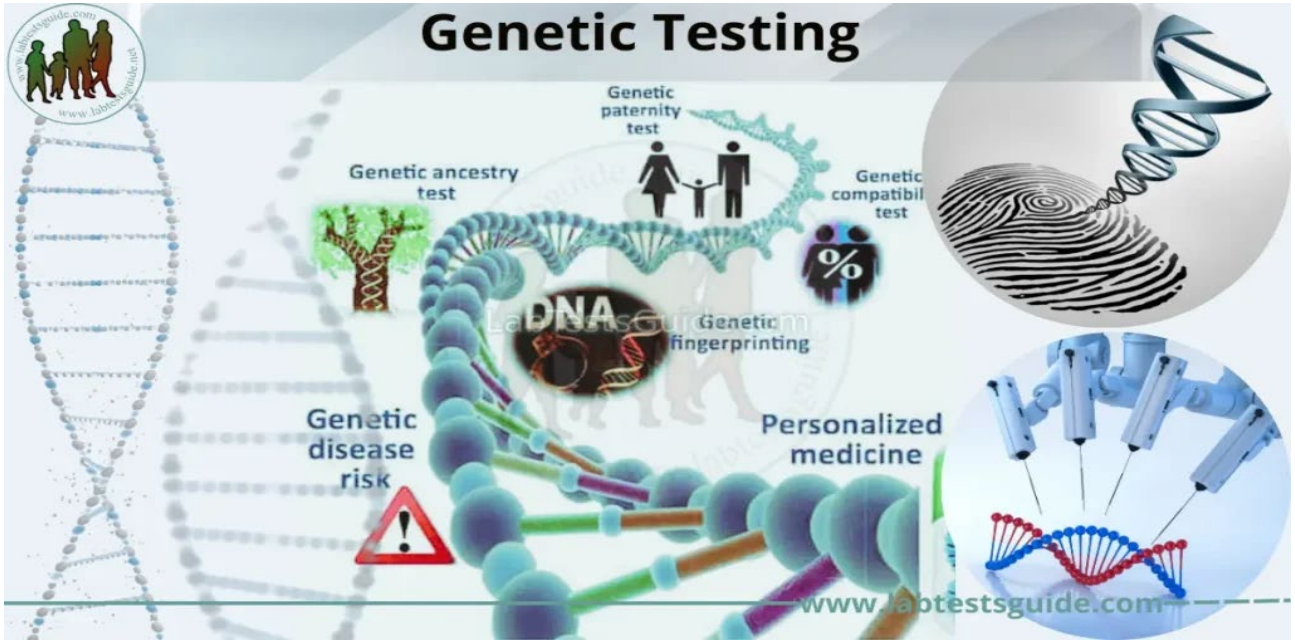
Exploring Diagnostic Approaches in Clinical Genetics: Is NGS Always the Best Choice?

Bahareh Rabbani, PhD



NGS

- NGS technology has the appeal of reducing **the time** and **cost of testing**, especially when the sequencing involves a **larger number of genes**, but is it the best diagnostic tool in all clinical scenarios?

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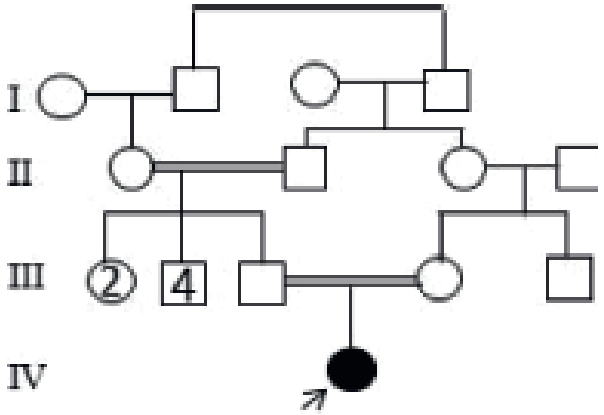
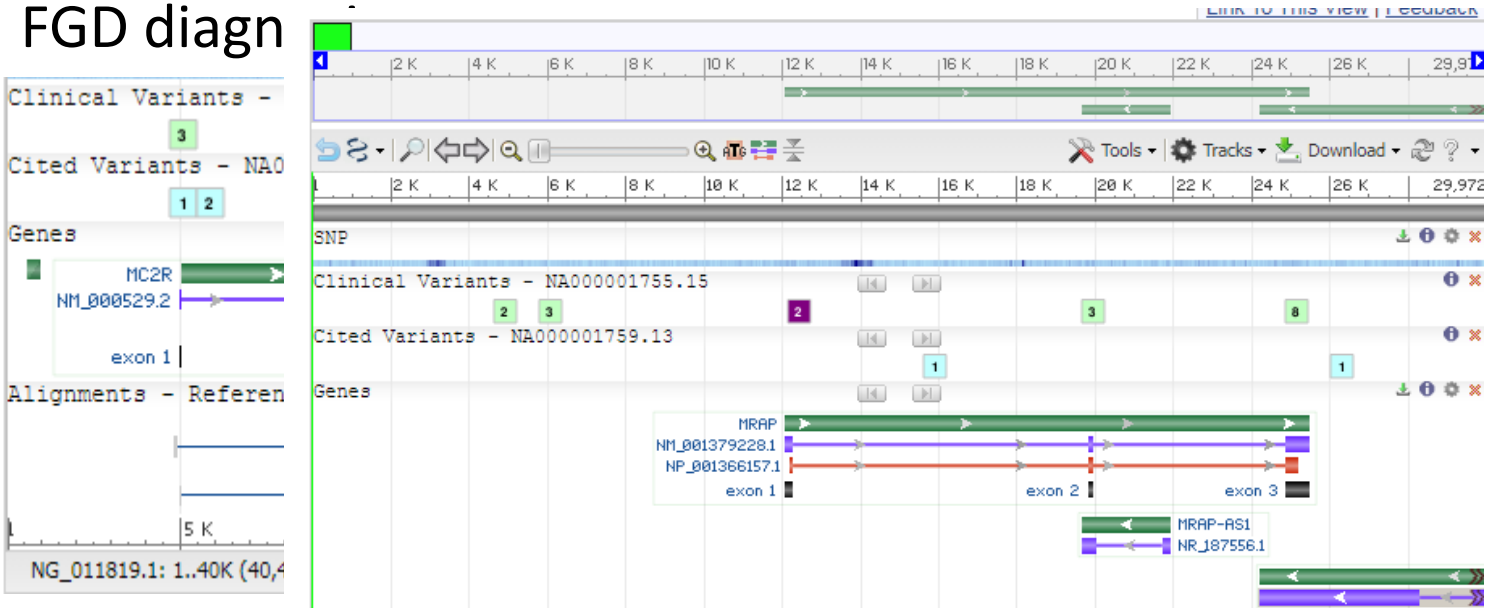


