מחלקת הכנסים
של ההסתדרות הרפואית בישראל

ההסתדרות הרפואית בישראל
Israeli Medical Association

האגודה הישראלית לגנטיקה רפואית
ISRAELI SOCIETY OF MEDICAL GENETICS

הכנס השנתי של

האיגוד הישראלי לגנטיקה רפואית

2-3 באפריל, 2025 | מלון וורט, ים המלח

יום רביעי | 2.4

09:00-10:00	פתיחת ההרשמה התכנסות וקפה
10:00-12:00	סדנת ביואינפורמטיקה צוות מרכז רפואי בילינסון - פרופ' לינה באסל-שלמון, ד"ר נועה רורמן שחר, ד"ר לילי בזק, ד"ר שרית פרג'-ברהום, ד"ר דנה ברבינג גולדשטיין, ד"ר גבי לידזבסרסקי, גב' מרינה ליפשיץ קליס, פרופ' עידית מאיה "Workshop" Mind the gaps - genome-wide sequencing pitfalls *מותנה ברישום מראש
12:30-13:00	**ארוחת צהריים**
13:00-13:15	מילות פתיחה וברכות **פרופ' לנה שגיא-דאין**, מרכז רפואי כרמל
13:15-15:05	**מושב ראשון - שיטות בגנטיקה קלינית** יו"ר - פרופ' אבי אור, פרופ' אילון פרס, ד"ר נורית מגל
13:15-13:30	**ד"ר עפר איסקוב**, המרכז הגנומי של כללית Incorporating Clinical and Molecular Variant Properties Improves Classification and Calibration of in silico Pathogenicity Prediction Tools
13:30-13:45	**גב' שלומית עזר**, מרכז רפואי הדסה Exploring the unique characteristics of genes with dual autosomal dominant and recessive inheritance: mechanisms, phenotypes and candidate identification
13:45-14:00	**ד"ר ליאת גנון**, המרכז הגנומי של כללית Accuracy and Reliability of Next Generation Sequencing (NGS) results is Validated by Sanger sequencing.
14:00-14:15	**ד"ר הילה לדרמן נחמיאס**, מרכז רפואי איכילוב Optical Genome Mapping of Bone-Marrow in Hematological Malignancies
14:15-14:30	**גב' שירה שביב**, מרכז רפואי שערי צדק Enhanced Haplotype Construction in Preimplantation Genetic Testing Using SNP Microarray Analysis
14:30-14:45	**ד"ר הגר מור שקד**, מרכז רפואי הדסה Long-read whole genome sequencing uncovers a deletion upstream to HOXD13 causing synpolydactyly
14:45-15:05	**Maria Martinez-Fresno**, PharmD Director, GDT&RH Medical Affairs, Europe Unleashing Genomics Past, present and future הרצאה בחסות Danyel Biotech & Illumina
15:05-15:30	**הפסקת קפה + קבלת חדרים**
15:30-17:00	**מושב שני - גנטיקה והריון** יו"ר - פרופ' צבי אפלמן, גב' אלן טאוב

15:30-15:45	**גב' ורד אופן גלסנר**, מרכז רפואי איכילוב Incremental Yield of Exome Sequencing in Fetuses with Isolated Polyhydramnios: Diagnostic Insights and Clinical Impact
15:45-16:00	**ד"ר איתי גת**, מרכז רפואי אסף הרופא Pathogenic genetic and clinical findings in sperm donors conceived offspring: A Single-Center Retrospective Study
16:00-16:30	דיבייט: **ד"ר נועה רורמן שחר**, מרכז רפואי רבין / **ד"ר אודליה חורין**, מרכז רפואי שיבא תל השומר Reporting fetal secondary (ACMG) findings in prenatal exome sequencing - pros and cones.
16:30-16:45	**ד"ר ריבל סגל-פלד**, מרכז רפואי שערי צדק Structural analysis of microduplications detected by chromosomal microarray analysis (CMA) for Preimplantation genetic testing (PGT)
16:45-17:00	**ד"ר נעם הדר**, מכון ויצמן למדע Seamless Genomic Analysis: From Candidate Variants to PGT Applications
17:00-18:00	**ד"ר צבי סער חסן** ד"ר לפילוסופיה, הרצאת אורח אתיקה ודילמות מוסריות בייעוץ גנטי בהריון
18:00-19:00	**הפסקה**
19:00-20:00	**ארוחת ערב**
20:00-21:00	**מר ליאור ליפשיץ**, הרצאת אורח כלים למנטליות מנצחת
21:00	מוזיקה

יום חמישי | 3.4

07:30-08:15	פעילות בוקר | צ'י קונג התעמלות בוקר סינית מודרכת *מותנה ברישום מראש
08:15-09:30	**ארוחת בוקר**
09:30-11:10	**מושב שלישי - גנטיקה קלינית וטיפולים** יו"ר - פרופ' אניק רוטשילד, פרופ' דבורה אבליוביץ
09:30-09:45	**ד"ר אבי פלנר**, מרכז רפואי בילינסון Using GPT-4 chatbot for genomic data interpretation in monogenic disorders
09:45-10:00	**גב' נועה הורביץ**, מרכז רפואי איכילוב Telomere Biology Disorders- clinical service, dilemmas and challenges
10:00-10:15	**ד"ר תמימה עבדאללה מועדי**, מרכז רפואי רמב"ם Impact of Rapid Exome Sequencing on Pediatric Patients with Cardiomyopathies and Acute Heart Failure
10:15-10:30	**פרופ' ליאור גרינבאום**, מרכז רפואי שיבא תל השומר The Benefit of Genetic testing for Parkinson's disease in Israel: Insights from the Rostock Parkinson's Disease (ROPAD) study
10:30-10:50	**ד"ר אבי פלנר**, מנהל המרפאה הנוירוגנטית, המכון הגנטי, מרכז רפואי רבין Following Friedreich's Footsteps: When Repeats Go Too Far הרצאה בחסות Medison Pharma
10:50-11:10	**Anny Goldman**, Lecturer on "Medication Adherence" for MS.c in Clinical Pharmacy at Ben Gurion University התמדה בטיפול התרופתי: מה כל הסיפור? הרצאה בחסות Takeda
11:10-11:40	**הפסקת קפה + עזיבת חדרים**

11:40-12:40	**מושב רביעי- חינוך בגנטיקה קלינית** יו"ר - ד"ר שירי שקדי-רפיד, פרופ' עידית מאיה, ד"ר אריה קויפמן
12:40-14:00	**מושב חמישי - אונקוגנטיקה** יו"ר - פרופ' איתן פרידמן, פרופ' ציפורה פליק-זכאי
12:45-13:00	**ד"ר עאסם אבו אשתייה**, מרכז רפואי רבין The genetic landscape of Lynch syndrome in the Israeli population
13:00-13:15	**ד"ר שרי ליברמן**, מרכז רפואי שערי צדק Comparative Analysis of BRCA Pathogenic Variant Detection: Population Screening Versus Genetic Counseling – in a Real-World Setting
13:15-13:30	**ד"ר מרים רגב**, מרכז רפואי שיבא תל השומר Five-year experience of genetic work-up in the Pediatric Oncogenetics joint clinic in Sheba Medical Center highlights WES as the optimal test
13:30-13:45	**פרופ' יעל גולדברג**, מרכז רפואי רבין A new gangster in town – POT1 founder pathogenic variants associated with long telomeres, recurrent melanoma, various solid malignancies and high tumor burden
13:45-14:00	**ד"ר שגיא יוספסברג**, קפלן פונדקאות - מי מחליט עלי סיכום הכנס
14:00-15:00	**ארוחת צהריים**

הכנס בחסות:

חסות פלטינום:

MEDISON illumina DanyelBIOTECH

חסות זהב:

EISENBERG ELDAN NEOPHARM GROUP

חסות כסף:

MSD INVENTING FOR LIFE AstraZeneca Blueprint Genetics gamidorDIAGNOSTICS

RHENIUM BIO SCIENCE Syntezza sanofi FUGENE GENETICS Takeda

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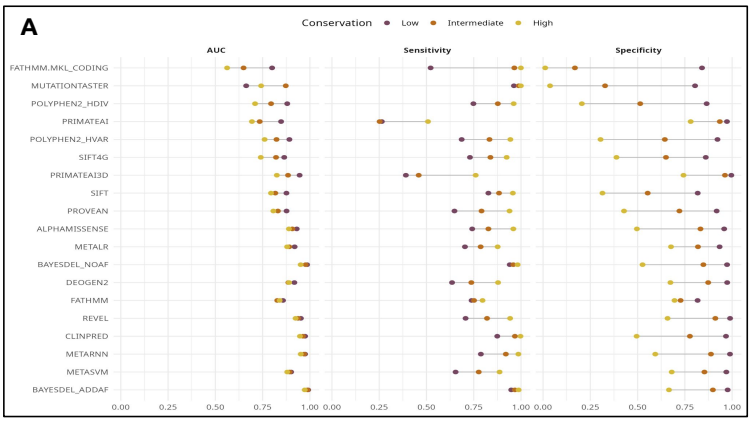
הרצאות

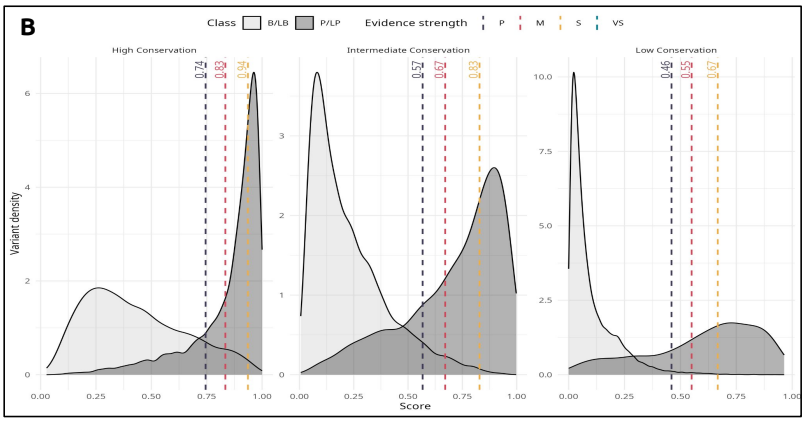
Incorporation of Clinical and Molecular Variant Properties Improves the Performance of in silico Pathogenicity Prediction Tools

Ofer Isakov[1,2,3,4], Reut Fluss[2], Dina Marek-Yagel[2], Shamil Sunyaev[5,6,7] and Shay Ben Shachar[2,3,4]

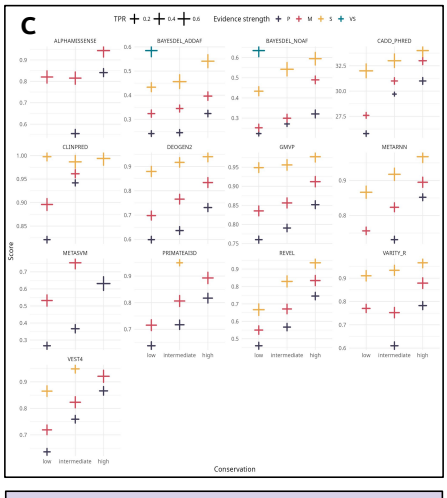
1 - Raphael Recanati Genetic Institute, Rabin Medical Center-Beilinson Hospital, Petach Tikva, Israel., 2 - Clalit Research Institute, Clalit Health Services, Ramat Gan, Israel., 3 - The Ivan and Francesca Berkowitz Family Living Laboratory Collaboration, at Harvard Medical School and Clalit Research Institute, Boston, MA, USA., 4 - Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel., 5 - Department of Biomedical Informatics, Harvard Medical School, Boston, MA, USA., 6 - Division of Genetics, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA., 7 - Broad Institute of MIT and Harvard, Cambridge, MA, USA.

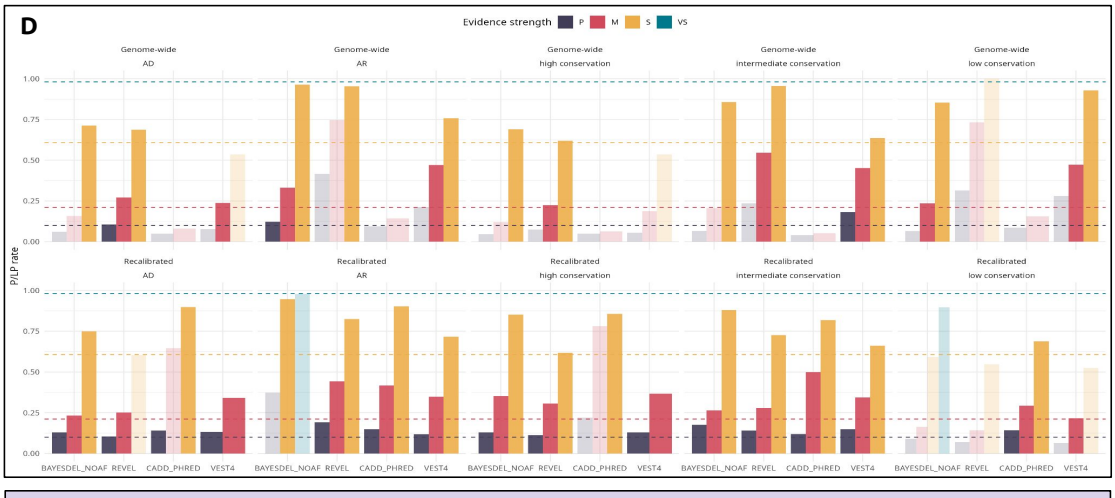
Background: In silico pathogenicity prediction tools are essential for determining the potential pathogenicity of genetic variants, aiding clinical diagnostics and genetic counseling. However, their performance can vary depending on molecular and clinical contexts, complicating variant classification. **Methods**: ClinVar variants classified as pathogenic (P), likely pathogenic (LP), benign (B), or likely benign (LB) were analyzed using 25 common tools. Tools were evaluated based on discriminatory performance. Data were stratified by variant creation date, allele frequency, conservation level, mode of inheritance (MOI), and disease category. For each subset, Bayesian methods were employed to recalibrate score thresholds corresponding to the levels of evidence defined by the American College of Medical Genetics (ACMG)





A) The effect of conservation on performance and calibration. Discriminatory performance metrics (AUC/Sensitivity/Specificity) in each level of conservation (B) Distribution of REVEL scores for P/LP (dark gray) and B/LB (light gray) variants stratified by conservation level with recalibrated score thresholds.

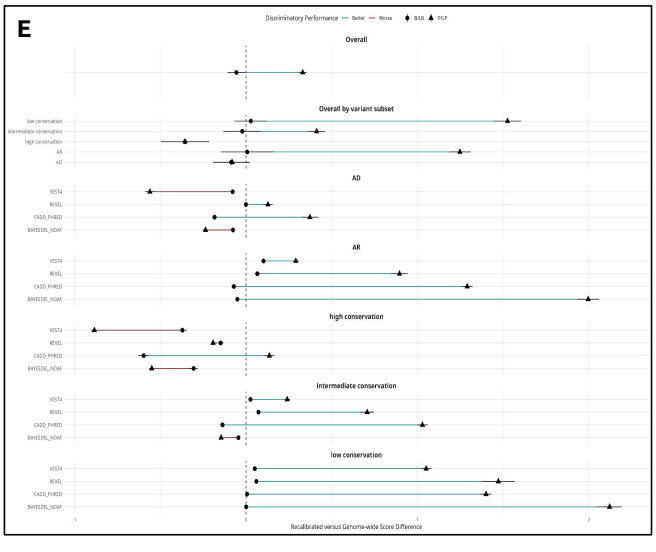




(C) Recalibrated score thresholds corresponding to various ACMG levels of evidence are added for each score in each conservation level

(D) Recalibrated versus genome-wide thresholds. For each variant subset the rate of pathogenic variants calculated using both the recalibrated and genome-wide thresholds. Rates estimates within the evidence strength range (dotted lines) are considered valid (bold color) and those outside are considered non-valid (light color).

(E) Comparing the recalibrated score thresholds with ClinGen's genome wide thresholds. Pathogenic variants received higher scores and benign variants received lower scores using the recalibrated score thresholds. Performance breakdown by variant subset demonstrated higher scores for pathogenic variants within low and intermediate regions and those affecting AR genes when using the recalibrated thresholds. Analyzing the effect of recalibration for each tool in each variant subset, we observed a significant improvement in discriminatory performance in the majority of cases.



Conclusion: This study emphasizes the importance of analyzing prediction tools in different clinically relevant contexts. By recalibrating score thresholds the tools can more accurately stratify variants according to their true pathogenic potential. This refinement enhances the confidence in variant classification and should be considered during training and evaluation of these tools.

Online webserver
https://c-gc.shinyapps.io/recalibrated_pp3_thresholds_browser/

GENOMIC CENTER | By CLALIT HEALTH

כללית CLALIT INNOVATION

Exploring the unique characteristics of genes with dual autosomal dominant and recessive inheritance: mechanisms, phenotypes and candidate identification

Shlomit Ezer,[1,2] Tal Sido,[1,2] Jonathan Rips,[1] Ronit Hoffman Lipschuetz,[1,2] Adina Fuchs,[1,2] Bassam Abu-Libdeh,[3] Elana Chervinsky,[4,5] Nadirah S Damseh,[3] Nada Danial-Farran,[4] Ilham Morani,[6] Ann Saada[1,2], Mohammed Al-Raqad,[7] Somaya Salah,[1,8] Shira Yanovsky-Dagan,[1] Nadra Samra,[6,9] Hanna Mandel,[10] Stavit Shalev,[4,5] Hagar Mor-Shaked,[1,2] Joël Zlotogora,[2] Tamar Harel[1,2]

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Introduction

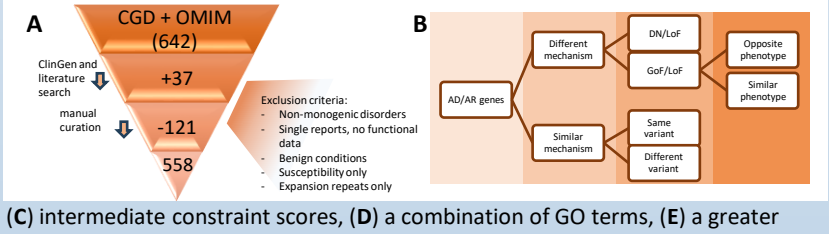
Autosomal dominant (AD) inheritance arises via mechanisms such as haploinsufficiency, dominant-negative (DN), or gain of function (GoF) effects, while autosomal recessive (AR) inheritance typically involves partial/complete loss of function (LoF). Yet, a subset of genes demonstrates both inheritance patterns. We aimed to curate and characterize a list of such 'AD/AR genes', and to suggest additional candidates.

Methods

A list of AD/AR genes was compiled through an extensive review of OMIM, the Clinical Genomic Database (CGD) and literature. Using bioinformatic analyses, we compared AD, AR and AD/AR genes across various metrics, including gnomAD constraint values, exon count, protein length, quaternary structure, and gene ontology (GO) terms. We searched a local dataset of >24,000 exomes for homozygous variants in AD genes to identify additional candidates.

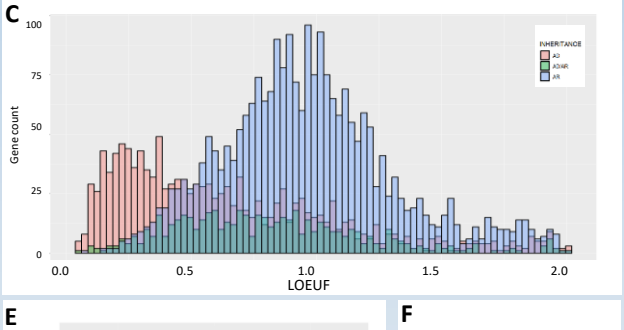
Results

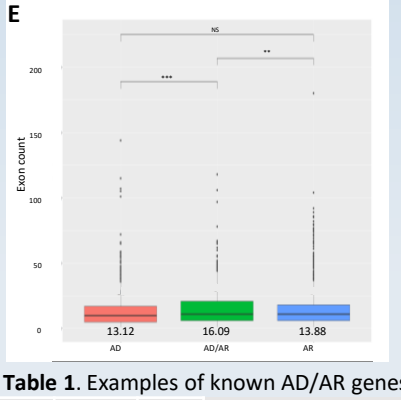
(**A**) AD/AR list was assembled; Approximately 12.5% of disease-associated genes have both dominant and recessive inheritance patterns. (**B**) genes were sub-classified by genotype-phenotype correlations and disease mechanisms. Pathogenic variants in AD/AR genes can cause distinct or similar phenotypes, depending on the molecular mechanism (**Table 1**).

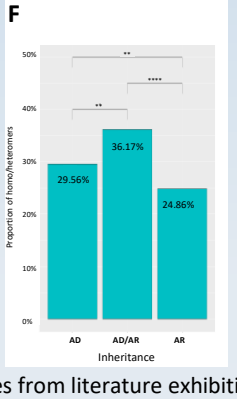


AD/AR genes exhibit unique bioinformatic properties, including: (**C**) intermediate constraint scores, (**D**) a combination of GO terms, (**E**) a greater average number of exons, and (**F**) an elevated propensity to form homo/heteromeric proteins.

We identified homozygous LoF or clinically reported variants in eight genes previously classified as AD only, and present a second confirmatory report for AR inheritance of a ninth gene.







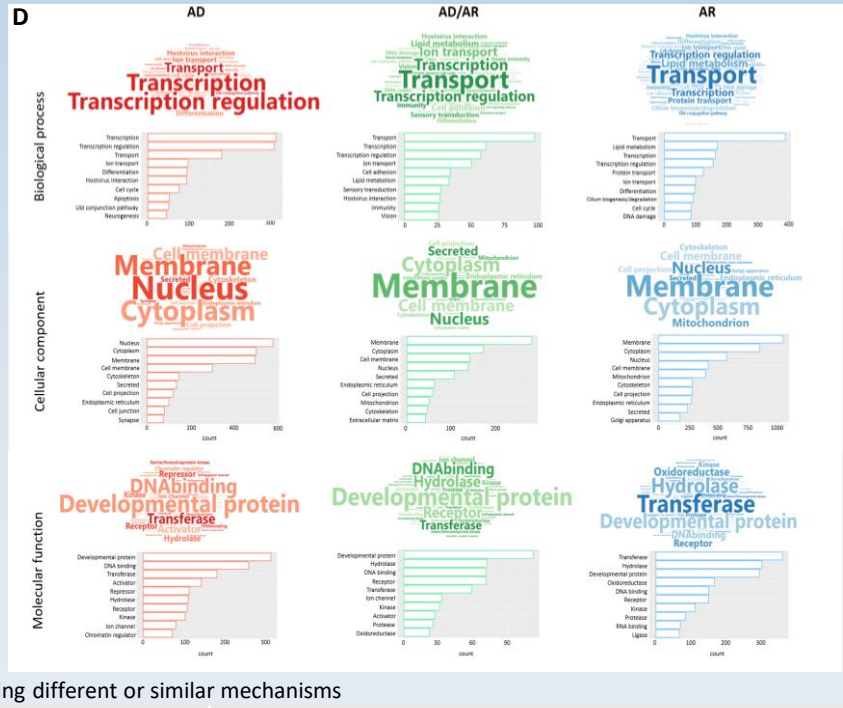


Table 1. Examples of known AD/AR genes from literature exhibiting different or similar mechanisms

	Gene	MIM	AD	AR
AD: GoF AR: LoF	***CACNA1A***	601011	Developmental and epileptic encephalopathy 42; Migraine, familial hemiplegic, 1; Spinocerebellar ataxia 6; Episodic ataxia, type 2	Early onset epileptic encephalopathy
	RYR1	180901	Congenital myopathy 1A, autosomal dominant, with susceptibility to malignant hyperthermia; King-Denborough syndrome; susceptibility to malignant hyperthermia	Congenital myopathy 1B, autosomal recessive; Neuromuscular disease, congenital, with uniform type 1 fiber
AD: DN AR: LoF	***SOD1***	147450	Amyotrophic lateral sclerosis 1	Spastic tetraplegia and axial hypotonia, progressive; ALS
	ATAD3A	612316	Harel-Yoon syndrome	Harel-Yoon syndrome; Pontocerebellar hypoplasia, hypotonia, and respiratory insufficiency syndrome, neonatal lethal
	COL7A1	120120	Epidermolysis bullosa dystrophica, autosomal dominant; Epidermolysis bullosa, pretibial	Epidermolysis bullosa dystrophica, autosomal recessive
AD,AR: LoF	***ALX4***	605420	Parietal foramina 2; susceptibility to craniosynostosis 5	Frontonasal dysplasia 2
	PALB2	610355	Susceptibility to breast cancer; susceptibility to pancreatic cancer	Fanconi anemia, complementation group N
	RTEL1	608833	Pulmonary fibrosis and/or bone marrow failure, telomere-related 3; Dyskeratosis congenita, autosomal dominant 4	Dyskeratosis congenita, autosomal recessive 5

Conclusions

AD/AR genes possess distinctive features that likely underpin their dual inheritance modes, and represent prime candidates for allele-specific RNA therapies targeting AD or GoF variants, given their tolerance to heterozygous LoF. We propose several candidate AD/AR genes, emphasize caution when filtering by inheritance type, and offer a framework to facilitate therapeutic development.

