NATIONWIDE CHILDREN'S
When your child needs a hospital, everything matters.℠

Nationwide Children's Laboratory Services
Institute for Genomic Medicine Clinical Laboratory
700 Children's Drive, Columbus, OH 43240
Phone: (614) 722-5321 / FAX: (614) 722-5471
NationwideChildrens.org/Lab

Whole Exome Sequencing (WES) Information Sheet

1. What is Whole Exome Sequencing (WES)?

Whole Exome Sequencing (WES) is a very large-scale genetic test that is designed to find changes (called variants) in a person's DNA that cause or may be related to his/her medical condition. This test is usually done when a person is suspected to have a genetic disorder. Most genetic disorders are caused by one or more variants in one or more genes. Genes are encoded by DNA, and each gene tells the body how to make a protein that is needed for the body to function properly. It is estimated that there are approximately 22,000 genes in human DNA, and at this time variants in about 5,000 of those genes are known to cause genetic disorders. Currently, most of the DNA variants that are known to cause genetic disorders occur in parts of the gene called "exons," which contain important DNA sequences needed to make a protein. The "exome" refers to the collection of all exons from the approximately 22,000 genes found in human DNA. The exome is estimated to make up approximately 2% of entire human DNA. In WES, a person's exome data are compared to that of standard human DNA (called the reference DNA sequence) to find variants in his/her DNA. WES can look for variants in the exome and other known important parts of these 22,000 genes all at the same time. Not all DNA variants cause disease. In fact, most variants in human DNA have no impact on one's health. Based on currently available information in the medical literature and in scientific databases, the laboratory will decide whether any of these variants found in the patient's DNA are predicted to be related to the patient's medical condition. It is important to note that WES may not find the underlying cause for the patient's medical condition. Even if WES finds the underlying genetic cause, it is possible that this information may not help in predicting the future disease course or change disease management.

2. WES Testing Available at Nationwide Children's Hospital

WES performed at Nationwide Children's Hospital can be done by either using a DNA sample from **only** the patient affected with a medical condition (called the proband), or by using DNA samples from both the patient **and** his/her biological parents. WES using only the patient's sample is typically performed when no biological parent is available to provide a sample. It is known that WES has a higher chance of finding disease-causing variant(s) in the patient when genetic data from both biological parents are available for comparison. If one or both of the biological parents are available for sample submission, the laboratory strongly recommends ordering the WES test using the patient sample **and** any available parental sample(s). This increases the chance of finding the disease-causing variant(s) in the patient's DNA and also allows for improved result interpretation of the patient's WES results. Samples from other family members (such as a grandparent or sibling) ***CANNOT*** be used in place of biological parents' samples.

3. Why is Submission of Biological Parents' Samples Recommended for WES?

WES using samples from both the patient **and** the patient's biological parents has a higher chance of finding disease-causing variant(s) compared to WES using only the patient's sample. For WES done at Nationwide Children's Hospital, when biological parents' samples are submitted, WES is also done on each parent's sample. Biological parents' WES data will be used to help interpret the patient's WES results. Biological parents' samples must be submitted to the laboratory before the patient's WES results are reported. If biological parents' samples are submitted after the patient's WES results are reported, re-analysis of the patient's WES data will be performed with additional charges. A separate report will be issued for each parent's sample submitted to the laboratory.

4. What Types of Results will be Reported from the Patient's WES?

The laboratory will issue a **"Focused"** report, which will contain variant(s) that may explain the cause of the patient's medical condition, if any are found. These findings are called "**primary findings**." In addition, the Focused report will also contain other types of results called "**secondary findings**," if you choose to receive these types of results. Secondary findings are not related to the patient's medical condition but may be meaningful for other reasons. For more detailed information on primary and secondary findings, please see sections 5 and 6 below. In the WES informed consent form, you will be asked to choose what types of secondary findings you would like to receive. After reviewing the Focused report, your (your child's) healthcare provider could request the laboratory to provide an **"Expanded"** report. The

Expanded report would list variants found in the patient's WES data that are not currently known to be associated with the patient's medical condition. Variants reported in the Expanded report may have unknown clinical significance or may be known to cause genetic disorders that are not associated with the patient's current medical condition.

