

WHOLE EXOME SEQUENCING (WES)

Whole Exome Sequencing (WES) is generally ordered when a patient's medical history and physical exam strongly suggest that there is an underlying genetic etiology. In some cases, the patient may have had an extensive evaluation consisting of multiple genetic tests, without identifying an etiology. In other cases, a physician may opt to order one of the Whole Exome Sequencing tests early in the patient's evaluation in an effort to expedite a possible diagnosis and reduce costs incurred by multiple tests.

Whole Exome Sequencing is a highly complex test that is newly developed for the identification of changes in a patient's DNA that are causative or related to their medical concerns. In contrast to current sequencing tests that analyze one gene or small groups of related genes at a time, Whole Exome Sequencing analyzes the exons or coding regions of thousands of genes simultaneously using next-generation sequencing techniques.

The exome refers to the portion of the human genome that contains functionally important sequences of DNA that direct the body to make proteins essential for the body to function properly. These regions of DNA are referred to as exons. There are approximately 180,000 exons in the human genome which represents about 3% of the genome. These 180,000 exons are arranged in about 22,000 genes. It is known that many of the errors that occur in DNA sequences that then lead to genetic disorders are located in the exons. Therefore, sequencing of the exome is thought to be an efficient method of analyzing a patient's DNA to discover the genetic cause of diseases or disabilities.

The principle of the test is to sequence nucleotide by nucleotide, the human exome of an individual to a depth of coverage necessary to determine the patient's sequence with high accuracy. The patient's sequence is then compared to the consensus and known variant sequences of the population and the results of these comparisons are interpreted by board-certified laboratory directors and clinicians. By sequencing the exome of a patient and comparing it to normal reference sequence, variations in an individual's DNA sequence can be identified and related back to the individual's medical concerns in an effort to discover the cause of their medical concerns.

WHOLE EXOME SEQUENCING PLATFORM TESTS

Test Name	Test Code	Turnaround Time	Parents
WES Trio	1600	10 weeks	Required
Critical WES Trio	1722	3 weeks	Required
Proband WES	1500	15 weeks	Recommended
Proband WES + CMA	1530	15 weeks for WES	Recommended
BluePrint Proband WES (reflex test, see FAQ #13 & # 31)	1399	10 weeks	Recommended
Prenatal WES Trio	1622	3 weeks	Required
Adult Screening Exome	1605	15 weeks	Recommended

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SECTION I: GENERAL INFORMATION

1. HOW DO I CHOOSE WHAT TEST TO ORDER?

Below is a table to help guide test selection. When deciding what test to order you may need to consider if appropriate family members or other control samples are available and how rapidly results are needed, as well as associated costs.

Test	Test Code	Considerations for Test Selection
WES Trio	1600	When both biological parents are available and desire reporting of all de novo, biallelic and hemizygous variants
Critical WES Trio	1722	When patient is critically ill and rapid turnaround time is desired and both biological parents are available
Proband WES	1500	When both biological parents are not available
BluePrint Proband WES	1399	Only available following testing of BluePrint panel; consider if BluePrint panel is negative
Prenatal WES Trio	1622	For ongoing pregnancies when prenatal imaging detects an anomaly that strongly suggests that there is an underlying genetic etiology and other testing/CMA has been non-diagnostic
Adult Screening Exome	1605	Adults who want information regarding potential future risks of developing a genetic disorder

2. CAN A PATIENT SUBMIT A SAMPLE WITHOUT A PHYSICIAN'S ORDER?

No, WES testing must be ordered by a physician and the results will only be reported to a physician.

3. WHAT TYPE OF CLINICAL INFORMATION DO YOU REQUEST?

A clinical information sheet and/or detailed clinic note in English must accompany each sample. A family pedigree is also requested. The appropriate completed and signed consent must accompany each sample. Results cannot be issued without a signed consent form on file and clinical information. Consent forms are not interchangeable between the different Whole Exome Sequencing options. Many of our consent form are also available in other languages.

4. WILL WE OFFER A RE-ANALYSIS PLAN?

We re-analyze past cases mostly for truncating and/or reported pathogenic variants in new disease genes. This review of past cases is performed periodically (about every 1-2 years) and/or prompted when a new disease gene was reported from a current patient. If a positive result is found, it will be reported to the referring physician in an addendum. There is the option to opt out of having this update released.