Optical genome mapping of bone-marrow in Hematological malignancies

Dr Hila Lederman Nachmias Genetic Institute Tel Aviv Sourasky Medical Center - Ichilov

Introduction:

Extreme genomic rearrangements are hallmarks of cancer. AML is characterized by genetic aberrations (chromosomal translocations, deletions, insertions) affecting its classification for risk of treatment. Optical Genome Mapping (OGM) detects structural variations in an unbiased manner and at much higher sensitivities than cytogenetic techniques used to

The OGM results were compared to the known aberrations as detected by karyotyping and

Figure1: Classic cytogenetic events Identified by OGM|

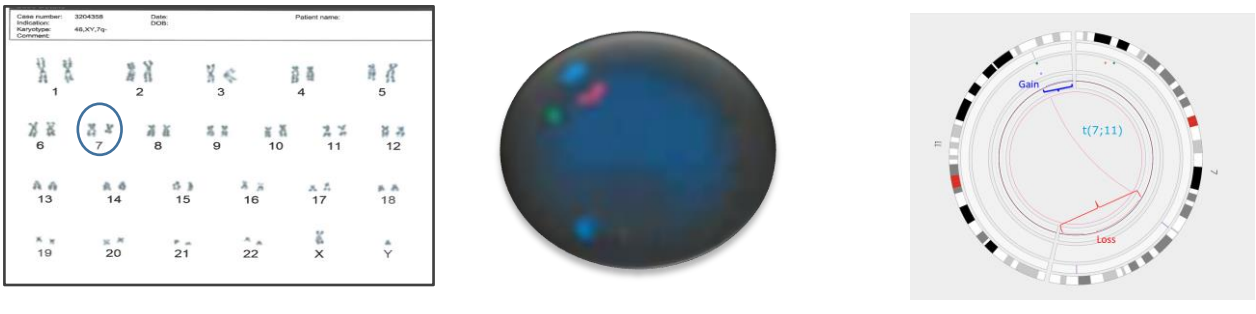


Figure2: OGM reveals more than classic cytogenetics for a 69 years old male patient with suspected AML

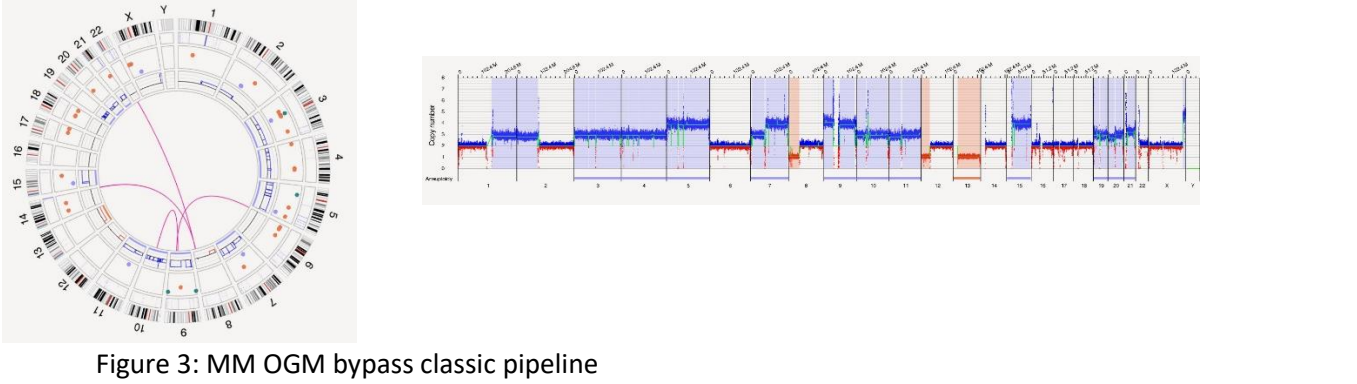


Figure 3: MM OGM bypass classic pipeline

Discussion:

It can be concluded that the results seen by the karyotype and the FISH were all observed using the OGM method; in addition, chromosomal aberrations that could not be diagnosed with low resolution were observed using the OGM.

Enhanced Haplotype Construction in Preimplantation Genetic Testing Using SNP Microarray Analysis

Shira Shaviv, Pinhas Renbaum, Sharon Zeligson, Reeval Segel, Tal Dror, Lihi Amit, David Zeevi, Elina Farhi, Merav Ben Shlomo, Elinor Hakam, Tali Azar, Anat Yadin, Rachel Beeri, Orit Freireich, Galit Lazer Derbeko, Hadar Bar-Noy, Gheona Altarescu

PGT Unit & Microarray Unit, Medical Genetics Institute,
Shaare Zedek Medical Center, Jerusalem

Objective: **Comprehensive PGT (Haploseek) aims to gather extensive genomic data from embryos, preventing transmission of hereditary genetic diseases and ensuring accurate chromosomal assessment. Standard STR methods face challenges in haplotype construction for de novo cases and frequently in cases of consanguinity.**

Methods: **We used a 750K microarray platform for each family member in our Haploseek system. By analyzing SNP data from the microarray, we determined allele linkage to the genetic variant.**

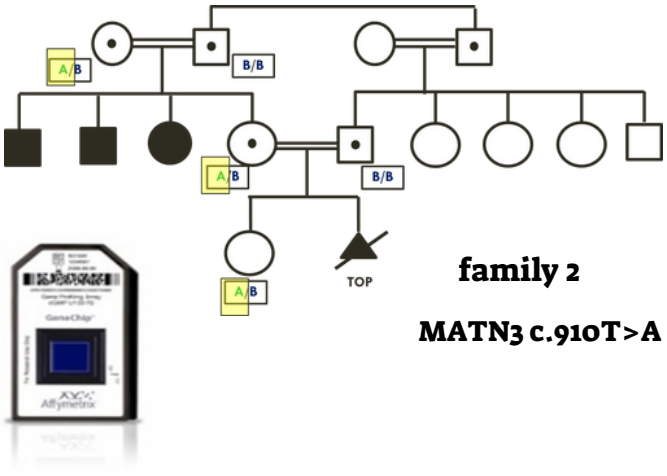
Results: **We present three families. The use of SNP data from microarrays enabled haplotype construction.**

· Family 1: A de novo maternal 1.3Mb deletion on 15q13, identified on the paternally transmitted allele.

· Family 2: A recessive pathogenic variant c.910T>A in the MATN3 gene in a consanguineous couple. A healthy child, used as a reference, showed recombination near the variant site. SNP data identified the wild-type alleles based on grandparents' haplotype.

· Family 3: A consanguineous couple, carriers of pathogenic variants in Joubert syndrome (TMEM216 gene) and Ichthyosis (CERS3 gene). One child was homozygous for TMEM216 and heterozygous for CERS3, while the other had the opposite genetic configuration. SNP comparison between parents and the two children allowed accurate analysis for both diseases.

Conclusions: **This method enhances genetic linkage analysis through abundant SNPs, particularly useful in de novo variants and consanguineous families where standard methods struggle. Microarray platform allows simultaneous SNP analysis across the genome for multiple diseases, eliminating the need for labor-intensive STR identification.**



family 2

MATN3 c.910T>A

dbSNP RS ID	Chr	Mother-Carrier	Father-Carrier	Child - Healthy	maternal grandmother Carrier	maternal grandfather Carrier
rs1489694	2	AB	BB	AB	AB	BB
rs1489696	2	AB	AA	AB	AB	AA
rs4233760	2	AB	AA	AB	AB	AA
rs75877558	2	AB	BB	AB	AB	BB
rs4666475	2	AB	BB	AB	AB	BB
rs9798024	2	AB	BB	AB	AB	BB
rs12612826	2	AB	BB	AB	AB	BB
rs17686549	2	AB	BB	AB	AB	BB
rs13011231	2	AB	BB	AB	AB	BB
rs12328613	2	AB	AA	AB	AB	AA
rs16987265	2	AB	AA	AB	AB	AA
rs10495701	2	AB	BB	AB	AB	BB
rs3924843	2	AB	BB	AB	AB	BB

Long-read whole genome sequencing uncovers a deletion upstream to HOXD13 causing synpolydactyly

Jonathan Rips,[1] Chaim Jalas,[2] Ayala Frumkin,[1,3] Shamir Zenvirt,[1] Avraham Shaag,[1] Tamar Harel,[1,3] Orly Elpeleg,[1,3] Hagar Mor-Shaked,[1,3]

1 Department of Genetics, Hadassah Medical Center, Jerusalem, Israel

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Introduction. Synpolydactyly (SPD) is a heterogeneous distal-limb malformation syndrome, characterized by webbing and duplication of adjacent digits. SPD1, the most common type, is attributed to disease-causing variants in HOXD13, a transcription factor in the HOXD cluster that is essential for limb development. Here, we present a challenging exome-negative case of familial SPD. The case was resolved using long-read genome sequencing, which revealed a microdeletion in a conserved non-coding regulatory region upstream of the HOXD cluster.

Methods. Exome-trio and standard chromosomal microarray analysis (CMA) on three affected siblings were negative. Oxford Nanopore whole-genome sequencing with long-read sequencing (WGS-LRS) was pursued on two affected sisters, followed by CMA-Cytoscan HT validation.

Results. WGS-LRS for two affected siblings revealed a shared microdeletion of ~5.6kb upstream of *EVX2* and *HOXD13* [chr2:176073523-176079120 (hg38)]. The deletion is predicted to affect an enhancer of several HOXD genes (EH38E2053988) and overlaps with a short stretch of high-sequence homology between human and mouse, called R3. CMA-Cytoscan HT analysis and a PCR assay confirmed segregation of the deletion in the siblings and revealed paternal inheritance.

Figure 1. Pedigree and clinical presentation of family with synpolydactyly (SPD)

A. Pedigree of the family. The proband (II-2) is marked with an arrow. del: heterozygous 5.6kbp deletion chr2:176073523-176079120 (hg38). B. photos of the feet of individuals II-2 (left) and II-4 (right): affected family members exhibit a significant bilateral medial deviation of the second and third toes. C. X-ray of the feet of individual II-2: The second and third toes have abnormally formed proximal through middle phalanges. The second toe has a proximal phalanx which is partially shared with the second and third metatarsals. The third toe has what appears to be a rudimentary proximal phalanx. The hindfoot and midfoot appear to be normal. D. X-ray of the hands of individual II-4: note the hypoplastic ring finger metacarpals, ring finger contracture.

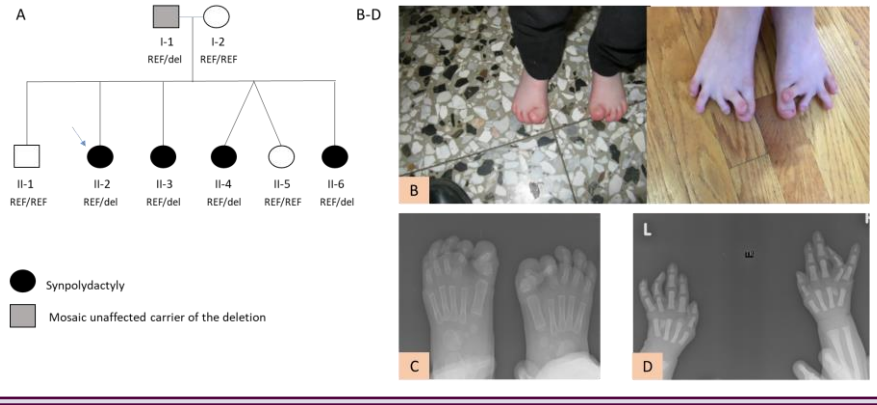
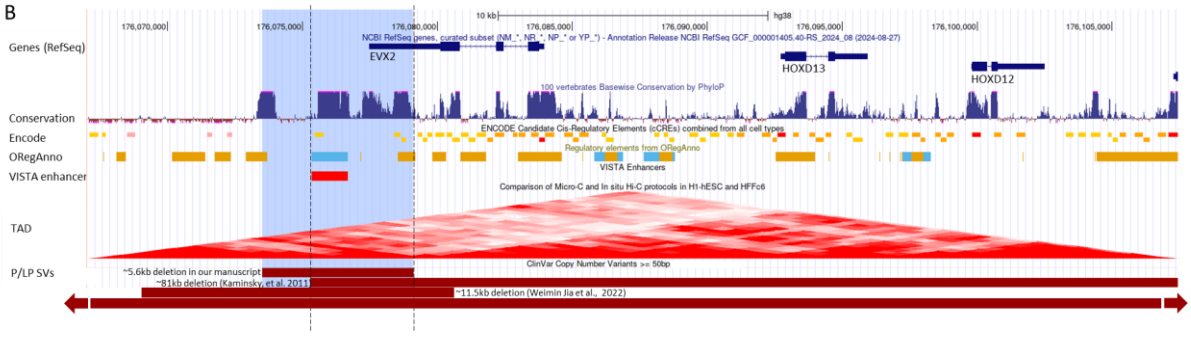


Figure 2. Detection of deletion event in EVX2

A. IGV view of the ~5.6kbp microdeletion identified using LRS in proband II-4, separated in two alleles [chr2:176073523-176079120 (hg38)].



B. UCSC screenshot of the HOXD gene cluster. Deletion with marked in light blue shading. Conservation track shows multiple alignments of 100 vertebrate species and measurements of evolutionary conservation. Encode, ORegAnno, and NCBI RefSeq Functional Elements enhancer analyses tracks are presented. P/LP SVs are presented from ClinVar database. TAD analysis Track provide heatmaps of chromatin folding data from in situ Hi-C. The dashed black lines represent the smallest region of overlap (SRO) described to date.

Discussion. Our study expands the genotype-phenotype correlation of the regulatory region upstream of HOXD13 in synpolydactyly (SPD), demonstrating its role in limb development. We highlight the utility of long-read WGS in identifying non-coding variants that are missed by traditional exome sequencing. Our findings emphasize the importance of analyzing regulatory elements, such as enhancers, which can impact gene expression and contribute to disease.

Incremental Yield of Exome Sequencing in Fetuses with Isolated Polyhydramnios: Diagnostic Insights and Clinical Impact

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Objective

To assess the diagnostic yield of exome sequencing (ES) in isolated polyhydramnios.

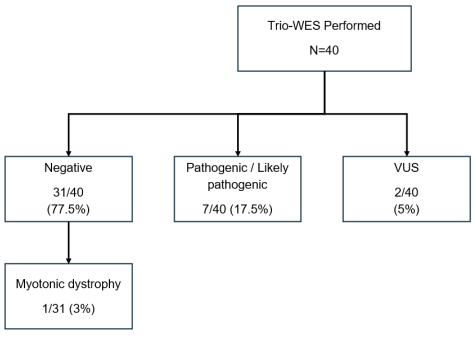
Methods

- The study included 40 cases diagnosed as isolated polyhydramnios, defined as an amniotic fluid index (AFI) ≥25 cm or a single deepest pocket (SDP) ≥ 8 cm.
- All cases had a normal CMA result on amniocentesis.
- All cases underwent screening for gestational diabetes mellitus (GDM). Four patients had GDM and one had type 1 diabetes mellitus.
- Polyhydramnios was diagnosed during the third trimester in 28 cases (70%) and during the second trimester in 12 cases (30%). The mean gestational age at diagnosis was 29.5 ± 4.2 weeks (range 21–36 weeks)
- The mean AFI at diagnosis was 31.7 ± 6.4 cm, with 23 cases (57.5%) classified as moderate-severe polyhydramnios and 17 cases (42.5%) classified as mild polyhydramnios.
- Clinical and postnatal outcomes were recorded.

Results

Seven cases demonstrated a causative a pathogenic/likely pathogenic (P/LP) variant. Two cases had variants of unclear significance (VUSs).

Gene	condition/OMIM	Inheritance pattern	ACMG classification	Origin	AFI	Early/ Late	Maternal diabetes	Pregnancy follow-up
AMER1	#300647 Osteopathia Striata with Cranial Sclerosis	XLD	LP	Inherited	moderate	Late	yes	Delivery
BSND	#606412 Bartter Syndrome Type 4A	AR	P	Inherited	mild	Early	no	TOP
KCNJ1	#600359 Bartter Syndrome Type 2	AR	P+P	Inherited	severe	Early	no	TOP
KCNJ1	#600359 Bartter Syndrome Type 2	AR	LP+LP	Inherited	severe	Late	no	TOP
MAGED2	#300823 Bartter Syndrome, X-Linked Transient	XLR	LP	Inherited	severe	Early	no	IUFD
RIT1	#609591 Noonan Syndrome 8	AD	LP	Inherited	moderate	Late	yes	Delivery
AUTS2	#607270 Autism Susceptibility 8	AD	P	De-novo	mild	Early	No	TOP
CHRND	#100720 Multiple Pterygium Syndrome, Lethal Type	AR	LP+VUS	Inherited	moderate	Late	No	TOP
DNAH11	#603339 Primary Ciliary Dyskinesia 7	AR	LP+VUS	Inherited	Severe	Early	NO	Delivery



Discussion

- In this study, we present preliminary data on the diagnostic yield of trio exome sequencing (ES) in 40 fetuses diagnosed with isolated polyhydramnios.
- Overall, a genetic diagnosis (P/LP variant) was obtained in 7/40 (17.5%) cases. Two variants (2/40, 5%) classified as VUSs and one case was positive for myotonic dystrophy diagnosed postnatal.
- Five of the positive cases presented with moderate-severe polyhydramnios and two with mild polyhydramnios.
- Two ES-positive cases also had GDM.
- Follow-up data were available for 20 out of 34 cases (59%), Mean age at follow-up was 23.9 months (range: 3–48 months)
- Normal development was observed in 85% of the negative ES cases.
- Among the ES-negative cases, one neonatal death at 2 months of age, One diagnosed at 21 months with global developmental delay, speech delay, strabismus, and failure to thrive, and one with speech delay at 24 months.

Conclusions

- We observed that mild polyhydramnios, late-onset cases, or the presence of GDM do not exclude genetic etiology, as these conditions may, in fact, coexist.
- **ES should be integrated into the diagnostic workup of isolated polyhydramnios**
- **ES may be considered as a first-tier test for late-detected cases**

OHAD BIRK'S LAB
THE MORRIS KAHN LABORATORY OF HUMAN GENETICS

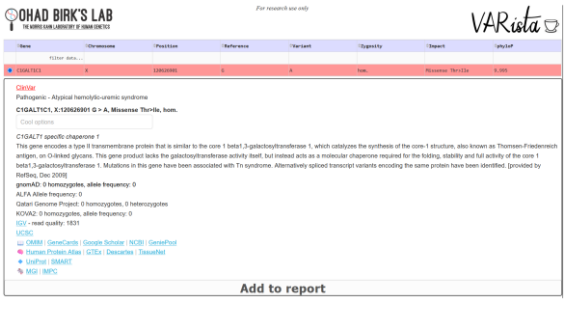
Seamless Genomic Analysis: From Candidate Variants to PGT Applications

Noam Hadar, Vadim Dolgin, Ginat Narkis, Shirly Amar, Grisha Weintraub, Ohad S. Birk

🧬😟 Genetic case unsolved by standard pipelines 😟🧬

VARista

Free and online streamline variant analysis platform



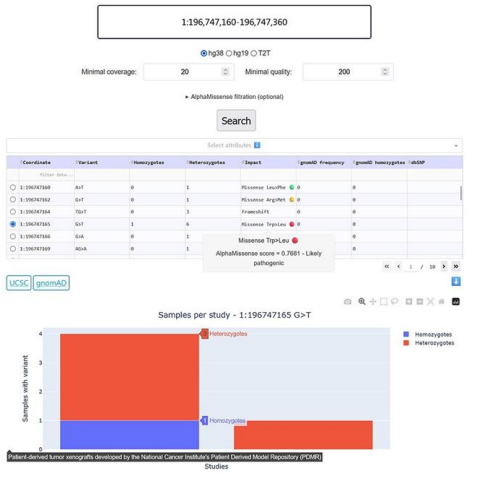
ACMG-criteria free!

https://VARista.link

Variant Analysis

GeniePool

Variant search engine in >60 WES samples with experimental attributions



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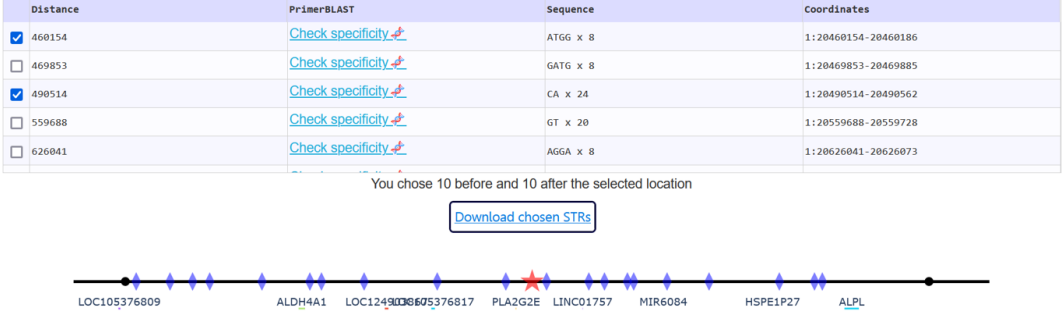
PGTailor



PGT-M assay design platform and STR database (*STRavinsky*)

https://fohs.bgu.ac.il/birklab/PGTailor

Poster Number: P11.036.D

Genome Sequencing Reanalysis Increases the Diagnostic Yield in Dystonia

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BACKGROUND AND OBJECTIVE

- Previous studies have variably yielded a molecular diagnosis in 11.7%-37.5% of dystonia patients, using gene panels, exome sequencing or genome sequencing.
- Systematic reanalysis of genomic sequencing data has been shown to increase the diagnostic yield in different Mendelian disorders. However, no previous studies evaluating reanalysis of genome sequencing data dedicated to individuals with dystonia are reported.
- ***We investigated the contribution of genome sequencing data reanalysis to the diagnostic yield of dystonia patients who remained undiagnosed after prior genome analysis.***

METHODS

- Initial genome sequencing and analysis were performed in 2019.[1]
- Genome sequencing reanalysis was performed through:

  A. Focused reanalysis for specific genes as part of gene discovery collaborations and clinical correlation efforts during 2020-2023.[2-4]

  B. Systematic updated genome sequencing data reanalysis (Ended January 2023).

RESULTS

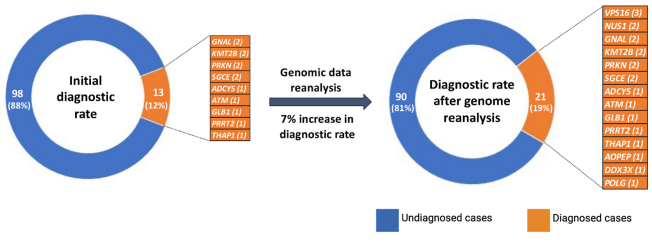

Figure 1. Improved diagnostic yield through genome sequencing data reanalysis. The number of individuals with and without a molecular diagnosis is shown for the initial genome sequencing analysis and after data reanalysis. The number of diagnosed individuals is shown for each gene in brackets.

Case	Sex ; Age at dystonia onset (yr.) ; Age at diagnosis (yr.)	Gene ; Inheritance	Reason for diagnosis through reanalysis
Diagnostic findings through gene-specific discovery collaborations and clinical correlation efforts[2-4]			
1	M ; 14 ; 62	*VPS16* ; AD	New dystonia gene
2	F ; 7 ; 69	*VPS16* ; AD	New dystonia gene
3	F ; 12 ; 61	*AOPEP* ; AR	New dystonia gene
4	M ; 23 ; 31	*POLG* ; AR	Evolving patient's phenotype
Diagnostic findings through systematic genome sequencing reanalysis			
5	F ; 19 ; 27	*NUS1* ; AD	Evolving phenotypic spectrum of *NUS1*
6	F ; 5 ; 54	*NUS1* ; AD	Evolving phenotypic spectrum of *NUS1*
7	F ; 5 ; 13	*DDX3X* ; XL	Reverse phenotyping
8	F ; 49 ; 59	1.2Mb chromosomal microdeletion encompassing the *VPS16 gene*	New dystonia gene + + Bioinformatic pipeline (Systematic reanalysis of CNV/SV detected with ClinSV[5])

Table 1. Diagnostic findings in genome sequencing data reanalysis. Summary of the findings in eight dystonia patients who remained undiagnosed after the initial genome sequencing analysis in 2019, and for whom a molecular diagnosis was found through genome sequencing reanalysis in this study.
AD - Autosomal dominant ; AR - Autosomal recessive ; CNV - Copy number variants ; F - Female ; M - Male ; SV - Structual variants ; XL - X-linked ; yr. - years.

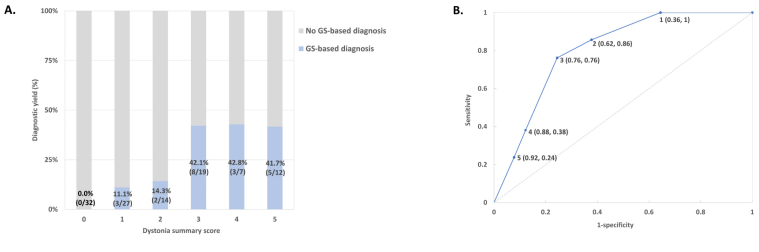

Figure 2. Association between the phenotype-based dystonia summary score and the molecular diagnostic yield.
A. Percentage of individuals with a molecular diagnosis found through genome sequencing among 111 individuals with dystonia is shown for patient subgroups according to the phenotype-based dystonia summary score.
B. Receiver operating characteristic curve plot shows the sensitivity and specificity for each score. Dystonia summary score was clacluated based on age at dystonia onset, body distribution and coexisting symptoms, as previously described.[6,7] GS - genome sequencing.

CONCLUSION

Periodic genome sequencing data reanalysis in dystonia can increase the diagnostic yield and provide patients with a genetic diagnosis, which may have significant implications for the patients and their families.

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Conflicts of interest: The authors report no conflicts of interest in relation to this work.

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Impact of Rapid Exome Sequencing on Pediatric Patients with Cardiomyopathies and Acute Heart Failure

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Introduction: Exome Sequencing (ES) is a comprehensive tool for the diagnosis of rare monogenic disorders, but scarce studies address its clinical impact on isolated pediatric cardiomyopathies (PCM) in urgent settings.

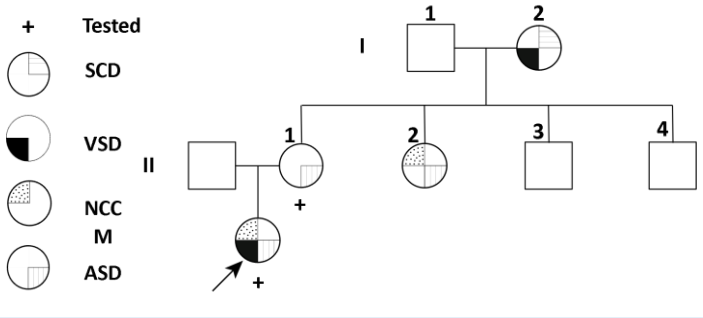
Methods: Data was collected retrospectively from medical records of pediatric patients presenting with acute heart failure and isolated cardiomyopathy who underwent singleton rapid ES between 2021 and 2023 at Rambam Medical Center.

Results: A total of nine patients were included. Eight patients (88.8%) presented in the first year of life. Age range 5days-11years, median-6 weeks. Family history was positive in only one case (11.1%). Turnaround Time was 5-14 days (median 9 days). The diagnostic yield was 5/9 (55.5%) confirming primary cardiomyopathy. The majority had dominant disorders (*ACTC1, MYBCP3, TNNI3, NKX2-5*) with two (22.2%) occurring *de novo*. One patient (11.1%) had a recessive condition (*MYBPC3)*. In Three patients (33.3%) who rapidly deteriorated during admission, a major impact on immediate medical management was imposed: palliative care in one and referral for heart transplantation in two. In most patients, the diagnosis led to avoidance of further metabolic or expert consultations, cardiac magnetic imaging or vitamin treatment. At-risk relatives in three families (33.3%) started cardiac follow-up. In five families accurate recurrence risk was provided with prenatal diagnosis and/or PGD offered.

