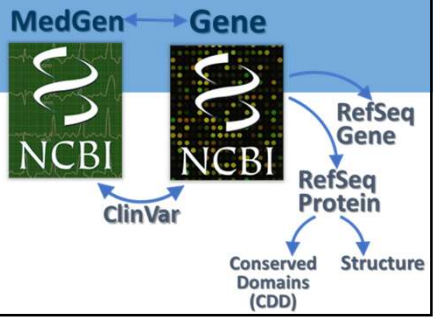
# Putting it all together!

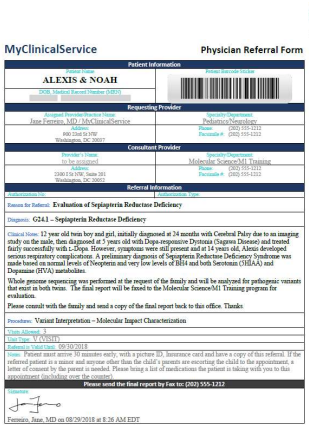
## USING NCBI RESOURCES TO ASSIST IN UNDERSTANDING, EXPLAINING, DIAGNOSING AND TREATING HUMAN DISEASE

- TRANSPARENT, SUSTAINABLE, RELIABLE, TRUSTED -
INFORMATION FOR CLINICAL DECISION SUPPORT




NIH U.S. National Library of Medicine
National Center for Biotechnology Information

## Here's where your cases begin:
## A Referral & a Genetic Test Result!


MyClinicalService — Physician Referral Form

Patient Information
ALEXIS & NOAH

Requesting Provider
Jane Ferreira, MD / MyClinicalService
Pediatrics/Neurology

Consultant Provider
to be assigned
Molecular Science/MI Training

Referral Information
Reason for Referral: Evaluation of Sepiapterin Reductase Deficiency
Diagnosis: G24.1 – Sepiapterin Reductase Deficiency

Clinical Notes: 12 year old twin boy and girl, initially diagnosed at 24 months with Cerebral Palsy due to an imaging study on the male, then diagnosed at 5 years old with Dopa-responsive Dystonia (Segawa Disease) and treated fairly successfully with L-Dopa. However, symptoms were still present and at 14 years old, Alexis developed serious respiratory complications. A preliminary diagnosis of Sepiapterin Reductase Deficiency Syndrome was made based on normal levels of Neopterin and very low levels of BH4 and both Serotonin (5HIAA) and Dopamine (HVA) metabolites.

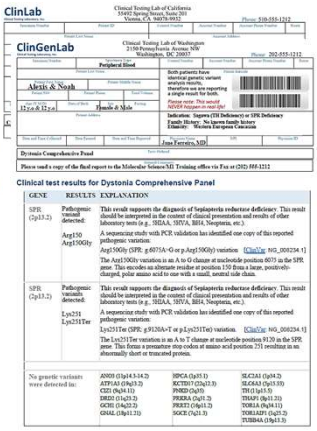
Whole genome sequencing was performed at the request of the family and will be analyzed for pathogenic variants that exist in both twins. The final report will be faxed to the Molecular Science/MI Training program for evaluation.

Please consult with the family and send a copy of the final report back to this office. Thanks

Procedure: Variant Interpretation – Molecular Impact Characterization

Please send the final report by Fax to: (202) 555-1212

Ferreira, Jane, MD on 08/29/2018 at 8:26 AM EDT


ClinLab
Clinical Testing Lab of California
5340E Spring Street, Suite 201
Vienna, CA 94078-9932
Phone: 510-555-1212

ClinGenLab
Clinical Testing Lab of Washington
2150 Pennsylvania Avenue NW
Washington, DC 20057
Phone: 202-555-1212

Alexis & Noah
Female & Male
Peripheral Blood
Both patients have identical genetic variant analysis results, therefore we are reporting a single result for both.
Please note: This would NEVER happen in real life!
Indication: Segawa (TH Deficiency) or SPR Deficiency
Family History: No known family history
Ethnicity: Hispanic, Mexico, Indigenous Caucasian
Jane Ferreira, MD
Dystonia Comprehensive Panel
Please send a copy of the final report to the Molecular Science/MI Training office via Fax at (202) 555-1212

Clinical test results for Dystonia Comprehensive Panel

| GENE | RESULTS | EXPLANATION |
|---|---|---|
| SPR (2p13.2) | Pathogenic variant detected: Arg150 Arg150Gly | This result supports the diagnosis of Sepiapterin reductase deficiency. This result should be interpreted in the context of clinical presentation and results of other laboratory tests (e.g., 5HIAA, 5HVA, BH4, Neopterin, etc.). A sequencing study with PCR validation has identified one copy of this reported pathogenic variation: Arg150Gly (SPR: g.6073A>G or p.Arg150Gly) variation. [ClinVar NG_008234.1] The Arg150Gly variation is an A to G change at nucleotide position 6073 in the SPR gene. This encodes an alternate residue at position 150 from a large, positively-charged, polar amino acid to one with a small, neutral side chain. |
| SPR (2p13.2) | Pathogenic variants detected: Lys251 Lys251Ter | This result supports the diagnosis of Sepiapterin reductase deficiency. This result should be interpreted in the context of clinical presentation and results of other laboratory tests (e.g., 5HIAA, 5HVA, BH4, Neopterin, etc.). A sequencing study with PCR validation has identified one copy of this reported pathogenic variation: Lys251Ter (SPR: g.9130A>T or p.Lys251Ter) variation. [ClinVar NG_008234.1] The Lys251Ter variation is an A to T change at nucleotide position 9130 in the SPR gene. This forms a premature stop codon at amino acid position 251 resulting in an abnormally short or truncated protein. |

No genetic variants were detected in:

| | | |
|---|---|---|
| ANO3 (1p14.3-14.2) | HPCA (1p35.1) | SLC2A1 (1p34.2) |
| ATP1A3 (19q13.2) | KCTD17 (22q13.3) | SLC6A3 (5p15.33) |
| CIZ1 (9q34.11) | PNKD (2q36) | TH (11p15.5) |
| DRD1 (5q35.2) | PRKRA (2q31.2) | THAP1 (8p11.21) |
| GCH1 (14q22.2) | PRRT2 (16p11.2) | TOR1A (9q34.11) |
| GNAL (18p11.21) | SGCE (7q21.3) | TOR1AIP1 (1q25.2) |
| | | TUBB4A (19p13.3) |

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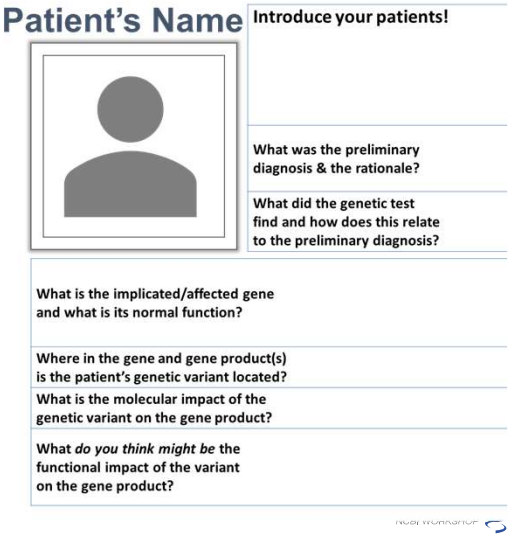
## Here's a case guide sheet
## to fill out as you go along.


Patient's Name

Introduce your patients!

What was the preliminary diagnosis & the rationale?

What did the genetic test find and how does this relate to the preliminary diagnosis?

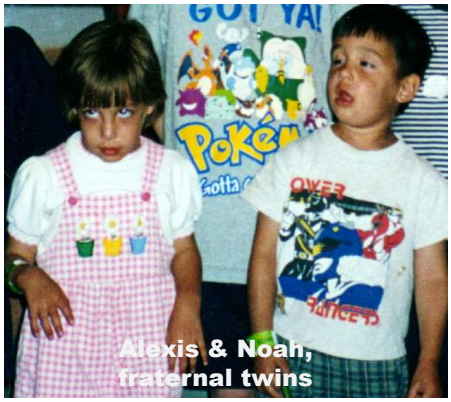
What is the implicated/affected gene and what is its normal function?

Where in the gene and gene product(s) is the patient's genetic variant located?

What is the molecular impact of the genetic variant on the gene product?

What *do you think might be* the functional impact of the variant on the gene product?

# Twins' diagnosis of Cerebral Palsy, then Segawa Dystonia, then…..




http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3314311/
http://dystonia.thebeerys.com/Home.aspx
http://dystonia.thebeerys.com/Video.aspx

**PRESENTING SYMPTOMS:**

developmental delay, dystonic movements, tremors, muscle hypotonia, unsteady gait, vomiting, drooling, sleep disturbances

**STRANGE FINDINGS**

conditions deteriorated and were temporal (significantly worse after 11am daily)

**CONTINUED ISSUES AFTER "SUCCESSFUL TREATMENT"**

tremors, drooling, sleep disturbances, and eventually unexplained respiratory issues in Alexis

TODAY



# Researching the Referral
## Wait! What is Sepiapterin Reductase Deficiency?

MyClinicalService — Physician Referral Form

Patient Information
ALEXIS & NOAH

Requesting Provider
Jane Ferreiro, MD / MyClinicalService

Consultant Provider

Referral Information
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Diagnosis: G24.1 – Sepiapterin Reductase Deficiency
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Whole genome sequencing was performed at the request of the family and will be analyzed for pathogenic variants that exist in both twins. The final report will be faxed to the Molecular Science/M1 Training program for evaluation.
Please consult with the family and send a copy of the final report back to this office. Thanks.
Procedures: Variant Interpretation – Molecular Impact Characterization

Ferreiro, Jane, MD on 08/29/2018 at 8:26 AM EDT

Referral Information
Authorization No: Authorization Type:
Reason for Referral: Evaluation of Sepiapterin Reductase Deficiency
Diagnosis: G24.1 – Sepiapterin Reductase Deficiency
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Please consult with the family and send a copy of the final report back to this office. Thanks.
Procedures: Variant Interpretation – Molecular Impact Characterization

# Researching the Referral

**INFORMATION ABOUT THE PROPOSED DISEASE/DISORDER FROM HUMAN-CURATED SOURCES:**

1. To learn more about the preliminary diagnosis, **go to the NCBI website** (**https://www.ncbi.nlm.nih.gov** or "google" NCBI to find the homepage) and **search NCBI's MedGen database with**: **`sepiapterin reductase deficiency`**

   **WHAT KEY THINGS DO YOU FIND IN THE SUMMARY SECTION?** (This is a place for *your* notes.)

   **WHAT OTHER DISORDERS COULD HAVE BEEN CONSIDERED AND WHICH GENES ARE ASSOCIATED WITH EACH?** (Hint: Check the **GeneReviews>Differential Diagnosis link**.)

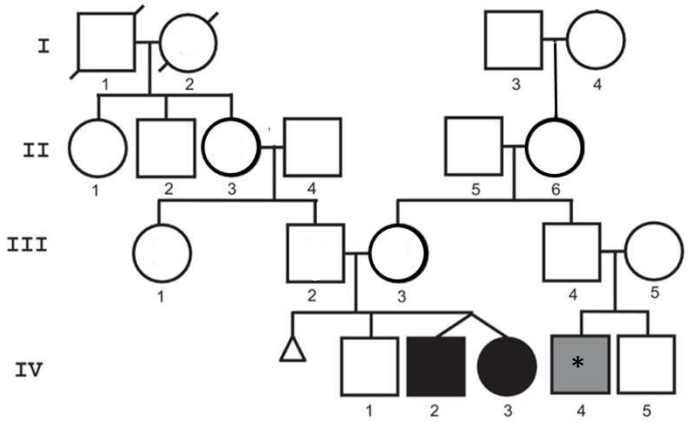
   **WHAT WOULD YOU LOOK FOR IN A LABORATORY TEST AS CONFIRMATION OF THE DIAGNOSIS?** (Hint: Check the **GeneReviews>Diagnosis Link** for what it says about CSF neurotransmitters and pterins.)

