ability to access the tests. (2) Early detection of genetic risk variants Early detection of genetic risk variants allows an individual to make appropriate informed lifestyle adjustments and to partner with healthcare professionals in early discussions regarding surveillance and management. Some positive test results provide an opportunity for diagnostic work-up prior to the development of signs and symptoms, as well as targeted prevention and early intervention efforts. As a result, the onset of a disease may potentially be delayed or avoided and morbidity and mortality may be reduced in the long term. (3) Promoting public awareness of genetic risks User education materials including FAQs are included in the device labelling. As more users are exposed to the topic of genetic risks, such educational materials could serve as a useful resource for education.
Summary of Risks	(1) Risks associated with false results In general, the risk associated with false results are mitigated by robust clinical and analytical performances of the device. False positive results may prompt unnecessary additional testing. As a measure of risk mitigation, the device label has provided recommendation for consulting healthcare professionals, genetic counselors, board-certified clinical molecular geneticist, or equivalent. Healthcare professionals routinely review a patient's personal and family medical history and perform physical examinations before ordering additional diagnostic tests. Therefore, the risk of unnecessary additional testing is low. False positive results can also lead to unnecessary dietary restriction, unwarranted prophylactic therapy, inappropriate lifestyle choices, anxiety, or depression. False negative results can delay the identification of genetic risks. Users may not be able to initiate appropriate lifestyle changes, therapeutic options, and targeted surveillance. Taken together, the risks associated with false results are adequately mitigated by the clinical and analytical performances, appropriate labeling, and relevant special controls.

	(2) Risks associated with erroneous interpretation of the results The risks of erroneous result interpretation are similar to those listed for false results. An accurate test result could be interpreted erroneously by the manufacturer or the user, or the healthcare professional. The risks of result misinterpretation by the manufacturer are mitigated by special controls for clinical performance. Therefore, the chances of result misinterpretation by the manufacturer are very low. The risks of erroneous result interpretation by the user are mitigated by a combination of properly designed user comprehension studies, adequate labeling, including an opt-in page and FAQs, and appropriate special controls. The users may discuss the results with healthcare professionals. The risks of result misinterpretation by the healthcare professionals are very low as the genetic risk test results are typically interpreted in combination with comprehensive clinical evaluations, which may include inquiry of medical and family history, physical examination, other laboratory tests and imaging. (3) Risks associated with genetic privacy violation The risks associated with genetic privacy violation are mitigated by the manufacturer providing a privacy statement on their website.
Summary of Other Factors	The studies also included precision/reproducibility, analytical sensitivity/limit of detection, and user comprehension.
Conclusions	Given the device's indications for use, required general controls and special controls established for this device, the probable benefits would outweigh the probable risks.

Patient Perspectives

This submission did not include specific information on patient perspectives for this device.

T. Conclusion:

The information provided in this *de novo* submission is sufficient to classify this device into class II under regulation 21 CFR 866.5950. FDA believes that special controls, along with the applicable general controls, provide reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Product Code: PTA

Device Type: Genetic Health Risk Assessment System

Class: II (special controls).

Regulation: 21 CFR 866.5950

(a) Identification. A genetic health risk assessment system is a qualitative in vitro molecular diagnostic system used for detecting variants in genomic DNA isolated from human specimens that will provide information to users about their genetic risk of developing a disease to inform lifestyle choices and/or conversations with a healthcare professional. This assessment system is for over-the-counter use. This device does not determine the person's overall risk of developing a disease.

(b) Classification. Class II (special controls). A genetic heath risk assessment system must comply with the following special controls:

(1) The 21 CFR 809.10 compliant labeling and any prepurchase page and test report generated, unless otherwise specified, must include:

(i) A section addressed to users with the following information:

(A) The limiting statement explaining that this test provides genetic risk information based on assessment of specific genetic variants but does not report on a user's entire genetic profile. This test [does not/may not, as appropriate] detect all genetic variants related to a given disease, and the absence of a variant tested does not rule out the presence of other genetic variants that may be related to the disease.

(B) The limiting statement explaining that other companies offering a genetic risk test may be detecting different genetic variants for the same disease, so the user may get different results using a test from a different company.