Case	Sex	Age	Cardiac phenotype	TAT	Gene	NM	Variant	Class	Zygosity	INH	FU
1	M	6W	DCM	8 D	ACTC1	005159.4	c.664G>A, p.Ala222Thr	P	HET	DN	** transferred to HT center, deceased 4 mo.
2	F	10D	HCM	7D	MYBPC3	000256.3	c.3491-2A>C, p.?	P	HOM	NT	**palliative care, deceased 45d
3	M	1 Y	DCM	10 D	IARS2	018060.4	c.3G>T, p.Met1?	VUS	HOM	NT	** successful HT
4	F	6M	DCM	14D	CDH2	001792.5	c.970A>T, p.(Asn324Tyr)	VUS	HET	Healthy Mother	waiting HT
5	F	11M	DCM	9 D	none	-	-	-	-	-	palliative
6	M	13D	HCM+arrythmia	5D	MYBPC3	000256.3	c.1504C>T, p.Arg502Trp	P	HET	Healthy Mother	cardiac FU
					CACNA1C	000719.7	c.1252C>T,p.(Arg418Trp)	VUS	HET		
7	M	11 Y	RCM+VF	14D	TNNI3	000363.5	c.509G>A;p.Arg170Gln	P	HET	DN	ICD+cardiac FU
8	F	6W	NC+VSD+ASD	9D	NKX2-5	004387.4	c.693dup,p.Gly232Argfs*20	P	HET	Affected Mother	cardiac FU
9	M	5D	VSD, myocarditis, LAE	12D	ACTN2	001103.3	c.2082G>C,p.Lys694Asn	VUS	HET	NT	resolved

Table 1: Patients demographics and Exome results. TAT: turnaround time, FU: follow up, M- male, F- Female, W- week D- days, M- months, DCM: dilated CM, HCM: hypertrophic CM, , RCV-restrictive NC: non-compaction , LAE: left atrial enlargement, VSD- ventricular septal defect, ASD- atrial septal defect, P-pathogenic, DN- De Novo, HET-heterozygous, HOM- homozygous, VUS- variant of unknown significance, Class- classification, HT- heart transplantation, INH-Inheritance, NT- not tested, ** rapidly deteriorated during admission and a major impact on immediate medical management was imposed

Conclusions: Our results show a high clinical impact of rapid ES in isolated PCM presenting in urgent settings, including shorter time to diagnosis, high diagnostic yield, improved patient management, and therapeutic approach. In addition to family members at risk segregation and genetic counseling regarding family planning.



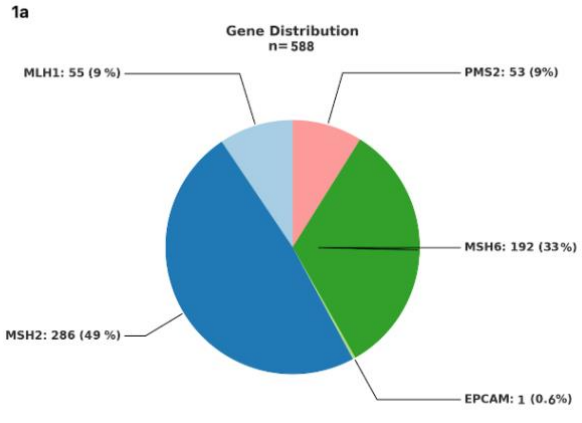
Family pedigree of **individual I#8** (arrow), presented neonatally with heart failure, non-compaction CM(NCCM), atrial and ventricular septal defects (ASD+VSD), her mother had ASD, maternal aunt had ASD+NCCM and the maternal grandmother had VSD and died at 54 years from sudden cardiac death (SCD). ES detected a pathogenic variant (c.693dup,p.Gly232Argfs*20) in the transcriptional factor NKX2-5 in the proband and her mother, other family members were not tested yet.

רמב"ם
הקריה הרפואית לבריאות האדם

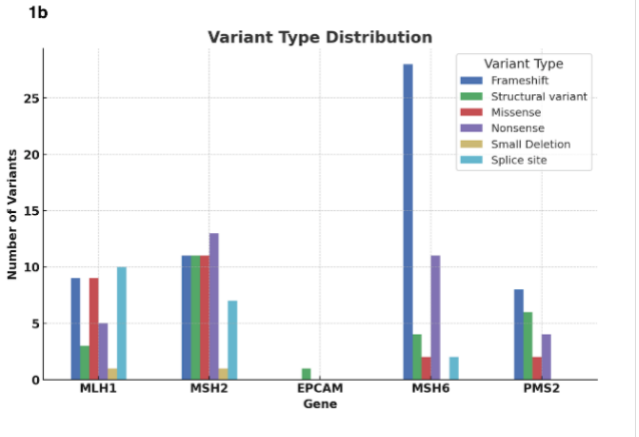
The genetic landscape of Lynch syndrome in the Israeli population

Aasem Abu Shtaya, Sofia Naftaly Nathan, Inbal Kedar, Eitan Friedman, Elizabeth Half, Gabi Lidzbarsky, Gili Reznick Levi, Ido Laish, Lior Katz, Lily Bazak, Lilach Peled Peretz, Lina Basel Salmon, Liza Douiev, Marina Lifshitc Kalis, Menachem Schechter, Michal Barzily-Rokni, Nadra Nasser Samra, Naim Abu-Freha, Ofir Hagari-Bechar, Ori Segol, Samar Mattar, Sarit Farage Barhom, Shikma Mordechai, Shiri Shkedi Rafid, Stavit A Shalev, Tamar Peretz-Yablonski, Zohar Levi, Revital Bruchim, Chana Vinkler Rinat Bernstein-Molho, Sari Lieberman, Yael Goldberg

Deciphering the spectrum and founder disease-causing variants (DCVs) in specific populations can shape and facilitate the diagnostic process of Lynch Syndrome (LS). The aim of this report was to comprehensively update on the genetic landscape of LS in the ethnically diverse Israeli-Jewish population. The cohort included 1080 carriers from 588 families; some from underrepresented, understudied Israeli ethnic groups recruited from 8 genetic institutes and high-risk clinics throughout the country.



Variant classification was performed according to the American College of Medical Genetics criteria. A total of 157 DCVs were identified, 12 are reported here for the first time, and 9 reclassified. *MSH2* DCVs were identified in 286 families (49%). Most DCVs (125/157, 80%) were noted in one or two families only. Sixteen DCVs, each detected in ≥5 families, and accounted for LS in 378/588 (64%) families. Constitutional mismatch repair deficiency (CMMRD) was diagnosed in 7 families. Twenty-five carriers (2.3%) had an additional DCV or risk alleles in another cancer susceptibility gene. In conclusion, MMR gene variant distribution in Israel is diverse. *MSH2* is most commonly mutated due to founder DCVs. Though the 16 prevalent LS-associated DCVs were frequently detected in our cohort, none of them is frequently reported in the general population. These data should facilitate variant interpretation, spouse and cascade testing.



Comparative Analysis of BRCA Pathogenic Variant Detection: Population Screening Versus Genetic Counseling in a Real-World Setting

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Importance: Israel is the first country to initiate BRCA population screening (PS) in the Ashkenazi Jewish population. PS is expected to reduce morbidity and mortality, but implementation presents unique challenges.

Objective: To examine the process and socio-psychosocial outcomes of carriers identified by PS vs. carriers identified in onco-genetics clinics following genetic counselling (GC).

Methods: All newly identified healthy BRCA1/2 carriers in 2021-2023 attending a high-risk surveillance clinic in SZMC; Data collected from medical records and self-reported questionnaires.

Results: PS group included 202 women and GC included 150. Mean age (38y,37y) and high-education level (82%) were comparable between groups. PS had a higher rate of BRCA2 (vs. BRCA1) carriers (65.1%) vs. GC (48.2%)(p=0.02). Family history motivated testing in 30.4% in PS vs. 72.7% in GC (p<0.001). PS Women were referred by: gynecologists (41.7%), breast surgeons (17.7%) and family physicians (6.2%).

Psychosocial and knowledge Assessment: by univariate analysis: Groups were similar for knowledge, STAI6 and PPC scores and both had high general satisfaction. PS reported lower satisfaction with provided information and result delivery (p=0.09,0.001). Satisfaction with health decision was high, but slightly lower among PS(p=0.014). Median IES was significantly higher PS, not achieving PTSD scores. Multivariate analysis: the mode of testing was not predictive of psychosocial outcomes.

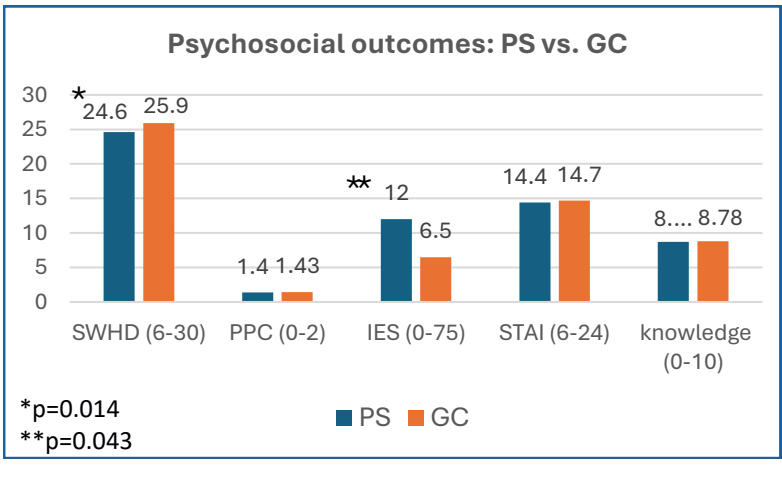
Preventative surgeries: risk-reducing salpingo-oophorectomy (RR-BSO) rates at the recommended age were similar between groups, in PS carriers: BRCA1≥40:79.2%, BRCA2≥45:81.4%.

Table 1: Characteristics and risk reduction surgery in BRCA carriers: PS vs. GC

		Population screening n=202	Genetic counseling n=150	p-value
Age at testing mean (y±SD)		38.39±10.9	36.63±11.2	0.15
Family history:	HBOC none- low	96/161 (59.6)	53/136 (38.9)	0.001
	HBOC mode-high	65/161 (40.4)	83/136 (61)	
Test results				
Founder mutation testing		198 (100)	117/149 (78.5)	0.001
BRCA gene	BRCA1	75/202 (37.1)	77/150 (51.3)	0.011
	BRCA2	127/202 (62.9)	73/150 (48.7)	
Risk reducing surgery				
RR-BSO		62/197 (31.47)	40/147 (27.2)	0.47
Age at BSO mean±SD		50.05±7.63	49.07 ±8.34	0.53
Time to RR-BSO (years) Median [IQR]		0.62 [0-1]	1 [0-1]	0.87
RR-BSO ≥40 for BRCA1		20/27 (74.1)	18/21 (85.7)	0.53
RR-BSO ≥45 for BRCA2		35/43 (81.4)	14/22 (63.5)	0.21

Table 2 Motivation for genetic testing: PS vs. GC

Reason for genetic testing n (%) (multiple choices)			
	PS	**GC**	
Cancer family history	50 (39.7)	86 (78.2)	<0.001
Known familial PV	32 (25.4)	83 (75.5)	<0.001
Physician recommendation	56 (44.4)	3 (2.7)	<0.001
Other	8 (6.3)	1 (0.9)	0.04



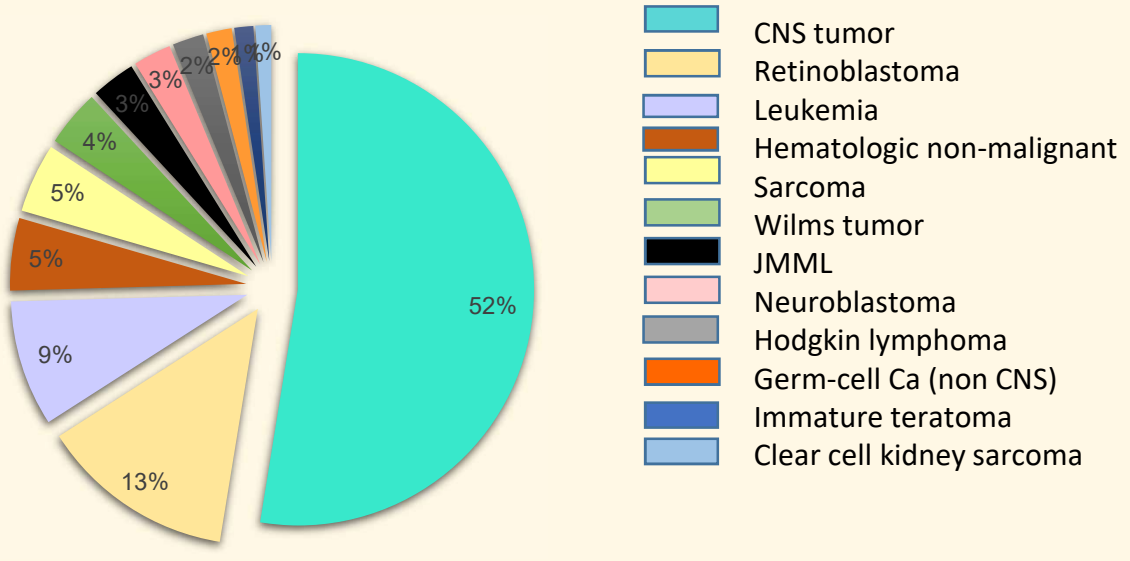
*p=0.014
**p=0.043

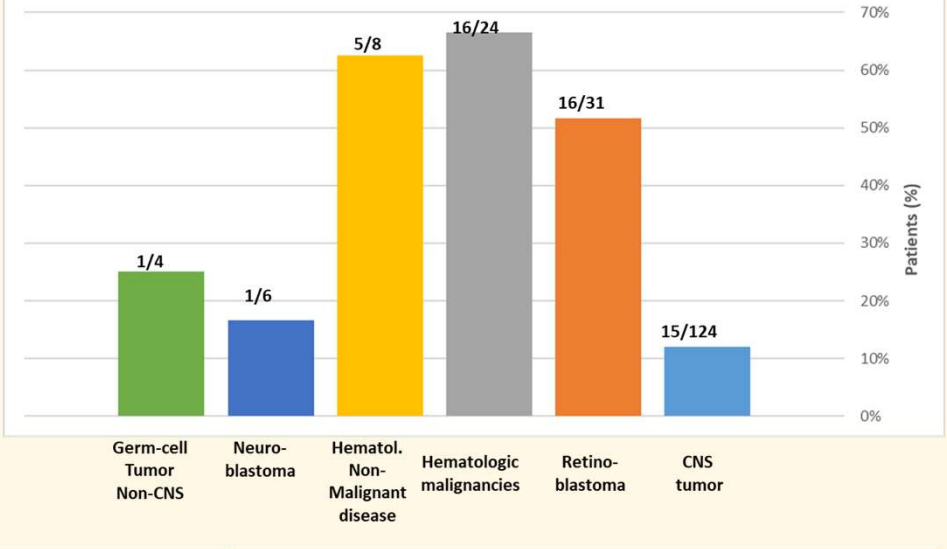
Conclusion and Relevance: "Real world" data demonstrate high satisfaction with favourable psychological outcomes in PS. PS resulted in actionable measures with high uptake of RR-BSO. The process needs refinement and long-term evaluation.

Five-year experience of genetic work-up in the Pediatric Oncogenetics joint clinic in Sheba Medical Center highlights WES as the optimal test

Miriam Regev, Michal Ylon, Shani Caspi, Gadi Abebe-Campino, Ortal Barel, Ohad Birk
Genetics Institute, Pediatric Hemato-Oncology Department and the Bioinformatics Unit, Sheba Medical Center

Oncologic diagnoses in our cohort (250 patients, 2020-2024)





Mutation frequency according to cancer subtype

Results	Diagnostic tools		
	Specific-problem designed gene panel	Expanded gene panel	Whole Exome Sequencing
Specific genetic diagnosis	12 out of 67 (18%)	4 out of 31 (13%)	37 out of 98 (37.7%)
VUS	0.85/patient	2.4/patient	0.2/patient
Incidental findings	8 out of 67 (12%)	4 out of 31 (13%)	7 out of 98 (7.1%)
Secondary findings	2 out of 67 (3%)	2 out of 31 (6.4%)	10 out of 98 (10.2%)

Genetic work-up results according to diagnostic tools

Conclusions

Our data demonstrate that in pediatric hemato-oncology, the most desirable diagnostic tool, which provides maximum diagnostic yield and minimum VUS findings, is WES. In our oncogenetics joint clinic, we achieved a significant higher genetic diagnostic yield (24.4%) relatively to the diagnostic yield mentioned in literature (up to 10%). The maximal diagnostic yield was obtained among the cases with CNS-tumors. The value of the inter-disciplinary collaboration will be pointed out.

מחלקת הכנסים
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2-3 באפריל, **2025** | מלון וורט, ים המלח

פוסטרים

Elevated soluble ACE2 in Gaucher patients - an evolutionary advantage?

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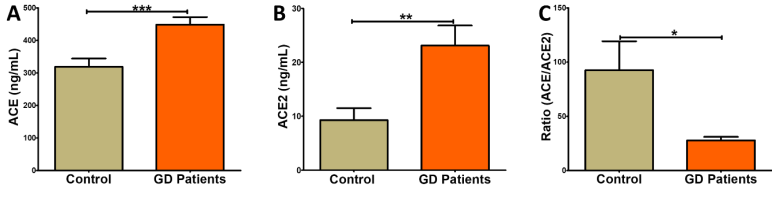
Background:

Gaucher disease (GD) has a high carrier rate among Ashkenazi Jews, although the most common disease-causing variant, N370S, is pan-ethnic. This has led to speculations of evolutionary advantage for carriers, particularly of this variant. Indeed, during the recent COVID-19 pandemic, GD patients reportedly had a surprisingly low infection rate and mild symptoms considering their disease status. As SARS-CoV-2 gains entry into the cell via membrane-bound angiotensin-converting enzyme 2 (ACE2), we speculated that differences in levels of soluble ACE2 in GD patients could contribute to this protective state. While ACE is a biomarker of GD, ACE2 levels have not been explored in GD.

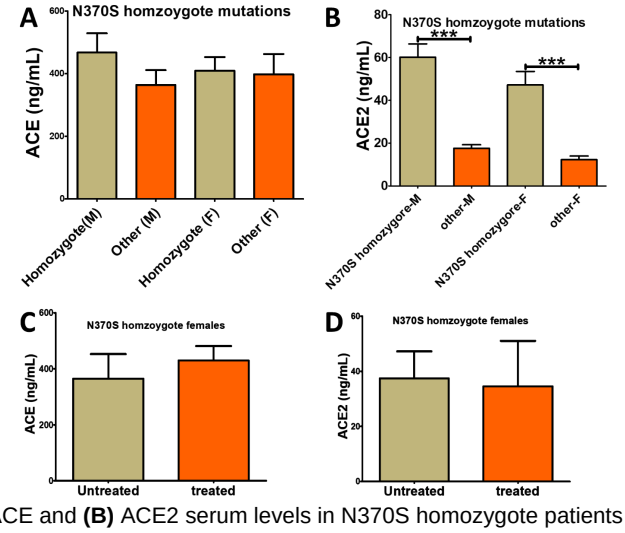
Method:

We measured serum levels of ACE and ACE2 by ELISA in 33 GD patients and 17 age- and sex-matched controls as well as macrophage bound ACE2 by western blot, in 7 GD and 7 age- and sex-matched controls.

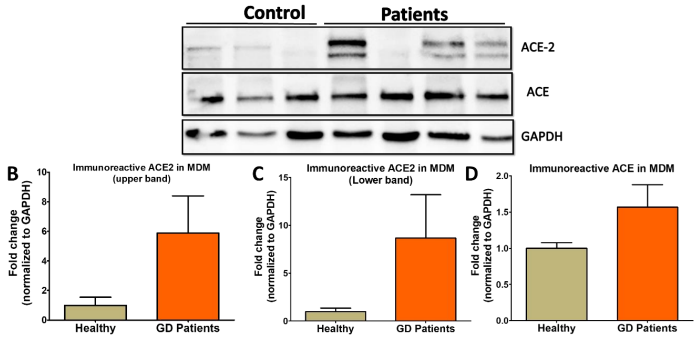


Circulating serum levels of (**A**) ACE and (**B**) ACE2 in GD patients and healthy subjects. (**C**) Calculated ratio between ACE and ACE2 serum levels in both subgroups.. *Indicates P ≤ 0.05; **, P ≤ 0.01; ***, P ≤ 0.001.

Figure 2 ..



(A) ACE and **(B)** ACE2 serum levels in N370S homozygote patients compared to other variants (compound heterozygotes). **(C)** ACE and **(D)** ACE2 circulating levels among treated and untreated N370S homozygote female patients.. ***, P ≤ 0.001.



(A) WB analysis of MDM cell lysates with antibodies against ACE2 and ACE. **(B-D**as mean±S. **)** Relative quantification of ACE2 (upper and lower bands) and ACE WB band intensities normalized to GAPDH

Results

Our results revealed a significant elevation of both serum and macrophage-bound ACE and ACE2 in GD patients compared to healthy controls. Moreover, the most robust ACE2 elevation was observed in N370S homozygotes (60ng/ml in homozygotes versus 20ng/ml in controls; P ≤ 0.001.), regardless of treatment status

Conclusions

We provide preliminary evidence for significant soluble ACE2 elevation in GD patients, particularly those with the N370S homozygous genotype. Since coronaviruses use the ACE2 receptor as a gateway for host cell entry, we speculate that elevated circulating ACE2 may serve as a decoy, giving advantage to GD patients during viral infections, of which SARS-Cov-2 serves as a contemporary example.

Familial Segregation of a 2q23.1 Deletion in the *MBD5* Gene: Reevaluation of Pathogenicity Based on Asymptomatic Carriers

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Introduction

The MBD5 gene, located on chromosome 2q23.1, encodes a protein involved in DNA methylation and chromatin remodeling. Disruption of MBD5 through heterozygous deletions or point mutations leads to MBD5-associated neurodevelopmental disorder (MAND), characterized by autosomal dominant inheritance. MAND manifests with universal intellectual disability, alongside common features such as seizures, dysmorphic traits (short stature, microcephaly), sleep disturbances, ataxia, aggressive behavior, and hyperactivity. MBD5 deletions are relatively rare, detected in approximately 0.05% of 17,477 individuals undergoing clinical microarray analysis. The majority of these cases are de novo, although rare instances of inheritance from mildly affected or mosaic parents have been described

Methods and results

We report a family that underwent prenatal diagnosis without a specific medical indication. Chromosomal microarray analysis (CMA) revealed a 172 kb deletion at the 2q23.1 locus encompassing exons 3 and 4 of MBD5 (Fig.2). Due to the potential clinical risk, the pregnancy was terminated. Subsequent parental testing identified paternal inheritance of the deletion. Family segregation analysis, including ten paternal relatives, revealed two additional asymptomatic carriers (the paternal grandfather and a brother) who harbor the same deletion (Fig1).

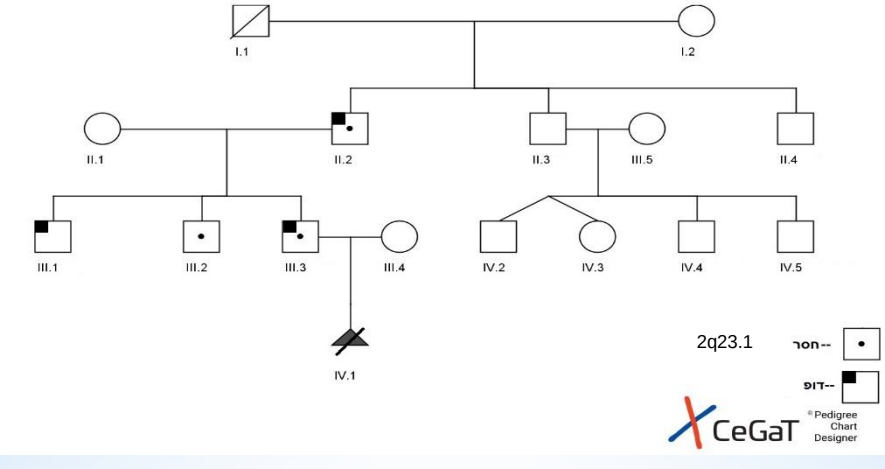

Fig.1.Family Pedigree

Conclusion

Segregation analysis identified three unaffected individuals carrying the 2q23.1 deletion. This observation suggests that, within this family, the deletion may not result in the severe phenotypic manifestations typically associated with MBD5 haploinsufficiency. However, a pathogenic effect cannot be definitively excluded, particularly in offspring. In light of these findings, the variant classification was re-evaluated and revised from 'Likely Pathogenic' (LP) to 'Variant of Uncertain Significance' (VOUS). This case highlights the importance of familial segregation studies in refining variant interpretation and underscores the challenges of classifying copy number variations in the context of variable expressivity and incomplete penetrance.

Fig.2. CMA analysis: arr[hg19] 2q23.1(148,938,765-149,110,309)x1

Identifying novel homologous recombination genes in ovarian cancer patients

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Introduction

DNA stability is maintained by an intact homologous recombination repair (HRR) mechanism which participates in DNA double strand breaks repair. Mutations in genes crucial for HRR function such as *BRCA1/2* disrupt DNA damage response, resulting in HRR deficiency. Carriers of a pathogenic variation in HRR genes have an increased risk for ovarian cancer. Mutational signature 3 is an indication for HRR deficient tumors and is found also in *BRCA1/2* wild type ovarian tumors, suggesting pathogenic variations or mutations in other HRR genes leading to ovarian cancer development. In this research we aim to identify candidate HRR genes that were mutated in ovarian cancer blood and tumor samples and to assess their mutational signature pattern and HRR deficiency state.

Methods

20 ovarian cancer patients with no known *BRCA1/2* mutation in the tumor consented to participate in this study (Fig. 1.A). Exome sequencing of DNA from blood and tumors samples was performed using Illumina NOVASEQ sequencer (Fig. 1.B). To identify pathogenic variation in *BRCA1/2* and HRR genes we used GATK HaplotypeCaller and mutect2 variant calling pipelines, Ensemble variant effect predictor – VEP (Fig. 1.C) and Franklin ACGM classification tools which allowed us to determine the germinal and the somatic variants' pathogenic level (Fig. 1.D). To state the tumors' mutational signature pattern, we will use COSMIC SigProfile packages. We run the variant calling tools on Hadassah patients' tumor samples which have been molecularly diagnosed by the pathology department. We ensured that the mutations found in the molecular diagnosis report appear in the variant calling results and by that we validated our pipeline quality and accuracy. To ensure the existence of the variants we found in the variant calling assay in our samples' DNA sequence, we used IGV and we aim to conduct PCR. We will use the Hadassah exome bank to identify other patients with a predisposition to be homologous recombination deficient and therefore developing ovarian cancer.