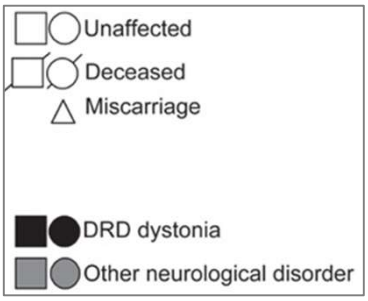
   **DO THE LAB RESULTS CONFIRM THE REFERRAL'S LISTED DIAGNOSES?** **We'll go over this together next!**

2. To assist you in learning more about this disorder **find links to**:

   - ☐ **PubMed** – to find some helpful recent publications.
   - ☐ **ClinicalTrials.gov** – to see if any are currently enrolling participants.
   - ☐ **Patient Education materials** – to provide as an additional resource for the family.
   - ☐ The **NIH Genetic Testing Registry -** where you could find a genetic test to order.

# MIGHT THIS BE GENETIC? HERE'S THE FAMILY PEDIGREE



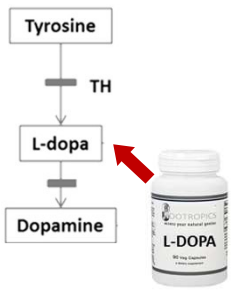

*Late discovery, an unrelated neurological disorder runs in the maternal line of this family unit.



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3314311/
*(graphic modified to highlight key points)*



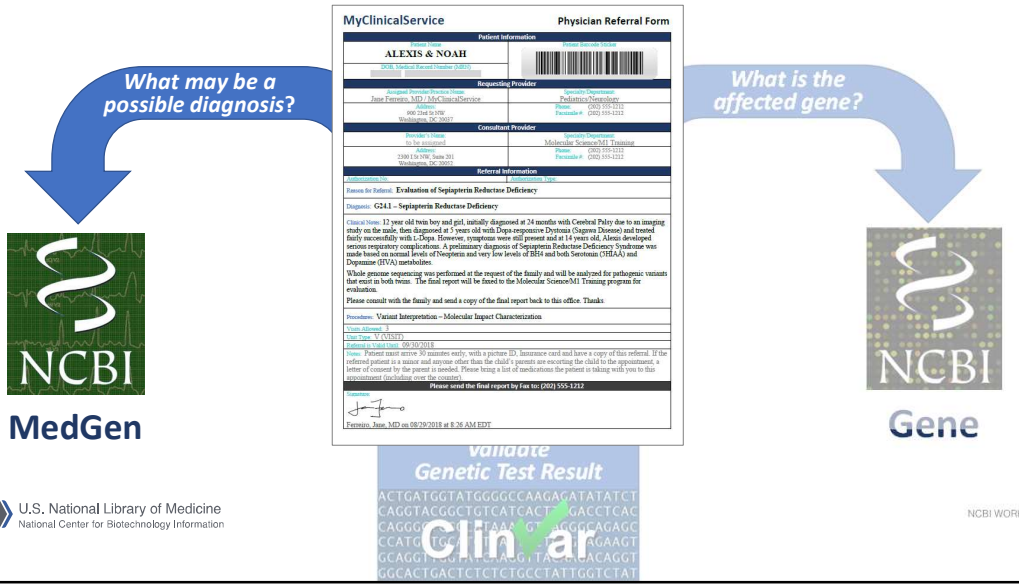
# INITIALLY, THEY WERE TREATED WITH L-DOPA & IT SEEMED TO WORK....ISH. WHAT DID THE DOCTOR THINK WAS WRONG? WHAT OTHER TARGETS SHOULD WE CONSIDER?



# MEDGEN

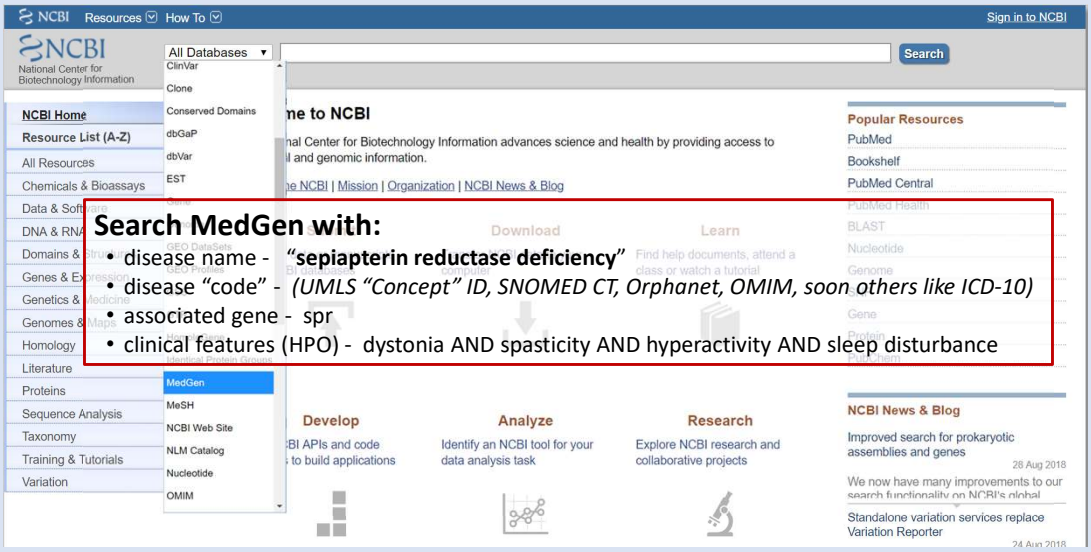
*AGGREGATED INFORMATION FOR GENETIC DISORDERS & HEALTH-RELATED PHENOTYPES*

https://www.ncbi.nlm.nih.gov/medgen






# Let's look it up! https://www.ncbi.nlm.nih.gov



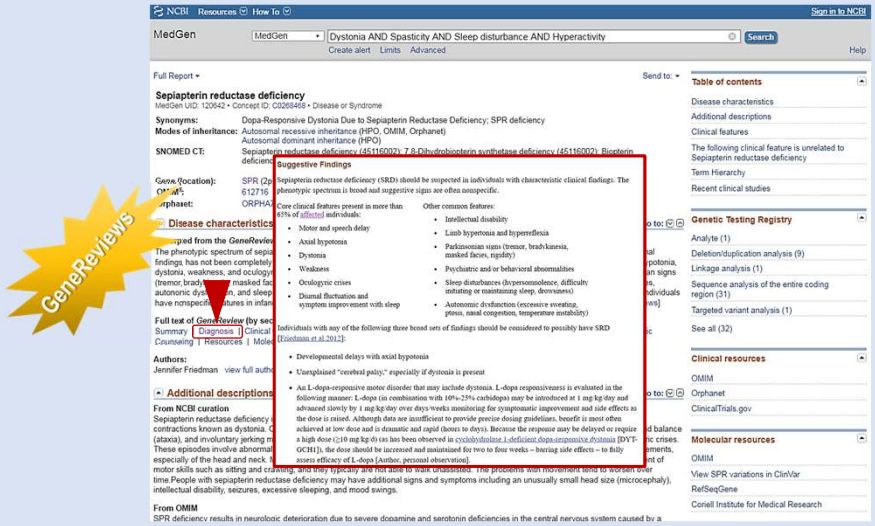

**Search MedGen with:**

- disease name - **"sepiapterin reductase deficiency"**
- disease "code" - *(UMLS "Concept" ID, SNOMED CT, Orphanet, OMIM, soon others like ICD-10)*
- associated gene - spr
- clinical features (HPO) - dystonia AND spasticity AND hyperactivity AND sleep disturbance

# Okay, let's get into this!




# MedGen (Medical Genetics Disorders): Sepiapterin Reductase Deficiency



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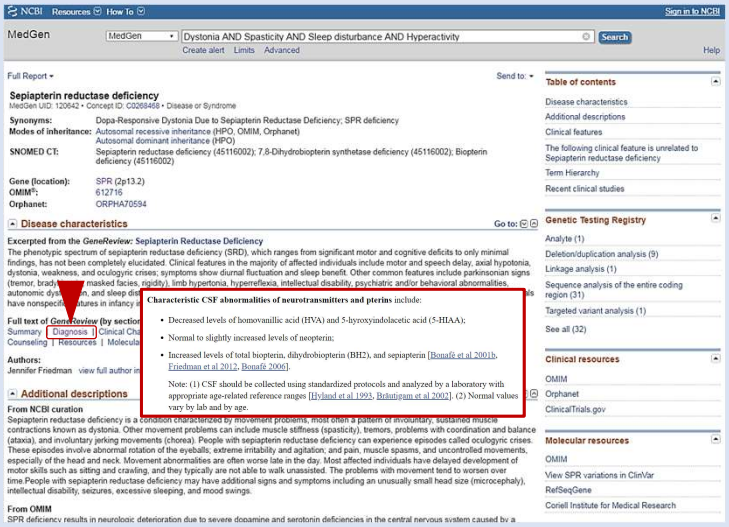





# DIAGNOSTIC LABORATORY TESTS

**WHAT WOULD YOU LOOK FOR IN A LABORATORY TEST AS CONFIRMATION OF THE DIAGNOSIS?**
(Hint: Check the **GeneReviews>Diagnosis** section for what it says about CSF neurotransmitters and pterins.)

"….**Misdiagnoses of cerebral palsy (CP) are common.** ….. **Cerebrospinal fluid findings are distinctive. Diagnosis is confirmed by mutation analysis** …."

Friedmen, et. al, "Sepiapterin reductase deficiency: a treatable mimic of cerebral palsy."
Ann Neurol. 2012 Apr;71(4):520-30. PMID: 22522443

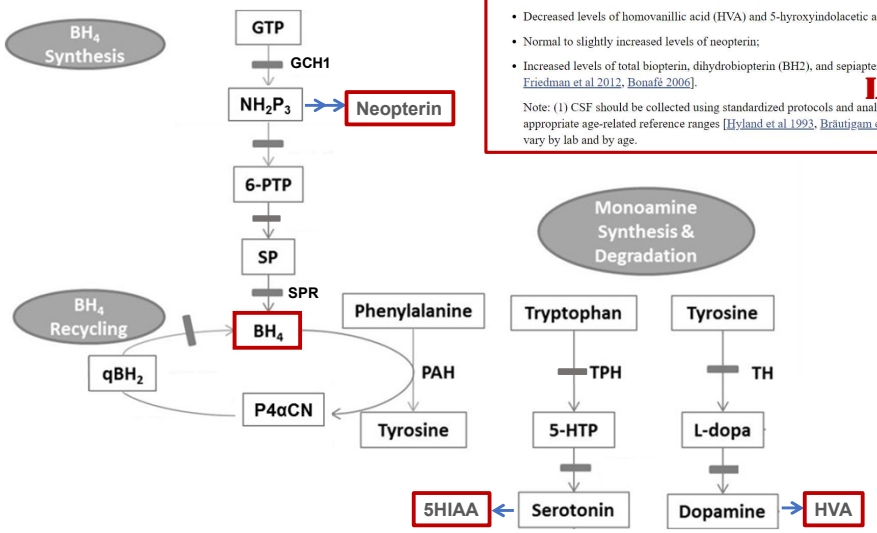
**Characteristic CSF abnormalities of neurotransmitters and pterins** include:

- Decreased levels of homovanillic acid (HVA) and 5-hyroxyindolacetic acid (5-HIAA);
- Normal to slightly increased levels of neopterin;
- Increased levels of total biopterin, dihydrobiopterin (BH2), and sepiapterin [Bonafé et al 2001b, Friedman et al 2012, Bonafé 2006].

  Note: (1) CSF should be collected using standardized protocols and analyzed by a laboratory with appropriate age-related reference ranges [Hyland et al 1993, Bräutigam et al 2002]. (2) Normal values vary by lab and by age.