5. ARE THERE ANY GENES THAT WILL NOT BE REPORTED?

Please see the below sections for details regarding reporting for each test. Our policy is to NOT report findings in genes causing adult onset dementia syndromes such as early onset Alzheimer, for which there is no treatment, with the exception of the adult sequencing exome (test code 1605). If the proband has a phenotype that clearly indicates such a disorder, we recommend pursuing targeted testing based on phenotype and not WES testing. However, please note that if the patient has a clinical presentation that could indicate such a disorder or a mixed neurological phenotype then results may be returned for genes that have an allelic association with dementia. If dementia is a component of the phenotype then results will be reported in the proband and the parents.

6. IS RAW DATA AVAILABLE TO REQUEST?

FASTQ data files (text-based format for storing nucleotide sequences) may be requested. Data will be made available through a secure FTP site. Release of data requires submission of the Raw Data Release form and “Authorization for Release of Protected Health Information”. Data can only be released to a physician or a researcher with properly completed consent forms and after the release of the BMGL clinical report. These additional documents are available for download on our website.

7. ARE RESEARCH STUDIES AVAILABLE?

Research studies through the Baylor College of Medicine may be available. As part of each consent, families are requested to indicate if they are willing to be contacted directly or through their physician regarding possible research opportunities. There is no obligation to participate.

8. HOW DO YOU HANDLE REPORTING OF INCIDENTAL FINDINGS?

Families may opt in via the consent form to receive incidental findings. The following are definitions of each of these categories. However, see the following pages for more details regarding which categories are available for which test as well as the timing of return of these results.

A Medically Actionable Finding is defined as a pathogenic change in a gene that will have a clear and immediate medical significance to the patient’s health or the immediate health of other family members. Knowledge that these conditions may be present in the patient would be expected to lead to increased surveillance or potential specific therapy. In addition, information in the medically actionable category may include adult onset conditions that have little relevance for a pediatric-aged proband, but may be relevant for their parent. There are two categories of medically actionable findings.

ACMG categorized incidental findings: The American College of Medical Genetics (ACMG) has issued guidelines for the reporting of incidental findings. ACMG recommends a minimally inclusive list of genes for which identified pathogenic variants be reported. Please visit the ACMG website at www.ACMG.net to review the list of genes (PMID: 23788249).

BMGL categorized incidental findings: In addition to the published list of genes recommended by ACMG as medically actionable, additional genes may also be reported as determined by our laboratory experts to meet the definition and criteria of medically actionable. We are unable to create a comprehensive list of all genes that could fall into this category of reporting. However, a few examples are listed in the following Table.

EXAMPLES OF MEDICALLY ACTIONABLE CONDITIONS NOT INCLUDED ON THE ACMG LIST	
Syndrome/Disorder	Gene
Progressive Familial Heart Block, Type 1B	TRPM4
Long QT type 4	ANK2
Familial Gastric Cancer	CDH1
Von Willebrand disease	VWF

Carrier Status for autosomal recessive conditions that are recommended by ACMG or ACOG (The American Congress of Obstetricians and Gynecologists) for reproductive carrier screening have the option to be reported. Examples include cystic fibrosis and Tay-Sachs disease (see the following table for a complete list).

AUTOSOMAL RECESSIVE CARRIER STATUS GENES INCLUDED IN THE FOCUSED REPORT			
Gene	Disorder	Gene	Disorder
CFTR	Cystic fibrosis	FANCC	Fanconi anemia group C
HBB	Sickle cell anemia	SMPD1	Niemann-Pick type A, B
IKBKAP	Familial dysautonomia	BLM	Bloom syndrome
HEXA	Tay-Sachs disease	MCOLN1	Mucopolidosis IV
ASPA	Canavan disease	GBA	Gaucher disease Type I
G6PD*	Hemolytic anemia due to G6PD deficiency		* X-linked inheritance

Pharmacogenetic information: For some tests, variants in genes involved in drug metabolism may also be opted for reporting. At this time, we will limit the reporting of pharmacogenetic variants to VKORC1/ CYP2C9 (altered warfarin metabolism) and CYP2C19 (altered Plavix metabolism). This data is reported in the proband for Proband WES (1500) and Adult Screening Exome (1605).