5. Primary Findings Reported on WES Focused Report

Variants found in the patient's DNA that are believed to be related to his/her medical condition are called "primary findings." When variants are found in a patient's DNA, the laboratory will categorize them into one of the following types:

- ***Pathogenic*** — A variant was found in the patient's DNA that is known to cause a disorder related to his/her medical condition. The variant has been reported before in human patients and is well described.
- ***Likely Pathogenic*** — A variant was found in the patient's DNA that is likely related to his/her medical condition. The variant may not be well known, but existing medical evidence suggests that it may cause a disorder.
- ***Variant of Uncertain Significance (VUS)*** — A variant was found in the patient's DNA, but it is uncertain whether or not it causes a disorder.
- ***Likely Benign*** — A variant was found in the patient's DNA. The medical literature and scientific databases suggest that this may be a variant present in the general population and is unlikely to cause a genetic disorder. Likely benign variants will NOT be listed on the laboratory report.
- ***Benign*** — A variant was found in the patient's DNA, but it is found frequently in the general population. It is known that this variant does not cause a genetic disorder. Benign variants will NOT be listed on the laboratory report.

It is possible that patient's WES results may come back ***NEGATIVE***, with no pathogenic variants, likely pathogenic variants, or VUS found in the patient's DNA that are related to his/her medical condition. Additionally, genetic test results may impact other family members. Based on the results, testing of other family members may be recommended.

6. Secondary Findings Reported on WES Focused Report

Variants found in the patient's DNA that are not related to his/her medical condition, but may still be reported are called "secondary findings." There are several categories of secondary findings. The Focused report may contain the following two categories of secondary findings, if you choose to receive these results:

(A) Medically Actionable Genes

The Focused report may contain information on genes and genetic disorders that are considered "medically actionable". Genes reported as medically actionable can cause serious medical conditions (such as cancer or heart failure), and there are medical interventions available that have a good chance of preventing or treating these conditions if they are diagnosed before a person develops symptoms. The American College of Medical Genetics and Genomics (ACMG) has published guidelines for reporting these types of medically actionable findings (PubMed ID#: 23788249, 25356965, and 27854360). These guidelines currently list 59 genes that have been determined to be medically actionable, and therefore laboratories should report pathogenic or likely pathogenic variants in these genes if detected by WES. **For the list of these 59 medically actionable genes, please see the table below**. The list of genes may be updated periodically by ACMG. Additionally, the laboratory directors may determine that additional genes meet the same criteria to be considered medically actionable and therefore warrant the same reporting as the genes included in the ACMG list. You may choose in the WES informed consent form whether or not you want information on variants found in the genes determined as medically actionable. **Note: only pathogenic or likely pathogenic variants will be reported for these medically actionable genes.** VUS, likely benign and benign variants will not be reported. If you choose not to receive information from medically actionable genes, the pathogenic or likely pathogenic variants in these listed genes will not be reported on either the Focused **or** the Expanded report, unless they relate to the patient's medical condition.

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List of 59 Medically Actionable Genes (ACMG 2016)							
BRCA1	*BRCA2*	*TP53*	*STK11*	*MLH1*	*MSH2*	*MSH6*	*PMS2*
APC	*MUTYH*	*BMPR1A*	*SMAD4*	*VHL*	*MEN1*	*RET*	*PTEN*
RB1	*SDHD*	*SDHAF2*	*SDHC*	*SDHB*	*TSC1*	*TSC2*	*WT1*
NF2	*COL3A1*	*FBN1*	*TGFBR1*	*TGFBR2*	*SMAD3*	*ACTA2*	*MYH11*
MYBPC3	*MYH7*	*TNNT2*	*TNNI3*	*TPM1*	*MYL3*	*ACTC1*	*PRKAG2*
GLA	*MYL2*	*LMNA*	*RYR2*	*PKP2*	*DSP*	*DSC2*	*TMEM43*
DSG2	*KCNQ1*	*KCNH2*	*SCN5A*	*LDLR*	*APOB*	*PCSK9*	*ATP7B*
OTC	*RYR1*	*CACNA1S*					