*How do these recommendations relate to a biological pathway related to the L-Dopa (which seemed to work for a while)?*

# BASED ON BIOLOGY, WHAT ANALYTES COULD HAVE BEEN MEASURED?

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**LAB TESTS**

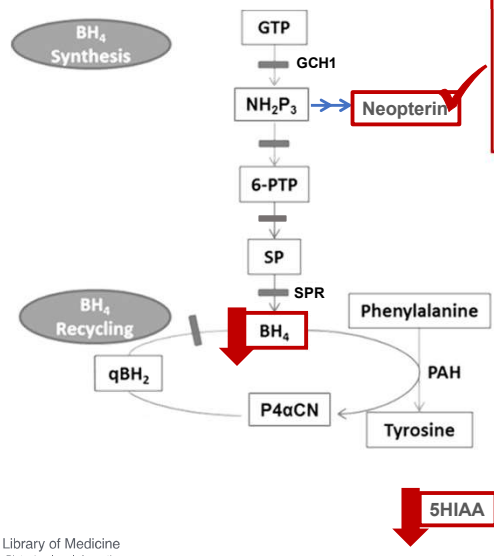
Note: (1) CSF should be collected using standardized protocols and analyzed by a laboratory with appropriate age-related reference ranges [Hyland et al 1993, Bräutigam et al 2002]. (2) Normal values vary by lab and by age.



| Peripheral Blood TESTS | RESULT | FLAG | UNITS | REFERENCE INTERVAL | RESULT | FLAG | UNITS | REFERENCE INTERVAL | LAB |
|---|---|---|---|---|---|---|---|---|---|
| | Alexis - 6 y.o. Female | | | | Noah - 6 m.o. Male | | | | |
| CBC, Platelet Ct, and Diff | | | | | | | | | |
| Hematocrit | 30.6 | | | 30.9 - 37.0 | 34.8 | | % | 30.9 - 37.0 | 07 |
| Hemoglobin | 10.1 | | | 10.3 - 12.4 | 11.7 | | g/dL | 10.3 - 12.4 | 07 |
| Red Blood Cell Count | 4.70 | | | 4.10 - 5.00 | 4.85 | | x10E6/uL | 4.10 - 5.00 | 07 |
| White Blood Cell Count | 14.3 | | | 6.2 - 14.5 | 12.2 | | x10E3/uL | 6.2 - 14.5 | 07 |
| RDW | nd | | | N/A | nd | | % | N/A | 07 |
| MCV | 71.2 | | | 70.5 - 81.2 | 73.7 | | fL | 70.5 - 81.2 | 07 |
| MCH | 14.5 | | | 23.2 - 27.5 | 26.2 | | pg | 23.2 - 27.5 | 07 |
| MCHC | 32.2 | | | 31.9 - 35.0 | 34.1 | | g/dL | 31.9 - 35.0 | 07 |
| Imm.Granulocytes (Absolute) | 4.9 | | | 1.6 - 8.3 | 7.2 | | x10E3uL | 1.6 - 8.3 | 07 |
| Granulocytes (Percent) | 29.4 | | | 21.3 - 66.7 | 45.3 | | % | 21.3 - 66.7 | 07 |
| Eosinophils (Absolute) | nd | | x10E3uL | NA | nd | | x10E3uL | NA | 07 |
| Eosinophils (Percent) | nd | | % | 0.0 - 3.3 | nd | | % | 0.0 - 3.3 | 07 |
| Basophil (Absolute) | nd | | x10E3uL | NA | nd | | x10E3uL | NA | 07 |
| Basophil (Percent) | nd | | % | 0 - 2 | nd | | % | 0 - 2 | 07 |
| Monocytes (Absolute) | nd | | x10E3uL | N/A | nd | | x10E3uL | N/A | 07 |
| Monocytes (Percent) | nd | | % | 5 - 11 | nd | | % | 5 - 11 | 07 |
| Lymphocytes (Absolute) | 5.8 | | x10E3uL | 1.9 - 6.8 | 4.7 | | x10E3uL | 1.9 - 6.8 | 07 |
| Lymphocytes (Percent) | 61 | | | 20 - 64 | 52 | | % | 20 - 64 | 07 |
| Platelets (Absolute) | 276 | | | 219 - 452 | 308 | | x10E3uL | 219 - 452 | 07 |

| CSF TESTS | RESULT | FLAG | UNITS | REFERENCE INTERVAL | RESULT | FLAG | UNITS | REFERENCE INTERVAL | LAB |
|---|---|---|---|---|---|---|---|---|---|
| | Alexis - 6 y.o. Female | | | | Noah - 6 m.o. Male | | | | |
| Selected Pterin/Neuro Basic | | | | | | | | | |
| Neopterin | 13 | | nmol/L | 7-40 | 38 | | nmol/L | 7-40 | 07 |
| Tetrahydrobiopterin (BH4) | 0.8 | Very low | nmol/L | 9-40 | 1.2 | Very low | nmol/L | 9-40 | 07 |
| 5HIAA (Serotonin metabolite) | 6 | Very low | nmol/L | 88-278 | 8 | Very low | nmol/L | 88-278 | 07 |
| HVA (Dopamine metabolite) | 28 | Very low | nmol/L | 200-800 | 31 | Very low | nmol/L | 200-800 | 07 |

# MAPPING THE LAB TEST RESULTS TO THE BIOLOGY

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**LAB TESTS**

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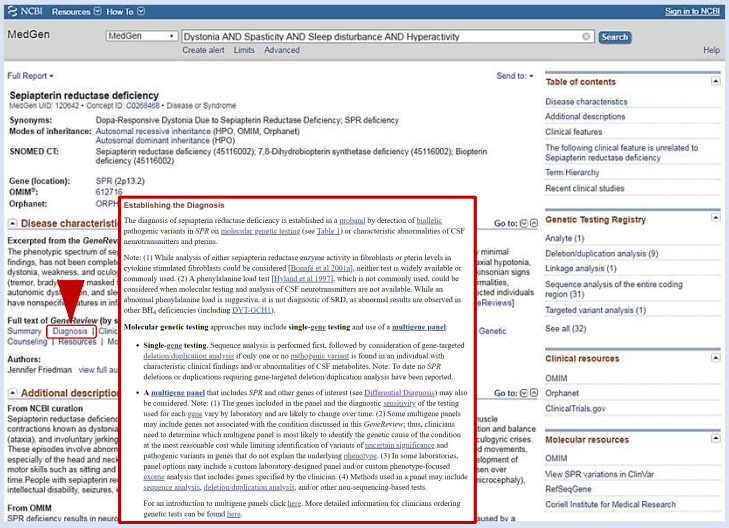
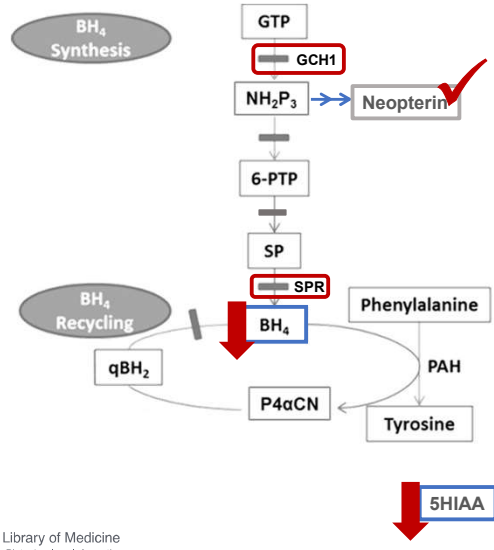
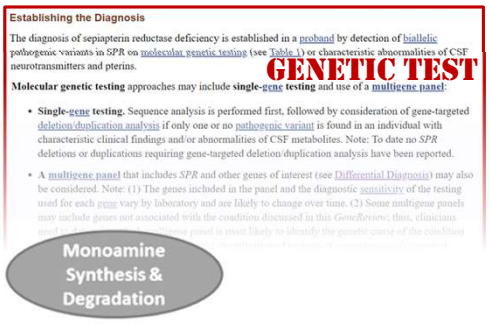
# MedGen (Medical Genetics Disorders): Sepiapterin Reductase Deficiency



# BASED ON BIOLOGY, WHICH GENES COULD BE ASSESSED?

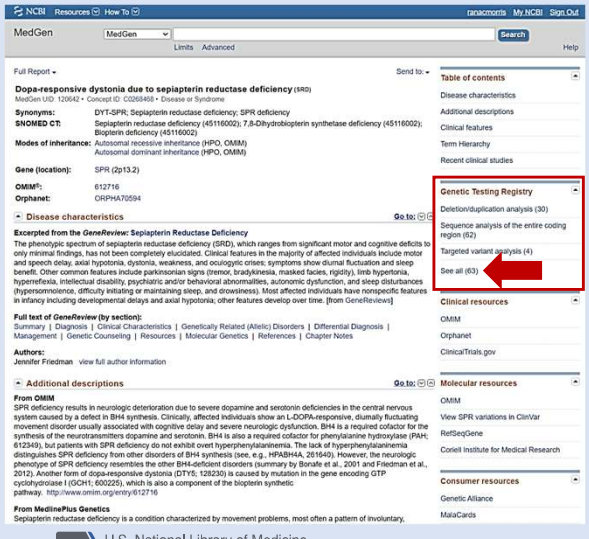


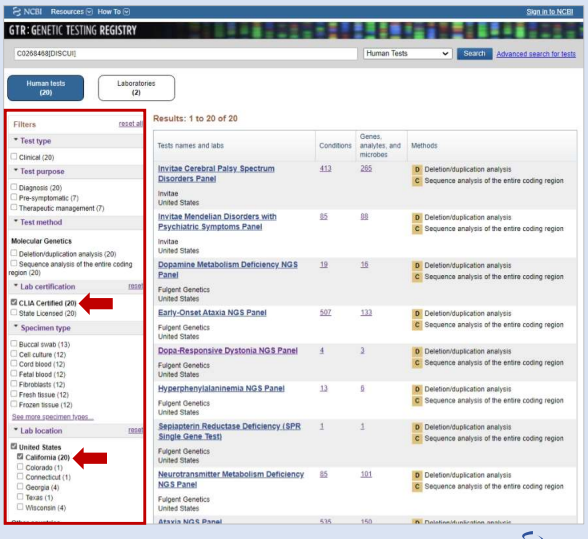

Monoamine Synthesis & Degradation



## MedGen (Medical Genetics Disorders): Sepiapterin Reductase Deficiency

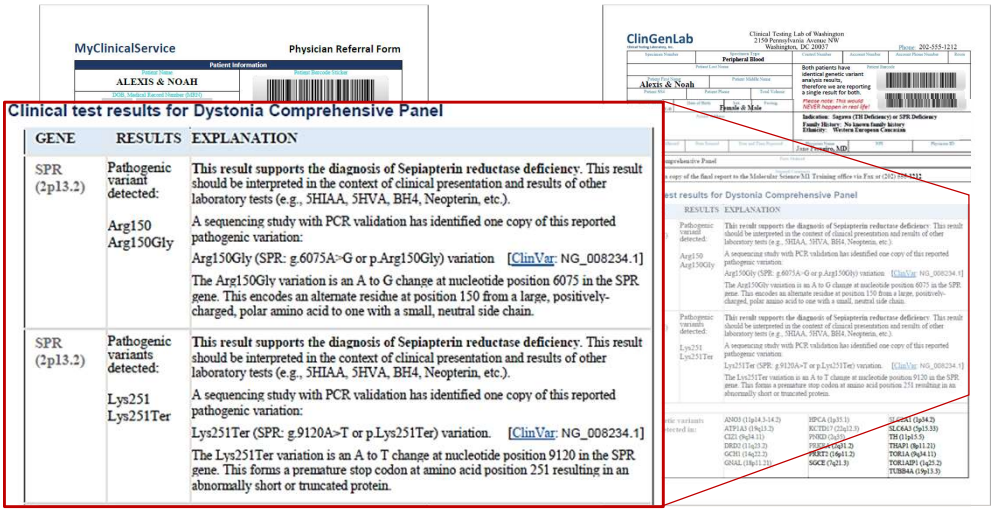



## Genetic Testing Registry (GTR): *Tests with SPR Deficiency listed as Condition*



# Understanding and Validating the Genetic Test Results

***Begin to learn the molecular aspects of your patients' situation.***



MyClinicalService — Physician Referral Form

Patient Information

ALEXIS & NOAH

ClinGenLab

Clinical Testing Lab of Washington
2150 Pennsylvania Avenue NW
Washington, DC 20037

Peripheral Blood

Alexis & Noah

Female & Male

Both patients have identical genetic variant analysis results, therefore we are reporting a single result for both.