SECTION II: PROBAND WHOLE EXOME SEQUENCING (FOR TEST CODES 1500, 1530, AND 1399)

9. WHAT CAN I EXPECT THE PROBAND WES FOCUSED REPORT TO CONTAIN?

The results of the WES test will be reported to the referring physician in two separate reports. All samples submitted for testing will receive the focused report. The focused report will contain information on pathogenic variants or variants of unknown clinical significance (VUS) in genes that are related to the clinical phenotype. It will also contain the option to opt in to receive information regarding incidental findings, including ACMG and BMGL medically actionable, reproductive carrier status, and select pharmacogenetic loci, see FAQ #8 for definitions of these categories.

10. WHAT CAN I EXPECT THE PROBAND WES EXPANDED REPORT TO CONTAIN?

After the focused report has been issued, an expanded report can be ordered separately by the referring physician, at no additional charge (test code 1510). This report is available for up to six months after the focused report is received. The turnaround time is 4 weeks for this report to be issued.

The expanded report will contain:

- Information about genes unrelated to the patient's phenotype
- Pathogenic variants and unclassified variants (VUS) in genes unrelated to the disease phenotype
- Deleterious mutations in genes with no currently known association with disease in humans.

Heterozygous unclassified variants associated with recessive disorders will not be reported unless a pathogenic variant or a second unclassified variant in the same gene is also detected. There is an opt-out option for the VUS in genes unrelated to the clinical phenotype. Please note that if there is a VUS in a gene that also has a pathogenic variant, the VUS will not be reported if the opt out option is chosen.

11. WHAT IS THE POLICY WITH REGARD TO PARENTAL SAMPLES FOR PROBAND WES TESTING?

Biological parental samples are requested to facilitate interpretation of the proband's WES results. Blood samples from the parents may accompany the proband's sample (preferred) or may be sent together at another time. The samples should be shipped within two weeks of the submission of the proband's sample. If blood cannot be obtained please contact the laboratory to arrange for a saliva kit. The parental samples will not be subjected to whole exome sequencing, but will be tested by targeted Sanger sequencing to confirm mode of inheritance, de novo status, etc. for pathogenic variants and/or VUS in genes that are highly likely to be causative of disease. Not all variants will be confirmed by Sanger sequencing; the laboratory will prioritize which variants to confirm based on the likelihood of being diagnostic in the case (see list below). These studies will be performed at no additional charge.

- Variants in genes related to patient phenotype will be confirmed by Sanger sequencing in proband and parental samples to confirm WES findings and determine inheritance, with the exception of single VUS alleles in autosomal recessive genes, those VUS will not be confirmed by Sanger sequencing in the proband or parents.
- If a medically actionable pathogenic variant is identified in the proband, we will not automatically test parents for this finding. After counseling, if such testing is desired by the parents, it can be completed at a later date at no additional charge with a completed new test order.
- We will report parental data for carrier status for pathogenic variants as recommended for reproductive screening, unless the proband sample has opted-out of this information.
- Parental inheritance information will not be included for any of the genes reported in the Expanded report and is not available for the pharmacogenetic loci.

12. WHAT IF ONE OR BOTH PARENTS ARE NOT AVAILABLE FOR TESTING?

If one or both of the biological parents are not available, Proband WES can still be ordered on the proband. However, there is a higher risk of ambiguous test results. The parental testing policy is limited to the parents of the proband; siblings or grandparents cannot be substituted. Please call the laboratory prior to submitting any additional family members other than the parents. There may be circumstances when the lab will request samples from additional family members (such as for X-linked conditions). If so, these studies will be performed at no additional charge.

13. WHAT IS THE DIFFERENCE BETWEEN PROBAND WES (TEST CODE 1500) AND BLUEPRINT PROBAND WES (TEST CODE 1399)

BluePrint proband WES (test code 1399) is only offered as a reflex test following the completion of a BluePrint panel (test code 1300). The BluePrint panel (test code 1300) is a genetic test that enables physicians to order a customized panel of up to 100 genes based on a patient's clinical symptoms (see BMGL website and FAQ #31 for further details). BluePrint WES (test code 1399) does NOT report pharmacogenetic information (see FAQ #8); all other reporting is the same as proband WES (see FAQ # 9). The expanded report (test code 1510) will be available for BluePrint WES as described in FAQ question #10.