- Case: 5 mo, Consanguineous family
- 17 days: Hypoglycemia, TSH: 14.2
- 4 mo:
  - low cortisol =<0.054 μg/dL (normal range: 7–25 μg/dL)
  - high ACTH= >2000 pg/mL (normal range: <50 pg/mL)
  - normal aldosterone=866 pg/mL (normal range: 20–1100 pg/mL)
  - Normal 17-OHP=0.74 ng/mL (normal range: <1 ng/mL)
  - TSH= 0.5 U/L (normal range: 0.5–5 U/L)
- Blood electrolytes were at normal range.
- Physical examination: hyperpigmentation was obvious with female external genitalia, and neurological development was normal. She was suspicious of a **type of PAI**. (Hydrocortisone)

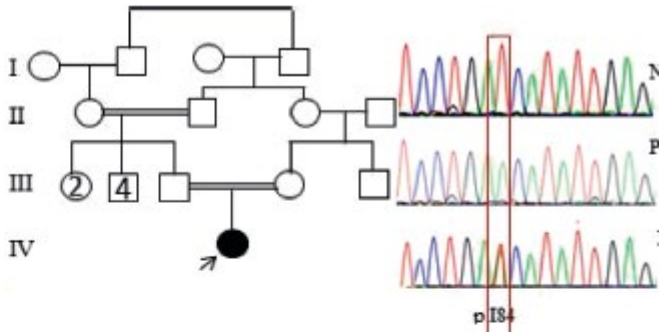
MC2R and MRAP gene

- Six-month-old:
  - 17OHP < 0.1 ng/mL (normal range: <1 ng/mL),
  - TSH 5.3 U/L.
  - She was 5.9 kg (3rd percentile), with height 64.3 cm (15th percentile). She underwent genetic testing to confirm FGD diagn



MC2R and *MRAP*

-*MC2R*: c.251T >A (p.Ile84Asn) homozygote variant
-Disease causing, likely pathogenic
-Segregation analysis: heterozygote parents



Q01718	ACTHR_HUMAN	30	IFFTISIVGVLENLIVLLAVFKNKNLQAPMYFFICSLAISDMLGSLYKILENIIIILRNM 89
Q64326	ACTHR_MOUSE	30	IFFTISVIGILENLIVLLAVIKNKNLQSPMYFFICSLAISDMLGSLYKILENIIIMFRNM 89
P34974	ACTHR_BOVIN	30	IFFTVSIVGVLENLMVLLAVAKNKSLQSPMYFFICSLAISDMLGSLYKILENVIIMFKNM 89
Q8HYN8	ACTHR_PIG	30	VFFTISVIGVLENLIVLLAVIKNKNLQSPMYFFICSLAISDMLGSLYKILENIIIIFRNM 89
Q9Z1S9	ACTHR_CAVPO	30	IFFIISITGVLENLIIILAVIKNKNLQFPMYFFICSLAISDMLGSLYKILESIIIMFRNM 89
P70115	ACTHR_MESAU	30	IFFTISIIGVLENLIVLLAVVKNKNLQCPMYFFICSLAISDMLGSLYKILENIIIMFRNR 89
Q9TU77	ACTHR_SHEEP	30	IFFTVSIVGVLENLMVLLAVAKNKSLQSPMYFFICSLAISDMLGSLYKILENVIIMFRNM 89
Q28928	ACTHR_PAPHA	6	IFFTISIVGVLENLIVLLAVFKNKNLQAPMYFFICSLAISDMLGSLYKILENIIIILRNM 65
Q544P9	Q544P9_MOUSE	30	IFFTISVIGILENLIVLLAVIKNKNLQSPMYFFICSLAISDMLGSLYKILENIIIMFRNM 89
A0A1L8FYF2	A0A1L8FYF2_XENLA	39	VYLTVSTVGLLENLLVLLAVIKNKNLHLPMYFFICSLAVSDMLFSLYKILETIIIILANI 98
A0A2I3T6A8	A0A2I3T6A8_PANTR	30	IFFTISIVGVLENLIVLLAVFKNKNLQAPVYFFICSLAISDMLGSLYKILENIIIILRNM 89
M3WZX2	M3WZX2_FELCA	30	IFFTISIIGVLENLMVLLAVMKNKNLQSPMYFFICSLAISDMLGSLYKILENIIIMFRNM 89
H2U7C8	H2U7C8_TAKRU	20	VFFTIGFVSLLENLLVIGAISWNRNLHSPMYCFIGSLAAFNTVASVTKTWENLMITFAEV 79
A0A1U7Q2G7	A0A1U7Q2G7_MESAU	30	IFFTISIIGVLENLIVLLAVVKNKNLQCPMYFFICSLAISDMLGSLYKILENIIIMFRNR 89
A0A1D5PF84	A0A1D5PF84_CHICK	45	VFFTVAAAGILENLLVLVAVIRNKNLHLPMYFFICSLAISDMLGSLYKTLENIFIILCKM 104
E2R4R4	E2R4R4_CANLF	30	IFFIISIIGVLENLMVLLAVIKNKNLQSPMYFFICSLAISDMMGSLYKILENIIIVFRNM 89
G1PVR8	G1PVR8_MYOLU	61	IFFTISIAGVLENLIVLLAVIKNKNLQAPMYFFICSLAISDMLGSLYKILENIIIMLRNK 120
H1ZYJ6	H1ZYJ6_DICLA	20	LFLAIGVVSLAENLLVVVAIIWNRNLHSPMYCFICSLAAFNTIASLSKTWETLMIVFADV 79
F6Y3T7	F6Y3T7_HORSE	30	IFFTISIIGVLENLMILLAVIKNKNLQSPMYFFICSLAISDMLGSLYKILENIIIMFRNT 89

Research Article

The Genetic Perspective of Familial Glucocorticoid Deficiency: *In Silico* Analysis of Two Novel Variants

Expanding the Phenotype of Congenital Glucocorticoid Deficiency: An Iranian Patient with Cholestasis due to Pathogenic Variants in the *MC2R* Gene

Shohreh Maleknejad,[1] **Setila Dalili**,[1] **Ameneh Sharifi**,[2] **Afagh Hassanzadeh Rad**,[1] **Reza Bayat**,[1] **Bahareh Rabbani**,[2] **and Nejat Mahdieh**[3]