Please note: This would NEVER happen in real life!

Indication: Sapiens (TH Deficiency) or SPR Deficiency
Family History: No known family history
Ethnicity: Western European Caucasian

Clinical test results for Dystonia Comprehensive Panel

| GENE | RESULTS | EXPLANATION |
|---|---|---|
| SPR (2p13.2) | Pathogenic variant detected:<br><br>Arg150 Arg150Gly | **This result supports the diagnosis of Sepiapterin reductase deficiency.** This result should be interpreted in the context of clinical presentation and results of other laboratory tests (e.g., 5HIAA, 5HVA, BH4, Neopterin, etc.).<br><br>A sequencing study with PCR validation has identified one copy of this reported pathogenic variation:<br><br>Arg150Gly (SPR: g.6075A>G or p.Arg150Gly) variation [ClinVar: NG_008234.1]<br><br>The Arg150Gly variation is an A to G change at nucleotide position 6075 in the SPR gene. This encodes an alternate residue at position 150 from a large, positively-charged, polar amino acid to one with a small, neutral side chain. |
| SPR (2p13.2) | Pathogenic variants detected:<br><br>Lys251 Lys251Ter | **This result supports the diagnosis of Sepiapterin reductase deficiency.** This result should be interpreted in the context of clinical presentation and results of other laboratory tests (e.g., 5HIAA, 5HVA, BH4, Neopterin, etc.).<br><br>A sequencing study with PCR validation has identified one copy of this reported pathogenic variation:<br><br>Lys251Ter (SPR: g.9120A>T or p.Lys251Ter) variation. [ClinVar: NG_008234.1]<br><br>The Lys251Ter variation is an A to T change at nucleotide position 9120 in the SPR gene. This forms a premature stop codon at amino acid position 251 resulting in an abnormally short or truncated protein. |

# Understanding and Validating the Genetic Test Results

**INFORMATION ABOUT THESE VARIANTS FROM HUMAN-CURATED SOURCES:**

3. **WHAT ARE THE SPECIFIC GENE AND VARIATIONS IDENTIFIED IN THE TWINS?**
   *(Read the test results, sometimes it is really helpful!)*

   **WHAT DOES THE GENETIC TEST RESULT SAY ABOUT THEIR DIAGNOSES?**

4. To validate what is asserted by this clinical testing laboratory, **search NCBI's ClinVar database with:**

   **`SPR Arg150Gly  OR  SPR Lys251Ter`**

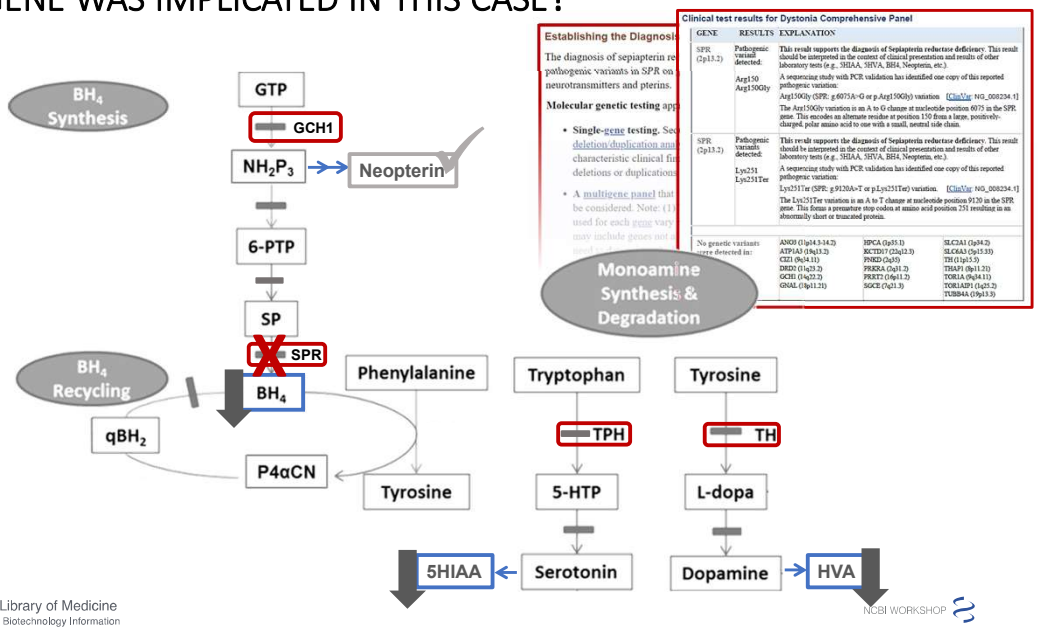
   **WHAT DOES CLINVAR SAY IN THE "INTERPRETATION" FIELD ABOUT EACH GENE VARIATION?**

   **Scroll down to the "Submitted interpretations and evidence" section** and look at what the various laboratories assert about the pathogenicity of each variation.

   **WHAT DO YOU CONCLUDE ABOUT THE VALIDITY OF THE GENETIC TEST RESULTS' CALLS WITH REGARD TO PATHOGENICITY FOR EACH VARIANT?**

5. In preparation for the next step, **find and write down the HGVS formatted information for each variant.** This will help to find the location of each of the variants in the gene (NG_ g.) & protein (NP_ p.).

# WHICH GENE WAS IMPLICATED IN THIS CASE?

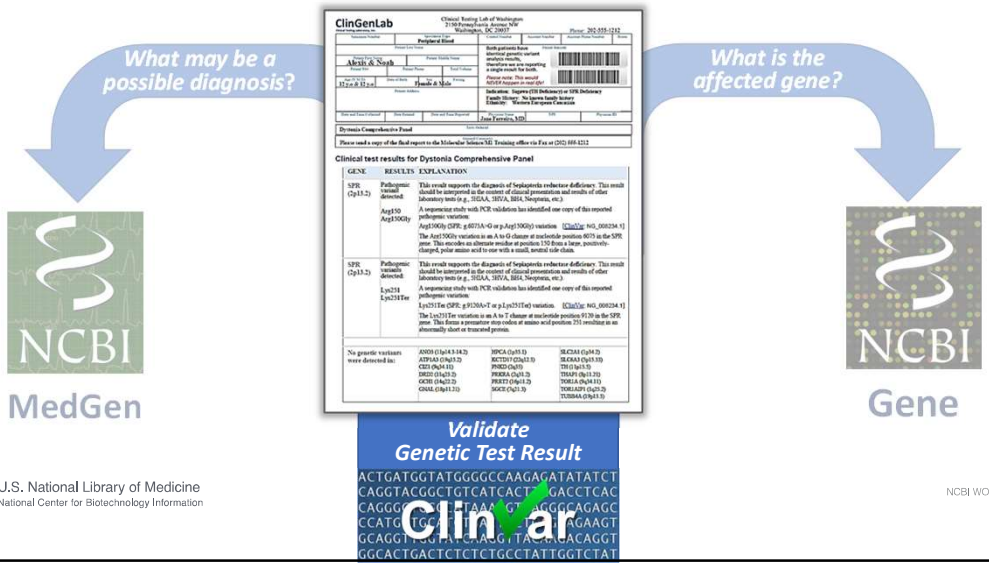





# CLINVAR

*REGISTRY OF HEALTH-RELATED ASSERTIONS FOR HUMAN GENETIC VARIATIONS*

https://www.ncbi.nlm.nih.gov/clinvar



# CLINVAR

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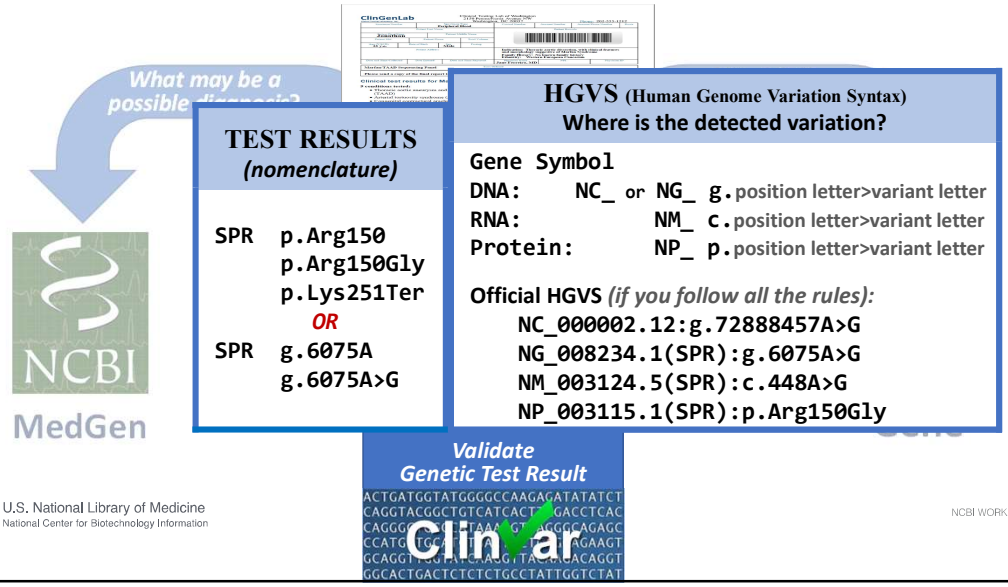
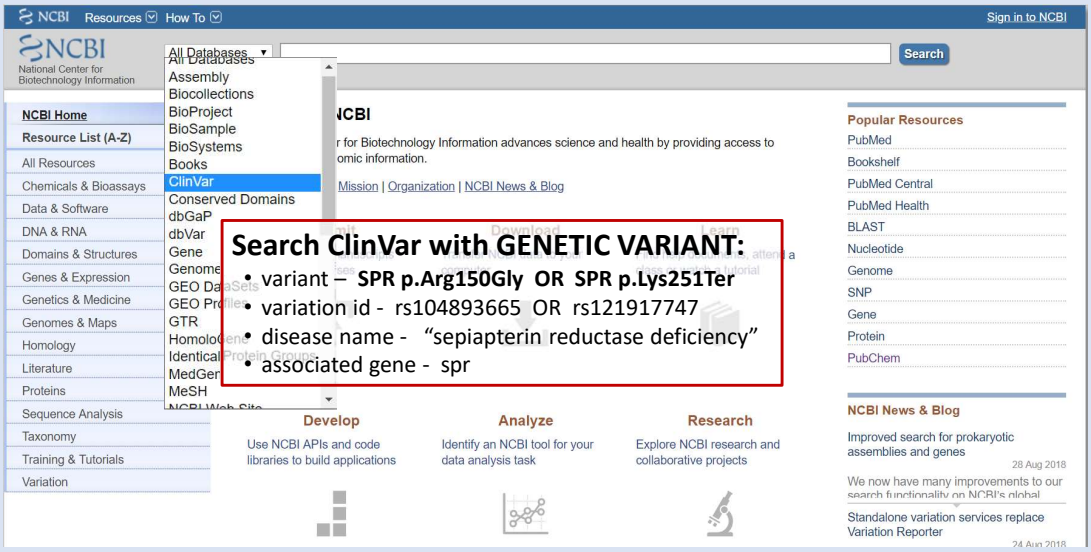
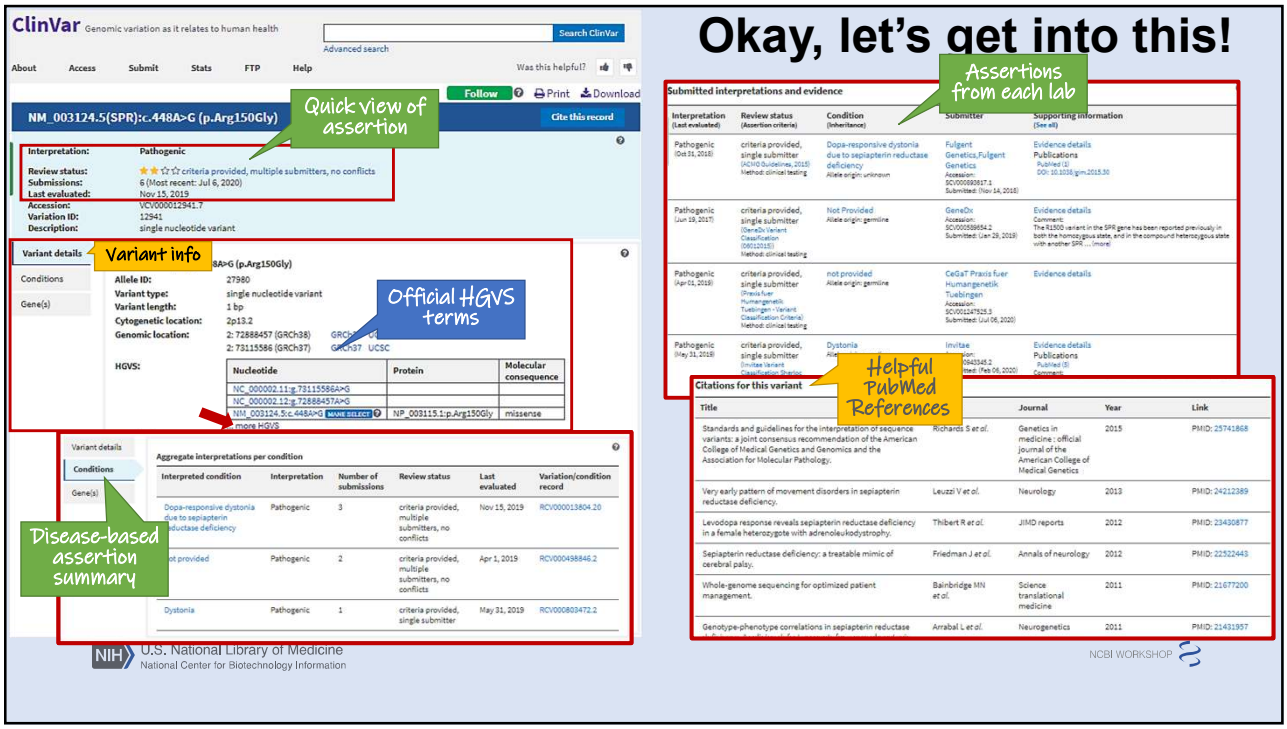