(C) The limiting statement explaining that other factors such as environmental and lifestyle risk factors may affect the risk of developing a given disease.

(D) The limiting statement explaining that some people may feel anxious about getting genetic test health results. This is normal. If the potential user feels very anxious, such user should speak to his or her doctor or other health care professional prior to collection of a sample for testing. This test is not a substitute for visits to a doctor or other health care professional. Users should consult with their doctor or other health care professional if they have any questions or concerns about the results of their test or their current state of health.

(E) Information about how to obtain access to a genetic counselor, board-certified clinical molecular geneticist, or equivalent health care professional about the results of a user's test.

(F) The limiting statement explaining that this test is not intended to diagnose a disease,

tell you anything about your current state of health, or be used to make medical decisions, including whether or not you should take a medication or how much of a medication you should take.

(G) A limiting statement explaining that the laboratory may not be able to process a sample, and a description of the next steps to be taken by the manufacturer and/or the customer, as applicable.

(ii) A section in your 21 CFR 809.10 labeling and any test report generated that is for health care professionals who may receive the test results from their patients with the following information:

(A) The limiting statement explaining that this test is not intended to diagnose a disease, determine medical treatment, or tell the user anything about their current state of health.

(B) The limiting statement explaining that this test is intended to provide users with their genetic information to inform lifestyle decisions and conversations with their doctor or other health care professional.

(C) The limiting statement explaining that any diagnostic or treatment decisions should be based on testing and/or other information that you determine to be appropriate for your patient.

(2) The genetic test must use a sample collection device that is FDA-cleared, -approved, or -classified as 510(k) exempt, with an indication for in vitro diagnostic use in over-the-counter DNA testing.

(3) The device's labeling must include a hyperlink to the manufacturer's public website where the manufacturer shall make the information identified in paragraph (b)(3) of this section publicly available. The manufacturer's home page, as well as the primary part of the

manufacturer's website that discusses the device, must provide a hyperlink to the web page containing this information and must allow unrestricted viewing access. If the device can be purchased from the website or testing using the device can be ordered from the website, the same information must be found on the web page for ordering the device or provided in a publicly accessible hyperlink on the web page for ordering the device. Any changes to the device that could significantly affect safety or effectiveness would require new data or information in support of such changes, which would also have to be posted on the manufacturer's website. The information must include:

(i) An index of the material being provided to meet the requirements in paragraph (b)(3) of this section and its location.

(ii) A section that highlights summary information that allows the user to understand how the test works and how to interpret the results of the test. This section must, at a minimum, be written in plain language understandable to a lay user and include:

(A) Consistent explanations of the risk of disease associated with all variants included in the test. If there are different categories of risk, the manufacturer must provide literature references that support the different risk categories. If there will be multiple test reports and multiple variants, the risk categories must be defined similarly among them. For example, "increased risk" must be defined similarly between different test reports and different variant combinations.

(B) Clear context for the user to understand the context in which the cited clinical performance data support the risk reported. This includes, but is not limited to, any risks that are influenced by ethnicity, age, gender, environment, and lifestyle choices.

(C) Materials that explain the main concepts and terminology used in the test that

include:

(*1*) Definitions: scientific terms that are used in the test reports.

(*2*) Prepurchase page: this page must contain information that informs the user about what information the test will provide. This includes, but is not limited to, variant information, the condition or disease associated with the variant(s), professional guideline recommendations for general genetic risk testing, the limitations associated with the test (e.g., test does not detect all variants related to the disease) and any precautionary information about the test the user should be aware of before purchase. When the test reports the risk of a life-threatening or irreversibly debilitating disease or condition for which there are few or no options to prevent, treat, or cure the disease, a user opt-in section must be provided. This opt-in page must be provided for each disease that falls into this category and must provide specific information relevant to each test result. The opt-in page must include:

(*i*) An option to accept or decline to receive this specific test result;

(*ii*) Specification of the risk involved if the user is found to have the specific genetic test result;

(*iii*) Professional guidelines that recommend when genetic testing for the associated target condition is or is not recommended; and

(*iv*) A recommendation to speak with a health care professional, genetic counselor, or equivalent professional before getting the results of the test.