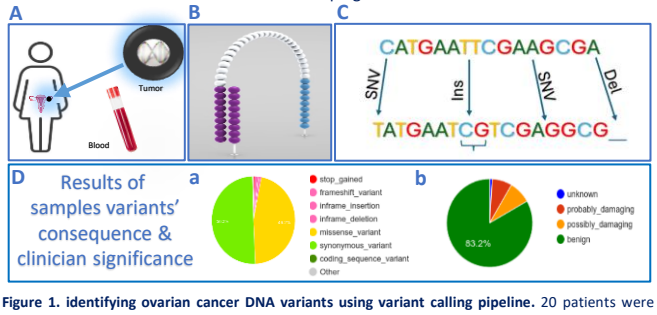


Figure 1. identifying ovarian cancer DNA variants using variant calling pipeline. 20 patients were diagnosed with ovarian cancer. **A:** DNA was extracted from patients' blood and formalin fixed tumor tissue. **B:** Exome sequencing was conducted on germinal and somatic DNA using Illumina NOVASEQ. **C:** Variant calling process was performed using GATK tools. Raw fastq files were aligned to Gcrh37/hg19 reference genome. DNA sequence was pre-processed and nucleotide quality scores were recalibrated. Using mutect2 pipeline, we detected all DNA single nucleotide variations and indels. **D:** A result of variants mapping of one ovarian cancer tumor sample. We used VEP to annotate the DNA variants of all 20 patients and to clarify their classification. We received the variants' consequence (a), pathogenicity prediction and clinical significance (a). We used Franklin database to distinguish between germinal and somatic variant annotations.

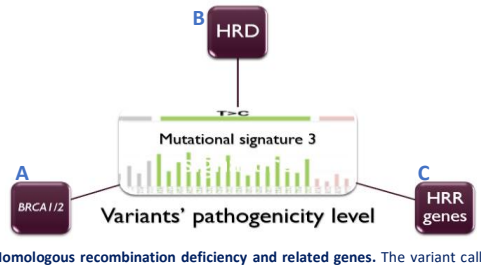


Figure 2. Homologous recombination deficiency and related genes. The variant calling results were analyzed. We filtered the germinal and somatic variants using Linux commands by their quality, coverage and classification. We regarded only: variants in sites having at least one allele that passes quality filters, reliable variants with coverage >10x read depth and automatically discarded intron and synonymous variants. **A:** We filtered *BRCA1/2* variants to determine their pathogenicity and discarded samples with pathogenic mutation in *BRCA1/2* genes. **B:** Mutational signature analysis will be conducted on somatic tumor samples by COSMIC Sigprofile tools, aiming to identify HRR deficient tumors with mutational signature 3 and removing samples with no indication for HRD. **C:** We filtered germinal and somatic variants among a list of known 115 genes[1] that are evolutionary associated with HRR pathway and determined their pathogenicity level using VEP and Franklin (Figure1.D).

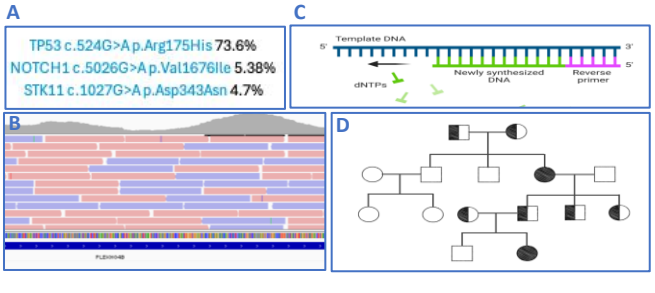


Figure 3. Method and results validation. A: We run the variant calling pipeline on cancer samples which exist in the Hadassah archive and have been molecularly diagnosed by the pathology department that determined which known variants are expressed in their tumors. **B:** We viewed the relevant variants through IGV to ensure their coverage is high as predicted. **C:** PCR will be conducted to validate the existence of the DNA variants in the DNA sequence. **D:** We aim to search the Hadassah exome bank for patients' relatives with pathogenic variations in the same candidate genes.

Results

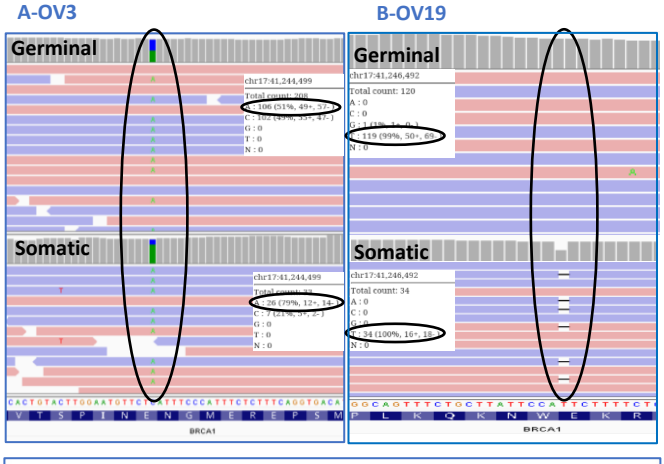
Variant calling scripts ran successfully after a technical adaptation to the Hebrew University of Jerusalem cluster computational system settings. Most of the reads had a high quality, however they had a very low coverage (~2x), as normally suits to background noise. High quality reads having also high coverage were considered as reliable variants.

Method validation: 19 out of 23 mutations that are reported in the molecular diagnosis summary as >20% of the total read count were efficiently identified using mutect2. They were detected in the variant calling results and appeared in IGV. Four mutations were not found in the variant calling results. Two of them are indicated in the molecular report as ~5% of the total allele count and the other two are reported as >20%.

BRCAness: Two patients are *BRCA1/2* carriers, one with a germline and one with a somatic mutation that also appeared in IGV. 18 ovarian cancer patients are *BRCA1/2* wild type.

Homologous recombination deficiency: Mutational signature analysis will be conducted.

Homologous recombination genes: Our initial findings include germinal and somatic pathogenic variations in HRR genes as described in table 1.



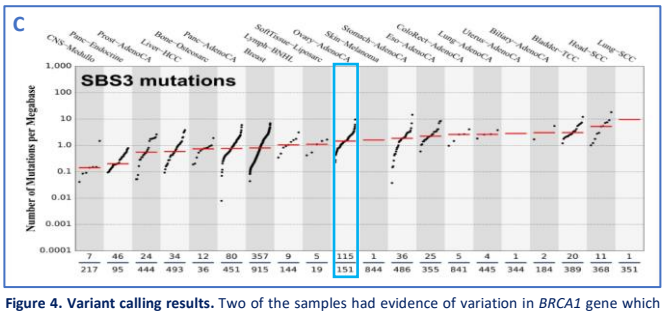


Figure 4. Variant calling results. Two of the samples had evidence of variation in *BRCA1* gene which caused a nonsense mutation of BRCA1 protein. Both samples were excluded from the cohort. **A:** Sample OV3 was found with germinal and somatic missense variation (G>T) in *BRCA1*. **B:** Sample OV19 was found with only somatic deletion variation (AT>A) in *BRCA1* involving 21 deletions out of 34 total reads which results in a decreased coverage of reference allele. **C:** An example of single base substitution 3 that is a mutational signature pattern that highly relates to ovarian cancer with HRR deficiency which we aim to identify in the HRR deficient tumor samples.

Sample	Gene	Variation type	
OV25	*FANCD2*	Coding sequence variant, c.1278+1del	Germinal variants
OV25, OV28, OV17	*RECQL4*	Splice donor variant, c.2296+1del	
OV17	*TTN*	Stop gained, termination	Somatic variants
OV17	*CHERP*	Stop gained, termination	
OV8, OV20	*TTN*	Frameshift, termination	
OV8	*TTN*	Stop gained, deletion insertion	
OV8	*POLE*	Stop gained, frameshift	

Table 1. HRR variant calling initial results. We meanwhile identified germinal pathogenic coding sequence and splice donor variants in the genes *FANCD2* in 2 of the samples and *RECQL4* in 3 of the samples, both caused by an indel that is in distance of one nucleotide after the end of an exon. We also found somatic pathogenic variations in genes *TTN*, *CHERP* and *POLE*. One sample had two stop gained mutations in *TTN* and *CHERP* genes. One sample had a del-ins and stop gained mutation and a frameshift mutation causing to a terminated protein in *TTN* gene, together with a stop gained mutation in *POLE* gene. One sample had an insertion mutation leading to a terminated protein in *TTN* gene.

Conclusion

FANCD2, RECQL4, TTN, CHERP and *POLE* are candidate genes which participate in the HRR pathway and might cause a malignant transformation of ovarian cancer patients. Further research is required to assess the mutated genes' molecular involvement in HRR deficiency and ovarian cancer. identifying these genes will allow a better understanding of HRR mechanism and a wider landscape of ovarian cancer patients who can be treated by PARP inhibitors. It will also improve medical preventative approaches available for the patients and the public and will enrich new personalized and targeted therapies' development.

Reference

1) Mapping global and local coevolution across 600 species to identify novel homologous recombination
repair genes (2019) Dana Sherill-Rofe. *Genome Research.*

2) COSMIC: a curated database of somatic variants and clinical data for cancer (2024) Zbyslaw Sondka. *Nucleic Acids Research 52:D1210–D1217.*

Mitral Valve Prolapse Caused by *TLL1* Gain-of-Function

Nadav Agam[a], Vadim Dolgin[a], Artyom Star[b], Ofek Freund[a], Matan M. Jean[a], Amit Safran[a], Tomer Poleg[a], Doron Zahger[b], and Ohad S. Birk[a, c, d]

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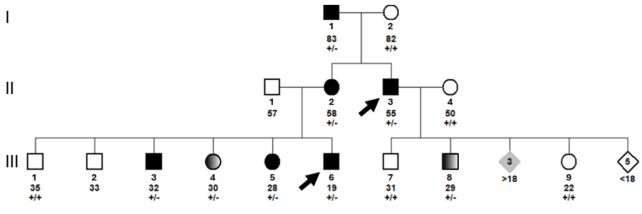
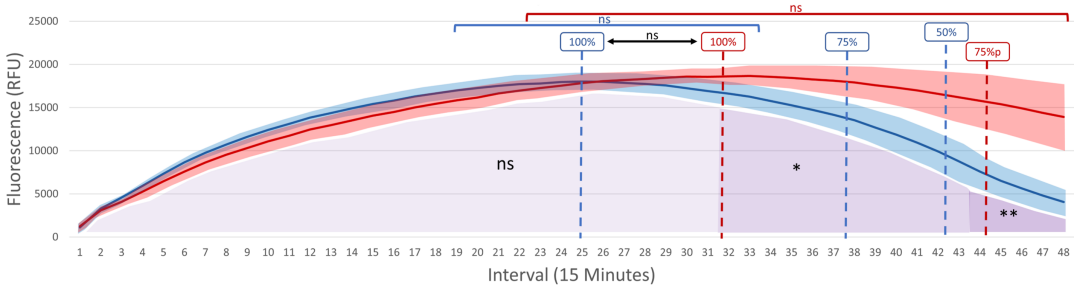
INTRODUCTION & METHODS

Mitral valve prolapse is a common cardiac valvular anomaly that can be caused by mutations in genes of various biological pathways. Individuals of three generations of a kindred presented with apparently dominant heredity of isolated MVP. Whole exome and genome sequencing data of two affected individuals were analyzed, delineating shared variants. TLL1 enzymatic activity was assayed in media of HEK293 cells transfected with wild-type versus mutant TLL1.



RESULTS

The only heterozygous variant segregating in the affected kindred as expected for dominant heredity of MVP was p.T253A, within the catalytic domain of TLL1. Of eight heterozygotes, six had MVP and two had trivial mitral regurgitation. Activity assay in extra-cellular media of HEK293-transfected cells showed that over time (12 hours), the enzymatic activity of the mutated TLL1 protein was X3.4 higher than that of the wild type.



DISCUSSION

Our genetic and biochemical studies show that a TLL1 Gain-of-Function mutation, prolonging the half-life of TLL1 active protein in the extracellular matrix (ECM), causes autosomal dominant MVP with variable expressivity. TLL1 encodes an extracellular metalloprotease regulating ECM composition and maintenance. Heterozygous Loss-of-Function TLL1 mutations have been previously shown to cause autosomal dominant atrial septal defects. Our findings enable novel insights into molecular pathways of valvular physiology and disease, the role of TLL1 in human development, and the differing phenotypes of Loss-of-Function and Gain-of-Function mutations of the same gene.

SEE PAPER

Identifying and characterizing the germinal genetic landscape of men with prostate cancer: a real-life multi-centered retrospective study

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Introduction: Epidemiological studies indicate that 5% to 10% of all prostate cancer (PCa) cases, and up to 40% of cases that occur earlier in life, are linked to dominantly inherited susceptibility genes with high penetrance.

Objectives: Characterizes the genetic landscape among a cohort of PCa patients in Israel and identifies demographic and clinical factors that influence the prevalence of disease-causing gene changes and increase the risk of malignant disease.

Methods: The study enrolled all men who were referred to genetic counseling due to prostate cancer in the genetic institutions of Beilinson Hospital and Assuta (Ashdod) Hospital. All patients were offered germline genetic testing using either the Israeli founder mutation panel or a multi-gene next-generation sequencing (NGS) commercial panel. Patients' demographic and clinical information were retrieved from medical records and computerized patient management software.

Results: 293 PCa patients were tested, 190 (64.8% of the tested cohort) were tested for the presence of known germline mutations, and the rest (n=103, 35.2%) underwent germline genetic testing using NGS-based gene panels. In 13.3% of patients, a genetic positive finding was reported: 18 (6.1% of the tested cohort) had a high penetrance pathogenic or likely pathogenic positive result, 21 (7.2%) had a result attributed to an increased risk allele. 50% of the patients diagnosed with pathogenic or likely pathogenic variants in high-penetrance genes using NGS panels had a founder mutation, while the other half (50%) consisted of unique variants.

Table 1: Demographic and clinical characteristics of participants categorized by ethnicity

		Ethnicity		
Variables	All (n=455)	Ashkenazi (n=232)	None Ashkenazi (n=223)	p
Age, years (M±SD)	67.8±8.6	68.4±8.3	67.1±18.9	0.110
Prostate cancer in the family (n, %)	124, 27.3	59, 25.4	65, 29.1	0.373
Any cancer in the family (n, %)	13, 2.9	9, 3.9	4, 1.8	0.182
Metastatic (n, %)	93, 20.4	40, 17.2	53, 23.8	0.084
Gleason score (Med, IQR)	8, 7-9	8, 7-9	8, 7-9	0.174
Gleason score (n, %)				
< 8	176, 40.6	95, 42.4	81, 38.6	0.416
≥ 8	258, 59.4	129, 57.6	129, 61.4	
Type of examination (n, %)				
Sequencing	103, 35.2	54, 32.7	49, 38.3	0.323
Founder mutations	190, 64.8	111, 37.3	79, 61.7	

Table 2: Genetic test results according to ethnicity, Gleason score and presence of cancer in the family

Variables	Negative or VOUS (n=254)	P / LP High penetrant (n=18)	Low penetrant (n=21)	p
Ethnicity (n, %)				
Ashkenazi	140, 55.1	10, 55.6	15, 71.4	0.350
Non-Ashkenazi	114, 44.9	8, 44.4	6, 28.6	
Gleason score (n, %) *				
< 8	103, 42.2	4, 26.7	6, 33.3	0.395
≥ 8	141, 57.8	11, 73.3	12, 66.7	
Prostate cancer in the family (n, %)	74, 29.1	5, 27.8	7, 33.3	0.910
Any cancer in the family (n, %)	8, 3.1	4, 22.2	0, 0	0.003

* Missing data of 16 patients

Table 3: Multivariate logistic regression model for the correlation between the genetic results of P+PL/Low and Ashkenazi ethnicity, cancer in the family with adjustment for age

Exposure variable	Type of variable and categories	OR	95% CI	p
Ethnicity	Binary - non vs. Ashkenazi	0.70	0.33-1.45	0.335
Any cancer in the family	Binary - Yes vs. No	3.81	1.05-13.77	0.041
Age	Continuous - years	0.99	0.94-1.03	0.490

OR - odds ratios, CI - confidence interval

Conclusions:

- Prostate cancer (PCa) patients in our cohort demonstrate 6.1% of pathogenic or likely pathogenic (P/LP) variants in high penetrance genes.
- The occurrence of P/LP variants is not affected by ethnicity, age, or disease progression.
- A significant number of these variants are unique to individual patients.
- Although further extensive studies are necessary, our findings highlight the importance of conducting genetic investigations in prostate cancer patients, irrespective of their ethnicity or the stage of the disease.

A variant in SPOUT1 is causing epileptic encephalopathy

Aviv Mesika[1,2*], Limor Kalfon[1*], Golan Nadav[1*], Adi Litmanovich[2], Efrat Shuster Biton[1], Hagai Ledany[2], David Savitzki[3], Bruria Ben-Zeev[4], Nehama Cohen Kfir[1], Rami Abou Jamra[5,6], Moshe Dessau[2], Tzipora C. Falik-Zaccai[1,2]

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Background:

SPOUT1, a member of the SPOUT family of methyltransferases, contains a catalytic domain crucial for RNA methylation. This study investigates the role of bi-allelic missense variants in SPOUT1 and their association with refractory seizures and severe global developmental delay in affected infants.

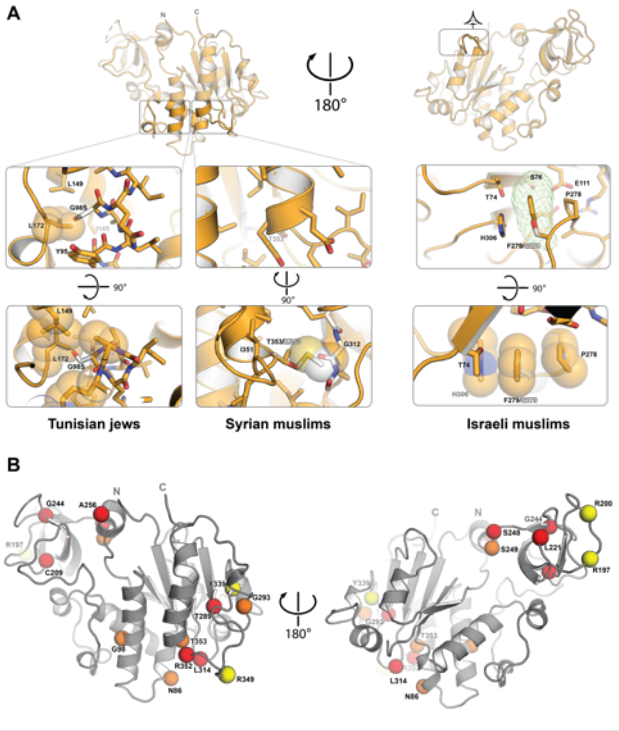
Results:

WGS identified three homozygous missense variants in SPOUT1 in three families: c.292G>A (p.Gly98Ser), c.1058C>T (p.Thr353Met), and c.836T>C (p.Phe279Ser). Structural modeling predicted that these variants destabilize the SPOUT1 protein, potentially leading to its degradation. Immunoblotting revealed significantly reduced SPOUT1 protein levels in fibroblasts carrying the G98S variant compared to controls. Immunofluorescence showed abnormal nuclear speckle organization in patient fibroblasts, likely contributing to the observed neurological symptoms.

Genetic and Clinical manifestation of SPOUT1 related disease

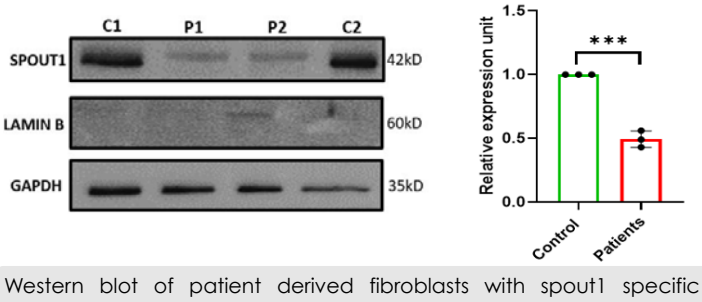
(A) Pedigree of the Tunisian Jewish family **(B)** Sanger sequencing of the c.292G>A; p.Gly98Ser variant **(C)** Clinical presentation of IV-5 patient

Structural analysis of the SPOUT1 variants



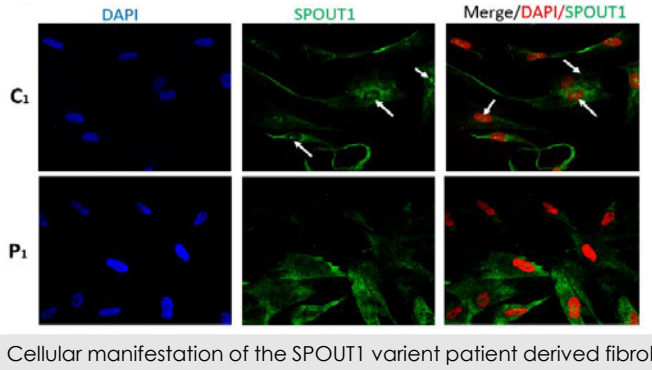
(A) A ribbon representation of the crystal structure of SPOUT1 protein (PDB ID: 4RG1), and the mutation's sites. In the magnification boxes is a stich representation of the relevant residues. Spheres represents VDW radius. **(B)** The distribution of the known mutations (including the ones from the literature) on the SPOUT1 structure.

SPOUT1 variants show low levels of spout1 protein



Western blot of patient derived fibroblasts with spout1 specific antibody **(A)** and spout1 protein level quantification **(B)**

SPOUT1 patient cells show unique nuclear speckle phenotype



Cellular manifestation of the SPOUT1 varient patient derived fibroblast. Abnormality nuclear speckles organization in SPOUT1 deficiency patients. White arroes indicate the cellular speckles

Conclusions:

This study links SPOUT1 deficiency to neurodevelopmental disorders with refractory seizures and severe developmental delay. It underscores SPOUT1's role in nuclear speckle organization and RNA processing, offering insights into epilepsy's molecular mechanisms. Adding SPOUT1 to the global developmental delay and seizure gene panel could aid in diagnosing affected infants.

The first nation-wide population screening program for BRCA pathogenic variants: qualitative analysis of the real-world experience

Rachel Michaelson-Cohen[1,2,3], Ephrat Levy-Lahad[1,2], Naama Srebnik[2,3], Ariela Tomer, Shunit Armon[2,3], Sari Lieberman[1,21]

1. Fuld Family Institute of Medical Genetics, Shaare Zedek Medical Center, Jerusalem, Israel. 2. Faculty of Medicine, Hebrew University of Jerusalem, Jerusalem, Israel. 3. Department of Obstetrics & Gynecology, Shaare Zedek Medical Center 4. BRCA High -Risk Surveillance Clinic, Shaare Zedek Medical Center, Jerusalem, Israel

Objective: The high BRCA1/BRCA2 pathogenic variant (PV) frequency in Ashkenazi-Jews, and observation that 50% of carriers have no family history [FH] of cancer, led to implementation of population screening (PS) in Israel, as the first national PS worldwide. We evaluated the PS process and its psychosocial outcomes in a real-world setting, comparing carriers identified by PS vs. genetic counseling (GC), using in-depth qualitative analysis.

Methods: Carriers identified through PS vs. GC attending the high-risk surveillance clinic (HRSC) in Shaare Zedek from 2021-2023 were interviewed (see **figure**). Transcripts were analyzed verbatim for recurring themes using an inductive-grounded theory approach.

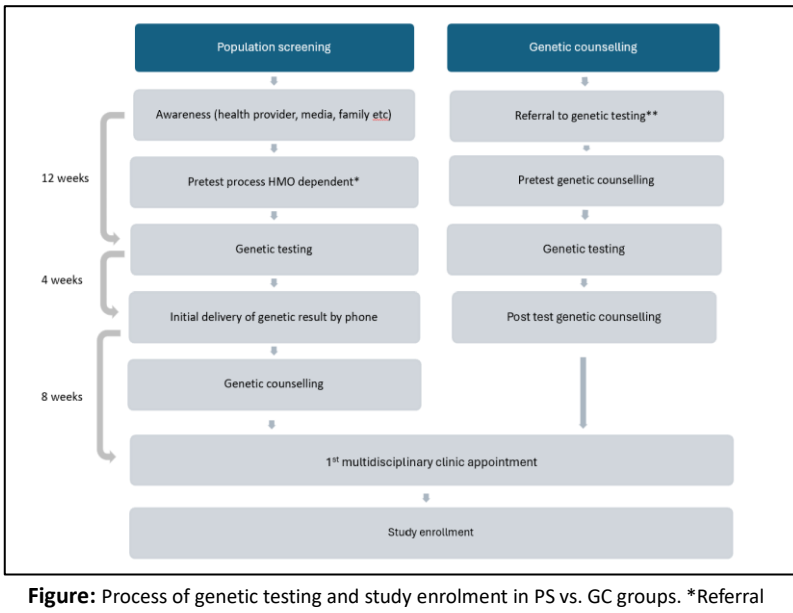


Figure: Process of genetic testing and study enrolment in PS vs. GC groups. *Referral differs by HMO- including referral by general practitioner, written information or information by dedicated nurse in community-based clinics. ** Referral can be made only by an oncologist physician or genetic counselor.

	PS	GC	p value
ge mean (y±SD)	38.9 (8.2)	42.1 (7.2)	NS
months testing till interview (m±SD)	13.8 (4.6)	17 (4.7)	NS
college education N(%)	20/21 (95.2)	11/11 (100)	NS
in partnership N(%)	20 (95.2)	11 (91.7)	NS
number children (m±SD)	3.1 (3.3)	3.2 (1.7)	NS
Mod-High FH risk N(%)	8 (40)	8 (66.7)	NS
low FH risk N(%)	12 (60)	4 (33.3)	
familial BRCA PV N(%)	5 (23.8)	11 (91.7)	<0.0005
BRCA1	7 (30)	4 (30)	NS
BRCA2	14 (70)	8 (70)	

Table: Sociodemographic characteristics of interview participants: PS vs. GC carriers. Chi-square, Exact tests used to calculate differences

Results: Mean testing ages were 38.9(SD 8.2) and 42.1(SD 7.1), college education 95.2% and 100%, and significant cancer FH 40% and 67%, for 21 PS vs. 12 GC carriers, respectively (see **table**).

The main drive for testing was "knowing cancer risk". "Being responsible" motivated carriers with familial PVs. Reactions to results included fear of cancer and sadness, yet most were satisfied with testing.

Themes unique to PS carriers were testing because it was "on a checklist", and initial shock receiving results. Carriers suggested improving awareness, pre-test information, and result delivery, which are currently provided in unscheduled phone calls. Some suggested result disclosure by treating physician or pre-scheduled calls, enabling preparedness. They preferred attending HRSCs rather than community-based clinics.

Conclusion and Relevance: Initial shock was described exclusively by PS-identified carriers, in addition to fear, guilt and sadness which also characterize GC carriers. Nonetheless, the process was viewed favourably. Outcomes from this first nation-wide BRCA-PS have importance for programs elsewhere.