(B) Carrier Status for Genetic Disorders Recommended for Reproductive Carrier Screening

The Focused report may contain information on carrier status for a limited number of genes that cause autosomal recessive or X-linked recessive genetic disorders. People who are "carriers" of a genetic disorder typically have no symptoms of the disorder, but they have a pathogenic variant in one of the two copies of the gene, and they have an increased risk to have an affected child. All of the carrier status genes reported in the Focused report cause autosomal recessive genetic disorders, except for the *G6PD* gene, which causes an X-linked recessive disorder.

- Autosomal recessive disorders affect both males and females, and both males and females can be carriers. When both members of the couple are carriers for the same autosomal recessive genetic disorder, the couple has a 1 in 4 (25%) chance in each pregnancy to have an affected child.
- X-linked recessive disorders usually affect only males, and females can be carriers. This is because genes that cause X-linked recessive disorders are located on the X chromosome, and males only have one X chromosome (one copy of the gene) while females have two X chromosomes (two copies of the gene). Female carriers have a 1 in 4 (25%) chance in each pregnancy to have an affected male child.

For the list of carrier status genes that will be reported by the laboratory, please see the table below. Offering carrier screening for certain genetic disorders (including those listed in below table) during pregnancy or before conceiving a pregnancy is recommended by professional medical organizations such as ACMG or the American College of Obstetricians and Gynecologists (ACOG). Carrier status information may provide helpful information for future reproductive planning for the carrier and his/her family members. However, carrier status usually does not affect the personal health management of the carrier. In the WES informed consent form, you may choose whether or not to receive information on carrier status. If you choose not to receive carrier status information, the carrier status will not be reported on either the Focused **or** the Expanded report, unless they relate to the patient's medical condition. **Please note that WES does not detect carrier status for all genetic disorders. Also, WES may not detect the carrier status for all of the disorders recommended by ACMG or ACOG, because this test does not guarantee to test all genes or all parts of the genes associated with these disorders. *This test DOES NOT replace the targeted reproductive carrier screening offered in preconceptional or prenatal care*.**

List of Carrier Status Genes Included in the Focused Report			
Gene	Disorder	Gene	Disorder
CFTR	Cystic fibrosis	***FANCC***	Fanconi anemia group C
HBB	Sickle cell anemia, other beta-globin hemoglobin disorders	***SMPD1***	Niemann-Pick disease type A, type B
HEXA	Tay-Sachs disease	***BLM***	Bloom syndrome
ASPA	Canavan disease	***MCOLN1***	Mucolipidosis IV
IKBKAP	Familial dysautonomia	***GBA***	Gaucher disease type I
G6PD	Hemolytic anemia due to G6PD deficiency (X-linked recessive inheritance)		

7. What Types of Results will NOT be Reported?

The following findings ***WILL NOT*** be reported in either the Focused **or** Expanded report:

- Variants in genes that cause adult-onset neurological disorders, such as dementia, for which there is presently no prevention or cure, unless the patient is of adult age (18 years or older) and currently having symptoms of such syndromes.
- Pharmacogenomic variants, which are findings in genes involved in determining how fast one's body can break down various drugs (drug metabolism).
- Benign and likely benign findings considered to be normal genetic variations in the population.

8. Who will Receive My (My Child's) WES Results?

When the results of this test become available, the laboratory will report out the results to the following people:

- the healthcare provider who ordered the test
- other healthcare provider(s) such as another physician and/or a genetic counselor named as additional report recipients on the test order form
- the hospital or medical office that was involved in ordering or billing for the test

All results are confidential and will be reported to others only with your written consent, unless otherwise required by law. However, your (your child's) WES results may become part of your (your child's) medical record at the ordering physician's hospital/office, which may be accessed by other healthcare providers in the same hospital/office who are involved in your (your child's) care.