(*3*) Frequently asked questions (FAQ) page: this page must provide information that is specific for each variant/disease pair that is reported. Information provided in this section must be scientifically valid and supported by corresponding publications. The FAQ page must explain the health condition/disease being tested, the purpose of the test, the information the test will and

will not provide, the relevance of race and ethnicity to the test results, information about the population to which the variants in the test is most applicable, the meaning of the result(s), other risk factors that contribute to disease, appropriate followup procedures, how the results of the test may affect the user's family, including children, and links to resources that provide additional information.

(iii) A technical information section containing the following information:

(A) Gene(s) and variant(s) the test detects using standardized nomenclature, Human Genome Organization nomenclature and coordinates as well as Single Nucleotide Polymorphism Database (dbSNP) reference SNP numbers (rs#).

(B) Scientifically established disease-risk association of each variant detected and reported by the test. This risk association information must include:

(*1*) Genotype-phenotype information for the reported variants.

(*2*) Table of expected frequency and risks of developing the disease in relevant ethnic populations and the general population.

(*3*) A statement about the current professional guidelines for testing these specific gene(s) and variant(s).

(*i*) If professional guidelines are available, provide the recommendations in the professional guideline for the gene, variant, and disease, for when genetic testing should or should not be performed, and cautionary information that should be communicated when a particular gene and variant is detected.

(*ii*) If professional guidelines are not available, provide a statement that the professional guidelines are not available for these specific gene(s) and variant(s).

(C) The specimen type (e.g., saliva, capillary whole blood).

(D) Assay steps and technology used.

(E) Specification of required ancillary reagents, instrumentation, and equipment.

(F) Specification of the specimen collection, processing, storage, and preparation methods.

(G) Specification of risk mitigation elements and description of all additional procedures, methods, and practices incorporated into the directions for use that mitigate risks associated with testing.

(H) Information pertaining to the probability of test failure (i.e., percentage of tests that failed quality control) based on data from clinical samples, a description of scenarios in which a test can fail (i.e., low sample volume, low DNA concentration, etc.), how users will be notified of a test failure, and the nature of followup actions on a failed test to be taken by the user and the manufacturer.

(I) Specification of the criteria for test result interpretation and reporting.

(J) Information that demonstrates the performance characteristics of the test, including:

(*1*) Accuracy of study results for each claimed specimen type.

(*i*) Accuracy of the test shall be evaluated with fresh clinical specimens collected and processed in a manner consistent with the test's instructions for use. If this is impractical, fresh clinical samples may be substituted or supplemented with archived clinical samples. Archived samples shall have been collected previously in accordance with the instructions for use, stored appropriately, and randomly selected. In some limited circumstances, use of contrived samples or human cell line samples may also be appropriate and used as an acceptable alternative. The contrived or human cell line samples shall mimic clinical specimens as much as is feasible and provide an unbiased evaluation of the device accuracy.

(*ii*) Accuracy must be evaluated by comparison to bidirectional Sanger sequencing or other methods identified as appropriate by FDA. Performance criteria for both the comparator method and the device must be predefined and appropriate to the device's intended use. Detailed study protocols must be provided.

(*iii*) Test specimens must include all genotypes that will be included in the tests and reports. The number of samples tested in the accuracy study for each variant reported must be based on the variant frequency using either the minimum numbers of samples identified in this paragraph or, when determined appropriate and identified by FDA, a minimum number of samples determined using an alternative method. When appropriate, the same samples may be used in testing to demonstrate the accuracy of testing for multiple genotypes by generating sequence information at multiple relevant genetic locations. At least 20 unique samples representing the wild-type genotype must be tested. To test samples that are heterozygous for the reported variant(s), common variants (>0.1 percent variant frequency in the relevant population) must be tested with at least 20 unique samples. Rare variants (≤0.1 percent variant frequency in the relevant population) must be tested with at least three unique samples. To test samples that are homozygous for the reported variant(s), variants with ≥2 percent variant frequency in a relevant population must be tested with at least 20 unique samples. Variants with a frequency in the relevant population <2 percent and ≥0.5 percent must be tested with at least 10 unique samples. Variants with a frequency in the relevant population <0.5 percent must be tested with at least three unique samples. If variants with a frequency of <0.5 percent are not found within the relevant population and homozygous samples are not tested, then the test results for this homozygous rare variant must not be reported to the user.