# Let's look it up! https://www.ncbi.nlm.nih.gov






# Review of Clinical Information

**What we've found so far:**

- Searched **MedGen** with Alexis & Noah's symptoms: *We've identified a preliminary diagnosis for the twins of **Sepiapterin Reductase Deficiency.***
  as well as specific information about what ***clinical lab (GeneReviews)*** and ***genetic tests (GTR)*** can be ordered to validate the proposed diagnosis.

  ➢ **Found sources for Clinical Decision Support Materials:**
  GeneReviews reports, PubMed articles & Clinical Trials

- The results of the *lab and genetic tests support the diagnosis and provide clues to finding an effective therapy*.

  ➢ **Identified sources for Patient Education Materials:**
  Links to Medline Plus, Genetics Home Reference, GeneReviews, & *more…*

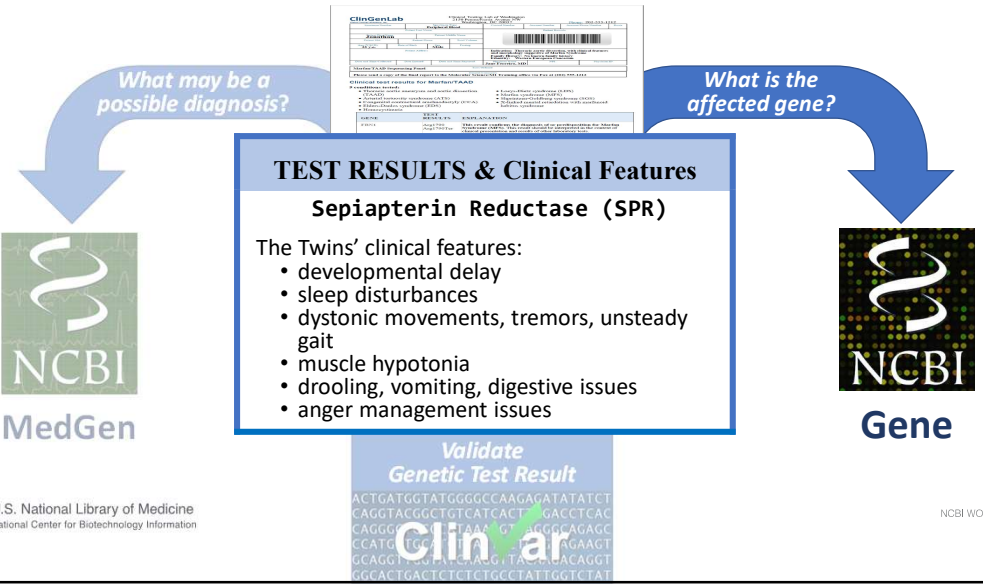
**Discovered two *specific hereditable pathogenic SPR genetic variants* (RefSeqGene NG_002234.1):**

**SPR:g.6075A>G = p.R150G (a.k.a. p.Arg150Gly) & SPR:g.9120T>A = p.K251X (a.k.a. p.Lys251Ter)**

# GENE

*AGGREGATED INFORMATION OF EVERYTHING WE KNOW ABOUT AN ORGANISM'S GENE*

https://www.ncbi.nlm.nih.gov/gene

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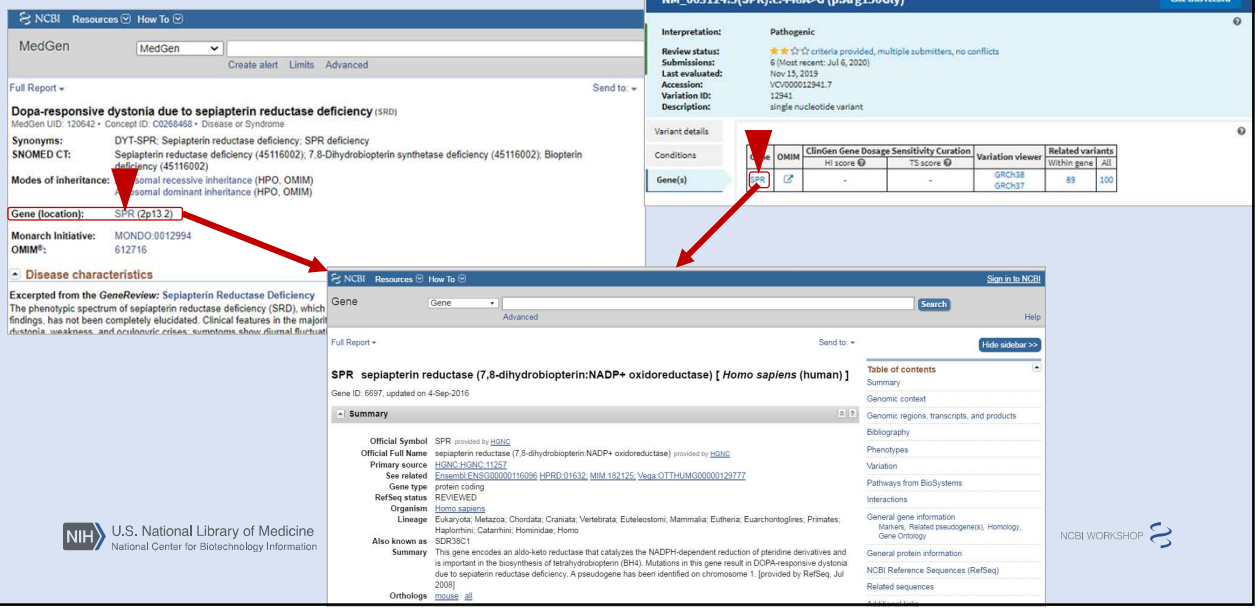
## MedGen or ClinVar → Gene

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# Molecular Biology Research – Normal gene function and expression

**INFORMATION ABOUT THIS GENE FROM HUMAN-CURATED SOURCES:**

6. On the MedGen or ClinVar record, **click the link for the gene** identified as having variants in the twins.
   **WHAT DOES THIS GENE NORMALLY DO?** *(summary from NCBI's RefSeq Group Curators)*

7. From the Gene record, **scroll down to the "General gene information">"Gene Ontology" section** to learn more about the protein produced from this gene. This section displays terms for where this gene product is likely to be found within a cell (Component), what processes it is often involved in (Process), and what it does (Function). *(terms assigned by the Gene Ontology Consortia's Curators)*

   **WHAT TYPE(S) OF PROCESS(ES) IS/ARE THIS PROTEIN NORMALLY INVOLVED WITH?**
   **DOES THIS MAKE SENSE BASED ON THE SUMMARY OF THE GENE THAT YOU JUST FOUND?**

   **WHAT SPECIFIC FUNCTION(S) DOES THIS PROTEIN HAVE?**
   *(Binding to ligands, substrates and/or cofactors; General and/or specific functional activities.)*
   **DOES THIS MAKE SENSE BASED ON THE SUMMARY OF THE GENE THAT YOU JUST FOUND?**

   **IN WHICH COMPONENT(S) (SUB-CELLULAR LOCATION) IS THIS PROTEIN NORMALLY FOUND?**

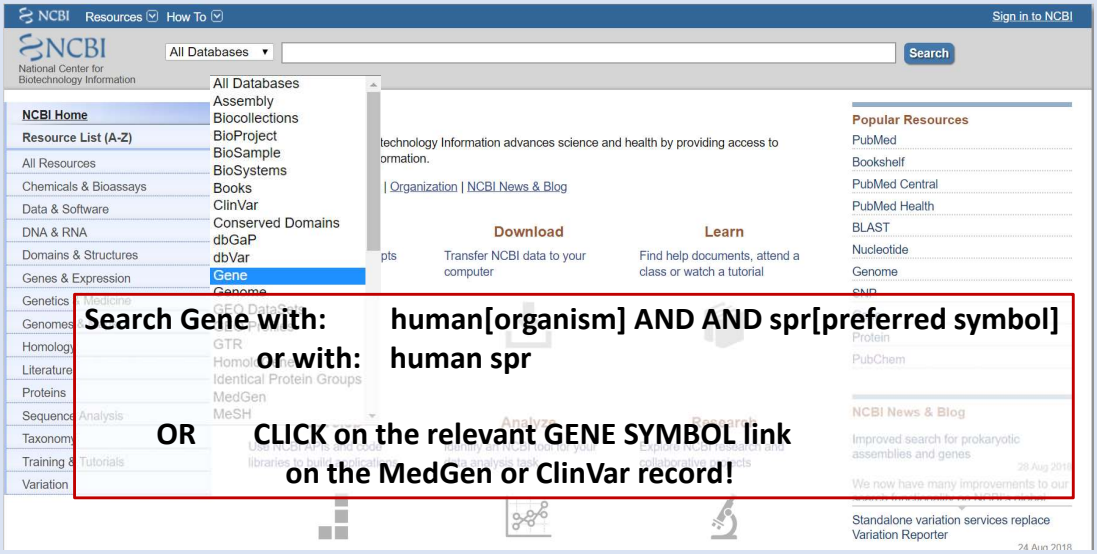
8. **Scroll down to the "Expression" section** to see in which tissues this gene is expressed and, since the protein is maintained within the cell, where it functions.