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Case2

- A 5-month-old infant wi
- Prenatal screening: Kary

he

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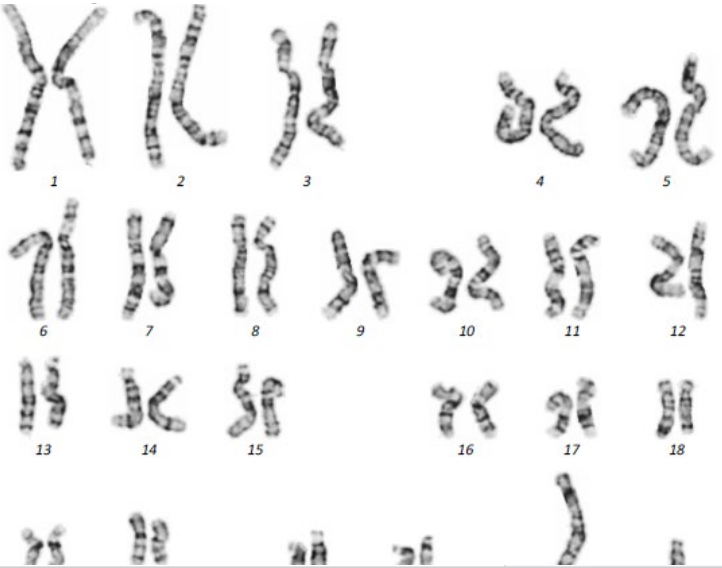
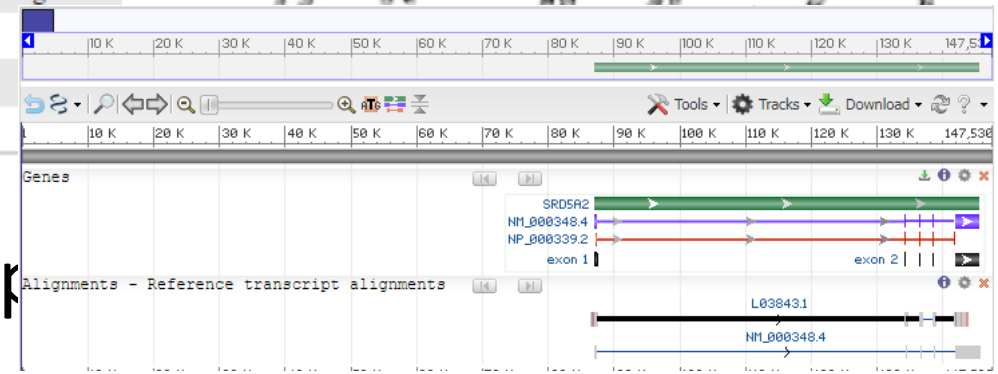
nc

Test	Result	Normal range		Unit
		Male	Female	
LH	1.02	1-9	2-10	IU/L
FSH	0.25	1-10	20-50	IU/L
17OHP	66.2	20-100	20-100	ng/dL
Testosterone/DHT	25	275-875	23-75	ng/dL
Free testosterone	0.0	0.4-0.9	0.15-0.6	
Na (Sodium)	130	135-145	135-145	
K (Potassium)	3.5	3.7-5.9	3.7-5.9	



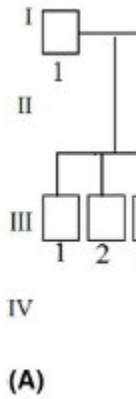


Male pseudoherma

DSD: SRD5A2 gene

5-alpha reductase deficiency

- male pseudohermaphroditism
- Sequencing *SRD5A2*: c.476T>G (p.Ile159Arg).



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CASE REPORT

Clinical Case Reports Open Access WILEY

A novel pathogenic variant of *SRD5A2* in an Iranian psuedohermaphrodite male

Setilla Dalili[1] | Bahareh Rabbani[2,3] | Afagh Hassanzadeh Rad[4] | Shaahin Koohmanaee[1] | Nejat Mahdieh

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Does single-gene analysis have a place?

- Yes, for well-characterized **monogenic conditions**
- Single-gene testing:
  - conditions with **distinctive clinical features:** achnodroplasia, PKU
  - **minimal locus heterogeneity:** CF
  - Limitation of NGS for trinucleotide repeat expansion and methylation/epigenetic abnormalities: Fragile X, PW, AS

Growth and Development Research Center

Disease	Gene	Disease	Gene
Congenital adrenal hyperplasia	CYP21A2, HSD3B2	Metachromatic leukodystrophy	ARSA
5 alpha reductase deficiency	SRD5A2	Androgen insensitivity syndrome	AR
Galactosemia	GALT		
Glutaric acidemia I	GCDH		
Familial glucocorticoid deficiency	MCR2, MRAP		
Achondroplasia	FGFR3		
hypochondroplasia	FGFR3		

[Elevated C3 Acylcarnitine] Propionic Acidemia (PA) and Methylmalonic Acidemia (MMA)

- **Par**
  ALDH
  DMG
  MMA
  MTHF
  PCCB
  TCN2
- **Mo**
  test
  to c

The patient was referred for genetic testing due to a clinical diagnosis of methylmalonic acidemia (MMA) and propionic acidemia.

Test Result
A pathogenic variant associated with methylmalonic acidemia (MMA) was detected.

Primary Findings:
Variants with possible relevance to patient's phenotype:
Please see table below for a detailed description of the detected variants.

Gene	Variant(NM#)	Location	Amino Acid Alteration	zygosity	Inheritance	Disease OMIM	Classification
MMUT	**Chr6:49425502-T-A NM_000255.4**	**c.655A>T**	**p.N219Y**	**Homozygote**	**AR**	**methylmalonic acidemia**	**Pathogenic**

Test Result	**The genetic diangnosis of methylmalonic acidemia is confirmed.**
	The identified variant, **c.655A>T (p.N219Y)**, is classified as **pathogenic** based on

Gene panel

- **Heterogeneity**: muscular dystrophy panel
- Disorders with **overlapping phenotypes**, differential diagnosis: cardiomyopathies
- Disorders **share one manifestation** but may have completely different overall presentation: Epilepsy
- Disease associated with genes from common **pathways**: MMA

Gene Panel Testing

Leukodystrophies

- **Why:** A gene panel is focused on **known leukodystrophy disease-associated genes, providing efficient and actionable results whil** **n who**

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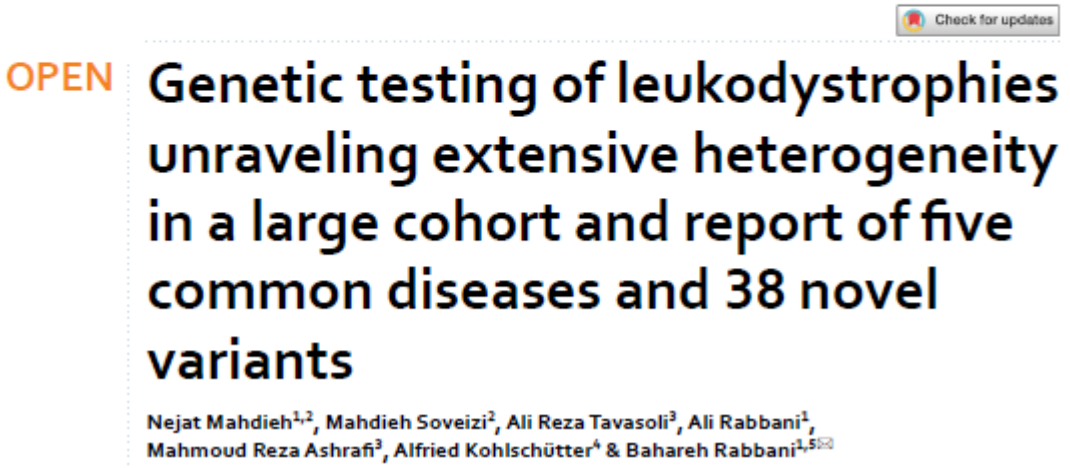
scientific reports