Bnai Zion Medical Center

High-Level Trisomy 8 Mosaicism in a Healthy Adult: A Rare Case with No Clinical Manifestations

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Introduction

Constitutional trisomy 8 mosaicism syndrome (T8MS), also known as Warkany syndrome, is a rare chromosomal disorder occurring in approximately 1 in 25,000–50,000 live births. It accounts for about 0.7% of spontaneous abortions and affects approximately 0.1% of recognized pregnancies. T8MS exhibits a broad phenotypic spectrum, with no established correlation between the degree of mosaicism and clinical severity. Typical features include intellectual disability, dysmorphic facial features, deep palmar and plantar creases, cardiac and renal anomalies, spinal deformities, and agenesis of the corpus callosum. In addition, T8MS has been associated with a risk of hematologic malignancies, including Wilms tumor, myelodysplasia, and myeloid leukemia. However, some individuals may present with normal cognitive function and no clinical signs.

Methods and results

A 60-year-old woman underwent genetic evaluation following the identification of a pathogenic deletion in her son. Chromosomal microarray (CMA) analysis confirmed that she carried the same deletion, establishing inheritance. Additionally, mosaic trisomy 8 was detected in 50–70% of cells arr[GRCh37] 8p23.3q24.3(158,049_146,295,771)x3 (Fig.1). Karyotype analysis revealed trisomy 8 in all examined cells (Fig.2), while fluorescence in situ hybridization (FISH) detected trisomy 8 in 49% of cells (Fig.3). A subsequent bone marrow examination confirmed complete trisomy 8 in karyotype analysis, with FISH showing trisomy 8 in 56% of cells. Despite the high proportion of trisomic cells, the patient exhibited no physical, cognitive, or hematologic abnormalities.

Fig.1. CMA analysis: arr[GRCh37] 8p23.3q24.3(158,049_146,295,771)x3

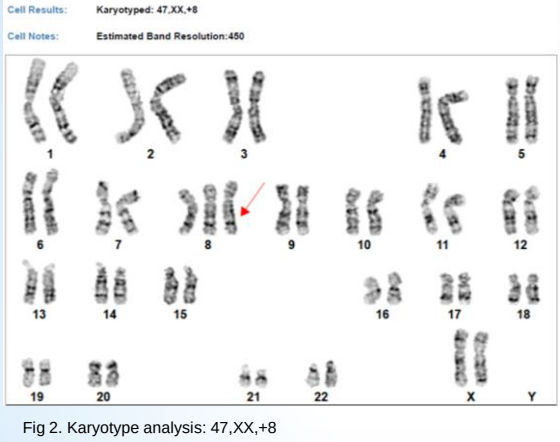

Fig 2. Karyotype analysis: 47,XX,+8

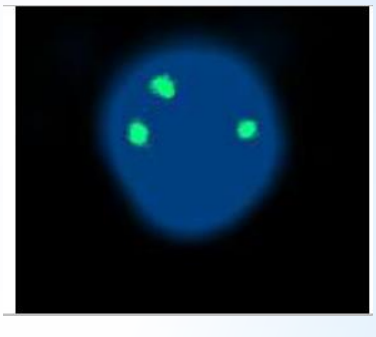

Fig 3. FISH analysis: nuc ish (D8Z1X3)

Conclusion

We report a rare instance of a healthy adult woman with a high percentage of trisomy 8 cells, illustrating that T8MS can be compatible with normal development and health. This case highlights the potential for high-level mosaic trisomy 8 to be clinically silent, raising considerations regarding its impact on genetic counseling and clinical management.

Exploring the role of SMCHD1 during early-stage development

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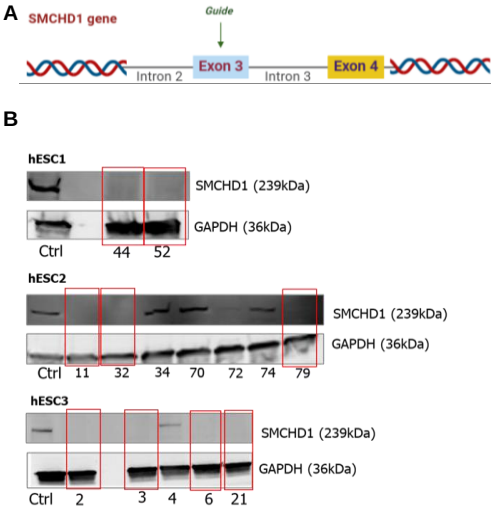
INTRODUCTION

SMCHD1 (Structural Maintenance Of Chromosomes Flexible Hinge Domain Containing 1) is a chromatin modifier implicated in epigenetic silencing and de novo DNA methylation during early embryonic development. It also mediates long-range chromatin interactions, potentially by antagonizing CTCF binding. Loss-of-function (LOF) mutations in SMCHD1, in the context of a permissive allele (4qA161) and partial shortening of the D4Z4 macrosatellite repeats (10-20 repeats) underlie facioscapulohumeral muscular dystrophy type 2 (FSHD2)—a late-onset, progressive neuromuscular disorder caused by heterochromatin relaxation at the D4Z4 repeats (4q35). How SMCHD1 leads to epigenetic silencing remains unknown.

AIM

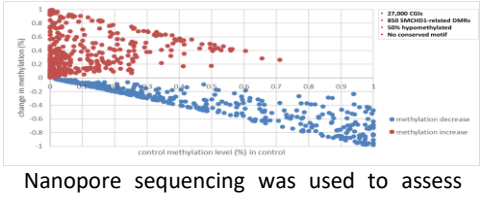
The aim of this research is to uncover the mechanism by which SMCHD1 haplo-insufficiency results in chromatin relaxation in the genome.

KNOCKING OUT SMCHD1 IN HUMAN EMBRYONIC STEM CELLS (hESCs)



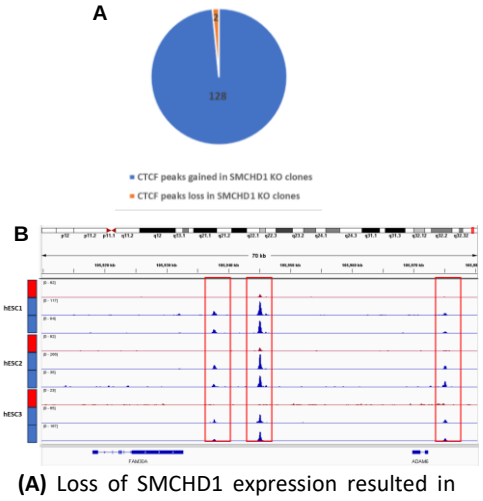
(A) CRISPR/Cas9 was used to target the exon 3 of SMCHD1 in three different human embryonic stem cell (hESCs) lines. A single guide RNA (gRNA) facilitated precise gene editing. **(B)** Western blot analysis confirmed efficient SMCHD1 targeting in all modified hESC lines. For each cell line, 2–4 knockout (KO) clones were successfully established, marked by red squares.

SMCHD1 KNOCK OUT HAS A LIMITED EFFECT ON CpG ISLAND DNA METHYLATION IN hESCs



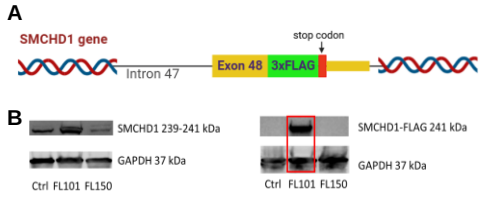
Nanopore sequencing was used to assess DNA methylation differences between control and SMCHD1 knockout (KO) hESCs. Differentially methylated regions (DMRs) within CpG islands are shown, with increased methylation represented by red dots and decreased methylation by blue dots. Among the 27,000 CpG islands identified, 850 exhibited changes in methylation, with a comparable number of hypermethylated and hypomethylated regions.

SMCHD1 ANTGONIZES CTCF BINDING IN hESCs



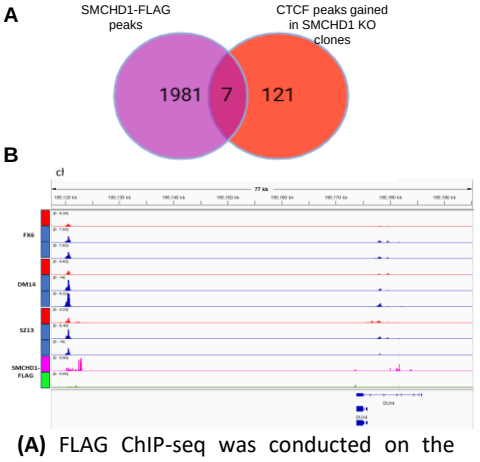
(A) Loss of SMCHD1 expression resulted in the formation of 128 new binding sites for CTCF, while only 2 were lost. **(B)** Representative **e**xample of a region with ectopic CTCF binding, as a result of SMCHD1 KO in the 3 hESCs lines (red- control, blue- SMCHD1 KO clones).

SMCHD1-3XFLAG KNOCK IN IN hESCs



(A) For creating hESCs expressing a 3xFLAG-tagged fused SMCHD1 protein, we have used CRISPR/Cas9-mediated homology-directed repair (HDR) with a donor oligonucleotide to efficiently tag the C-terminal of SMCHD1. **(B)** Western blot analysis with anti-SMCHD1/FLAG validated the expression of an SMCHD1 FLAG-tagged protein in one of the hESCs manipulated clones (FL101).

SMCHD1 AND CTCF BINDING SITES DO NOT OVERLAP IN hESCs



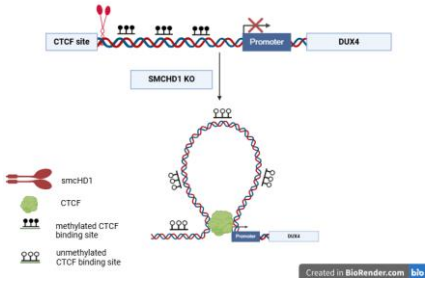
(A) FLAG ChIP-seq was conducted on the SMCHD1-FLAG expressing hESC clone (FL-101). Of the 1988 significant SMCHD1-FLAG binding sites identified, only 7 were in proximity to ectopic CTCF binding in the SMCHD KO clones. Two of those were within or nearby the D4Z4 region. **(B)** CTCF ChIP-seq of all lines an SMCHD1 KO clones (control-red, KO-blue) and SMCHD1-FLAG ChIP-seq in FL-101 (in pink, input-green), at the D4Z4 region. Notably, in SMCHD1 KO cells, there is consistent ectopic binding of CTCF 53 kb upstream of the DUX4 promoter. Additionally, 1.5 kb downstream of the DUX4 promoter, CTCF is differentially bound in all the SMCHD1 KO hESC clones.

CONCLUSIONS

Our study demonstrates that SMCHD1 knockout (KO) in hESCs:

- Modifies DNA methylation at a limited set of CpG islands without a consistent trend.
- Antagonizes CTCF binding at discrete sites in the genome of hESCs, including upstream to the D4Z4 repeats.
- SMCHD1 and CTCF binding sites do not overlap.

HYPOTHESIS



Given that SMCHD1 normally binds upstream of the D4Z4 region in undifferentiated hESCs, and that its absence (SMCHD1 KOs) results in ectopic CTCF binding, we hypothesize that repeat contraction to fewer than 20 units, as seen in FSHD2, similarly induces ectopic CTCF binding, leading to de-methylation and de-repression of the otherwise epigenetically silenced DUX4 retro-element.

Diagnostic Yield and Recognized Barriers of a Pediatric Multidisciplinary Neurogenetics Clinic

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Introduction

Recent advances in molecular genetics have revolutionized the diagnosis of neurogenetic disorders, yet the diagnostic journey for many pediatric patients remains prolonged and complex.

Our objective was to evaluate the clinical and diagnostic outcomes of a multidisciplinary pediatric neurogenetics clinic, emphasizing the role of advanced genetic testing and collaborative care and identifying potential barriers for reaching the genetic diagnosis.

Methods

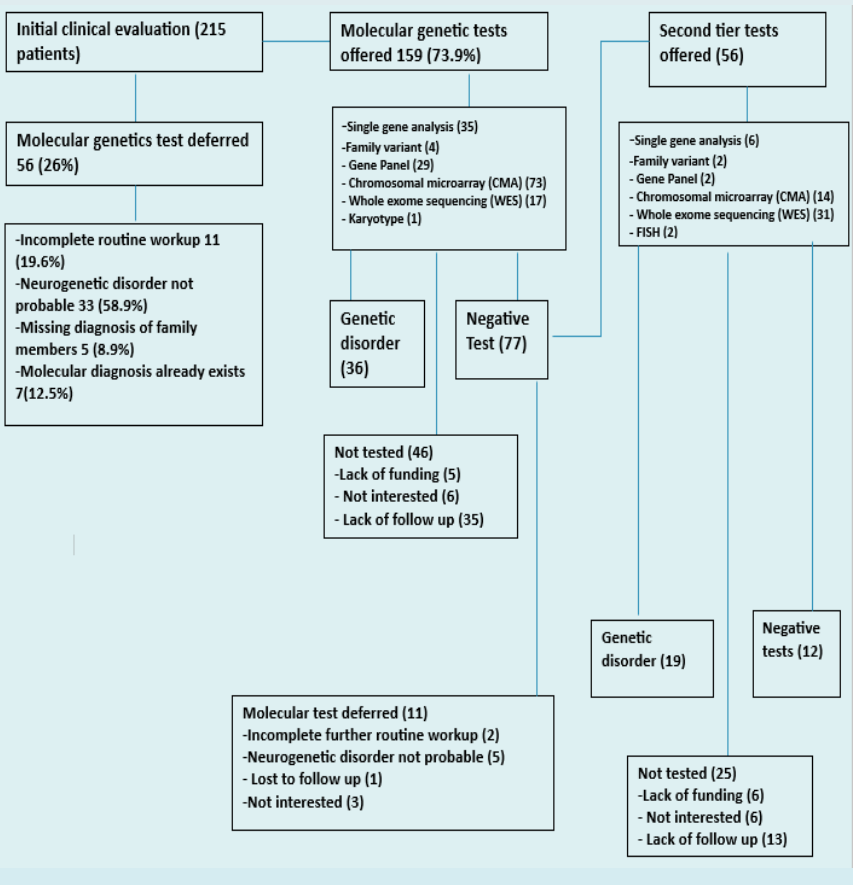
We conducted a retrospective review of pediatric patients referred to a multidisciplinary neurogenetics clinic between May 2014 and September 2021. Patients underwent joint evaluations by a pediatric neurologist and a geneticist, with genetic testing recommended based on clinical findings. Diagnostic yield and adherence to recommendations were analysed.

Results

215 patients were evaluated. The average age was 70±64 months (median 46 months, ranging from 0 to 252), 125 (58.1%) patients were males. The mean duration of symptoms before diagnosis was 36.6±47 months (median 16 months, ranging from 0 to 205).

A definitive neurogenetic diagnosis was established in 55 (34.6%) patients who were offered genetic testing, and 48.7% of these who performed the recommended genetic testing.

The diagnostic yield of specific tests was 65.5% for whole exome sequencing (WES), 17.8% for chromosomal microarray analysis (CMA), 46.4% for specific gene sequencing, 35.0% for gene panels.



Conclusions

The multidisciplinary approach demonstrated a high diagnostic yield and provided significant benefits for patient care, earlier and more accurate diagnoses and family planning. However, the limitation of public funding of some tests, the disparities in access to advanced genetic testing and follow-up adherence remain significant barriers.

Familial Hypogonadotropic Hypogonadism Linked to a Novel Pathogenic Intronic Variant in *FGFR1* Gene

Michal Yacobi-Bach,[1,2] Alina Kurolap,[1] Rona Hagin,[1] Adi Botvinik,[1] Yael Lebenthal,[3,4] Sigal Shaklai,[2] Hagit Baris Feldman[1,3]

[1]The Genetics Institute and Genomics Center, Tel Aviv Sourasky Medical Center; [2]Institute of Endocrinology, Metabolism and Hypertension, Tel Aviv Sourasky Medical Center; [3]Faculty of Medical & Health Sciences, Tel Aviv University; [4]The Institute of Pediatric Endocrinology, Diabetes and Metabolism, Tel Aviv Sourasky Medical Center

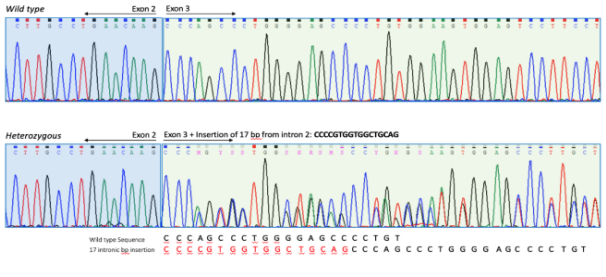
Introduction

Hypogonadotropic hypogonadism (HH) is a rare endocrine disorder characterized by insufficient secretion of gonadotropin-releasing hormone (GnRH), leading to impaired gonadal development, delayed or absent puberty, and infertility. Over 20 genes play a role in its pathogenesis, particularly in hypothalamic neuron development, migration, and GnRH signaling. We describe a familial case of HH caused by a novel pathogenic intronic variant in *FGFR1*, leading to aberrant gene splicing and subsequent gonadal dysfunction

RNA analysis

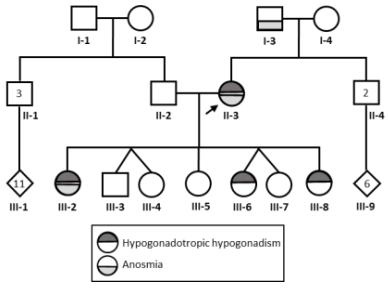
SpliceAI suggested disruption of the canonical splice acceptor site in exon 3 (score 0.97).

Analysis of RNA extracted from patient PBMCs confirmed aberrant splicing. The G-to-A substitution results in the inclusion of 17 nucleotides from intron 2 into exon 3, leading to a frameshift and a premature stop codon.

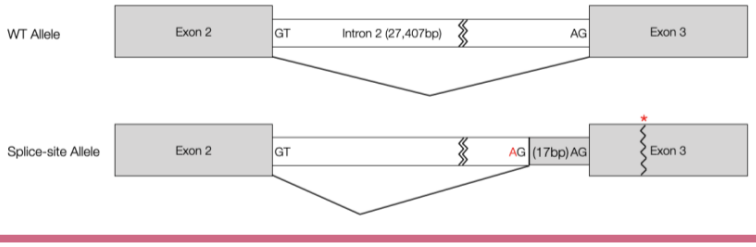


Case Presentation

A 53-year-old Bukharan Jewish woman presented with primary amenorrhea, delayed sexual development, and anosmia. Hormonal evaluations showed low follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels, consistent with HH. She had received gonadotropin treatment for fertility. A prior genetic panel was negative. Family history revealed that her three daughters (aged 24, 19, and 17) exhibited similar symptoms of delayed puberty and reproductive dysfunction, though with variable severity.



Exome sequencing Identified a novel intronic ***FGFR1*** variant: **c.92-19G>A** shared by all affected family members. The variant is located in intron 2 and was absent from gnomAD and internal databases but previously classified as a variant of unknown significance (ClinVar: VCV000430230.3).



Conclusion

We present a familial HH case in a mother and her three daughters caused by a **novel pathogenic *FGFR1* non-canonical splice-site variant**. This case underscores the importance of **non-coding regions** in gene regulation and disease pathogenesis. Our findings provide new insights into *FGFR1*-related HH and emphasize the clinical significance of intronic variants in genetic diagnosis.

It's all about the VOUS: inconclusive prenatal CMA results, and the role of the genetic counselor

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[1]Medical Genetics Institute, Shaare Zedek Medical Center, Jerusalem, Israel [2] Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel

Background

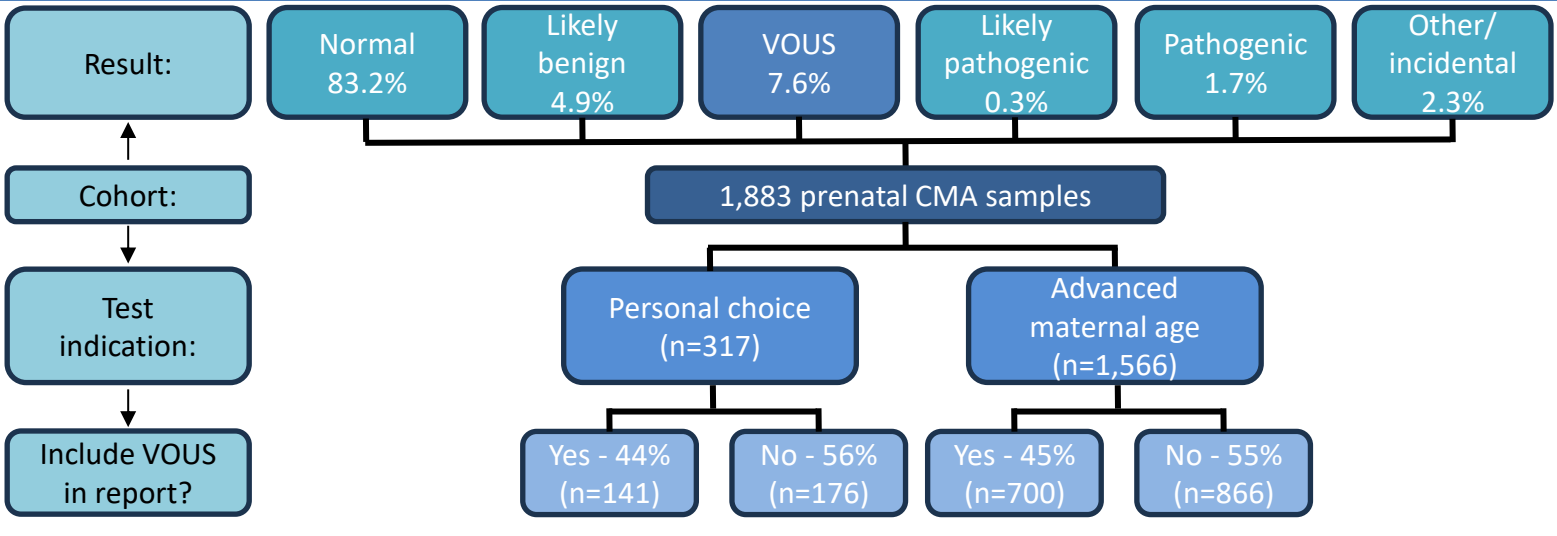
Chromosomal microarray analysis (CMA) replaced karyotyping as the standard prenatal genetic test in Israel on 01/02/2019. As a result, genetic counselors and their counselees needed to contend with variants of unknown significance (VOUS). If no sonographic fetal structural malformations are detected, most medical centers in Israel allow counselees to decide whether they want VOUS to be reported, preventing dilemmas caused by such results.

The purpose of this study is to estimate the frequency of VOUS in prenatal CMA, and to evaluate factors influencing counselees' decision.

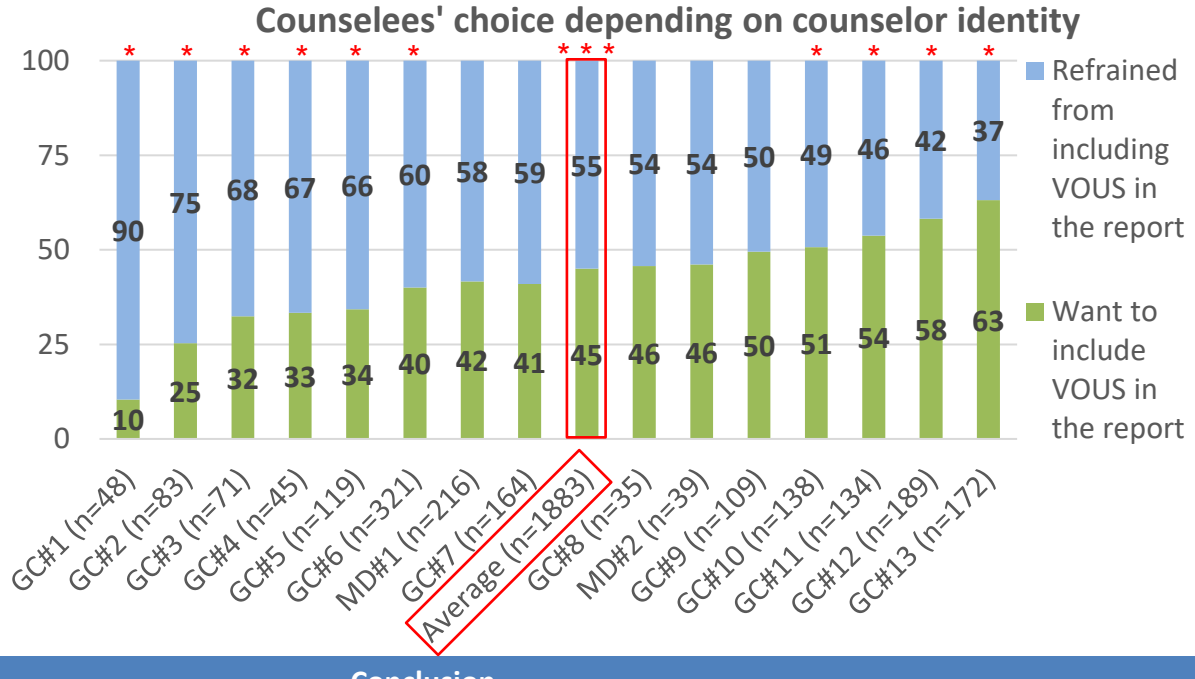
Methods

A 6-year retrospective analysis (02/2019-01/2025) of 1,883 prenatal CMA samples was performed regarding test indication (advanced maternal age or personal choice), medical center (two institutes), and genetics professional identity (13 genetic counselors - GC and 2 medical geneticists - MD).

Results



Of the three parameters tested, counselor identity was the only variable that significantly influenced the counselees decision:



Conclusion

Despite rapid progress in the ability to accurately classify CMA variants, many variants are still classified as VOUS. To alleviate additional uncertainty and stress in pregnancies, medical centers allow counselees to decide whether to receive uncertain results or not. Our study showed that counselees tend to opt out of receiving VOUS. In our cohort, of the factors tested, counselor identity was the only one to significantly influence the counselee's decision, emphasizing the importance of genetic counseling.

Conflict of interest: None

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Deciphering Elusive Structural Variants through Short-Read Mapping Interpretation: Focus on Rett Syndrome

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[2] Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Ramat Gan, Israel. Tel Aviv University School of Medicine, Tel-Aviv, Israel
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Objectives

Rett syndrome (RTT) is primarily caused by MECP2 mutations. However, a significant number of classical and atypical RTT patients lack genetic diagnosis despite thorough analysis. We aimed to solve the genetic riddle of three RTT cases, in which standard extensive testing failed to identify MECP2 aberrations.

Methods

We developed and applied a thorough strategy to address the complexities of identifying and characterizing SVs. We conducted Whole Genome Sequencing (WGS) and used MANTA software to identify potential SV breakpoints.

We utilized Integrative Genomic Viewer (IGV) to visualize read mapping, identify abnormal split reads, interpret unusual distances and orientations between paired-end reads, and assess read depth. This enabled us to hypothesize the SV types and accurately determine their boundaries. We then verified the borders of identified SVs using PCR and Sanger sequencing.