Clinical information and test results/data from your (your child's) WES may be recorded in a HIPAA-compliant public database after identifying information is removed (such as name and date of birth). An example of such public database is the ClinVar database maintained by the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/clinvar/). This is done to improve genetic test result interpretation for future patients and to help understand how different DNA variants relate to medical conditions. Comparing information about DNA variants between many different patients and many different laboratories is the best way to gather additional information about whether or not these variants cause disease.

The results from WES data of a group of patients tested at the Nationwide Children's Hospital may be published in the medical literature, including results from you (your child). These publications will not include any personal information, such as name or date of birth, that would enable others to link the published result to your (your child's) identity.

9. What are the Accuracy and Limitations of WES?

Accuracy of test results depends on:

- the quality and type of sample submitted to the laboratory,
- the way the test is done in the laboratory,
- the accuracy of information about the patient's symptoms and medical history provided to the laboratory,
- the accuracy of medical information about the patient's family members provided to the laboratory, and
- the accuracy of biological relationships between the patient and family members provided to the laboratory.

Errors in the medical information about the patient, his/her parents, and/or family members may lead to inaccurate result interpretation. As with all complex testing, there is always a chance of error or test failure. In many cases, WES will not be able to find a DNA variant related to the patient's medical condition. This may be due to a true negative result (patient does not have a genetic disorder), a current lack of knowledge about all genes involved in human genetic disorders, or an inability of the current technology to identify certain types of changes in a gene. While this test is designed to find most disease-causing variants in the majority of human genes, this test does not examine 100% of the genes present in human DNA, does not test for all parts of known human genes, and does not test for changes in mitochondrial DNA. In addition, certain types of genetic abnormalities are not detectable by WES but still may cause disease. Examples of such genetic abnormalities include large deletions and duplications in DNA, DNA methylation abnormalities, variants in genes with highly homologous pseudogenes, and trinucleotide repeat expansions. ***Medical information continues to advance, so it is important to know that the interpretation of the genetic variants is based on information available at the time of testing and may change in the future.***

10. Which Variants Found by WES will be Confirmed by a Second Methodology (Sanger Sequencing)?

If WES finds a DNA variant in the patient that may be clinically important, then a second testing methodology called "Sanger sequencing" may be used to confirm that the variant is truly present in the patient. Sanger sequencing is an older genetic testing methodology compared to Next Generation Sequencing (NGS) that is used for WES, but it is highly accurate and is currently considered the gold standard for confirming DNA sequence changes. However, Sanger sequencing can only test one exon at a time, unlike NGS that can test many exons at once. This is why NGS is used to perform WES rather than Sanger sequencing. If the following variants are detected in the patient's WES, they may be confirmed by Sanger sequencing at the discretion of the laboratory:

- Pathogenic variants, likely pathogenic variants, and variants of uncertain clinical significance (VUS) related to the proband's medical condition.
- Pathogenic or likely pathogenic variants in medically actionable genes, if you choose to receive these results.
- Carrier status variants for a set of genetic disorders, if you choose to receive these results.
- As determined by the laboratory, additional confirmation beyond these categories may also be performed.

Variants listed in the Expanded report (only issued at the request of the ordering healthcare provider) will not be confirmed by Sanger sequencing, unless determined necessary by the laboratory. Also, variants found in the parents will not be confirmed by Sanger sequencing — please see “What Testing will be Performed on Parental Samples?" in section 11 below.