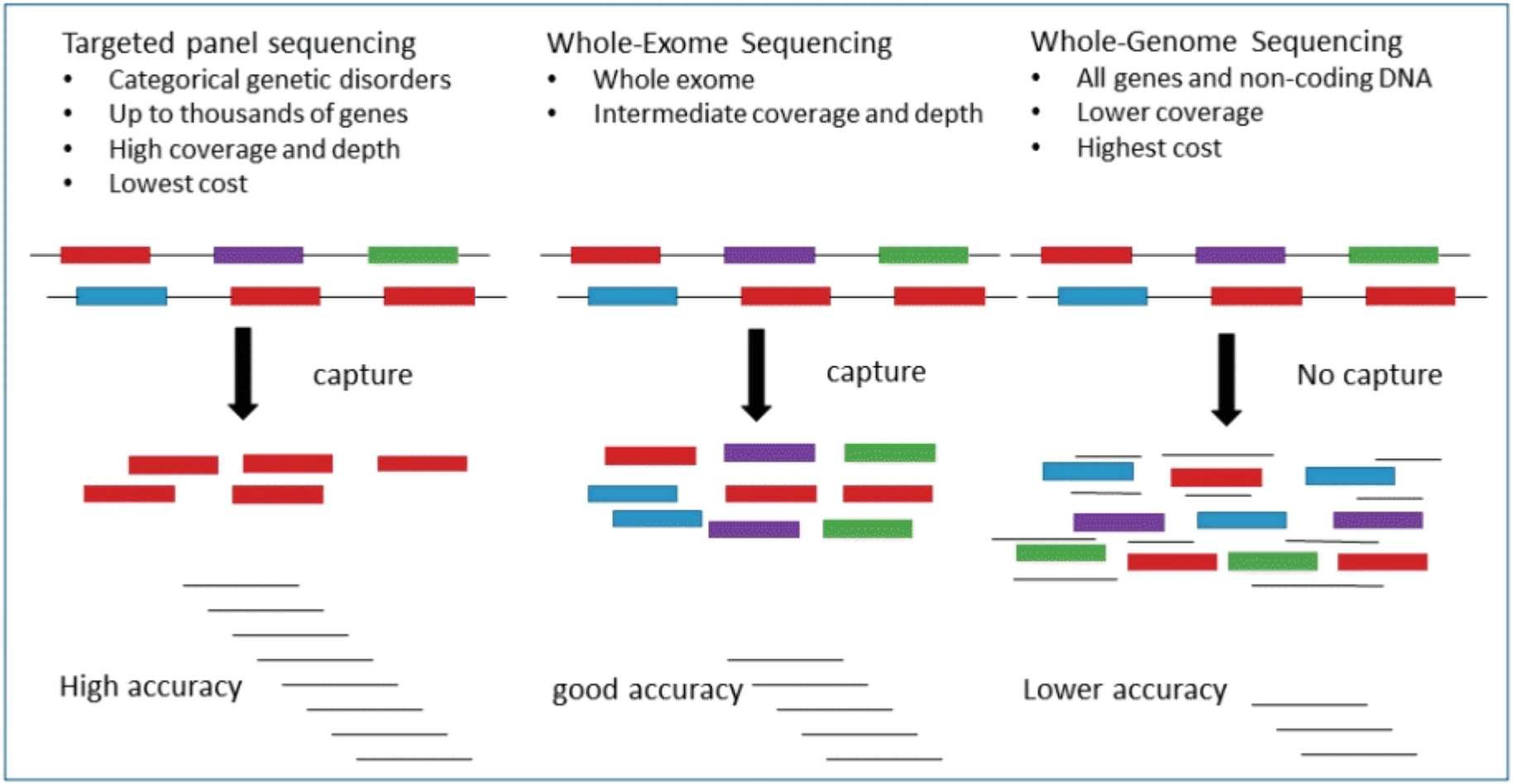


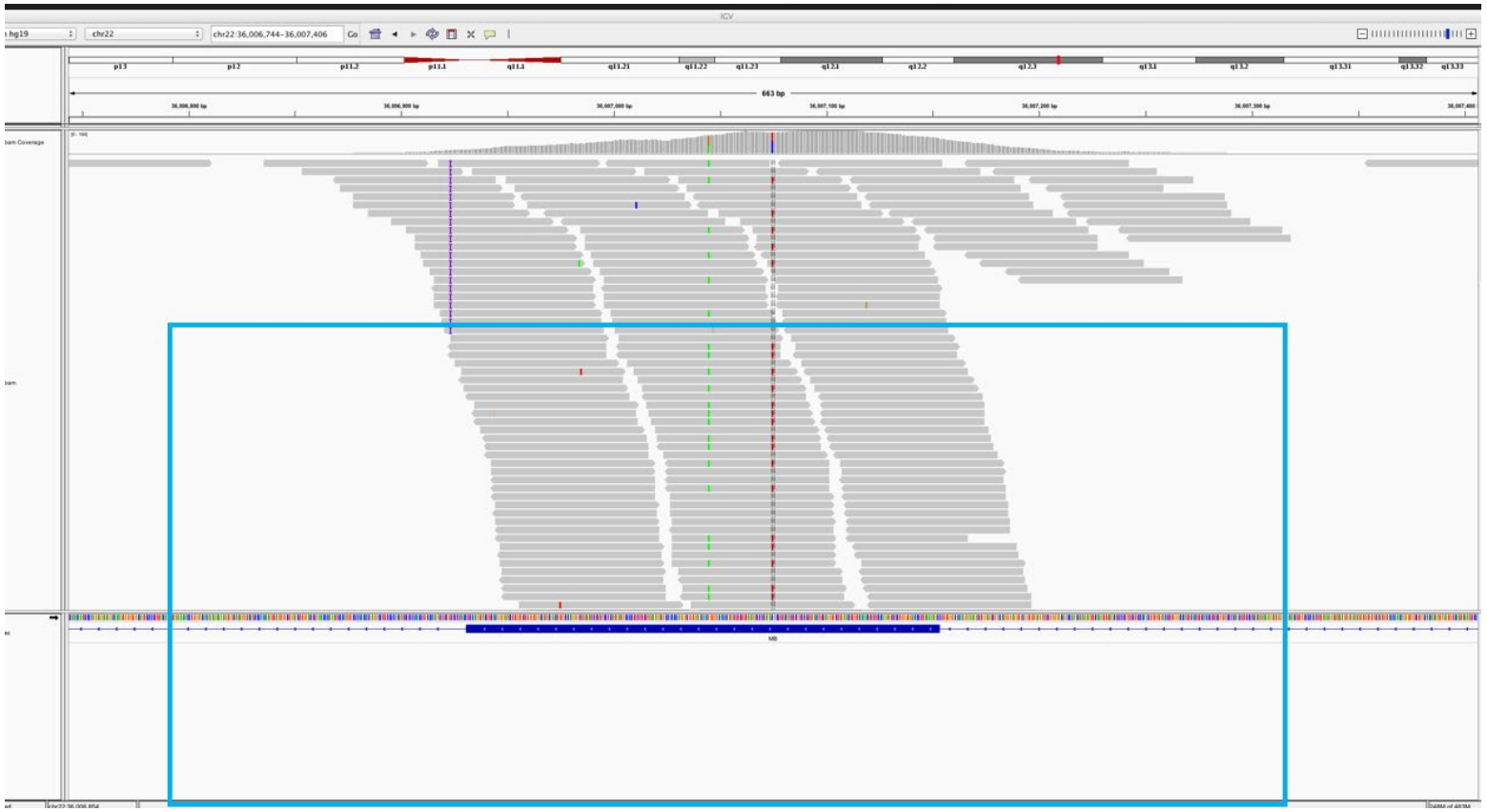
Check for updates

OPEN

Genetic testing of leukodystrophies unraveling extensive heterogeneity in a large cohort and report of five common diseases and 38 novel variants

Nejat Mahdieh[1,2], Mahdieh Soveizi[2], Ali Reza Tavasoli[3], Ali Rabbani[1], Mahmoud Reza Ashrafi[3], Alfried Kohlschütter[4] & Bahareh Rabbani[1,5]✉


Targeted panel sequencing
• Categorical genetic disorders
• Up to thousands of genes
• High coverage and depth
• Lowest cost
Whole-Exome Sequencing
• Whole exome
• Intermediate coverage and depth
Whole-Genome Sequencing
• All genes and non-coding DNA
• Lower coverage
• Highest cost
capture
capture
No capture
High accuracy
good accuracy
Lower accuracy

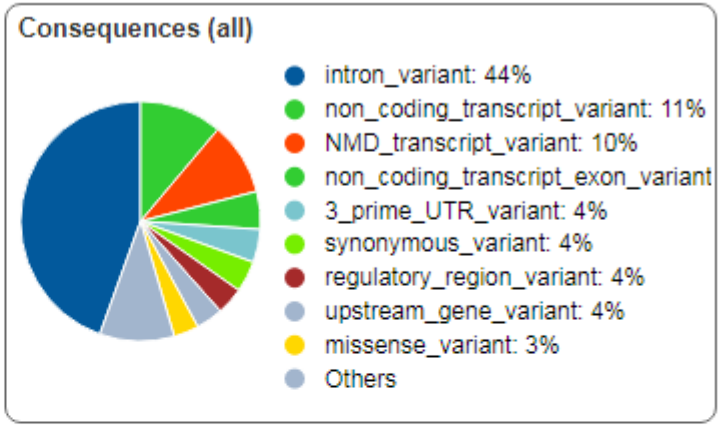

IGV
hg19
chr22
chr22:36,006,744-36,007,406
Go
663 bp

Why Choose Gene Panel Testing When Exome Sequencing (ES) Can Cover All Coding Regions?

- G… h
  ca…
  w… ter
  su…

Summary statistics

Category	Count
Variants processed	101746
Variants filtered out	0
Novel / existing variants	0 (0.0) / 101746 (100.0)
Overlapped genes	22514
Overlapped transcripts	137412
Overlapped regulatory features	22277