Results

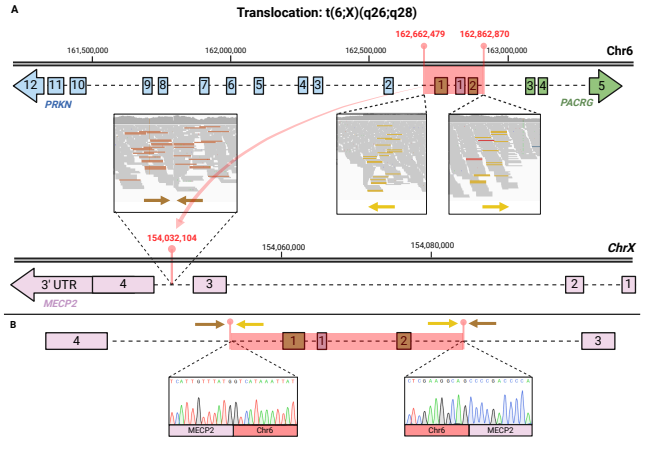


Fig. 1: Case 1: A ~ 200 kbp translocation from chromosome 6 into *MECP2*. **A** Schematic representation of the translocation breakpoints in the reference wild-type genome. *Red square* marks the translocated region from chromosome 6. IGV visualization shows: *Brown reads* span the translocation entry site on chromosome X, *Yellow reads* flank the translocated borders on chromosome 6. **B** Schematic representation of the predicted mutated patient genome. Paired-read visualization illustrates the directions of the aligned reads: *brown reads* (aligned to ChrX) are adjacent to and directed toward *yellow reads* (aligned to Chr6). Sanger sequencing confirms the SV breakpoints.

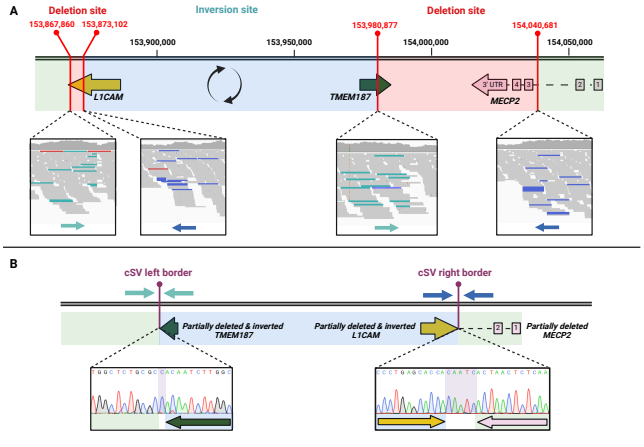


Fig. 2: Case 2: A complex structural variant (cSV) disrupting *MECP2*. **A** Schematic representation of the cSV breakpoints in wild-type genome. IGV visualization shows the analyzed reads directions by Cyan, and Blue arrows. **B** Schematic representation of the predicted mutated patient genome. Visualization of the paired-read directions aligned to the mutated allele. Sanger sequencing confirms the cSV breakpoints. Purple squares indicate unrelated insertions within the cSV breakpoints.

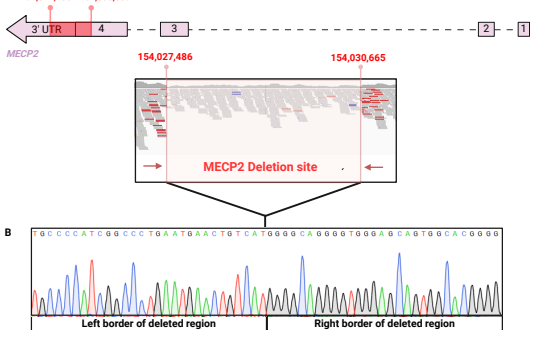
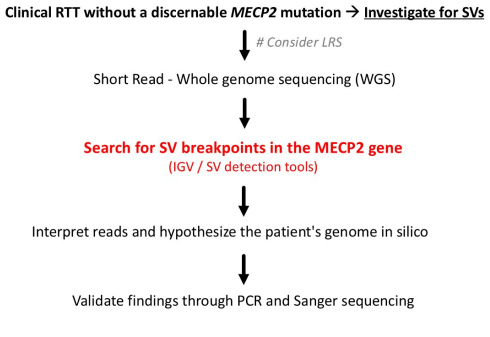


Fig. 3: Case 3: A ~3Kbp heterozygous deletion within *MECP2*. **A** *Red square* illustrates the c-terminal deletion site within *MECP2*. IGV visualization shows the deletion borders. *Brown reads* represents the paired-reads encompassing the deleted area. **B** Sanger sequencing validated the deleted region borders.



Conclusions

Our findings demonstrate that SVs can be resolved through IGV interpretation of cost-effective short-read sequencing. The high diagnostic yield in our RTT cases suggests that *MECP2* SVs may be a significant cause of RTT in previously unresolved cases. These results highlight the importance of incorporating SV analysis into routine genetic testing for RTT and other genetic disorders.

Impaired Wnt/planar cell polarity signaling in yellow nail syndrome

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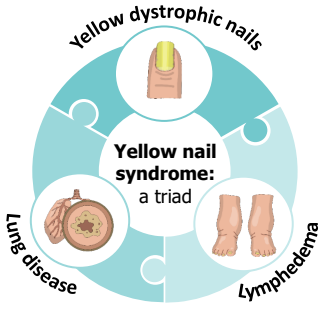
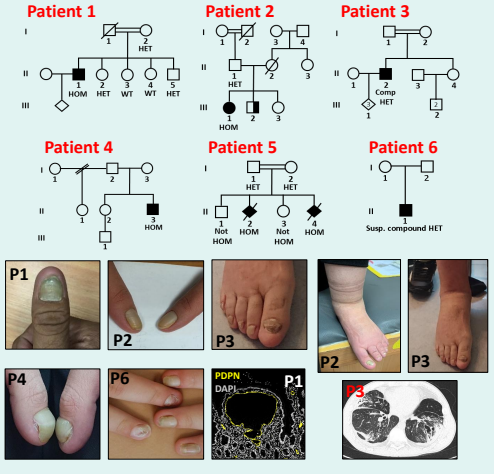
Yellow nail syndrome

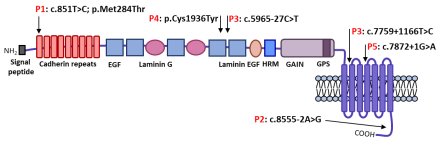


- Rare disorder (~400 cases published to date)
- 2 out of 3 symptoms are required for YNS diagnosis
- Additional symptoms, e.g. protein-losing enteropathy (PLE), primary intestinal lymphangiectasia (PIL) – in some cases
- Symptoms appear asynchronously
- Two subtypes:
  - Sporadic (sYNS) – mostly late onset
  - Congenital (cYNS)– extremely rare
- Etiology unknown
- Lymphatic abnormalities found in most patients

Congenital YNS (cYNS), n=6



Genetic analyses



- Rare variants (MAF < 1%)
- Effect on protein
- All inheritance modes

➔ **Candidate gene/s**

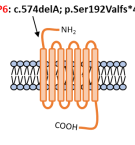
CELSR1 bi-allelic (n=5)

WES

Supplemented in patients with only one variant in the candidate gene

WGS

FZD6 Susp. bi-allelic (n=1)



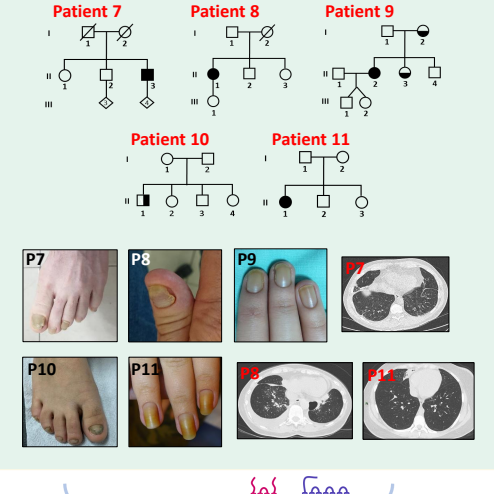
GENEMATCHER

Recruit additional patients

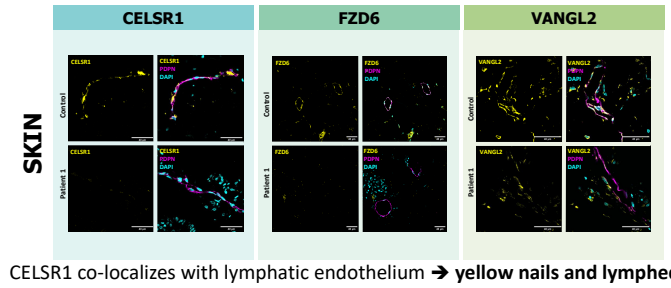
***CELSR1* or *FZD6* variants identified only in cYNS (n=6)**

No candidate variants were identified in sYNS patients (n=5)

Sporadic YNS (sYNS), n=5

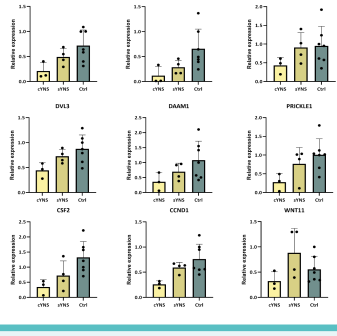


The Wnt/PCP pathway is downregulated in cYNS and sYNS patients

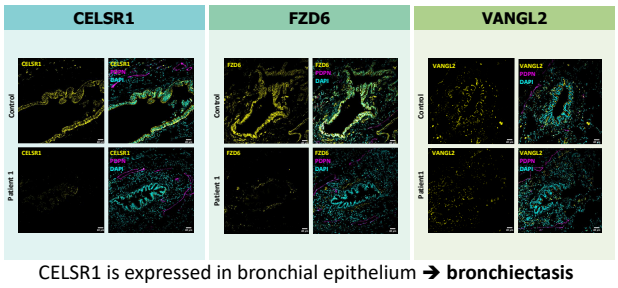


Protein expression (immunostaining)

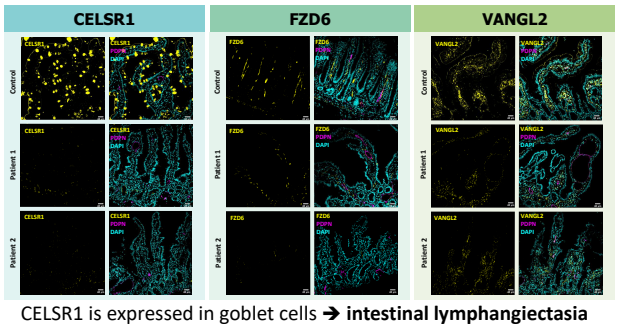
Gene expression (RT-qPCR)



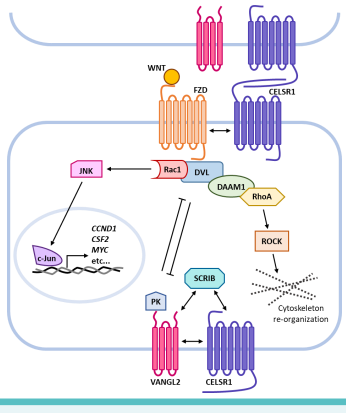
CELSR1 co-localizes with lymphatic endothelium ➔ **yellow nails and lymphedema**



CELSR1 is expressed in bronchial epithelium ➔ **bronchiectasis**



CELSR1 is expressed in goblet cells ➔ **intestinal lymphangiectasia**



The Wnt/PCP pathway

- ❖ **Defects in PCP organization play a major role in the pathogenesis of YNS**
- ❖ **This is the first demonstration of a mechanism explaining YNS development**
- ❖ The outcome of CELSR1 disruption corresponds to the defects observed in YNS, e.g. lymphatic involvement leading to yellow nails and lymphedema, bronchiectasis and PLE/PIL
- ❖ Although the Wnt/PCP gene expression defect in sYNS patients is relatively mild compared to cYNS and controls, it may suggest susceptibility to YNS development, together with other multifactorial factors not yet discovered

Not just Turner – rare X chromosome rearrangements causing infertility

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Introduction:

Premature ovarian failure (POF) characterized by absent menarche (primary amenorrhea) or premature depletion of ovarian follicles before the age of 40 years (secondary amenorrhea). Biochemically, it is characterized by low levels of gonadal hormones (estrogens and inhibins) and high levels of gonadotropins (LH and FSH) (hypergonadotropic amenorrhea).

POF is heterogeneous and could be caused by a variety of etiologies. Here, we present three patients diagnosed with POF and rare rearrangements on X chromosome.

Methods:

We clinically evaluated the three patients and performed chromosomal microarray (CMA) and Karyotype.

Results:

Patient #1 was a 26-year-old referred for abnormal fragile X testing suggestive of triple X syndrome. The initial Karyotype was reported as normal, but the CMA revealed a deletion of 30 Mb in Xq25q28 at the positions 124357762 to 155233098 and duplication of 36 Mb in Xp22.33p21 at the positions 168552 to 36909027. On revision, the duplicated segment was located in the deletion area, thereby masking this finding (Figure 1). On clinical evaluation, the patient was healthy with normal height but had infertility and elevated FSH levels.

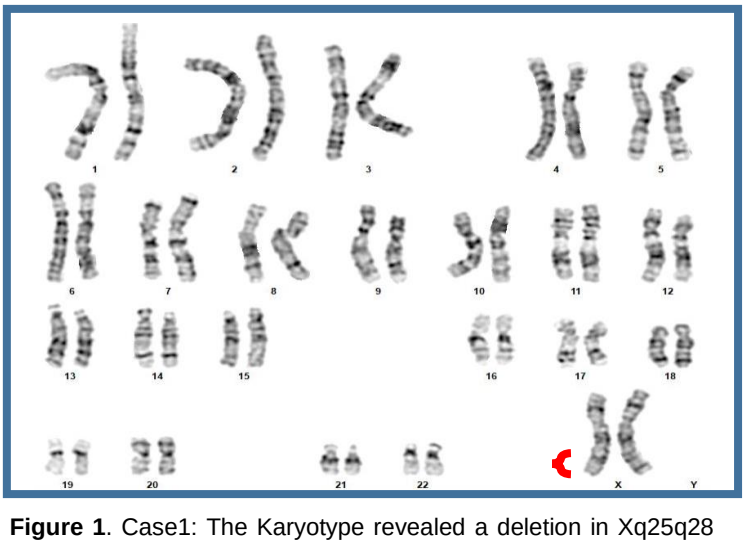

Figure 1. Case1: The Karyotype revealed a deletion in Xq25q28 and duplication of Xp22.33p21.

Patient #2 was a 16-year-old girl with secondary amenorrhea and increased levels of FSH. The Karyotype and CMA revealed a large de novo 54 Mb deletion on Xq13.2q25, spanning positions 73197850 to 127201959, that includes MECP2 (Figure 2). She was healthy with a normal height and physical examination but with suspected borderline intelligence.

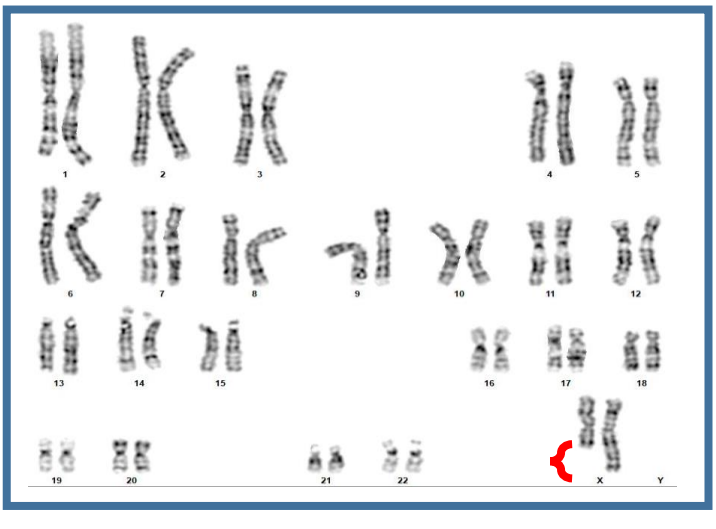

Figure 2. Case2: The Karyotype revealed a deletion on Xq13.2q25.

Patient #3 was 35 years old with low oocyte quality after ovum aspiration. CMA revealed a pathogenic 110kb deletion at Xq27.3 at the positions 146967352 to 147077423 including FMR1 and FMR1-AS genes. She had short stature with suspected borderline intelligence.

Conclusions:

We describe ultra-rare chromosome X rearrangements associated with apparently isolated POF. We found scarce data in the literature on these abnormalities. A full medical history and physical examination together with Karyotype and CMA, should be performed in such cases to fully understand the phenotype and genetic diagnosis.

Recurring de novo mutations in PSMB10: A novel cause of SCID-Omenn syndrome with revertant mosaicism as a possible rescue mechanism

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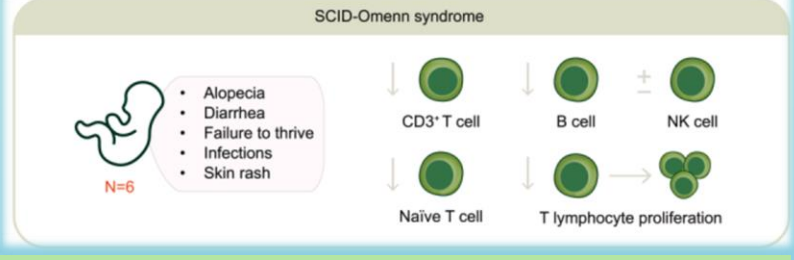
1 Department of Human Genetics, Radboud University Medical Center and Radboud Institute for Molecular Life Sciences, Nijmegen, the Netherlands; 2 Department of Internal Medicine and Radboud Center for Infectious Diseases (RCI), Radboud University Medical Centre and Radboud Institute for Molecular Life Sciences, Nijmegen, the Netherlands; 3 Institute of Rare Diseases, Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Tel Hashomer, Israel; 4 Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel; 5 Newcastle University Translational and Clinical Research Institute, Newcastle upon Tyne, UK; 6 Department of Medical BioSciences, Radboud University Medical Center, Nijmegen, the Netherlands; 7 Paediatric Immunology and Infectious Diseases, Great North Children's Hospital, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK; 8 Laboratory of Cancer Genetics and Tumor Biology, Research Unit of Translational Medicine and Biocenter Oulu, University of Oulu, Oulu, Finland; 9 Maastricht University Medical Centre MUMCp, Department of Clinical Genetics, Maastricht, the Netherlands; 10GROW School for Oncology and Developmental Biology, Department of Genetics and Cell Biology, Maastricht, the Netherlands; 11Department of Pathology, Radboud University Medical Center, Nijmegen, the Netherlands; 12Department of Cellular Pathology, Royal Victoria Infirmary, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK; 13MRC Centre for Medical Mycology, University of Exeter, Exeter, UK; 14Department of Paediatric Infectious Diseases, Great Ormond Street Hospital, London, UK; 15Department of Dermatology, Radboud University Medical Center, Nijmegen, the Netherlands; 16Pediatric Department A and the Immunology Service, Jeffrey Modell Foundation Center, Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Faculty of Medicine, Tel Aviv University, Tel-Aviv, Israel; 17The Wohl Institute for Translational Medicine and Cancer Research Center, Sheba Medical Center, Ramat Gan, Israel; 18Department of Pediatrics, Laboratory for Pediatric Immunology, Willem-Alexander Children's Hospital, Leiden University Medical Center, Leiden, the Netherlands; 19Department of Pediatric Infectious Diseases and Immunology, Amalia Children's Hospital, Radboud University Medical Center, Nijmegen, the Netherlands

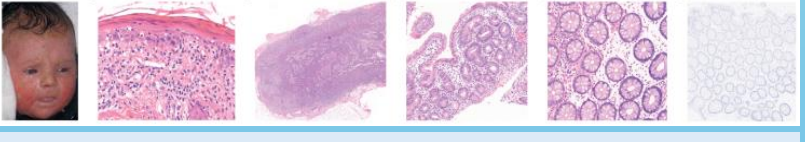
*equal contribution

Background: Severe combined immunodeficiency (SCID) manifests in early infancy with recurrent opportunistic infections and failure to thrive (FTT), with absence of T cells. Omenn syndrome (OS) is an atypical form of SCID, manifested by a generalized erythrodermic rash, lymphadenopathy, and hepatosplenomegaly. Distinguishing laboratory findings include oligoclonal T cell expansion, hypereosonophilia and elevated IgE levels. Both disorders can be detected clinically or in newborn screening (NBS) programs for SCID.

The human proteasome facilitates the controlled degradation of intracellular proteins that are targeted for breakdown by ubiquitination. Mutations in proteasome b-subunits or their chaperone and regulatory proteins are implicated in proteasome-associated autoinflammatory disorders (PRAAS). These conditions manifest with recurrent fevers, dermatitis, lipodystrophy, and immune dysregulation.

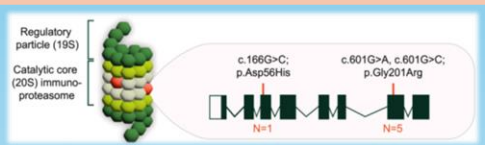


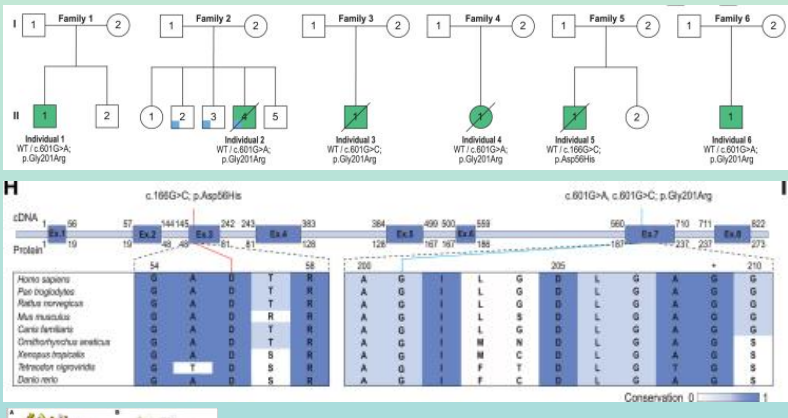
Objectives: We studied six unrelated infants, who shared features of SCID, with limited T cell repertoires, and clinical findings suggestive of OS. All patients lacked typical manifestations of PRAAS and underwent HSCT with poor outcomes.

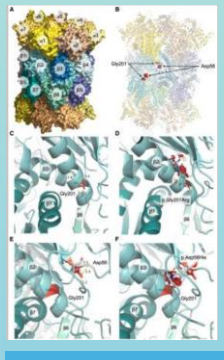


1. Erythematosquamous rash in individual 1 at 12 weeks after birth. 2. Histology of initial skin biopsy of individual 1 showed a graft-versus-host-disease-like pattern with vacuolar interface inflammation, multiple scattered apoptotic keratinocytes, and involvement of the adnexal structures, in the presence of a limited lymphocytic infiltrate (hematoxylin and eosin staining; original magnification ×41). 3. Histopathological evaluation of an inguinal lymph node extracted from individual 5 showed a paucicellular, stroma-rich lymph node. 4. A jejunal biopsy from individual 3 was hallmarked by partial villous atrophy and crypt hyperplasia with relatively few lymphocytes. 5. Colonic mucosa biopsied from individual 5 showed preserved crypt architecture with an empty lamina propria with few lymphocytes, in keeping with an immunodeficiency-related enteropathy. 6. Immunohistochemistry showed the absence of $CD3^+$ and $CD20^+$ positive cells in the colon samples from individual 5, although significant numbers of $CD4^+$ cells were observed that may be of a macrophage/monocyte lineage.

Methods and Results: Exome sequencing revealed de novo pathogenic variations within PSMB10 among the six infants consequently shared through the Genematcher platform: p.Gly201Arg (n=5) and p.Asp56His (n=1). Both variants were predicted to profoundly disrupt 20S immunoproteasome structure through impaired b-ring/b-ring interaction.



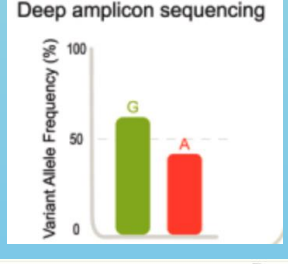


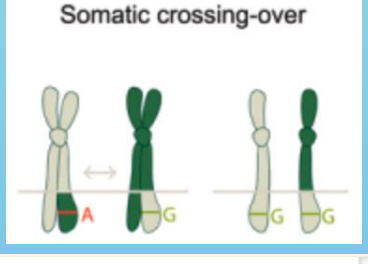


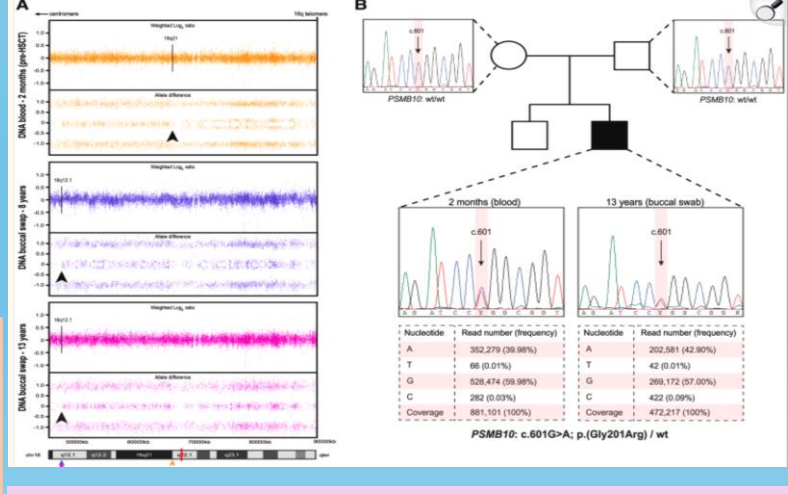
(A)Crystal structure of human IP 20S particle (PDB: 6E5B) is shown with its alpha subunits in shades of yellow/orange and beta subunits in shades of blue/green. (B) Positions of interest, Gly201 and Asp56, in the β2i subunits are highlighted as red spheres. The local structural environment of Gly201 (sticks) is depicted in (C) including distances from its interacting residues in the α-helix, which is located close to the β-ring interface. (D). Local structural clashes (in red discs) potentially brought about by p.Gly201Arg are shown. Together with the drastic changes in free energy of the complex, p.Gly201Arg appears to be structurally damaging. Similar structural representations are illustrated for the variant p.Asp56His in (E) and (F), which also include the position Gly201 for visual reference

Revertant mosaicism

The single long-term transplant survivor showed evidence for genetic rescue through revertant mosaicism overlapping the affected PSMB10 locus.







Conclusions: De novo mutations in *PSMB10* are a novel cause of SCID-OS. Revertant mosacism detected in the single long term survivor suggests a novel mechanism of genetic rescue. These findings highlight the connection between PRAAS-related diseases and SCID. *PSMB10* testing should be incoprporated into SCID newborn screening.