(*iv*) Information about the accuracy study shall include the number and type of samples

that were compared to bidirectional Sanger sequencing or other methods identified as appropriate by FDA. This information must either be reported in tabular format and arranged by clinically relevant variants or reported using another method identified as appropriate by FDA. As an example, for samples with different genotypes DD, Dd, and dd, the following table represents data from the accuracy study presented in tabular format:

		Comparator		
		DD	Dd	dd
Device	DD	A_1	B_1	C_1
	Dd	A_2	B_2	C_2
	Dd	A_3	B_3	C_3
	no calls or *invalid*	A_4	B_4	C_4
Total		N_{DD}	N_{Dd}	N_{dd}

where:

D and d = Variants; d = Risk variant;

A_1, A_2, A_3, A_4 are numbers of samples with DD result by the comparator and DD, Dd, dd, or 'no calls' or 'invalid' results by the device correspondingly and N_{DD} is the total number of samples with DD result by the comparator ($N_{DD}=A_1+A_2+A_3+A_4$);

B_1, B_2, B_3, B_4 are numbers of samples with Dd result by the comparator and DD, Dd, dd, or 'no calls' or 'invalid' results by the device correspondingly and N_{Dd} is the total number of samples with Dd result by the comparator ($N_{Dd}=B_1+B_2+B_3+B_4$);

C_1, C_2, C_3, C_4 are numbers of samples with dd result by the comparator and DD, Dd, dd, or 'no calls' or 'invalid' results by the device correspondingly and N_{dd} is the total number of samples with dd result by the comparator ($N_{dd}=C_1+C_2+C_3+C_4$);

(*v*) The accuracy represents the degrees of agreement between the device results and the comparator results. The accuracy must be evaluated by measuring different percent agreements (PA) of device results with the comparator results and percent of 'no calls' or 'invalid calls.' Calculate the rate of 'no calls' and 'invalid calls' for each comparator output as %Inv(DD) = A_4/N_{DD}, %Inv(Dd) = B_4/N_{Dd}, %Inv(dd) = C_4/N_{dd}. If 'no calls' or 'invalid calls' are required to

be retested according to the device instructions for use, the percent of final 'no calls' or 'invalid calls' must be provided. In the table presenting the results of the accuracy study, use only the final results (i.e., after retesting the initial 'no calls' or 'invalid calls', if required according to the instructions for use). Samples that resulted in a 'no call' or 'invalid call' after retesting must not be included in the final calculations of agreement. If the percentages of 'no calls' or 'invalid calls' for each comparator output are similar, combine these estimates as $(A_4 + B_4 + C_4)/(N_{DD} + N_{Dd} + N_{dd})$ and provide a 95 percent two-sided confidence interval. The percent of final 'no calls' or 'invalid calls' must be clinically acceptable.

(*vi*) Point estimates of percent agreement for each genotype must be calculated as the number of correct calls for that genotype divided by the number of samples known to contain that genotype excluding 'no calls' or 'invalid calls'. The calculations must be performed as follows:

$PA(DD|DD)=A_1/(A_1+A_2+A_3)$;

$PA(Dd|DD)=A_2/(A_1+A_2+A_3)$; and $PA(dd|DD)=1- PA(DD|DD)- PA(Dd|DD)$.

$PA(Dd|Dd)=B_2/(B_1+B_2+B_3)$;

$PA(DD|Dd)=B_1/(B_1+B_2+B_3)$; and $PA(dd|Dd)=1-PA(DD|Dd)-PA(Dd|Dd)$.

$PA(dd|dd)=C_3/(C_1+C_2+C_3)$;

$PA(Dd|dd)=C_2/(C_1+C_2+C_3)$ and $PA(DD|dd)=1-P(Dd|dd)-PA(dd|dd)$.