- ES…
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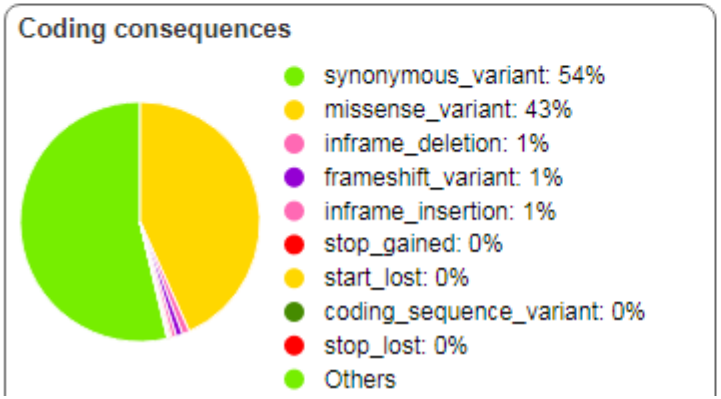
  re… s.



Gene Panels, Is Including More Genes Always Better?

Not necessarily. While larger panels increase the likelihood of identifying a relevant variant, they also increase the risk of finding **incidental findings** or variants of uncertain significance **(VUS),** which may not help the patient.

WES

- Developmental Delay?

A pathogenic variant is detected that is relevant to the patient's clinical presentation, specifically related to developmental delay.

Primary Findings:

Variants with possible relevance to patient's phenotype:

Please see table below for a detailed description of the detected variants.

Gene	Variant(NM#)	Location	Amino Acid Alteration	zygosity	Inheritance	Disease OMIM	Classification
IRF2BPL	**14:77493815T>TGC NM_024496.4**	**c.320_321insGC**	**p.Q108Hfs*45**	**Heterozygote**	**AD**	NEDAMSS	**Pathogenic**

Neurodevelopmental disorder with regression, abnormal movements, loss of speech, and seizures Interferon regulatory factor 2 binding protein-like (IRF2BPL)

Exome Sequencing Becoming the First-Tier Test in Some Scenarios?