   **IN WHICH TISSUES HAS THIS GENE BEEN FOUND TO BE EXPRESSED?**

   **DO ANY OF THESE CORRELATE WITH SOME OF THE TWINS' SYMPTOMS?**

   Based on your understanding of the complexity of gene expression, how might you **EXPLAIN SOME OF THE DIFFERENCES IN SYMPTOMS OBSERVED IN THE TWINS?**

# Or look it up! https://www.ncbi.nlm.nih.gov



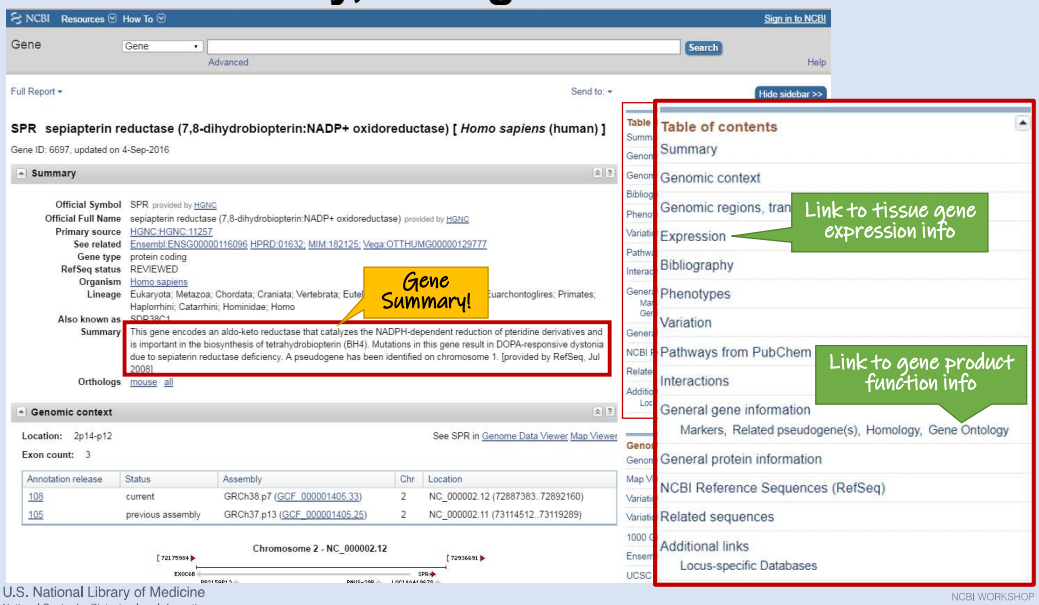

**Search Gene with:** human[organism] AND AND spr[preferred symbol]

**or with:** human spr

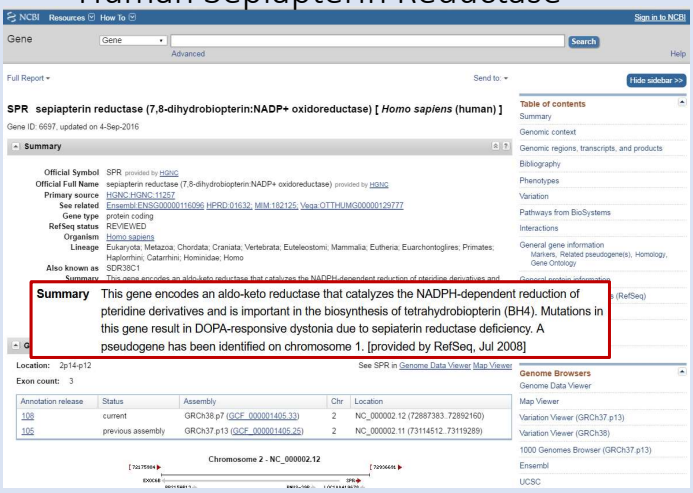
**OR** CLICK on the relevant GENE SYMBOL link on the MedGen or ClinVar record!



# Okay, let's get into this!



# Gene (Gene Summary Hub): Human Sepiapterin Reductase



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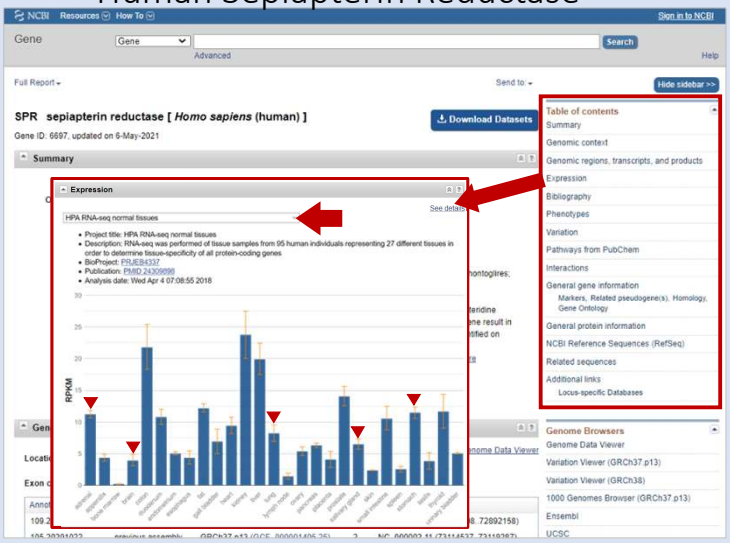

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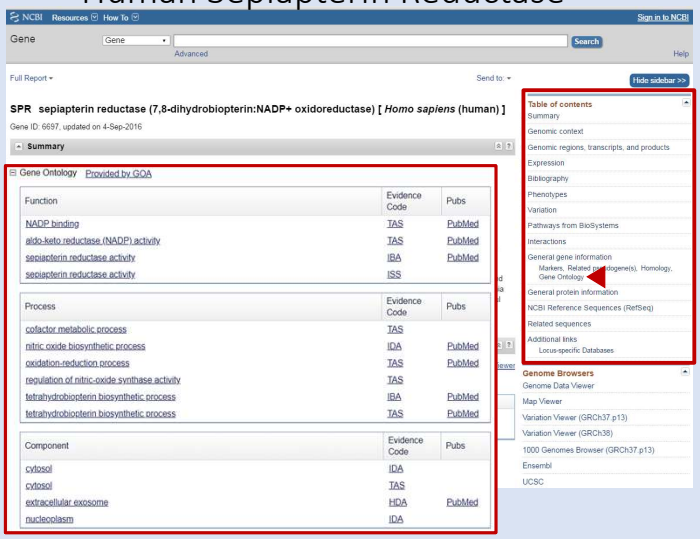
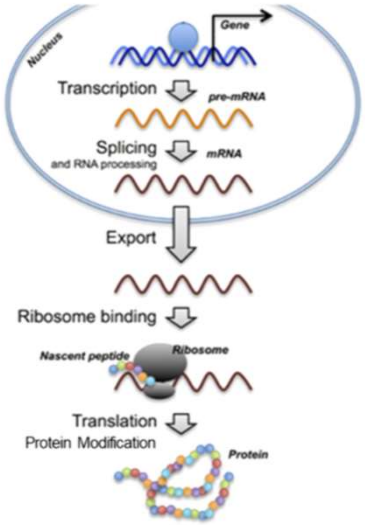





# A Review of Molecular Biology & UNDERSTANDING VARIANT IMPACT




Genome, assembly level → GRCh38

Chromosome/DNA → NC_ (RefSeq)

**HGVS Variant Example: NC_000002.12:g.72888457A>G**
***might impact chromatin structure or function***

Gene/DNA, gene *region* level → NG_* (RefSeqGene)

**HGVS Variant Example: NG_008234.1(SPR):g.6075A>G**
***might impact expression for or splicing of a gene transcript***

Transcript/RNA → NM_ , NR_ ("RefSeq RNA"|RefSeq Select)

**HGVS Variant Example: NM_003124.5(SPR):c.448A>G**
***might impact transcript processing and/or translation***

Protein, mRNA-encoded sequence → NP_* ("RefSeq Protein")

**HGVS Variant Example: NP_003115.1(SPR):p.Arg150Gly**
***might impact protein folding, processing or function***

Structure

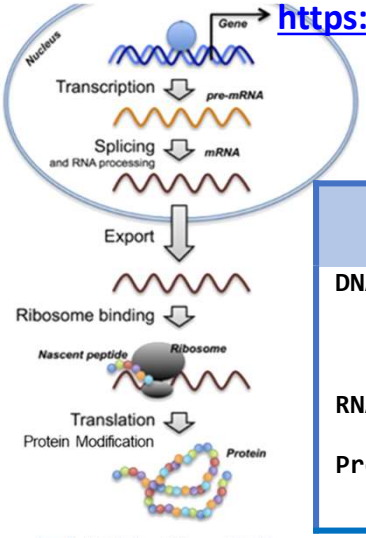


For more information about our RefSeq curation project:
https://www.ncbi.nlm.nih.gov/refseq/

# GENE

*AGGREGATED INFORMATION OF EVERYTHING WE KNOW ABOUT AN ORGANISM'S GENE*

https://www.ncbi.nlm.nih.gov/gene




*What is the affected gene?*

**TEST RESULTS in HGVS Format**
***(& why this is helpful)***

**DNA:** `NC_000002.12:g.72888457A>G`
*might impact chromatin structure or function*
`NG_008234.1(SPR):g.6075A>G`
*might impact gene expression for a particular gene*

**RNA:** `NM_003124.5(SPR):c.448A>G`
*might impact splicing and/or translation*

**Protein:** `NP_003115.1(SPR):p.Arg150Gly`
*might impact protein folding, processing or function*

NCBI

**Gene**

ClinVar

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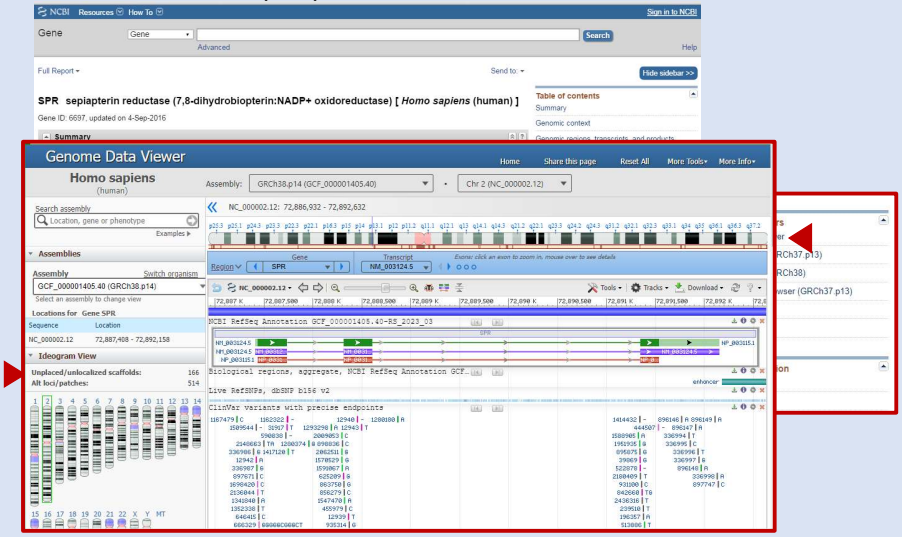
# Chromosome - Gene Region shown in GDV
## Human Sepiapterin Reductase

Chromosome
Gene
Transcript (mRNA)
Protein
*Structure*

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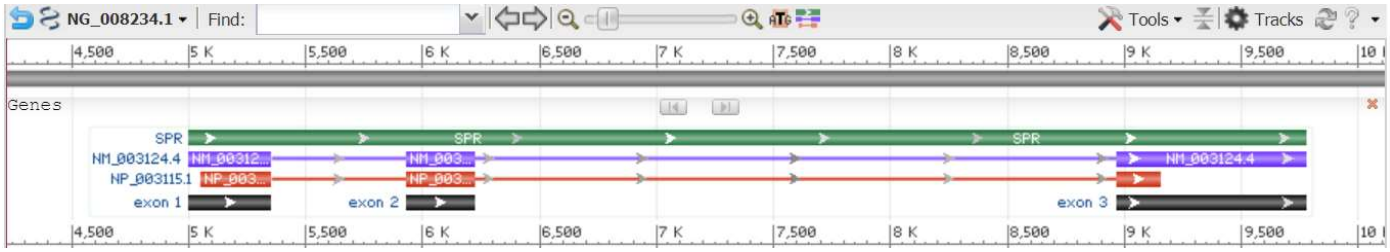
# Molecular Biology Research – Variant location and potential impact

**INFORMATION ABOUT THIS GENE DETERMINED FROM SEQUENCE-BASED SOURCES:**

9. On the right-hand side of the Gene record, **click the "RefSeqGene" link** to see the "Graphic" view of the gene structure defined on the chromosome on a RefSeqGene nucleotide page.




**HOW MANY TRANSCRIPT VARIANTS AND ENCODED PROTEINS ARE KNOWN TO BE PRODUCED BY THIS GENE?**

**There are several different "Tracks" available in this view, with names shown on the left.** (You can manipulate what is shown by the "Tracks" button in the upper-right.)