First patient cohort with a novel syndromic neurodevelopmental disorder caused by the c.231+4A>C hotspot variant in the *LSM1* gene

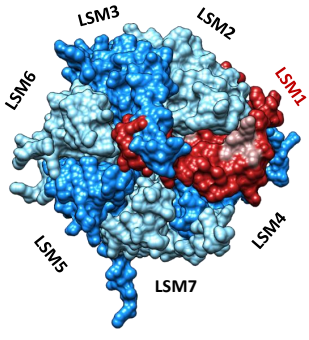
Sivan Reytan Miron,[1,*] Alina Kurolap,[1,*] Bassam Abu-Libdeh,[2] Abdel Salam Abu-Libdeh,[2] Clara Velmans,[3] Florian Erger,[3] Vera Riehmer,[3] Tzung-Chien Hsieh,[4] Hellen Lesmann,[4,5] Adi Reches,[1] Chofit Chai Gadot,[1] Adi Mory,[1] Motee Ashhab,[2] Christian Netzer,[3] Nadirah Sadi Damseh,[2] Hagit Baris Feldman[1,6]

[1]The Genetics Institute and Genomics Center, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; [2]Department of Pediatrics and Genetics, Makassed Hospital, Al-Quds Medical School, East Jerusalem, Palestine; [3]Institute of Human Genetics, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany; [4]Institute for Genomic Statistics and Bioinformatics, University Hospital Bonn, Rheinische Friedrich-Wilhelms-Universität Bonn, Bonn, Germany; [5]Institute of Human Genetics, University Hospital Bonn, Rheinische Friedrich-Wilhelms-Universität Bonn, Bonn, Germany; [6]School of Medicine, Faculty of Medical and Health Sciences, Tel Aviv University, Tel Aviv, Israel; *Equal contribution

Background

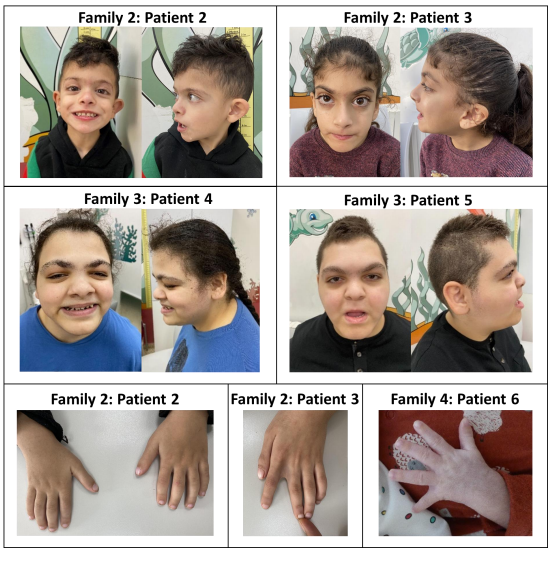
The LSM1 gene encodes an Sm-like protein, belonging to a family of proteins that typically function in hexameric or heptameric complexes that bind to RNA molecules. These complexes are involved in mRNA regulation, such as de-capping of the pre-mRNA. The LSM1 protein is a key member of a large protein complex known as the LSM1-7-PAT1 complex.

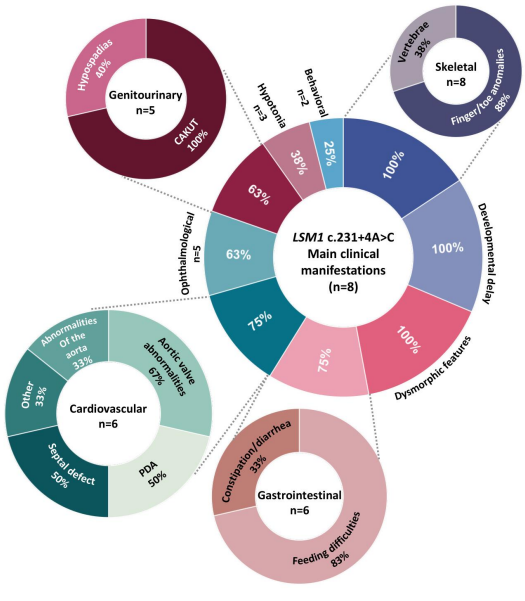
Variants in *LSM1* gene have been described in two separate case reports. The first published report identified the homozygous splice-site variant c.231+4A>C, while the second reported a homozygous missense variant (c.118A>T; p.Asn40Tyr). In addition, three individuals with leukodystrophy were recently identified with bi-allelic missense variants in the *LSM7* gene. Nevertheless, variation in *LSM1* has yet to be established as disease-causing in humans.

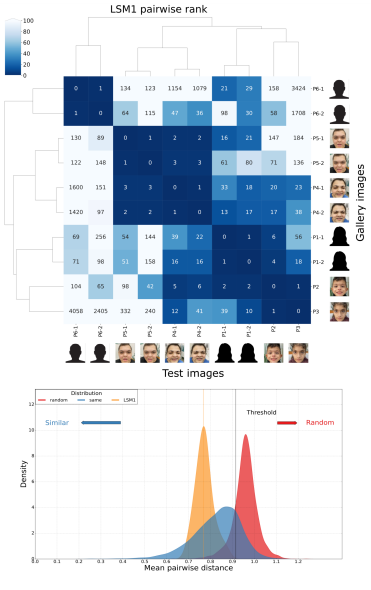


The patients

we report six pediatric patients from four different families and ethnic backgrounds (Ashkenazi Jewish and Muslim Arabs), harboring the previously reported homozygous splice-site variant, c.231+4A>C, in LSM1. All patients demonstrate developmental delay (except for the newborn who was too young for evaluation), dysmorphic facial features and abnormalities across diverse body systems, including skeletal, gastrointestinal, cardiovascular, ophthalmological and genitourinary involvement. GestaltMatcher analysis indicated that *LSM1* patients present with a similar facial gestalt.

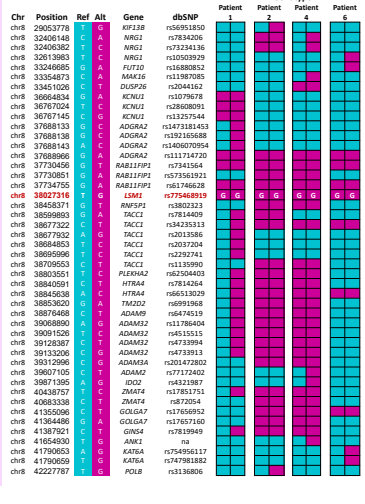






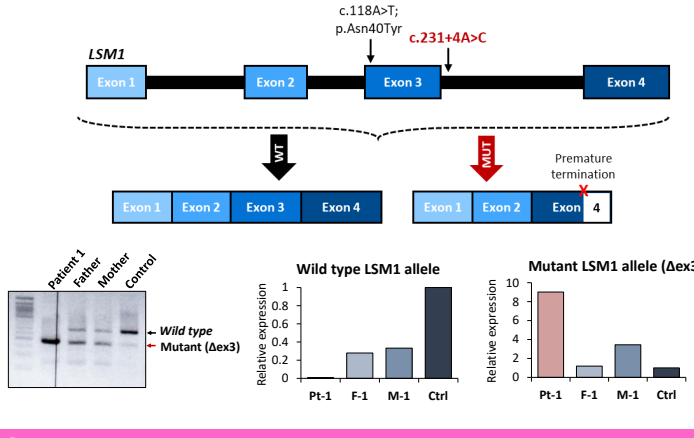
Haplotype analysis

Haplotype analysis using exome sequencing data showed independent occurrences of the variant between different ancestries (Ashkenazi Jewish and Muslim Arab). Moreover, while both Muslim Arab patients from Eastern Jerusalem share the same haplotype encompassing the *LSM1* variant, suggesting a founder effect, the Muslim Arab patient of Syrian origin exhibits a different haplotype. These results suggest that the variant occurs independently and may reflect a mutational hot spot.



RNA studies

Gel electrophoresis and RT-qPCR confirmed that the c.231+4A>C variant causes exon 3 skipping, producing negligible wild-type *LSM1* mRNA expression in patient cells compared to the parents and control.



Conclusions

Our findings establish LSM1, and specifically the c.231+4A>C homozygous variant, as causative for a novel autosomal recessive syndromic neurodevelopmental disorder. These results expand the understanding of LSM1-related diseases and provide a foundation for further investigation of its molecular mechanisms.

Endo: Genetic Clinic as a Tool for better Clinical Yield in Genetic Evaluation of Endocrine Cases

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Abstract

Endocrine diseases often have a genetic basis, and identifying this can improve diagnosis and guide treatment. This study evaluated the genetic diagnostic yield of an integrated endo-genetic clinic (EGC) at Carmel Medical Center. In a retrospective review (2014–2021), 210 endocrine patients were assessed. The EGC achieved a genetic diagnosis rate of 34.28%, exceeding the reported yield of standard genetic clinics (<15%). These findings suggest that an integrated EGC improves genetic diagnosis rates in endocrine patients, though larger-scale studies are needed to confirm this effect.

Background

Genetic factors play a significant role in many endocrine diseases, and identifying these genetic causes can improve diagnosis and guide targeted treatments. Traditionally, genetic testing in endocrinology focused on conditions linked to abnormal sex chromosomes (e.g., Turner and Klinefelter syndromes) or specific hereditary syndromes like multiple endocrine neoplasia (MEN1 and MEN2). Advances in genetic technology, particularly Next-Generation Sequencing (NGS), have expanded the scope of genetic testing, enabling more comprehensive evaluation of inherited and de novo endocrine disorders.

Study Goals

We propose an "Endo-genetic clinic" (EGC) model, integrating endocrinologists and medical genetics specialists to improve the diagnostic process. This multidisciplinary team evaluates patients across three phases: pre-evaluation (medical history review), clinical assessment (anamnesis and physical examination), and post-evaluation (future planning). This collaborative approach aims to enhance diagnostic accuracy, reduce unnecessary testing, and provide comprehensive genetic counseling alongside endocrine care.

EGC Design

Significantly higher diagnosis rate than in standard genetic clinics!

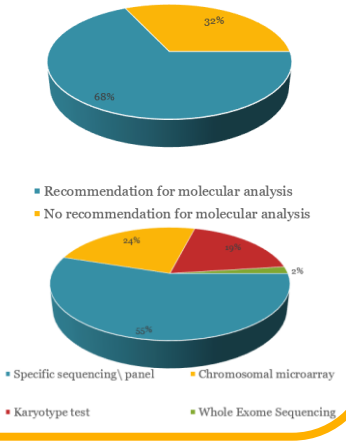
Study Goals

the most common reasons for referral were short stature (33.75%), obesity/underweight (15.38%), and dysmorphism or suspected syndrome (14.96%). This diverse patient population reflects the broad utility of the EGC across various endocrine conditions.

Characteristic	Patients (n=210)	%
Gender		
male	113	54%
female	97	46%
Age (years)	9.87 ± 6.66	
Reason for consultation		
Short stature	96	33.75%
Obesity/underweight	44	15.38%
Dysmorphism/ suspected syndrome	42	14.96%
Developmental delay	40	13.99%
Delayed puberty	22	7.69%
Adrenal insufficiency	12	4.20%
Glycemic control	9	3.15%
Hypothyroidism	7	2.45%
Abnormal laboratory results	7	2.45%
Family history	7	2.45%

Follow-Up Testing

68% of patients received recommendations for further genetic testing. Of these, 51.05% proceeded with the recommended tests. The most commonly suggested tests were specific sequencing panels (55%), followed by chromosomal microarray (24%) and karyotype analysis (19%).



Conclusions

The integrated Endo-genetic clinic (EGC) achieved a higher genetic diagnostic yield (34.28%) compared to standard genetic clinics (<15%). This multidisciplinary approach improves diagnostic accuracy by optimizing patient selection and test recommendations. Further research is needed to evaluate its impact on a larger scale and address barriers to test completion.

A Maternal Polygenic Risk Score for Preeclampsia Toxemia and a Systematic Review of the Literature

Ariel Zimerman[1,2], **Orit Reish, Tzila Reich**[1], **Ron Unger**[1]

1 The Mina Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel
2 Institute of Medical Genetics, Shamir Medical Center, Tzriffin, Israel

Backgrond

- Preeclampsia toxemia (PET) is a severe multisystem gestational disorder of unknown etiology with complex genetic architecture characterized by hypertension and organ dysfunction which affects 2-8% of pregnancies worldwide.

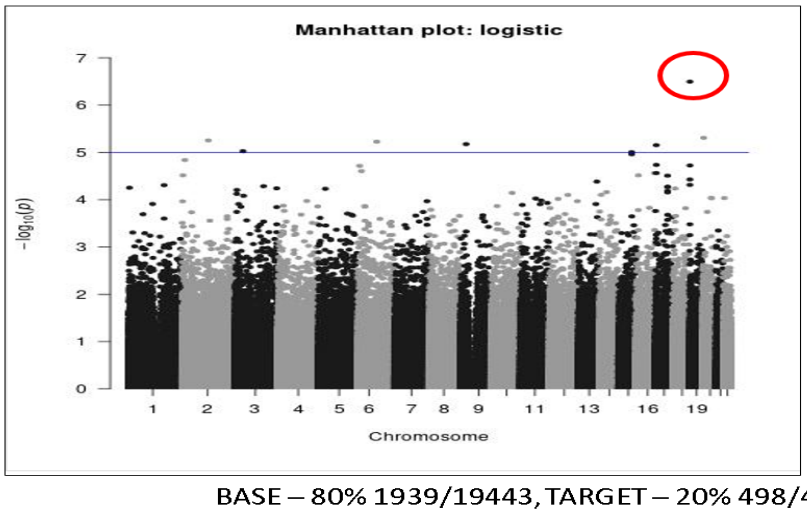
Objective

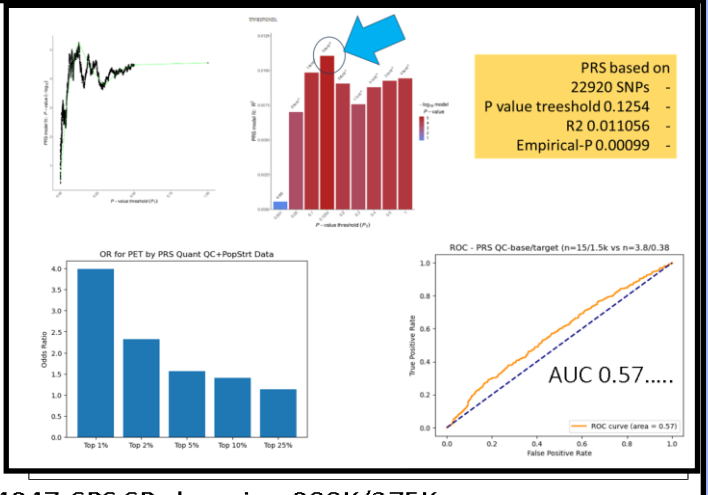
- To develop a new Polygenic Risk Score (PRS) based on UK-Biobank (UKB) data following established protocols and compare it to a systematic review of the literature

Materials and Methods

1- Preeclampsia-Toxemia GWAS and PRS -
Using UKB raw data and following published protocols (Marees 2021, Choi 2020) we produced a GWAS for PET vs. healthy controls (PLINK 1.9 software, missingness >0.02, MAF > 0.05, pi-hat >0.2, population stratification using HapMap1KG data). And a PRS using GWAS summary statistic and UKB target and validation cohorts (PRSice-2 software, clumping and threshold - C+T method).
2- Systematic Review of the literature-
Using predefined criteria following PRISMA 2020 guidelines (Page 2021) a PubMed comprehensive literature search up to March 2024. Studies quality assessment, data extraction, and comparison were conducted using a modified Polygenic Risk Score Report Standards guidelines (PRS-RS) criteria (Wand 2021).

Results





BASE – 80% 1939/19443, TARGET – 20% 498/4847, SPS SP clumping-800K/275K

Results 2 – PRS construction and target cohort

n	Description	Target c.	ancestry	population	cases	controls	c/c	PRS characteristics	PRS method	SNPs	p treshold	LD data
1	Kovacheva VP, 2024	y	multiancestry, 32.7% non-White	Mass General Brigham Biobank,	87	800	0.10	BP PRS using GWAS statistics from diff study	PRS- CS	not specifed	n	y
2	Honigberg MC, 2023	y	EUR	UKB	2.7K	4.4K	0.61	PRS from different biobank by authors	PRS- CS	1.08M	n	not reported
3	Xiao B, 2023	y	EUR + AFR	PMBB and eMERGE cohorts	1.1K	71K	0.02	GWAS from diff biobanks for cardiometabolic traits in same population	PRS -CS	1M	n	not reported
4	Nurkkala J,2023	y	FINISH	FinnGen Biobank	6K	134k	0.04	BP and PET PRS using diff cohort same population	PRS-CS	1.1M	n	y
6	Periji MM, 2022	y	EUR	UKB	2.7K	13K	0.21	PRS using SNPs form UKB population and SNPS reported in other studies	machine learning	115	n	not reported
7	Kivioja A, 2022	y	FINISH	FINNPEC	1.5k	1.8k	0.83	BP PRS using GWAS statistics from diff study	PRS-CS	1.08M	n	y
9	Steinthorsdottir V, 2020	y	ICELANDIC	deCODE cohort	2.4K	144K	0.02	BP PRS using different biobank	Ldpred (Bayesian)	not specifed	n	not reported
11	UKB 2024	y	EUR	UKB	0.5K	5K	0.10	PET PRS from diferent cases same population	PRS P+T	22920	0.01	not reported

- 6/8 studies used Bayesian PRS constrction(PRS-CS and Ldpred)
- 5/8 used summary statistics of Hypertension GWAS (no PET)
- Large variation in cases/controls ratios (01-0.04)

Results 3- Validation and PRS performance

n	Description	R2	beta	AUC	C-statist	Deciles comp details	Deciles comp -resul	Remarks
1	Kovacheva VP, 2024			0.62				report Xboostt (with clinical data) AUC=0.74
2	Honigberg MC, 2023					bottom vs top decile	4% vs 10%	PRS_PET+SBP increased the C-statistic from 0.690 to 0.701 (ΔC-statistic = 0.011, 95% CI: 0.001-0.021, P = 3.7 × 10^-2) C-statistic PRS_PET alone, not reported
3	Xiao B, 2023		0.2			quintiles		
4	Nurkkala J,2023				0.649			addition of the blood pressure PRS to the model containing both clinical variables and the preeclampsia PRS improved the c-statistic to 0.661
6	Periji MM, 2022			0.64		lower 50% vs top 1%	OR=12.76	
7	Kivioja A, 2022							Women with high BP-PRS (>95th percentile) had an increased risk of preeclampsia compared to those with lower BP-PRS (OR 1.7, 95% CI 1.1-2.5). Women with low BP-PRS (≤5th percentile) had a decreased risk of preeclampsia compared to those with higher BP-PRS (OR 0.51, 95% CI 0.35-0.73)
9	Steinthorsdottir V, 2020	0.02-0.006						proportion of variance in preeclampsia risk explained by hypertension HT-PRS R2 in the Icelandic deCODE cohort (R2=0.0006).
11	UKB 2024	0.011		0.57		50% vs top 1%	OR=4	see figure 3

- 3/8 (including ours) reported AUC (0.57-0.62)
- 4/8 reported deciles comparison
- 3/8 reported association R2 (0.01-0.02) and beta 0.2

Conclusions

- ❑ Reported PRS differ significantly in sample size, source data, GWAS and PRS methodology, and endpoints, therefore was not possible to perform a meta-analysis
- ❑ Despite the heterogeneous methodology the three ROC-AUC reported, included ours, were in the same low discriminatory range
- ❑ PET-PRS show limited clinical utility. Including maternal, paternal and fetal clinical and genetic data may improve current models.

GRID1/GluD1 homozygous variants linked to intellectual disability and spastic paraplegia impair mGlu1/5 receptor signaling and excitatory synapses

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Institut des neurosciences Cognitives et Intégratives d'Aquitaine, Bordeaux University, CNRS, EPHE, 33000 Bordeaux, France; 12) Service de Génétique, Centre Hospitalier Universitaire, 37044 Tours, France ; 13) These authors contributed equally to this work.

Introduction

- The inotropic glutamate delta receptor GluD1, encoded by the *GRID1* gene, is involved in synapse formation, function, and plasticity. GluD1 does not bind glutamate, but instead cerebellin and D-serine, which allow the formation of trans-synaptic bridges, and trigger transmembrane signaling.
- Despite wide expression in the nervous system, and data from *Grid1*$^{-/-}$ mice exhibiting behavioral, cognitive, synaptic and mGlu1/5 signaling level alterations, pathogenic *GRID1* variants have not yet been associated with a distinct human phenotype.
- We report homozygous missense *GRID1* variants in five individuals from two unrelated families, presenting with a novel genetic disorder characterized by intellectual disability (ID) and spastic paraplegia (SPG), with or without glaucoma. We further demonstrate the pathophysiological effects of GluD1 mutants on metabotropic glutamate receptor signaling and neuronal connectivity.

Homozygous pathogenic variants in *GRID1* cause ID and SPG, with or without glaucoma, in two unrelated families

- Using exome sequencing (ES), we identified five individuals of two unrelated consanguineous families (Fig. 1), harboring biallelic variants in *GRID1*, and sharing overlapping phenotypes:
- **Family A** – Three affected male siblings (ages 40y, 37y, 24y), born to consanguineous (first-degree cousin) parents of Algerian descent, were evaluated due to non- or slowly-progressive SPG, mild/moderate ID with normal occipitofrontal circumference, and juvenile open-angle glaucoma causing severe visual impairment. Brain MRI and EMG were normal, and initial metabolic and genetic investigations did not yield a diagnosis.
- **Family B** – A 6-years old female proband, born to consanguineous parents of Arab-Muslim descent, presented for evaluation due to global developmental delay (GDD), SPG, craniosynostosis, dysmorphic features (brachycephaly, bilateral ptosis) and minor skeletal anomalies. Her 24y sister was similarly affected, but also showed kyphosis of the cervical spine and required a wheelchair. Initial genetic tests were not diagnostic.

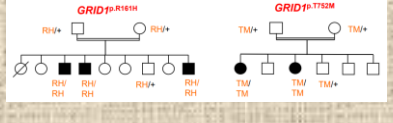


Figure 1- Pedigrees of families harboring biallelic *GRID1* variants. Full symbols indicate individuals with ID, SPG and glaucoma (left), or ID and SPG (right). Segregation analysis for *GRID1* variants shown in orange.

- Genome-wide single nucleotide polymorphism (SNP) genotyping (Family A) and ES (Families A, B) revealed each sibship to share a distinct homozygous missense variant in *GRID1*: p.Arg161His in Family A, and p.Thr752Met in Family B. Each of these variants segregated with the disease in an autosomal-recessive manner in the families. Both variants were predicted to be disease-causing, and only found in heterozygous state in 4 and 11 individuals, respectively, in the gnomAD database.

Structural impact of Arg161His and Thr752Met mutations on GluD1 extracellular domains

- Based on GluD1 sequence and cryo-EM 3D structure (Burada *et al.* 2020), we assigned the R^{161} and T^{752} residues to the extracellular Amino Terminal Domain (ATD) and Ligand Binding Domain (LBD) of GluD1, which bind cerebellin and D-serine, respectively (Fig. 2A, 2C).
- We modelled the complete structure by generation of unresolved 3D loops crucial for GluD1 activation. In this full-length model, we characterized the possible impact of the mutations on the ATD, LBD, and their coordination, based on the 3D structure of GluD1.
- R^{161}H is indicated to impact interactions of the hinge between ATD and LBD by modifying the binding pattern with the Q^{416}, D^{417} and P^{419} residues of the loop linking the two domains. T^{752}M results in additional interactions between lateral chains of M^{752}, Y^{748} and I^{729} that could lead to stiffening of the structure.
- These results suggest that both mutations can affect GluD1 function by altering the transduction of ligand binding to transmembrane/intracellular signaling of this receptor.

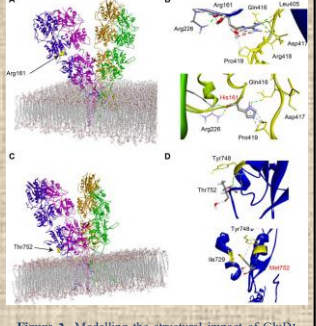


Figure 2- Modelling the structural impact of GluD1 R^{161}H and T^{752}M mutations on cerebellin-binding and D-serine-binding domains. (*A*) Structure of the GluD1 homotetramer sitting above the plasma membrane (adapted from Burada *et al.* 2020). Mutations affect residues situated at the interface (R^{161}) between ATD and LBD extracellular domains, or within LBD (T^{752}). (B) Predicted interactions of WT R^{161} and T^{752} residues, and of mutant H^{161} and M^{752} residues. Note that the R^{161}H mutation suppresses interaction with D^{417} residue of the loop linking ATD to LBD, thereby changing loop conformation, whereas the T^{752}M mutation results in supplementary interaction with I^{729} and Y^{748} residues of the LBD, thereby rigidifying this latter domain (C, D).

R^{161}H and T^{752}M mutations do not hamper cerebellin binding to GluD1

- To gain further insights into the impact of the mutations on GluD1 function, we pursued comparison of GluD1^{WT}, GluD1^{R161H} and GluD1^{T752M} expression in HEK cells through plasmid transfection; and transiently overexpressed these in mature hippocampal primary neuronal cultures from *Grid1*$^{+/+}$ mice (data not shown). We found that the mutations' pathogenic effects do not result from deficits in their expression, stability or trafficking.
- Further experiments into the ability of GluD1^{WT}, GluD1^{R161H} and GluD1^{T752M} to bind cerebellins, showed that cerebellin binding, and thus trans-synaptic scaffolding ability, is essentially preserved in GluD1 mutants.

R^{161}H and T^{752}M mutations impair the modulation of mGlu1/5 signaling by GluD1

- As GluD1 associates both physically and functionally with mGlu1/5 receptors, we tested the impact of GluD1 mutations on mGlu1/5 signaling via the ERK pathway.
- Using primary cultures of cortical cells from *Grid1*$^{-/-}$ mice, we showed GluD1-immunopositivity in all GFP-labelled cells transduced with GluD1^{WT}, GluD^{R161H} or GluD^{T752M} (Fig. 3A). Following incubation with mGlu1/5 agonist DHPG, ERK signaling was stimulated in GluD1^{WT}, with a stronger increase of pERK/ERK ratio relative to mock-treated control cultures, and a weaker stimulation in cultures expressing either mutation (Fig. 3C).
- These data indicate that the modulation by GluD1 of mGlu1/5 signaling via the ERK pathway is impaired by R^{161}H and T^{752}M.