14. WHAT IF WE WANT TO ORDER A CHROMOSOMAL MICROARRAY ANALYSIS (CMA) AND WES?

Under test code 1530, we offer Proband WES plus CMA. This CMA (test code 8665) is a custom designed 400k microarray that targets over 4,200 genes at the exon level. In addition to exon level copy number, this array also includes 60,000 probes used for SNP analysis for the detection of uniparental disomy (UPD) and absence of heterozygosity (AOH). Results from the CMA are available in 2 weeks and the Proband WES results are available

in the standard 15 weeks. Please note that the WES cannot be cancelled after CMA results are received. Once the WES data has been generated, variants related to patient phenotype with an autosomal recessive inheritance pattern will be checked with the CMA data to determine if a deletion or duplication is present in that gene. However, not all genes of interest will have exonic coverage on the CMA platform.

SECTION III: WHOLE EXOME (FOR TEST CODES 1600 AND 1722)

15. WHAT CAN I EXPECT THE WES TRIO REPORT TO CONTAIN?

The WES Trio report will contain information on variants found in genes that are related to the clinical phenotype. It will also contain the option to opt in to receive information regarding incidental findings, including ACMG medically actionable and reproductive carrier status, see FAQ #8 for definitions. It will also contain a list of de novo, biallelic and hemizygous variants in genes unrelated to the patient's phenotype or variants in genes with no currently known disease association.

16. WHAT IS THE POLICY WITH REGARD TO PARENTAL SAMPLES FOR WES TRIO TESTING?

Both biological parental samples are required in order to order WES Trio. Blood samples from the parents must accompany the proband's sample. Saliva samples are not acceptable for this test. The parental samples will have whole exome sequencing in order to confirm mode of inheritance, de novo status, etc. The parental data will appear in the proband's WES Trio report. Parents also have the option to receive information regarding ACMG medically actionable findings and reproductive carrier status (See FAQ #8). This information will be issued in a separate parental report and will not be stated in the proband's report.

17. WHAT IF ONE OR BOTH PARENTS ARE NOT AVAILABLE FOR WES TRIO TESTING?

If one or both of the biological parents are not available, then WES Trio testing cannot be ordered. The parental testing policy is limited to the parents of the proband; siblings or grandparents cannot be substituted. Please call the laboratory prior to submitting any additional family members other than the parents. We would recommend considering Proband WES (test code 1500) instead of WES Trio if both parents are not available. If parental samples and the proband's sample cannot be shipped together then there is the option to request the samples to have DNA prep and hold only. It is then the client's responsibility to fax a completed requisition indicating WES Trio should be initiated once all three family members' samples have been received at the BMGL.

18. WHAT IS THE DIFFERENCE BETWEEN CRITICAL WES TRIO (1722) AND WES TRIO (1600)?

Critical WES Trio offers a rapid turnaround time of 3 weeks. This option is suggested for patients who are critically ill. It is only available for institutional or self-pay cases. Insurance billing is not accepted for Critical WES Trio. The incidental findings report will be issued separately with a turnaround time of 10 weeks.

SECTION IV: WES TESTING IN THE PRENATAL SETTING

19. DO YOU OFFER PRENATAL WES TESTING?

Testing of prenatal samples requires discussion with one of the BMGL genetic counselors. This is to discuss if WES testing is appropriate, sample requirements, and coordination of testing. For ongoing or continuing pregnancies, we offer Prenatal WES Trio (test code 1622). For testing of prenatal samples from a fetal demise or from products of conception, we offer testing through our usual postnatal options of Proband WES (1500) or WES Trio (1600). Reasons to consider prenatal WES in an ongoing pregnancy are due to a fetus who has/d complex fetal anomalies detected by imaging (such as ultrasound).

20. WHAT CAN I EXPECT THE PRENATAL WES TRIO REPORT TO CONTAIN (TEST CODE 1622)?

The Prenatal WES Trio report will contain information on pathogenic variants and variants of unknown clinical significance (VUS) found in genes that are related to the clinical phenotype. It will also contain variants in disease genes unrelated to the prenatal indications but likely to cause significant disorders during childhood. An incidental findings report can be requested after birth with an additional test order at no additional charge for the ACMG categorized medically actionable changes and the carrier status, see FAQ #8 for definitions. See FAQ #17 for information regarding parental sample requirements and reporting.

SECTION V: EXOME METHODOLOGY

21. WHAT PLATFORM ARE YOU USING?

We are using the Illumina platform for next-generation sequencing. The exome capture is performed with Nimblegen reagents using a HGSC custom-designed capture reagent called VCRome 2.1. Starting in September 2014, all WES samples will be analyzed using version 3 of the capture reagent, which contains the original VCRome 2.1 as well as additional probes for about 3,650 clinically relevant disease genes in order to further improve the exome coverage.