(*vii*) For percent agreements for DD, Dd and dd (PA(DD|DD), PA(Dd|Dd) and PA(dd|dd)) as described in paragraph (b)(3)(iii)(J)(*1*)(*vi*) of this section, the 95 percent two-sided confidence intervals must be provided. The accuracy point estimates for percent agreements for DD, Dd and dd must be ≥99 percent per reported variant and overall. Any variants that have a point estimate for either PA(DD|DD), PA(Dd|Dd), or PA(dd|dd) of <99 percent compared to

bidirectional sequencing or other methods identified as appropriate by FDA must not be incorporated into test claims and reports. Accuracy results generated from clinical specimens versus contrived samples or cell lines must be presented separately. Results must be summarized and presented in tabular format by sample type and by genotype or must be reported using another method identified as appropriate by FDA (see paragraph (b)(3)(iii)(J)(*1*)(*iv*) of this section).

(*viii*) Information must be reported on the Technical Positive Predictive Value (TPPV) related to the analytical (technical) performance of the device for genotypes in each relevant subpopulation (e.g., ethnicity, gender, age, geographical location, etc.). TPPV is the percentage of individuals with the genotype truly present among individuals whose test reports indicate that this genotype is present. The TPPV depends on the accuracy measures of percent agreements and on the frequency of the genotypes in the subpopulation being studied. The f(DD) is the frequency of DD and f(Dd) is the frequency of Dd in the subpopulation being studied; TPPV must be calculated as described in paragraphs (b)(3)(iii)(J)(*1*)(*ix*) through (*xi*) of this section.

(*ix*) For variants where the point estimates of PA(DD|DD), PA(Dd|Dd) and PA(dd|dd) are less than 100 percent, use these point estimates in TPPV calculations.

(*x*) Point estimates of 100 percent in the accuracy study may have high uncertainty about performance of the test in the population. If these variants are measured using highly multiplexed technology, calculate the random error rate for the overall device. The accuracy study described in paragraph (b)(3)(iii)(J) of this section in those cases is more to determine that there is no systematic error in such devices. In those cases, incorporate that rate in the estimation of the percent agreements as calculated in paragraph (b)(3)(iii)(J)(*1*)(*vi*) of this section and include it in TPPV calculations.

(*xi*) The TPPV for subpopulations with genotype frequencies of f(dd), f(Dd) and f(DD) = 1 - f(dd) - f(Dd) in the subpopulation is calculated as:

The TPPV for subpopulations with genotype frequencies of f(dd), f(Dd) and f(DD)=1-f(dd)-f(Dd) in the subpopulation is calculated as:

TPPV for a device result of dd = [PA(dd|dd)•f(dd)]/[PA(dd|dd)•f(dd)+ PA(dd|Dd)•f(Dd)+PA(dd|DD)•f(DD)]

TPPV for a device result of Dd =[PA(Dd|Dd)•f(Dd)]/[PA(Dd|DD)•f(DD)+ PA(Dd|Dd)•f(Dd)+PA(Dd|dd)•f(dd)]

(*2*) Precision and reproducibility data must be provided using multiple instruments and multiple operators, on multiple non-consecutive days, and using multiple reagent lots. The sample panel must either include specimens from the claimed sample type (e.g., saliva) representing all genotypes for each variant (e.g., wild type, heterozygous, and homozygous) or, if an alternative panel composition of specimens is identified by FDA as appropriate, a panel composed of those specimens FDA identified as appropriate. A detailed study protocol must be created in advance of the study and must include predetermined acceptance criteria for performance results. The percentage of samples that failed quality control must be indicated (i.e., the total number of sample replicates for which a sequence variant cannot be called (no calls) or that fail sequencing quality control criteria divided by the total number of replicates tested). It must be clearly documented whether results were generated from clinical specimens, contrived samples, or cell lines. The study results shall report the variants tested in the study and the number of replicates for each variant, and what conditions were tested (i.e., number of runs, days, instruments, reagent lots, operators, specimens/type, etc.). Results must be evaluated and

presented in tabular format and stratified by study parameter (e.g., by site, instrument(s), reagent lot, operator, and sample variant). The study must include all extraction steps from the claimed specimen type or matrix, unless a separate extraction reproducibility study for the claimed sample type is performed. If the device is to be used at more than one laboratory, different laboratories must be included in the reproducibility study and reproducibility across sites must be evaluated. Any no calls or invalid calls in the study must be listed as a part of the precision and reproducibility study results.