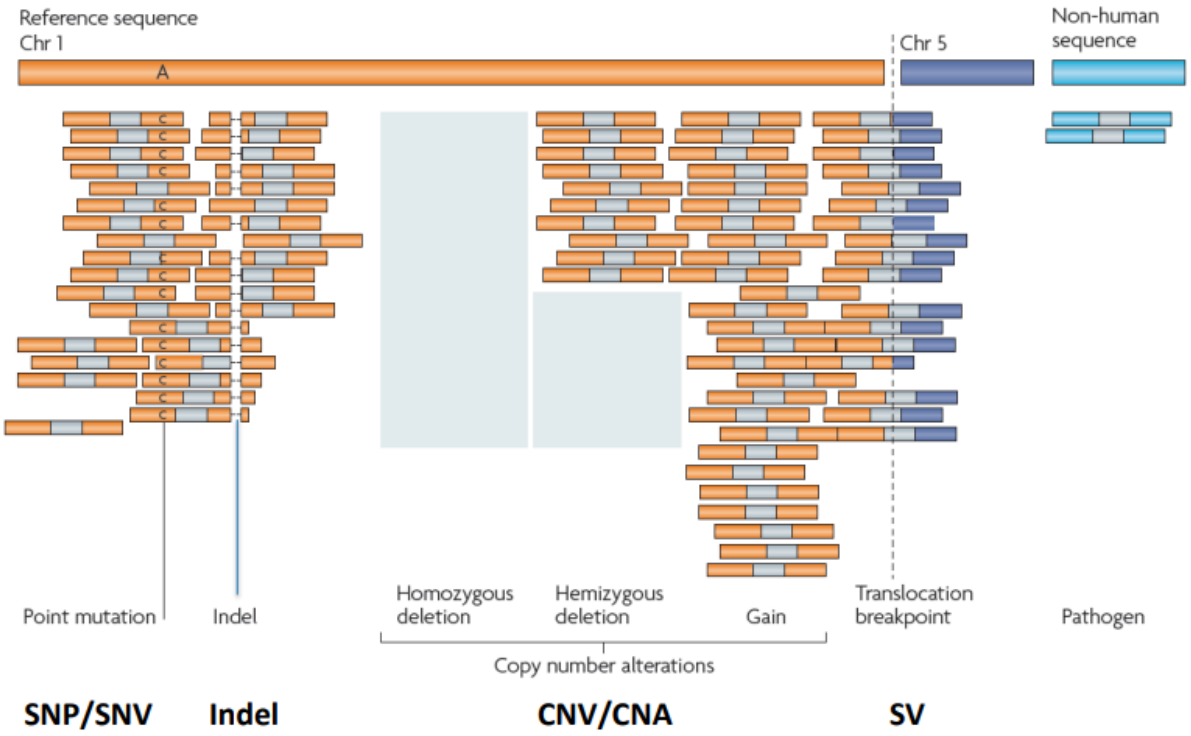
- ES is increasingly used as a first-tier test, particularly for conditions with **broad phenotypic presentations or unclear genetic etiology**.
- **Extreme heterogeneity** and **De novo changes**: autism
- No key phenotypic feature: Kabuki syndrome

- ES has become more affordable and accessible, and its ability to analyze all coding regions makes it ideal for cases where a panel might miss a relevant gene.

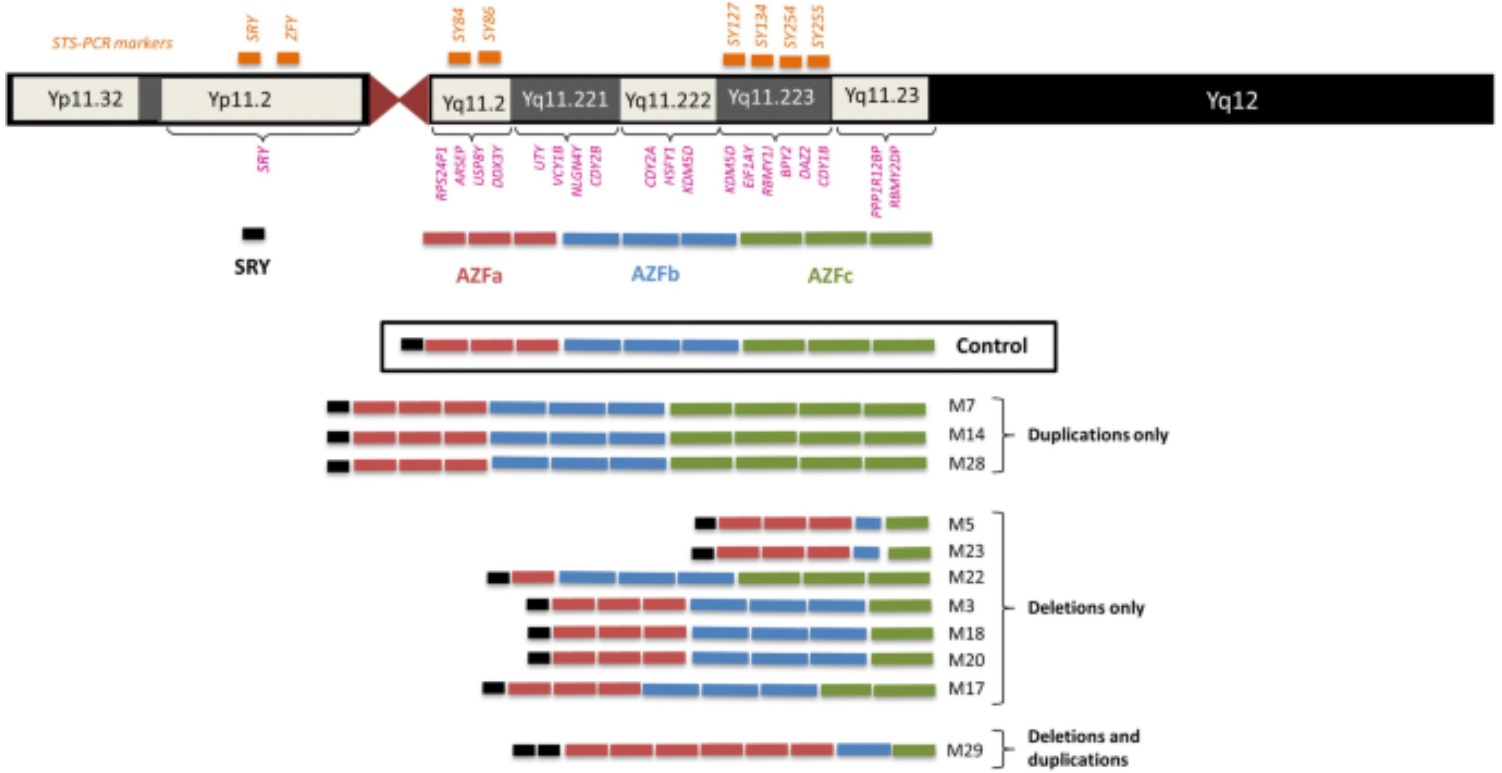
Genome is recommended as a first-line test

- **The American College of Medical Genetics and Genomics (ACMG)** recommends genome or exome as a first-line test for **developmental delay, intellectual disability, and congenital anomalies.**
- **The International Precision Child Health Partnership (IPCHiP)** recommends genome or exome as a first-line test for NICU patients **with unexplained hypotonia.**
- **The National Society of Genetic Counselors (NSGC)** recommends genome or exome as a first-line test for all individuals with **unexplained epilepsy** and this guideline is endorsed by the **American Epilepsy Society (AES)**.

Types of variants



Copy Number Analysis (e.g., CMA or MLPA)



Test of Choice: MLPA or array CGH to detect large deletions in the **GALT**, CYP21A2 gene.