22. WHAT ARE THE STATISTICS FOR COVERAGE/QUALITY?

There is approximately 13Gb of sequencing data generated for each patient. The mean coverage of the exome is 100 – 120X, 95% of the exome is covered at >20X coverage.

23. HOW DO I KNOW IF MY GENE OF INTEREST HAS GOOD COVERAGE ON EXOME PLATFORM?

The BMGL website includes a WES coverage search tool. This search tool provides an estimated coverage for the entered gene(s). However, actual coverage can vary for individual samples.

24. HOW DO YOU FILTER THE VARIANTS?

Variants are filtered using various stringencies of minor allele frequencies, as well as mutation databases and disease specific databases (N Engl J Med. 2013 Oct 17;369(16):1502). A team of ABMG certified molecular lab directors and medical directors interpret the results based on disease phenotype. Parental results of potentially causative variants may yield additional information useful in predicting clinical significance.

25. WHAT TYPES OF MUTATIONS ARE NOT WELL DETECTED BY THIS METHODOLOGY?

Triplet repeat expansions, large deletions and duplications are better detected by methods other than next generation sequencing. Detection of small indels may not be as accurate as detection of base substitutions.

26. ARE THERE GENES THAT ARE NOT WELL COVERED BY THIS METHOD?

Genes that have closely related pseudogenes or copy genes may not be uniquely captured by this method. Please see our website for a WES coverage search tool. This tool provides a representation of coverage that can be expected for WES testing on a gene by gene basis.

SECTION VI: OTHER TESTS

27. WHAT IS THE ADULT SCREENING EXOME SEQUENCING (TEST CODE 1605)?

The Adult Screening Exome Sequencing test is ordered by a physician and must be submitted with a signed consent form from the individual being tested and detailed clinical information. The test is used when a patient's medical history and physical exam findings are normal, but the patient desires information about potential future risk of developing a genetic disorder. The patient must be 18 years of age or older with no significant active or past personal or family history of genetic disorders.

29. WHAT IF EXTENDED FAMILY MEMBERS WANT TO BE TESTED?

Extended family members can be tested on a fee-for-service basis for variants in genes identified by WES through the custom sequencing tests (test codes 1560-1589). Please consult with a BMGL genetic counselor for assistance in selecting the correct testing.

30. WHAT ARE THE CUSTOM SEQUENCING TESTING OPTIONS (TEST CODES 1560-1589)?

Custom Proband Sequence Analysis is for Sanger confirmation of targeted variants. The patient may have had the variant identified in a research setting or by a clinical WES test that did not include Sanger confirmation. These test codes should only be used for genes in which the BMGL does not routinely offer testing. Records indicating the targeted variant must be available in order to offer testing. For Autosomal Dominant, Homozygous, or X-linked Targeted Gene Testing: Use test codes 1560-1569 for requests when confirmation of only ONE sequence change is being requested for that gene. For Autosomal Recessive Targeted Gene Testing: Use test codes 1570-1579 for requests when confirmation of TWO sequence changes are being requested for that gene.

Custom Family Sequence Analysis is for Sanger testing of targeted variants previously confirmed by Sanger sequencing in the proband in a CLIA/CAP lab. These test codes (1580-1589) should only be used for genes in which the BMGL does not routinely offer testing. This testing is commonly used for parental or sibling testing following WES. Only one 1580 order is needed per gene (even if there are two changes in the gene).