At the very top of this view, the **grey bar** shows the region corresponding to the genome with a "ruler" above showing nucleotide or base/residue.

Below this is the "**Genes**" track with the following:

A **green bar** with the gene symbol (SPR) shown is the full length of the gene region with little arrow-heads indicating the 5' to 3' direction of the coding. There is a single "green" gene region for each gene annotated on the genome.

Underneath the **green bar** may be shown the gene products of the gene:

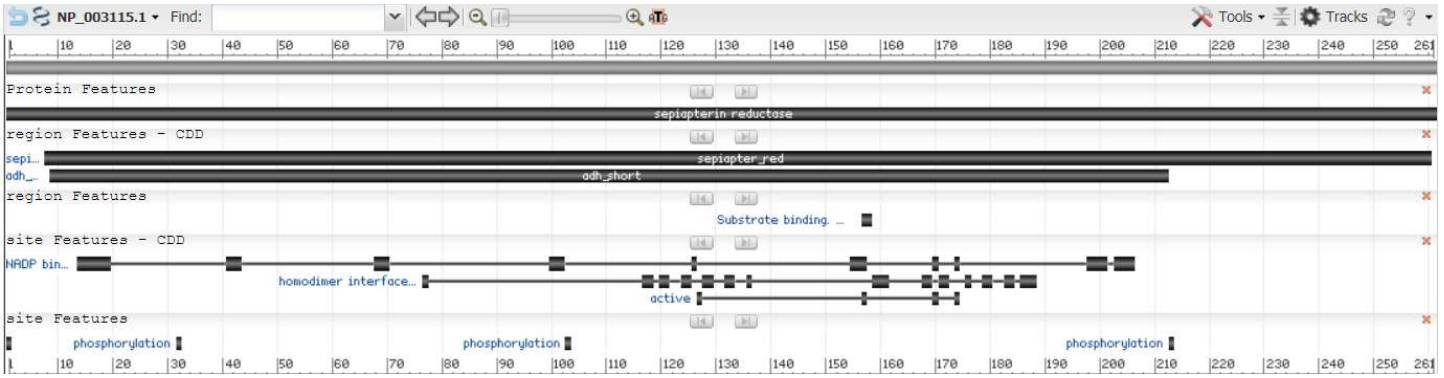
The **purple bar** with the accession indicates regions that are transcribed into RNAs. For mRNAs, thicker regions are the designated "exons" and the connecting lines (with directional arrow-heads) representing the connecting "introns" (which will be removed by splicing) and the **red bar** indicates the regions on the mRNA exons that encode the protein (also called coding sequence or CDS).

For some genes, it is known that there are multiple transcript splice-variants formed due to RNA processing (splicing), these would be shown as addition purple (and corresponding red – if protein coding) tracks. In order to help to establish a common numbering system of exons (since there may be different combinations in different transcript variants), the **black bar** below shows numbered boxes corresponding to each possible exon identified for the particular gene.

**WHERE IS/ARE THE TWINS' GENETIC VARIANTS LOCATED IN THIS GENE AND IN THE mRNA?**
*(on the picture above or on your screen – draw or visualize a vertical line at the position of each if the variants)*

**BASED ON THE POSITION(S) OF THE VARIANT(S) IN THE GENE, WHAT IS THE MOST LIKELY MECHANISM FOR IMPACTING THE FINAL GENE PRODUCT?** *(alter gene expression, influence transcript processing, or change encoded protein sequence)*

10. On the right-hand side of the RefSeqGene page, **can click the "Protein" link** or go back to the Gene record and **click the "RefSeq Proteins" link**. **Click "Graphics"** to see a graphical view of the annotated regions curated on the protein sequence. The information shown in in these "tracks" of this view can help you to learn more about this protein.




**WHERE IN THE PROTEIN SEQUENCE IS/ARE THE TWINS' GENETIC VARIANTS LOCATED?**
*(on the picture above or on your screen – draw or visualize a vertical line at the position of each if the variants)*

Take a look at the annotations shown in the Graphic view. **BASED ON WHERE IT THE VARIANT(S) IS/ARE LOCATED, WHICH MIGHT THE VARIANT(S) ALTER:**

- ☐ the protein's **location** *(signal peptide)*
- ☐ post-translational **processing** of the protein *(cleavage site)*
- ☐ **post-translational modification** of the protein *(phosphorylation or methylation site, for example)*
- ☐ the **functional activity** of the protein (domain, motif, and/or specific site/"key" residue – *binding, active site, catalysis, for example*)

to learn more about the main functional regions of the protein **click "Identify Conserved Domains".**

**There are several different "Tracks" available in this view, with names shown on the left.** (You can manipulate what is shown by the "Tracks" button in the upper-right.)

At the very top of this view, the **grey bar** shows the length of the protein with a "ruler" above showing amino acid position/residue from N-terminus to C-terminus. The "**Protein Features**" bar mimics the grey bar, above, and displays the gene name.

**Other "Features" tracks** indicate important residues for the function of the protein.

**- Mouse-over the features to learn more about each -**

The "**region Features - CDD**" track shows bars corresponding to functional domains that have been annotated in this protein sequence by the NCBI Conserved Domain Curation Staff.

**"region Features"** indicates critical amino acid positions *involved* in the activity of the protein

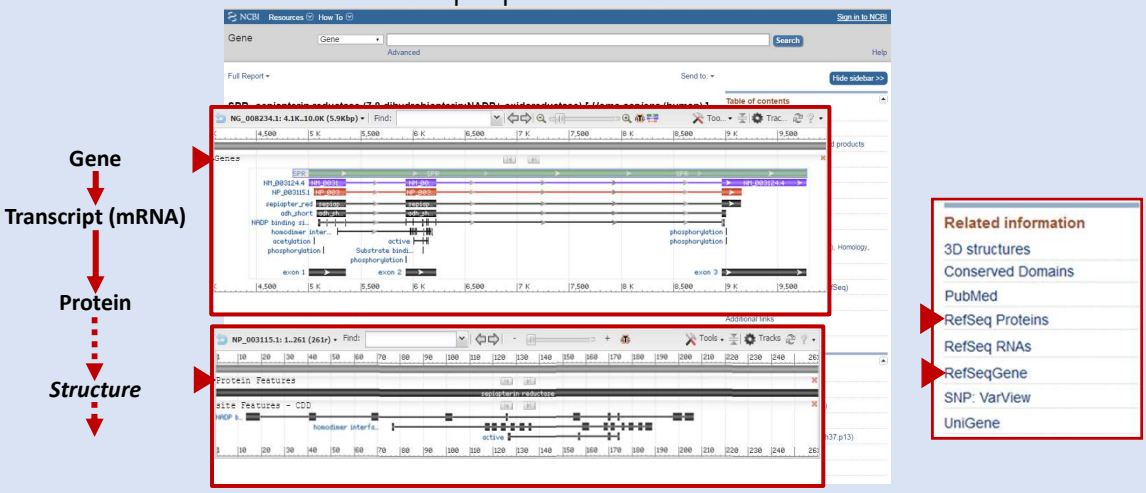
**"site Features – CDD"** shows specific amino acid positions that are critical for various functions of this protein (including binding, catalysis, active site, interface & protein-protein interactions, etc.)

**"site Features"** identifies other important amino acid positions which are predicted to be targets of other cellular processes (including sites for targeting with post-translational modifications or targets for proteolytic cleavage).

When there is evidence of Other tracks that are shown here when there is information to support it, include "signal peptide" if the protein is to be targeted to a specific subcellular location other than the cytosol.

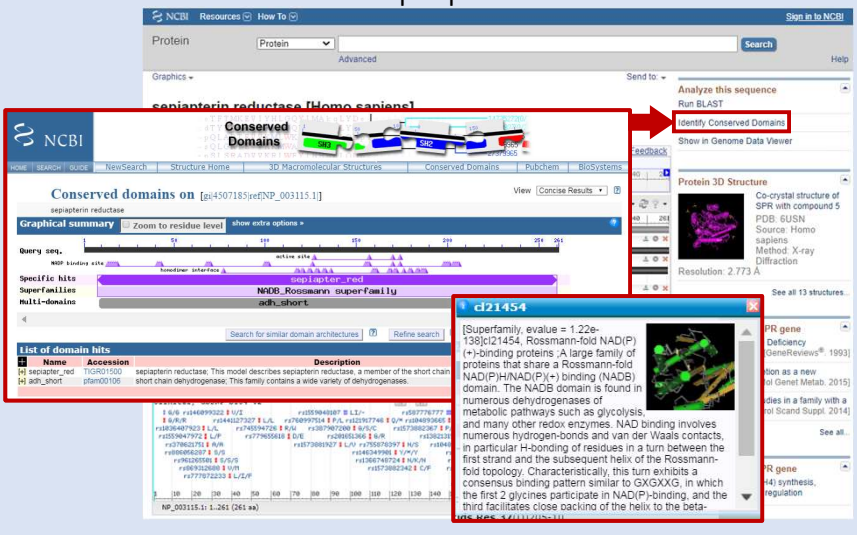
**WHAT MIGHT BE THE IMPACT OF THE GENETIC VARIATIONS ON THE PROTEIN'S FUNCTION?**