(*3*) Analytical specificity data: data must be provided that evaluates the effect of potential endogenous and exogenous interferents on test performance, including specimen extraction and variant detection. Interferents tested must include those reasonably likely to be potentially relevant to the sample type used for the device.

(*4*) Interfering variant data: nucleotide mutations that can interfere with the technology must be cited and evaluated. Data must be provided to demonstrate the effect of the interfering variant(s) on the performance of the correct calls. Alternatively, for each suspected interfering mutation for which data is not provided demonstrating the effect of the interfering variant, the manufacturer must identify the suspected interfering variants in the labeling and indicate that the impact that the interfering variants may have on the assay's performance has not been studied by providing a statement that reads "It is possible that the presence of [insert clearly identifying information for the suspected interfering variant] in a sample may interfere with the performance of this test. However, its effect on the performance of this test has not been studied."

(*5*) Analytical sensitivity data: data must be provided demonstrating the minimum amount of DNA that will enable the test to perform correctly in 95 percent of runs.

(*6*) Reagent stability: the manufacturer must evaluate reagent stability using wild-type,

heterozygous, and homozygous samples. Reagent stability data must demonstrate that the reagents maintain the claimed accuracy and reproducibility. Data supporting such claims must be provided.

(*7*) Specimen type and matrix comparison data: specimen type and matrix comparison data must be generated if more than one specimen type can be tested with this device, including failure rates for the different specimens.

(K) Clinical performance summary.

(*1*) Information to support the clinical performance of each variant reported by the test must be provided.

(*2*) Manufacturers must organize information by the specific variant combination as appropriate (e.g., wild type, heterozygous, homozygous, compound heterozygous, hemizygous genotypes). For each variant combination, information must be provided in the clinical performance section to support clinical performance for the risk category (e.g., not at risk, increased risk). For each variant combination, a summary of key results must be provided in tabular format or using another method identified as appropriate by FDA to include the appropriate information regarding variant type, data source, definition of the target condition (e.g., disease), clinical criteria for determining whether the target disease is present or absent, description of subjects with the target disease present and target disease absent (exclusion or inclusion criteria), and technical method for genotyping. When available, information on the effect of the variant on risk must be provided as the risk of a disease (lifetime risk or lifetime incidences) for an individual compared with the general population risk.

(*i*) If odds ratios are available, using information about the genotype distribution either among individuals with the target disease absent, or in the general population, or information

about the risk variant frequency and odds ratios, the likelihood ratios for the corresponding device results along with 95 percent confidence intervals must be calculated. Using information about pretest risk (π), an estimate of likelihood ratio (LR), and a relationship between post-test risk R as $R/(1 - R) = LR \bullet \pi/(1 - \pi)$, the post-test risk R must be calculated.

(*ii*) When available, likelihood ratios (LR) for different test results must be presented in a tabular format along with references to the source data or using another method identified as appropriate by FDA as stated in paragraph (b)(3)(iii)(K)(2) of this section. When these values are not directly available in published literature, likelihood ratios can be separately calculated along with the 95 percent confidence interval with references to the source data. Note that a minimum requirement for the presence of the variant's effect on the risk is that a corresponding LR is statistically higher than 1 (a lower bound of 95 percent two-sided confidence interval is larger than 1). It means that the post-test risk is statistically higher than the pretest risk (an observed value of the difference between the post-test and pretest risks).

(L) Materials that explain the main concepts and terminology used in the test that includes, but is not limited to:

(*1*) Definitions: scientific terms that are used in the test reports.

(*2*) Prepurchase page: this page must contain information that informs the user about what the test will provide. This includes, but is not limited to, variant information, the condition or disease associated with the variant(s), professional guideline recommendations for general genetic risk testing, the limitations associated with the test (e.g., test does not detect all variants related to the disease) and any precautionary information about the test the user should be aware of before purchase. When the test reports the risk of a life-threatening or irreversibly debilitating disease or condition for which there are few or no options to prevent, treat, or cure the disease, a

user opt-in section must be provided. This opt-in page must be provided for each disease that falls into this category and must provide specific information relevant to each test result. The opt-in page must include:

(*i*) An option to accept or decline to receive this specific test result;

(*ii*) Specification of the risk involved if the user is found to have the specific genetic test result;

(*iii*) Professional guidelines that recommend when genetic testing for the associated target condition is or is not recommended; and

(*iv*) A recommendation to speak with a health care professional, genetic counselor, or equivalent professional before getting the results of the test.