31. WHAT IS THE BLUEPRINT PANEL (TEST CODE 1300)?

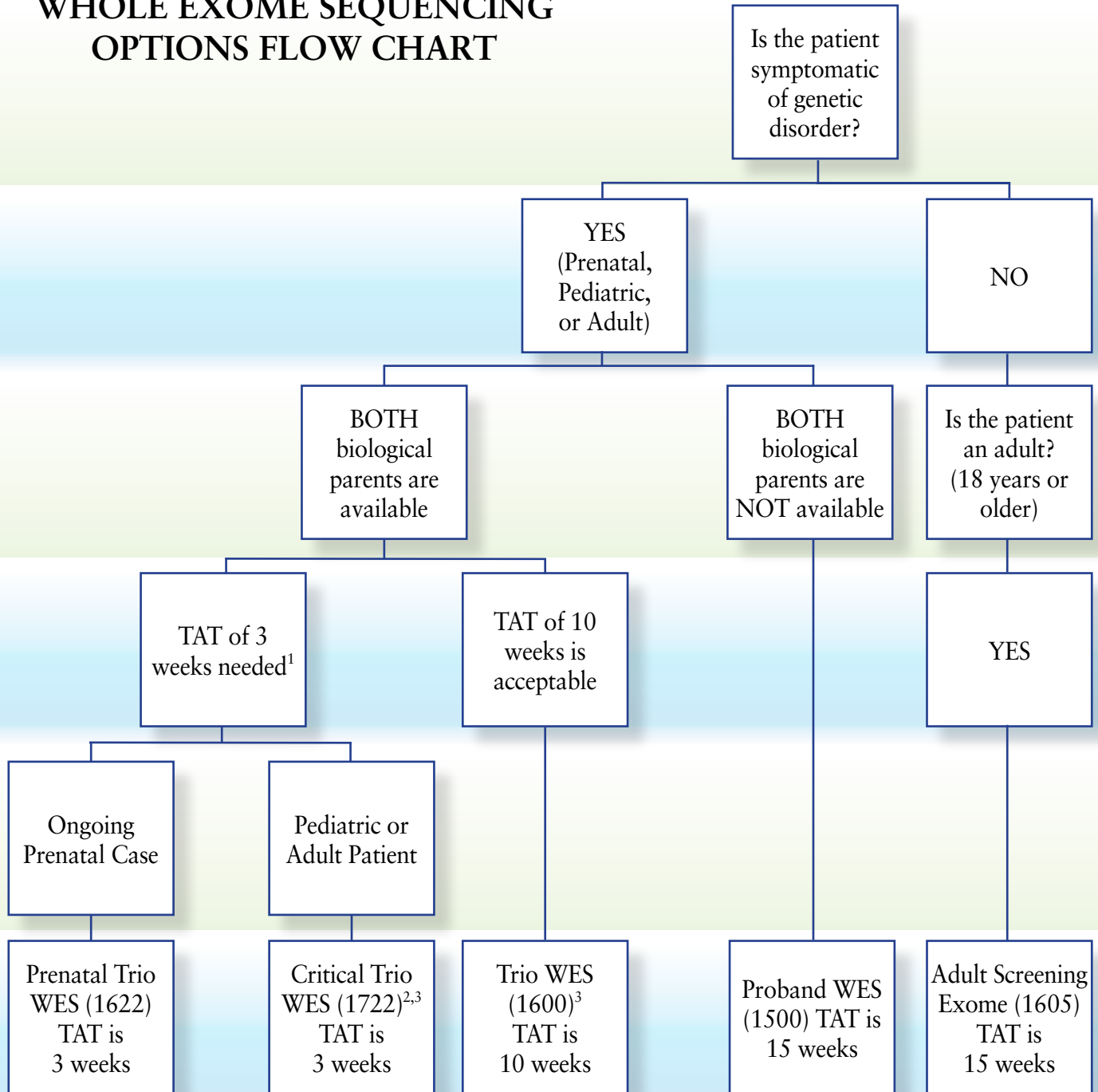
The BluePrint panel is a genetic test that enables physicians to order a panel of customized genes based on a patient's clinical symptoms. Up to 100 genes can be included in the panel. The genes of interest can be automatically generated by entering the clinical phenotype of the patient, or by manually entering a list of genes. Due to the nature of the methodology used for this test, complete sequencing coverage of the selected genes may not be available. The coverage tool built into the test order provides an estimate of the coverage expected for each gene; however, exact coverage may vary for each individual sample.

32. WHAT IF I WANT TO ORDER MITOCHONDRIAL GENOME TESTING AND WES?

Please speak with a BMGL genetic counselor for guidance on patient-specific test selection. However generally, clients may wish to consider ordering either Comprehensive mtDNA Analysis by Massively Parallel Sequencing (test code 2055) or Advanced mtDNA Point Mutations and Deletions by Massively Parallel Sequencing (test code 2010) in addition to one of the Whole Exome Sequencing Tests.



WHOLE EXOME SEQUENCING OPTIONS FLOW CHART



Additional Information

Detailed clinical information and signed consent form is required for all test options. Prenatal cases require discussion with BMGL genetic counselor prior to submission.

¹Turnaround time (TAT) is independent of required culture time.

²Institution and self-pay are the only acceptable billing options.

³Consider Proband WES (1500) if lower cost option is needed and longer TAT is acceptable.