11. What Testing will be Performed on Biological Parents' Samples?

When a sample from one or both biological parents is submitted at the same time as the patient's sample (or submitted before the patient's WES results are reported), WES will be performed on each biological parent's DNA sample as well as on the patient's DNA sample. WES data from the biological parent(s) will be used to help interpret the patient's variants that are highly likely to be related to his/her medical condition, whether these variants were inherited from a parent or occurred as a new change just in the patient. Although WES will be performed on each biological parent’s sample, only variants related to the child’s medical condition and selected secondary findings (medically actionable and carrier status genes) selected by each biological parent will be evaluated.

Please note that variant confirmation by Sanger sequencing will not be performed on biological parents' samples without a separate clinical test order for the parent(s). If clinically significant variants are detected in biological parent's WES data, follow-up variant confirmation testing should be offered to the parent to confirm their WES finding(s). Follow-up variant confirmation test on parent(s) will be performed with additional charges, and a separate test report for the parent(s) will be issued by the laboratory.

12. Will a Lab Report be Issued for Each Biological Parent?

Yes, a separate test report will be issued for each biological parent submitting a sample. The biological parents' lab reports will be sent to the healthcare provider who ordered the patient's (child's) WES testing. Each biological parent's report ***will include*** whether he/she carries the same variant(s) found in the child that are related to the child's medical condition. For secondary findings that are not related to the patient's medical condition, such as variants in medically actionable genes or reproductive carrier screening genes, biological parents may choose whether or not they wish to be informed of these findings in their report. In the WES informed consent form, biological parents submitting a sample will be asked to indicate their choice of reporting preference for secondary findings.

Each biological parent's report will include information on ***only*** the variants identified in that parent. The following information will ***NOT*** be included in the biological parent's report: variants found in the patient (child) that was not inherited from that parent, the patient's (child's) secondary findings, and variants found in the other biological parent.

Please note: If patient's WES finds variants that could be related to his/her medical condition, then the patient's (child's) WES report ***will include*** whether each variant was inherited from the mother, the father, or was not seen in either parent.

13. Will the Laboratory Issue an Updated WES Report?

The laboratory currently does not perform a scheduled periodic review of all previous WES cases. However, if the laboratory becomes aware of new information regarding the significance of a particular variant that was observed in the patient, then the laboratory can issue an updated report to the healthcare provider who ordered the patient's WES. The review does not include a complete review of all of patient or biological parents' WES data. Review of entire WES data (re-analysis) is only available with additional charges. If the patient's healthcare provider orders re-analysis of WES data to be done with additional charges, then the laboratory will issue an updated WES report, even if no new variants are found by re-analysis.

14. What are Potential Consequences of WES?

- There is a possibility that the patient and his/her biological parents will learn genetic information that is not directly related to the reason why WES was ordered. This information might relate to diseases with symptoms that may develop in the future in the patient or his/her family members as well as conditions that currently have no treatment available.
- It is possible for WES to reveal a discrepancy between the reported familial relationship and the genetic relationship between the patient and his/her biological parents. One example is non-paternity, which reveals that the person identified as a father is not the biological father. Such discrepancies ***will be*** reported to the ordering healthcare provider, because they may directly affect result interpretation or implications for other family members.
- Testing may suggest that patient's biological parents are related by blood, and this result ***will be*** reported to the ordering healthcare provider.
- WES data from the patient and his/her biological parents will be kept in a secure computer environment, where only limited, authorized personnel have access to these data. Every care will be taken to make sure that this information stays in the secure environment, but a very small risk exists for these data to unintentionally be released to an unsecure environment.

15. What Laws Exist to Protect Against Genetic Discrimination?

There are federal laws in place that prevent health insurers and employers from discriminating based on genetic information , such as the Genetic Information Nondiscrimination Act (GINA) of 2008 (Public Law 110-233). There are currently no federal laws that prohibit life insurance, long-term care, or disability insurance companies from discriminating based on genetic information. Different states may have more comprehensive laws in this area. The Genetics Public & Policy Center's website (at www.dnapolicy.org) is available for additional information on the protections provided by GINA and the limitations of current legislation. The results of genetic testing are considered "Protected Health Information (PHI)" as described in the Health Insurance Portability and Accountability Act (HIPAA) of 1996 (Public Law 104-191). The release of test results is limited to authorized personnel, such as the ordering healthcare provider, and to other parties as required by law.