(*3*) Frequently asked questions (FAQ) page: this page must provide information that is specific for each variant/disease pair that is reported. Information provided in this section must be scientifically valid and supported by corresponding publications. The FAQ page must explain the health condition/disease being tested, the purpose of the test, the information the test will and will not provide, the relevance of race and ethnicity on the test results, information about the population to which the variants in the test is most applicable, the meaning of the result(s), other risks factors that contribute to disease, appropriate followup procedures, how the results of the test may affect the user's family, including children, and links to resources that provide additional information.

(M) User comprehension study: information on a study that assesses comprehension of the test process and results by potential users of the test must be provided.

(*1*) The test manufacturer must provide a genetic risk education module to naïve user comprehension study participants prior to their participation in the user comprehension study.

The module must define terms that are used in the test reports and explain the significance of genetic risk reports.

(*2*) The test manufacturer must perform pre- and post-test user comprehension studies. The comprehension test questions must include directly evaluating a representative sample of the material being presented to the user as described in paragraph (b)(3)(ii) of this section.

(*3*) The manufacturer must provide a justification from a physician and/or genetic counselor that identifies the appropriate general and variant-specific concepts contained within the material being tested in the user comprehension study to ensure that all relevant concepts are incorporated in the study.

(*4*) The user study must meet the following criteria:

(*i*) The study participants must comprise a statistically sufficient sample size and demographically diverse population (determined using methods such as quota-based sampling) that is representative of the intended user population. Furthermore, the study participants must comprise a diverse range of age and educational levels and have no prior experience with the test or its manufacturer. These factors shall be well defined in the inclusion and exclusion criteria.

(*ii*) All sources of bias must be predefined and accounted for in the study results with regard to both responders and non-responders.

(*iii*) The testing must follow a format where users have limited time to complete the studies (such as an onsite survey format and a one-time visit with a cap on the maximum amount of time that a participant has to complete the tests).

(*iv*) Users must be randomly assigned to study arms. Test reports in the user comprehension study given to users must define the target condition being tested and related symptoms, explain the intended use and limitations of the test, explain the relevant ethnicities in

regard to the variant tested, explain genetic health risks and relevance to the user's ethnicity, and assess participants' ability to understand the following comprehension concepts: the test's limitations, purpose, appropriate action, test results, and other factors that may have an impact on the test results.

(*v*) Study participants must be untrained, be naïve to the test subject of the study, and be provided the labeling prior to the start of the user comprehension study.

(*vi*) The user comprehension study must meet the predefined primary endpoint criteria, including a minimum of a 90 percent or greater overall comprehension rate (i.e., selection of the correct answer) for each comprehension concept. Other acceptance criteria may be acceptable depending on the concept being tested. Meeting or exceeding this overall comprehension rate demonstrates that the materials presented to the user are adequate for over-the-counter use.

(*vii*) The analysis of the user comprehension results must include results regarding reports that are provided for each gene/variant/ethnicity tested, statistical methods used to analyze all data sets, and completion rate, non-responder rate, and reasons for nonresponse/data exclusion. A summary table of comprehension rates regarding comprehension concepts (e.g., purpose of test, test results, test limitations, ethnicity relevance for the test results, etc.) for each study report must be included.

(4) The intended use of the device must not include the following indications for use:

(i) Prenatal testing;

(ii) Determining predisposition for cancer where the result of the test may lead to prophylactic screening, confirmatory procedures, or treatments that may incur morbidity or mortality to the patient;

(iii) Assessing the presence of genetic variants that impact the metabolism, exposure,

response, risk of adverse events, dosing, or mechanisms of prescription or over-the-counter medications; or

(iv) Assessing the presence of deterministic autosomal dominant variants.