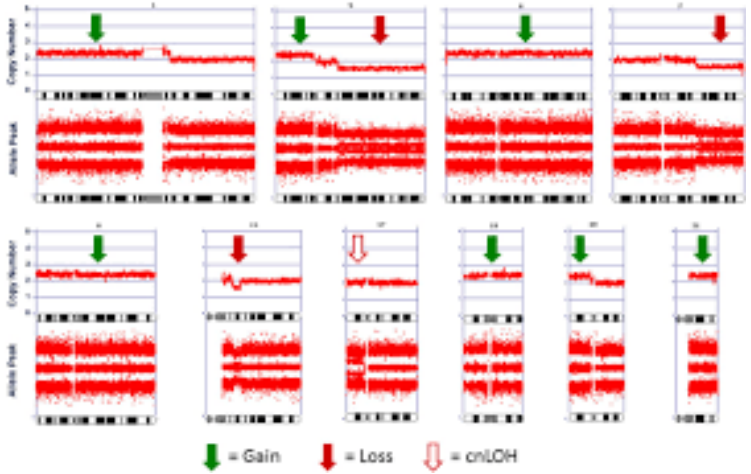
Why: Copy number analysis identifies large deletions or duplications that single-gene sequencing cannot detect.

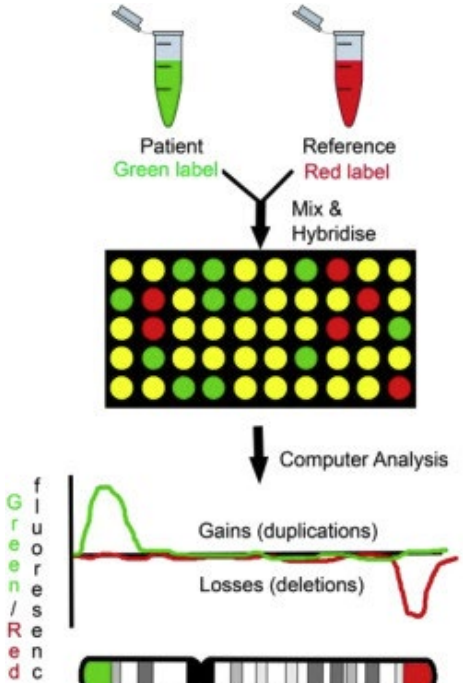
MLPA

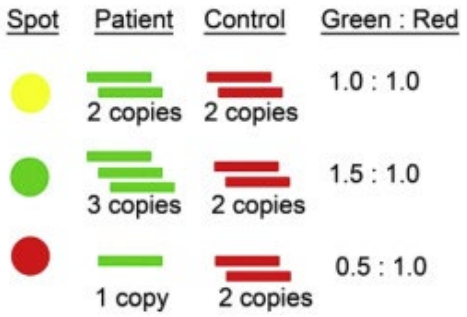
Clinical Features: Clinically diagnosed with Williams syndrome (WS).	
Referral Reason: Referred for MLPA screening analysis of microdeletions causing WS.	
Result	**Decreased dosage of the probe sets for *ELN* was detected.**
Summary	**MLPA assessment showed deletion in *ELN* gene.**
Interpretation and Comments	• Multiplex Ligation-dependent Probe Amplification (MLPA) assessment showed a heterozygous deletion in *ELN* gene. • *ELN* gene is located in 7q11.23 so deletion of this gene confirmed Williams Syndrome in this patient. • As this result has important implication for the family, a referral to a clinical genetic service is recommended.
Analysis Method	• DNA was extracted using salting out method. • Screening for microdeletions was performed using MLPA probe set P245-B1 (MRC-Holland) fragment

Chromosomal Microarray (CMA)

- Copy number variation: first-tier approach for the postnatal evaluation of individuals with **intellectual disability, developmental delay, autism spectrum disorder,** and/or **multiple congenital anomalies**, as well as for prenatal evaluation of fetuses with **structural anomalies** observed by ultrasound**.**







WGS

- **Why:** WGS captures all types of genetic variants, including **structural changes** or **non-coding variants**, that may not be detected by WES or panel testing.

What Role Does the Clinician Play in Choosing the Best Diagnostic Tool?

- Performing a detailed clinical assessment to guide test selection.
- The clinician's understanding of the patient's phenotype and family history helps prioritize testing strategies, ensuring both clinical and financial efficiency. (tested individual, family, cost, time, test quality/access)

Conclusion

- Almost half of the patients were diagnosed using **the traditional approach**, most at the initial visit. For those **remaining undiagnosed**, next-generation sequencing may be clinically and economically beneficial. Estimating a 50% success rate for next-generation Sequencing in **undiagnosed genetic disorders**, its application after the first clinical visit could result in a higher rate of genetic diagnosis at a considerable cost savings per successful diagnosis.