# Gene (Gene Summary Hub): Human Sepiapterin Reductase

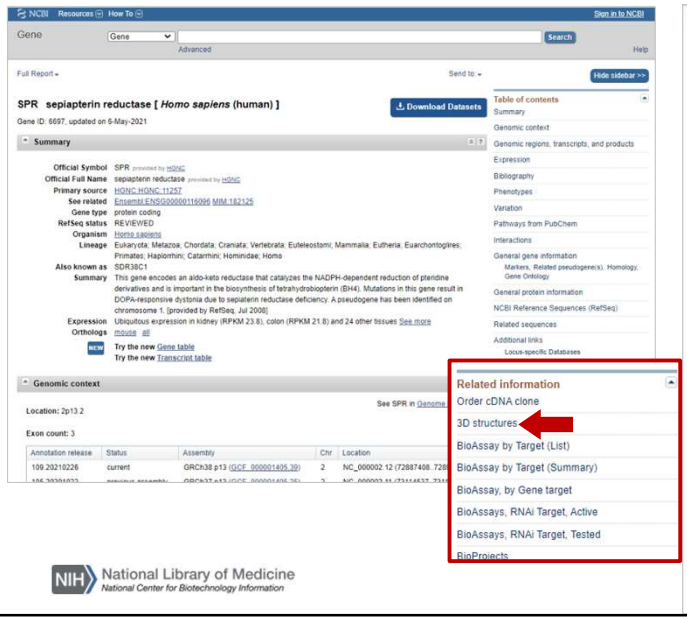





# Protein (RefSeq Protein & CDD records): Human Sepiapterin Reductase



# Gene (Gene Summary Hub)
## Human Sepiapterin Reductase

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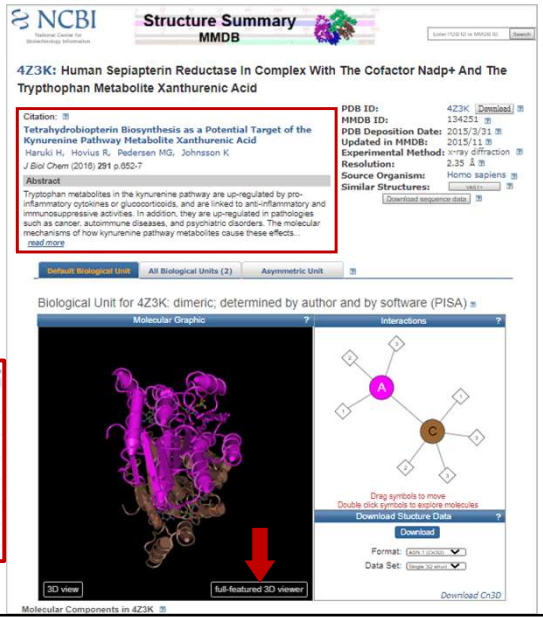
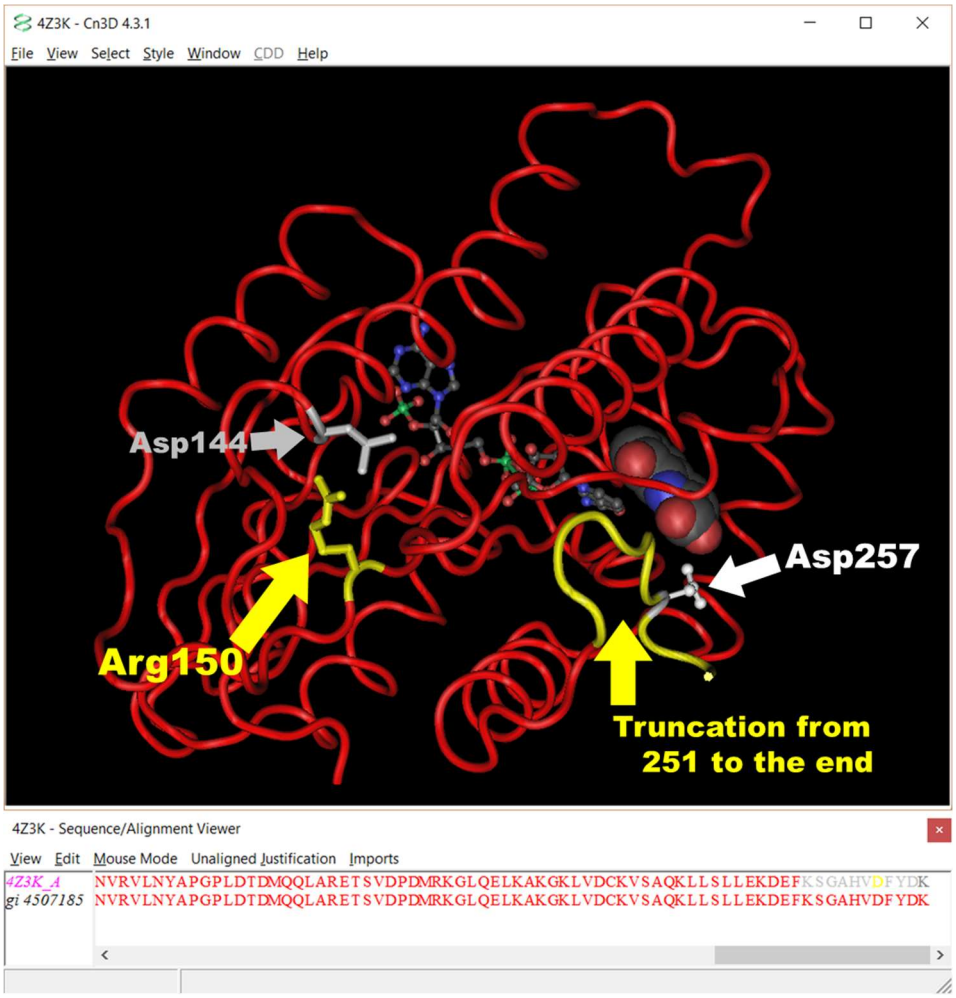
# Structure (Curated PDB Records)
## Human Sepiapterin Reductase



11. From either the Gene or Protein record, **can click a link to 3D Structure** to visualize experimentally-determined molecular structures for this protein. In the 3D structure you can see precisely the locations of the amino acids affected by the genetic variations.




To make things easier for you right now….

**here's a picture of the 3D crystal structure** monomer of the Human SPR protein complex (PDB accession: 4Z3K) as displayed in NCBI's Cn3D Viewer.

The protein backbone is showed in a long **red tube**, with two bound substrates (NADPH & a sepiapterin analog) for the SPR reaction shown as ball-and-stick and spacefill rendering, respectively.

The position of where the variants would exist (Arg150 & Lys251) are highlighted in **yellow** and two additional important residues displayed in **grey** (Asp144) and white (Asp257).

**WHAT DO YOU THINK THE CHANGE IN AMINO ACIDS MIGHT DO TO THE 3D STRUCTURE AND FUNCTION OF THE PROTEIN?**

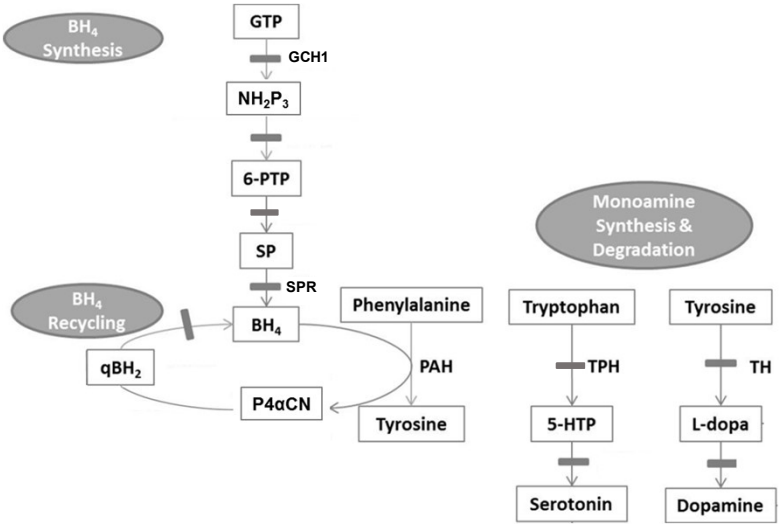
**Arg150** normally forms a salt-bridge with Asp144 to stabilize the protein's 3D structure.

**Loss of this salt-bridge, due to replacement of the negatively-charged arginine with a neutral glycine (Arg150Gly), causes the enzyme to unfold and lose all activity.**

**The C-terminal end of the protein and Asp257**, in particular, stabilizes the binding and locks the sepiapterin substrate into the active site of the enzyme.

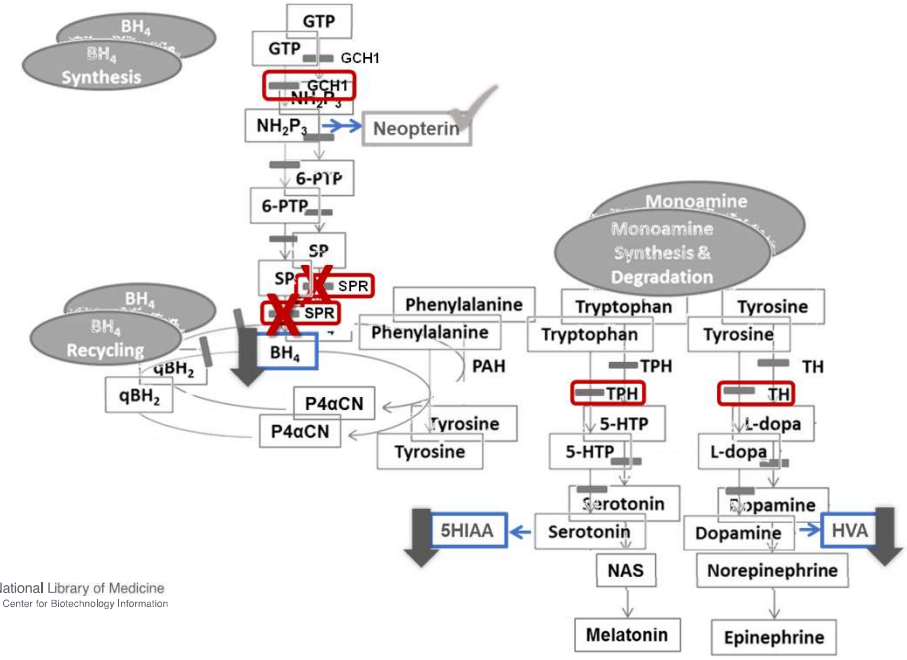
**Loss of the C-terminal tail and this particular residue (as in the truncation variant Lys251Ter) causes loss of the ability of the enzyme to efficiently bind and lock-in its substrate – resulting in the loss of all activity.**

## Review of the biological system: lab & genetic test info mapping

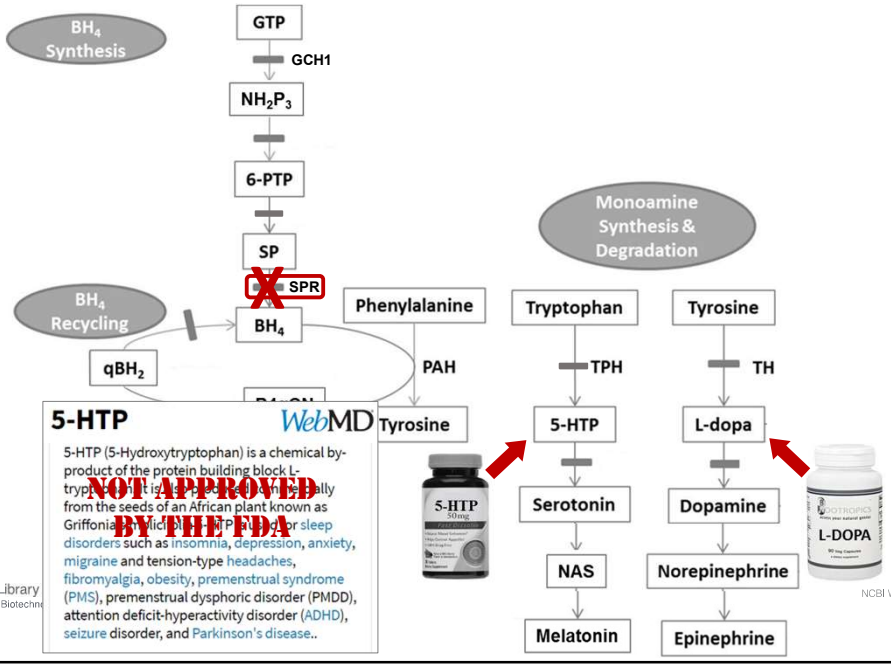





## How can Alexis & Noah's symptoms be managed?



# How can Alexis & Noah's symptoms be managed?

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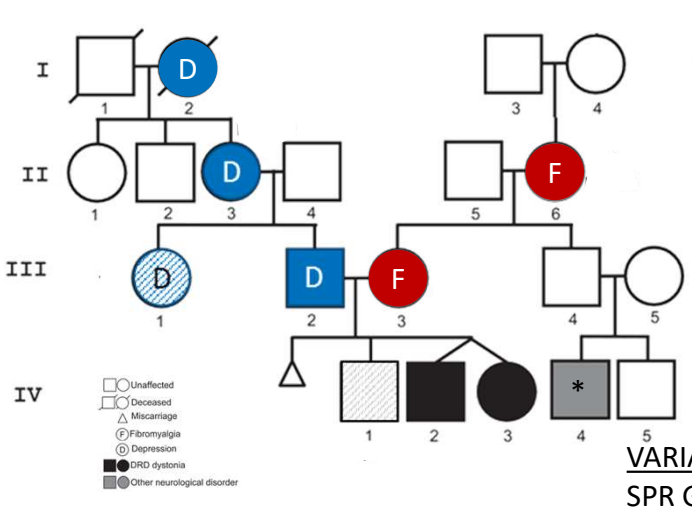
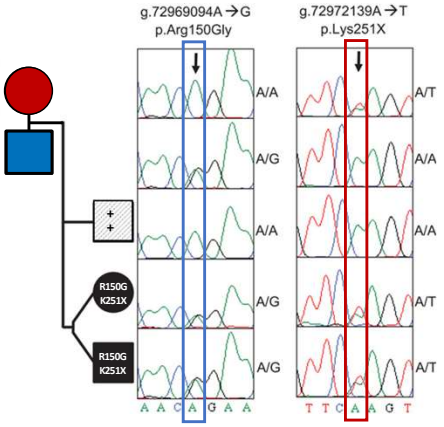
# REAL DATA!




VARIATIONS FOUND

SPR Gene: p.Arg150Gly & p.Lys251Ter

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https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3314311/
*(graphic modified to highlight key points)*

NCBI WORKSHOP

# How are the Beerys doing now?

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TODAY

***Advocacy for:***

- Collaborative Patient Care
- Genetic Testing
- NIH Funding

**RARE DISEASE DAY – 2/17/2022**

https://www.lunadna.com/rare-disease-day-personal-experience-into-technology/