16. Will My (My Child's) Health Insurance Plan Cover WES?

You should discuss with the patient's healthcare provider about the approximate cost of WES, the billing process, and the option of obtaining insurance prior authorization. It is highly recommended to obtain insurance prior authorization before WES is ordered on the patient. Insurance plans may or may not cover genetic testing depending on the patient's insurance plan. **Even if the patient's insurance plan covers WES, you may still have to pay a co-insurance, co-pay, or meet a deductible if required by the insurance plan**. Although obtaining insurance prior authorization maximizes the chance of insurance payment for the test, it is not a guarantee of payment by insurance. If the patient's insurance plan indicates that "prior authorization / pre-determination not required," this does not necessarily mean that WES is a covered benefit. Some insurance plans do not cover genetic testing at all.

17. Will Biological Parents be Billed for Participating in Child's WES?

The test charges for the biological parents' WES analyses are added to the patient's WES charges and are billed to the *patient's (child's) insurance*. Therefore, biological parents will not receive a separate bill for their WES testing. However, blood collection fee for each biological parent may be billed to each parent's insurance.

18. What will Happen to My (My Child's) DNA Sample Remaining from WES?

Any unused portion of the DNA sample from WES done on the patient and his/her parents will be kept by the clinical laboratory with identifying information attached on the sample. It may be made available for more testing (clinical or research test) as ordered by a healthcare provider. This should not be considered as a DNA banking or storage service, and the laboratory does not guarantee that the sample will be available for future use. If you choose that your (your child's) remaining DNA sample can be used for research purposes, then the direct identifiers (such as name, date of birth, medical record number) will be removed from the DNA sample before research testing is performed. However, results from the research testing may be communicated to the ordering healthcare provider at the laboratory's discretion.

19. What will Happen to My (My Child's) WES Data?

DNA sequence data from WES done on the patient and his/her biological parents will be stored in the clinical laboratory and will be available for more testing (clinical or research test) as ordered by a healthcare provider. These WES data will be kept in a secure computer environment, including cloud computer environments. Cloud computer environments are commonly used to examine genetic data and to store genetic data on remote computer servers hosted by a third party. Nationwide Children's Hospital contracts with reputable companies that provide these computing services. Only limited, authorized personnel have access to the patient and biological parents' genetic data. Every precaution will be taken to make sure that this information stays in the secure environment, but a very small risk exists for these data to unintentionally be released to an unsecure environment. If you choose that your (your child's) WES data can be used for research purposes, then the direct identifiers (such as name, date of birth, medical record number) will be removed from the data before research studies are performed. However, results from the research study may be communicated to the ordering healthcare provider at the discretion of the laboratory.

20. How will My (My Child's) DNA Sample and/or WES Data be Used for Research?

In the WES informed consent form, you will be asked whether or not you want your (your child's) DNA sample and data from WES (such as DNA sequence data) used for research done at Nationwide Children's Hospital. If you agree to have your (your child's) sample and data used for research, then your (your child's) DNA sample, clinical information, and DNA sequence data from WES may be kept by Nationwide Children's Hospital and used for development of new tests or for research, with privacy assured. No compensation will be given for any additional tests or products that result from research and development using these specimens. It is unlikely that you (your child) will directly benefit from this research, but the information may be used to help future patients. At the discretion of the laboratory, results from research studies may be communicated to the ordering healthcare provider. You may refuse to allow your (your child's) DNA sample and data used in this way. You can withdraw consent for the use of DNA sample and WES data for research purposes by calling the clinical laboratory at 1-800-934-6575 and speaking with a laboratory genetic counselor. ***Refusal to consent to medical research will not affect your (your child's) results or medical care.*** Within the WES informed consent form, you will be asked to consent or refuse to have your (your child's) DNA sample and data used for research purposes.