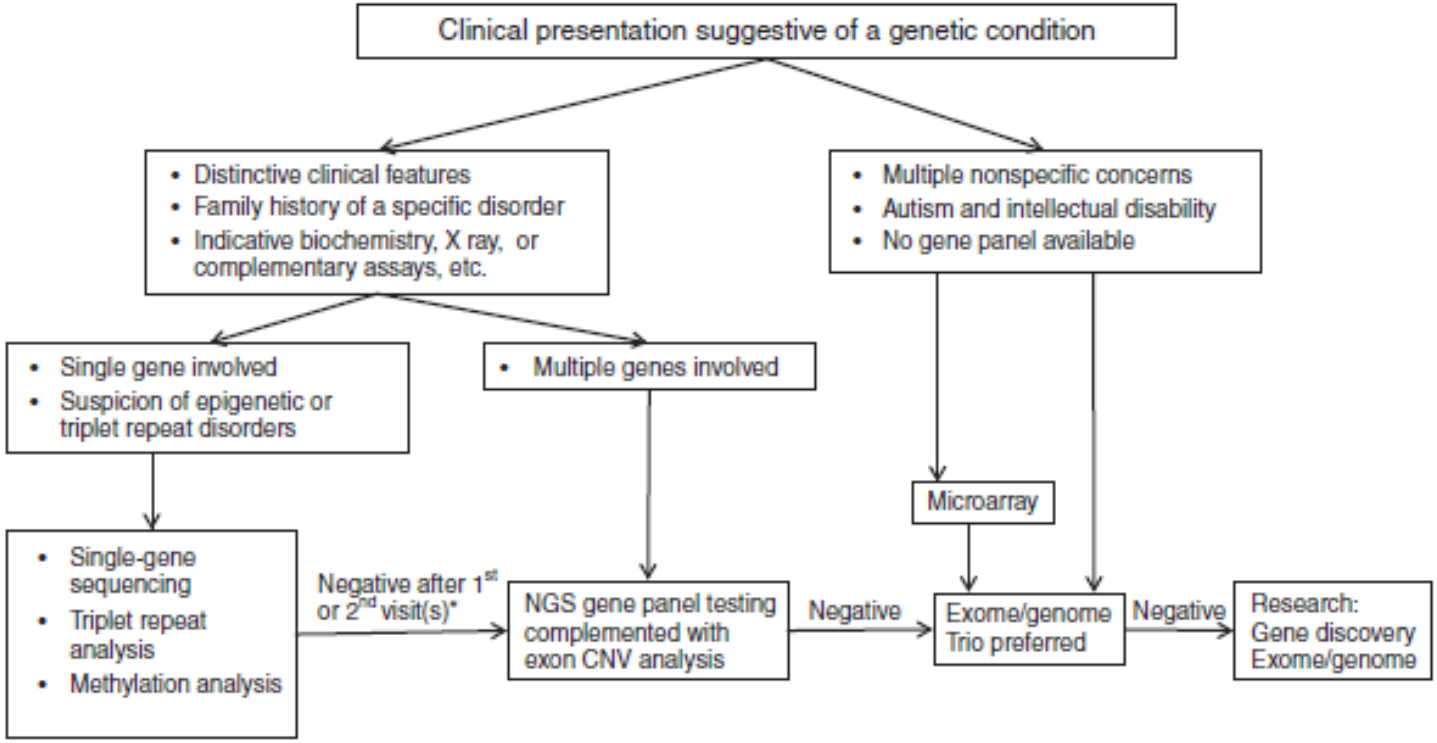
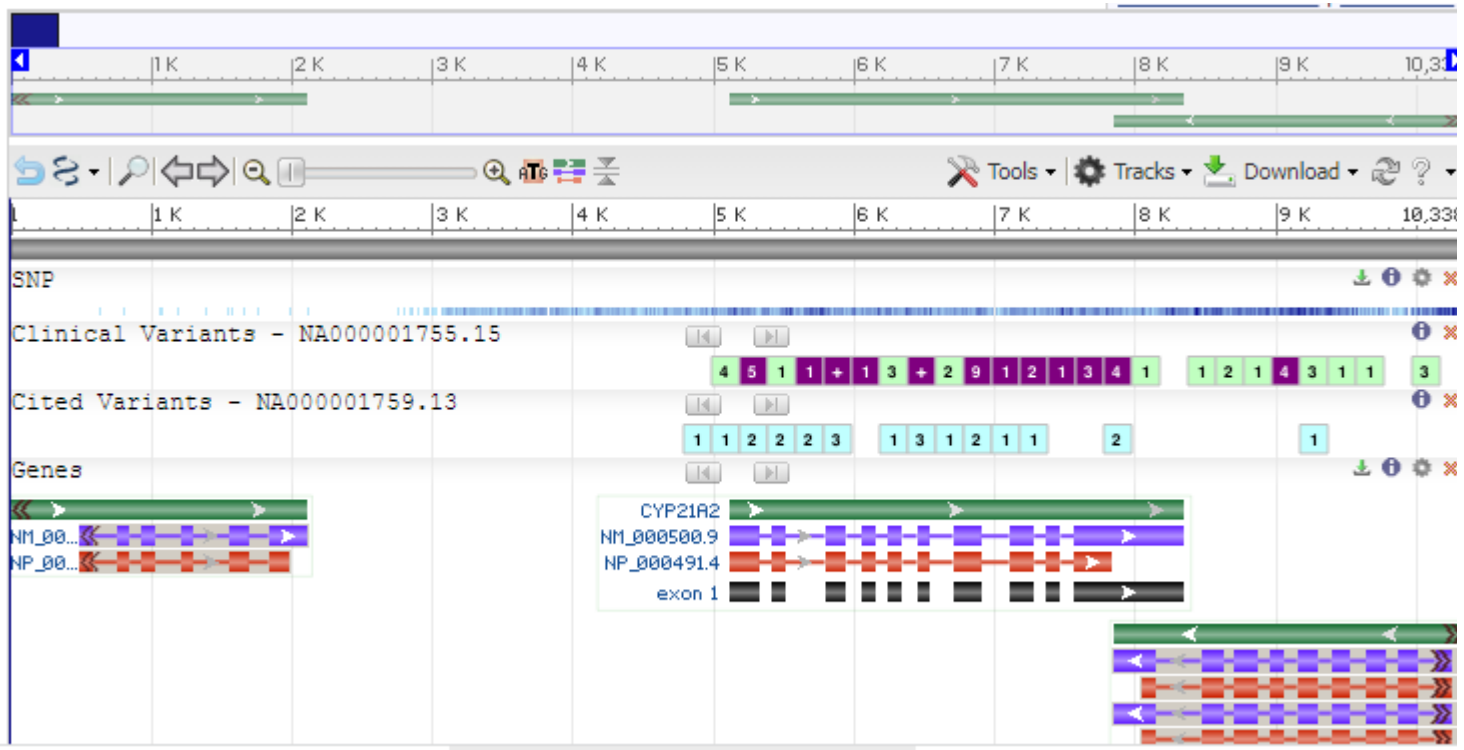


Figure 1 Molecular genetic testing algorithm. *This suggestion is based on the study by Shashi et al.[2]

Congenital adrenal hyperplasia

- Molecular genetic testing : support a diagnosis of CAH and may help to determine the type of 21-hydroxylase deficiency.



A. Whole Exome Sequencing (WES)

Selection (capture):

All exons of all known genes (1.5–2% of all human DNA)

- Variable read depth at boundaries
- Greater sequencing depth
- More cost effective

B. Whole Genome Sequencing (WGS)

No selection:

Entire human DNA analyzed including introns, RNA genes, etc.

- Moderate read depth
- Similar read depth across the genome
- Can identify copy number variants, repeat expansions
- Higher price

Hypospadias

- Genetically male, male external genitals his body did not respond to male sex hormones. caused problems during puberty.

The patient presents with clinical features consistent with congenital adrenal hyperplasia. Whole Exome Sequencing (WES) was performed to identify potential genetic causes for the patient's condition.

Test Result

Pathogenic variation detected related to Androgen Insensitivity.

Primary Findings:

Variants with possible relevance to patient's phenotype:

Gene	Variant(NM#)	Location	Amino Acid Alteration	zygosity	Inheritance	Disease OMIM	Classification
AR	**X:66942818 G>T NM_000044.6**	**c.2599G>T**	**p.Val867Leu**	**Hemizygote**	**XLD**	**Androgen insensitivity, Hypospadias 1**	**Pathogenic**

Please see table below for a detailed description of the detected variants.

Test Result	**The genetic diangnosis of androgen receptor variation is confirmed. Congenital adrenal hyperplasia is not confirmed.**
	The variant c.2599G>T in the AR gene results in a missense mutation leading to the substitution of Valine by Leu at amino acid position 867 (p.Val867Leu). The *AR* gene is