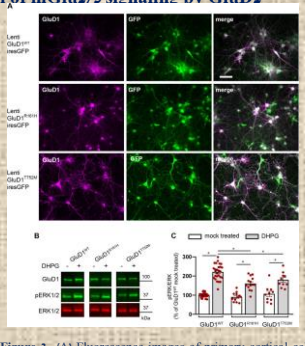
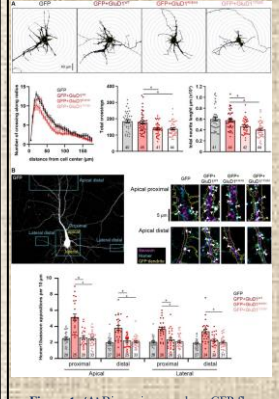


Figure 3- (*A*) Fluorescence images of primary cortical cell cultures from *Grid1-/-* mouse co-expressing GluD1^{WT}/GluD1^{R161H}/GluD1^{T752M} and GFP following lentiviral transfer. (B) Western blot analysis of virally transduced cortical cultures following incubation with the NMDAR antagonist APV, with or without mGlu1/5 agonist DHPG. Note the higher intensity of phosphoERK (pERK1/2), indicative of ERK signaling activation, following incubation with DHPG. (C) Mock-treated or DHPG-treated primary cortical cell cultures expressing GluD1^{WT}, GluD1^{R161H} or GluD1^{T752M}. Note that the increase of pERK/ERK ratio by DHPG was significantly larger in GluD1^{WT}- than GluD1^{R161H}- or GluD1^{T752M}-expressing cultures.

Impaired dendrite morphology and excitatory synapse density in *GluD1* mutants



- Morphological studies of WT murine mature hippocampal primary neuronal cultures, co-transfected with GluD1^{WT} / GluD^{R161H} / GluD^{T752M} and GFP, show that the R^{161}H and T^{752}M mutations perturb neurite outgrowth and architecture in neurons (Fig. 4A).
- As GluD1 is present at excitatory synaptic sites, and since excitatory synapses are localized on dendritic spines of hippocampal principal neurons, we analyzed spine density and morphology. Apical and basal dendrites of GluD1^{WT}-transfected neurons showed significantly higher density of putative excitatory synapses compared to controls, or to those overexpressing either mutation (Fig. 4B).

Figure 4- (A) Binary images show GFP fluorescence of cultured hippocampal neurons expressing GFP alone, or GFP and indicated GluD1 variants, after plasmid transfection. (B) Squares on the GFP fluorescence image of a pyramidal-shaped hippocampal neuron in transfected culture (*upper left*) exemplify regions where excitatory putative synapses, revealed by overlap of presynaptic Bassoon and postsynaptic Homer immunostaining on GFP positive dendrites (*upper right*), were counted. Note the enhancement of excitatory putative synapse density in GluD1^{WT}, but not GluD1 mutant conditions.

Discussion

- We report molecular and functional evidence of the association between homozygous missense variants p.Arg161His and p.Thr752Met in *GRID1*, encoding the GluD1 receptor, and a disease phenotype including ID, SPG and glaucoma.
- Our experimental findings indicate that both *GRID1* variants impair mGlu1/5 signaling via the ERK pathway, as well as dendritic morphology and excitatory synapse density, in neurons of primary cultures. While our study does not exhaust possible pathophysiological effects of these mutants, we demonstrate that their expression has deleterious consequences on neurons and circuits that can cause ID, SPG and glaucoma.

Conclusions

- We describe for the first time a human phenotype associated with biallelic *GRID1* variants, manifesting with ID and SPG, with or without glaucoma.
- Our findings unravel the importance of GluD1 receptor signaling in sensory, cognitive and motor functions of the human nervous system.

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Unveiling Genetic Causes of Failure to Thrive: A New Genetic-Pediatric Clinic`s Experience

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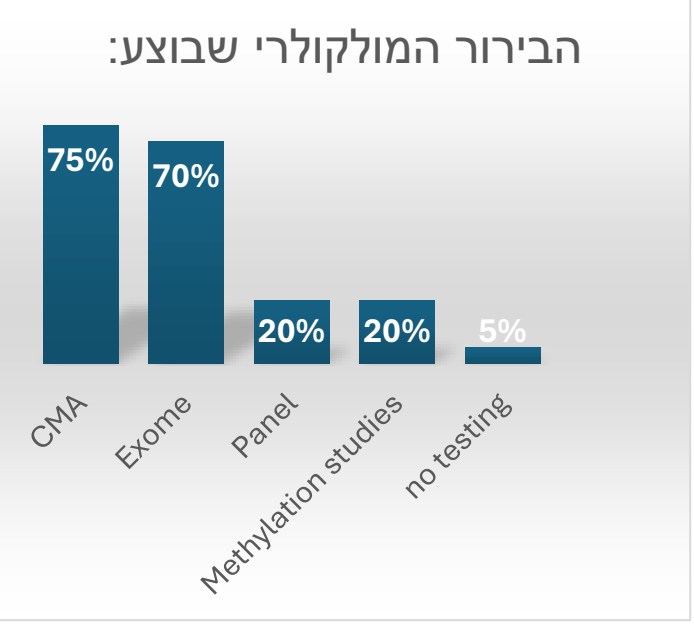
רקע: Failure to Thrive (weight faltering, poor weight gain או growth faltering) או כשל בשגשוג, הינו מושג המתאר מצב בו המשקל נמוך לגיל או לגובה, וישנה פגיעה בדפוס הגדילה של הילד. קיימות מספר הגדרות בספרות הרפואית. ההיארעות אינה ידועה במדויק, ולפי הספרות עשוי להימצא בעד 5-10% מהילדים במרפאות ובבתי החולים בארה"ב. מתוך המקרים של כשל בשגשוג, השכיחות של אטיולוגיות "אורגניות" ובינהן האבחנות הגנטיות אינה ידועה במדויק

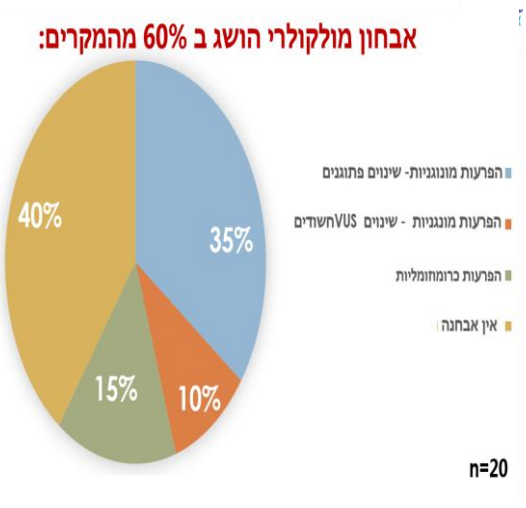
מטרות ושיטות: ב2021 הוקמה מרפאה גנטית ייעודית להפרעות בשגשוג בשיתוף פעולה עם המרפאה לתזונה ומחלקת ילדים בבי"ח דנה במטרה להגיע לאבחון גנטי מהיר. המרפאה משותפת לרופא ילדים עם התמחות בהפרעות בשגשוג/רופא גסטרואנטרולוגיה ילדים ומומחה לגנטיקה רפואית. ההפניה בוצעה לפי שיקול דעת קליני ולא לפי קריטריונים מוגדרים או הגדרות אנטרופומטריות. הבירור הגנטי בוצע באשפוז או אמבולטורית.

תוצאות: בין השנים 2021-2023 הופנו 22 ילדים: מתוכם 20 ילדים (91%) נבדקו במסגרת המרפאה, 12 בנות (60%) ו 8 בנים (40%)



מאפיינים קלינים	
גיל הפנייה	1.4 ± 1.3 שנים
Small for gestational age	35% (7/20)
משקל בעת הערכה	90% (18/20) < אחוזון 3
גבוה בעת הערכה	75% (15/20) < אחוזון 3
היקף ראש בעת הערכה	70% (14/20) < אחוזון 5
דיסמורפיזם משמעותי	45% (9/20)
עיכוב התפתחותי	40% (8/20)
מומים מולדים	20% (4/20)
בסך הכל: ב 50% מהילדים מופע תסמונתי וב 50% הפרעה מבודדת במדדי גדילה	

אבחנה	זמן עד קבלת תשובה ראשונה	זמן עד אבחון	השפעה
Cardiofaciocutaneous Syndrome	1 month	1 month	אפשרויות טיפולית, שינוי דרך מתן תזונה, סיכון הישנות
Sifrim-Hitz-Weiss syndrome, most likely	3.5 months	3.5 months	סיכון הישנות
	-		
Cardiofaciocutaneous Syndrome,	2 months	2 months	אפשרויות טיפולית, שינוי דרך מתן תזונה, סיכון הישנות
Deletion 10q26.2q26.3 / Duplication 10p15.3p12.33	3 months	3 months	סיכון הישנות
Trichohepatoenteric syndrome	1 month	1 month	השפעה טיפולית, שינוי דרך מתן התזונה, סיכון הישנות
Cornelia de Lange syndrome 2	0.5 months	0.5 months	שינוי דרך מתן תזונה, סיכון הישנות (בתאחיזה ל X)
Helsmoortel-van der Aa syndrome	4 months	10 months	אפשרות טיפולית, סיכון הישנות
Costello Syndrome	7 months	7 months	מעקב, סיכון הישנות
	4.5 months		
1q21.1 microdeletion syndrome	1.5 months	1.5 months	סיכון הישנות
	3 months		
	-		
16p13.11 microdeletion syndrome	3 months	3 months	סיכון הישנות
	-		
	2.5 months		
	-		
	2 months		
Renpenning syndrome	6 months	13 months	סיכון הישנות



מסקנות: בקרב מטופלים שנבחרו על ידי רופאים מומחים בהפרעות בשגשוג היתה היענות גבוהה לביקור במכון הגנטי (90%), ושיעור היענות גבוה לבירור גנטי - (בדיקת CMA/פאנל/אקסום) בוצעה ב95% (19/20 מטופלים).
שיעור האבחון הגנטי היה גבוה מאוד 60%, יותר במטופלים תסמונתיים לעומת לא תסמונתיים.
בחלק מהמטופלים האבחון אפשר שינויים טיפוליים.
נדרשות עבודות נוספות, רב מרכזיות, על מנת להכניס בעתיד בירור גנטי להפרעות בשגשוג לסל הבריאות

A missense variant in KIF14 results in two gene isoforms by affecting normal gene splicing

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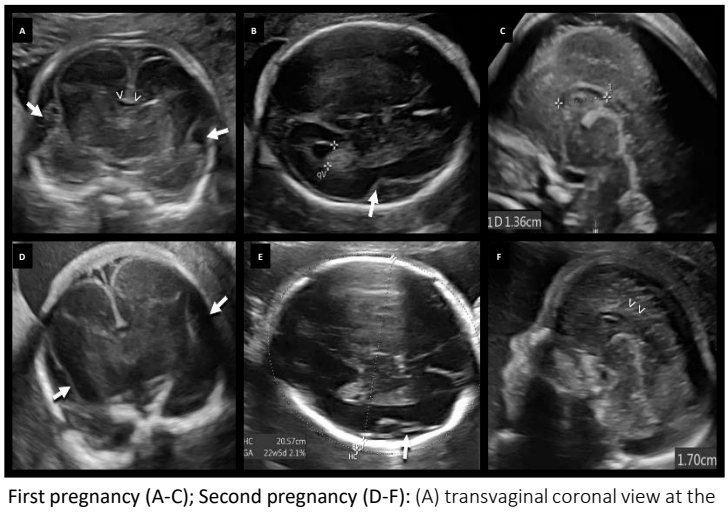
Background

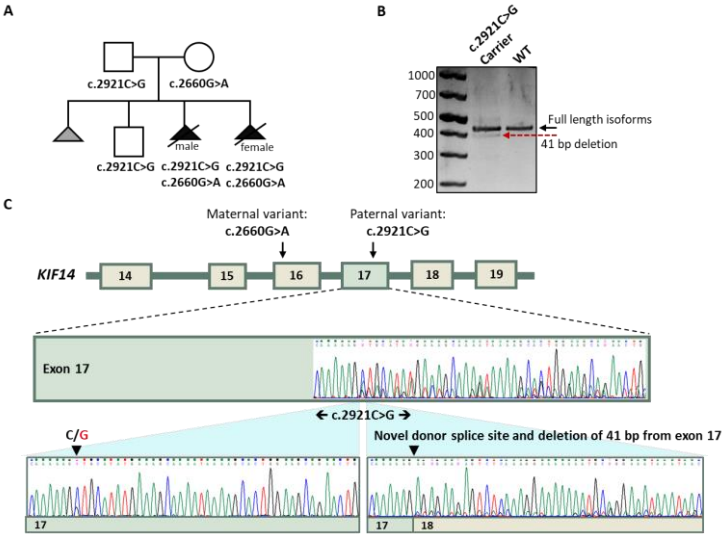
A couple of Ashkenazi Jewish descent was referred for genetic counseling following two terminations for pregnancy due to recurrent microcephaly with brain anomalies. (Figure 1). Quattro exome sequencing (ES) revealed compound heterozygosity for two previously undescribed missense variants of unknown significance (VUS) variants in the *KIF14* gene. Bi-allelic loss-of-function variants in *KIF14* are known to cause autosomal recessive Meckel syndrome type 12 (OMIM #616258). Missense and loss of function *KIF14* variants have also been described in primary microcephaly 20 (OMIM #617914).

Figure 1: Neurosonography at 24 weeks of gestation



First pregnancy (A-C); Second pregnancy (D-F): (A) transvaginal coronal view at the level of the basal ganglia: obliterated cavum septui pellucidui, asymmetric corpus callosum (open arrowheads), Sylvian fissures with delayed operculization (arrows); (B) transabdominal axial transthalamic view: aberrant Sylvian fissure, with complete flattening of the frontal component (arrow); (C) TV midsagittal view: dysgenetic and very short corpus callosum. (D) transabdominal coronal view at the level of the caudate nuclei: obliterated cavum septui pellucidui, Sylvian fissures with aberrant morphology (arrows); (E) transabdominal axial transthalamic view: small head circumference (2nd percentile), an aberrant Sylvian fissure, complete flattening of the frontal component (arrow) and absent fluid in the cavum septui pellucidui; (F) transabdominal midsagittal view: dysgenetic and very short corpus callosum (open arrowheads).

Figure 2: A missense KIF14 variant affects normal gene splicing.



(A) Pedigree of the studied family showing the segregation of both paternal and maternal KIF14 variants. (B) Gel electrophoresis of the PCR amplification of KIF14 exons 16–19 in paternal (c.2921C>G carrier) versus WT control mRNA, showing in addition to a normal sized isoform (upper band), the carrier has a second isoform which is smaller in size (lower band). (C) Schematic representation and Sanger sequencing chromatograms confirming that the paternal c.2921C>G variant results in two isoforms, one with the single nucleotide change, and the other introduces a novel donor splice site that leads to deletion of 41 bp from exon 17.

Diagnostic results and interpretation

ES showed that both fetuses were compound heterozygotes for two missense variants in *KIF14* (NM_014875.3), while each of the parents is a heterozygous carrier of one of these variants. Both variants were initially classified as VUS.

The **maternally-inherited** variant **c.2660G>A; p.Arg887Gln** was predicted to have a deleterious effect on the protein, and has been previously classified as a *VUS* in ClinVar (VCV002603121.2).

The **paternally-inherited** variant, **c.2921C>G; p.Ala974Gly**, is located in exon 17 of *KIF14*. SpliceAI predicted that this variant may have a strong splice-altering effect, with a Δ score of 0.98 of donor splice-site gain at position 1 bp from the nucleotide change, and a Δ score of 0.68 for a potential donor splice site loss at position -40 bp from the variant. RNA analysis confirmed a "leaky" abnormal splicing effect, corresponding to the donor site gain prediction and resulting in two isoforms: one with the C>G substitution (leading to p.Ala974Gly), the other introducing a novel donor splice-site within the exon, resulting in a deletion of 41 bp from exon 17 and a loss-of-function allele (p.Ala974Glufs*14) (Figure 2). The variant was, therefore, reclassified as likely pathogenic (LP).

This permitted the upgrade of the maternal variant from VUS to LP, due to it being in trans with the paternal variant, in two affected fetuses with phenotype consistency and an autosomal recessive disorder. Both reclassifications were done according to the American College of Medical Genetics and Genomics (ACMG) guidelines. This has empowered the couple to make informed reproductive choices and opt for preimplantation genetic testing (PGT) for future pregnancies.

Conclusions

- Missense variants in *KIF14* have been shown to cause primary microcephaly with developmental delay.
- In our case, the RNA analysis of the paternally-inherited variant lead to its reclassification as likely pathogenic (LP). This also permitted the upgrade of the maternal variant from VUS to LP.
- This highlights the importance of following up on VUSs in genes that could be related to the fetal phenotype and warrants careful consideration during the variant interpretation and reporting process.

רמב"ם
הקריה הרפואית לבריאות האדם

Novel RAG1 pathogenic variant in an adult with immune-mediated encephalopathy: Impact of Rapid exome sequencing during admission

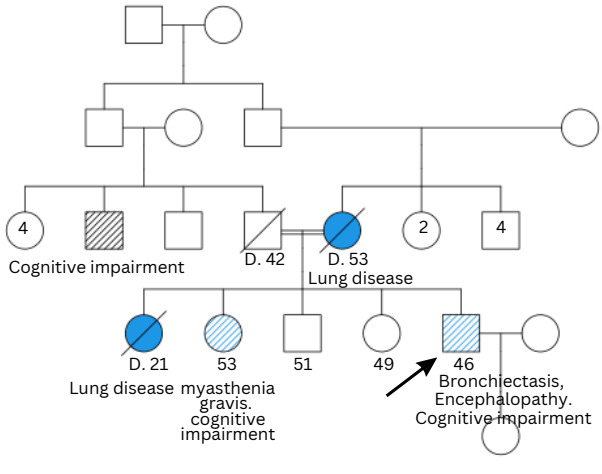
Sharon Bratman-Morag [1], Tamimah Abdallah Moady [1], Wisam Andrawis [2], Raz Winer [3], Fares Mazzawi [4], Abdalla Hamoudi [4], Tamar Paperna [1], Shahar Shelly [3,5], Ami Neuberger [4], Fabian Glaser [6], Karin Weiss [1,5]

1 Genetic Institute, Rambam Health Care Campus, Israel. 2 Clinical Immunology Unit, Rambam Health Care Campus, Israel. 3 Department of Neurology, Rambam Health Care Campus, Israel. 4 Department of Internal Medicine D, Rambam Health Care Campus, Israel. 5 Ruth and Bruce Rappaport Faculty of Medicine, Technion - Israel Institute of Technology, Israel. 6 Technion Center for Structural Biology (TCSB), Technion Human Health Initiative, Technion - Israel Institute of Technology, Israel.

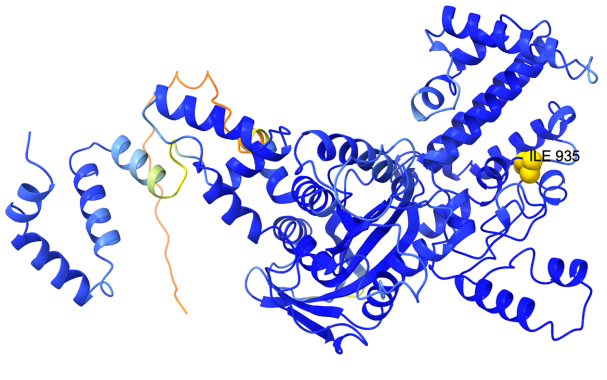
Objective: To describe the utility of rapid exome sequencing in hospitalized adult patients. We present a 46-year-old patient with a medical history of adult-onset bronchiectasis, vitiligo and mild thrombocytopenia admitted with progressive weakness, cognitive regression, weight loss and dysarthria. Laboratory studies revealed hypogammaglobulinemia and switched B cells. On imaging; hepatosplenomegaly, multiple abnormal T2 signals in both hemispheres. A brain biopsy was consistent with an inflammatory process. The family history was positive for consanguinity, a sister and a mother who died at an early age with lung disease hinted a possible genetic explanation.

Methods: Rapid exome sequencing was performed according to standard methods. 3D modelling of the missense substitution was performed using previously published crystal structure data.

Results: Initial exome results were reported after 5 days. We identified a homozygous novel variant in RAG1 (NM_000448.3): c.2803A>T, p.Ile935Phe. RAG1 encodes a protein involved in the immune system recombination process. Bi-allelic pathogenic variants were described in various types of primary immune deficiencies mostly presenting in childhood. In adults, there are a few reports describing combined immunodeficiency, granulomas, autoimmune conditions with systemic or organ-specific manifestations, and inflammatory phenomena. On 3D modelling, Ile935 is a buried hydrophobic residue in a protein binding domain. The larger Phe residue predicted to destabilize the region and make structural changes at the local environment supporting the pathogenicity of this variant. The molecular diagnosis prompted treatment with steroids and subcutaneous immunoglobulins with marked improvement.



Patient Pedigree: numbers - age (years), D. age of death. Arrow - proband. Blue - immune system disorder, stripes - neurodevelopmental disorder.



3D Modeling of RAG1: recombination-activating protein 1 consists 1043 amino acids. Yellow - hydrophobic Ile935 in protein binding domain.

Conclusion: RAG1 is associated with a wide age of onset and phenotypic spectrum that includes adult-onset chronic lung disease, autoimmune thrombocytopenia and inflammatory encephalopathy. In selected cases, rapid exome sequencing may improve the management of acutely ill patients.

A gain of function *SLC4A3* mutation causes short QT syndrome: From molecular analysis to phenotypic expression and novel diagnostic testing

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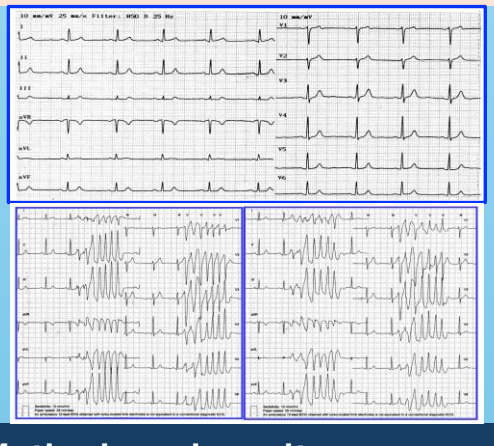
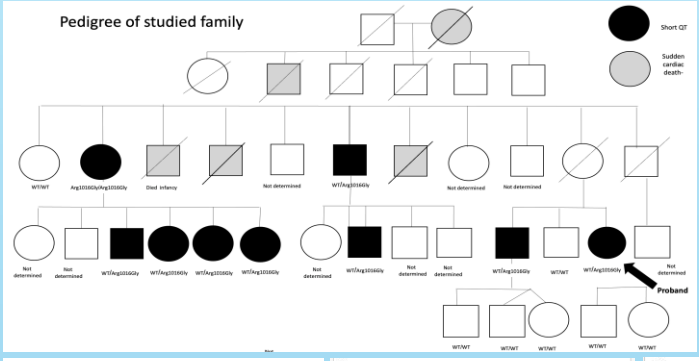
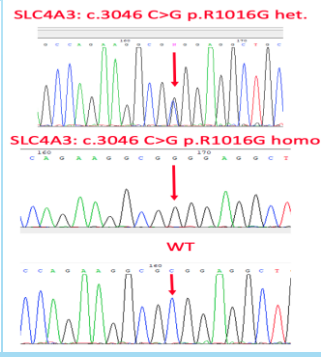
*Internal Medicine Division, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel ‡School of Medicine, Faculty of Health Sciences, Tel Aviv University, Israel ¶Department of Cardiology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel #The Danek Gertner Institute of Human Genetics, Tel Hashomer, Israel χ Institute of Rare Diseases, Edmond and Lily Safra Children's Hospital, Tel Hashomer, Israel δGenomics Unit, Sheba Cancer Research Center, Sheba Medical Center, Tel-Hashomer, Israel ø Department of Physiology and Cell Biology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel μDepartment of Biomedical Sciences and Public Health, School of Medicine, University "Politecnica delle Marche", Italy

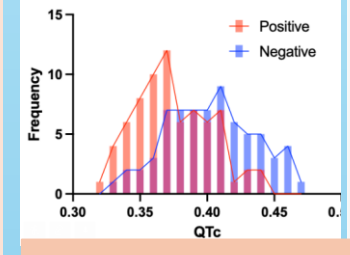
Background and aims: Congenital short QT syndrome (SQTS) is a genetic disorder characterized by short QT interval in the electrocardiogram (ECG) and a high risk of malignant ventricular tachyarrhythmias. We aimed to elucidate the genetic molecular cause of short QT in a family with six cases of sudden cardiac death and a case of documented polymorphic VT (proband) in five generations.

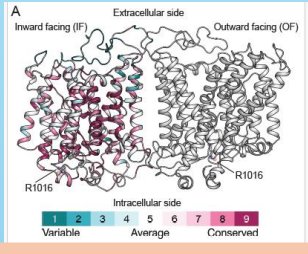


Methods and results: Exome sequencing for the proband and two affected family members, along with one unaffected parent was conducted with consequent segregation analysis . Testing revealed a novel missense variant in the *SLC4A3* gene, encoding the cardiac anion exchanger 3 (AE3): p.Arg1016Gly. This heterozygous variant segregated within the family, whereas one family member was homozygous.

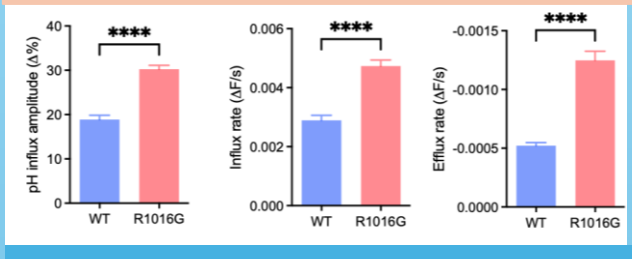


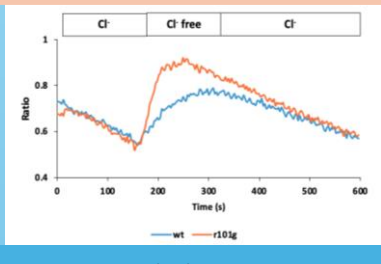






Functional analysis: Functional analyses in HEK293 cells revealed gain-of-function rather than the loss-of-function suggested for previously identified SQTS-associated SLC4A3 variants.





Discussion While affected family members exhibited a shorter QTc in resting ECG compared with non-affected (0.36 ±0.02 vs. 0.38 ±0.03 sec, p=0.0068) or 12-lead Holter (0.35 ±0.02 vs. 0.38 ±0.03 sec, p=0.0013), significant overlap exists. The Ippon test- a modified Valsalva maneuver, that incorporates supine passive leg rising, is a novel diagnostic procedure that was able to distinguish b/w carriers and non carriers, with inappropriately short QTc among affected vs. non-affected individuals (0.34 ±0.02 vs. 0.37 ±0.01, respectively, p=0.0003). The homozygous individual presented with a milder phenotype, which may be attributed to homodimerization of AE3, whereas dimerization of two mutants, leads to milder effect.

Conclusions: We describe herein a novel SQTS-variant associated with a GOF effect, with intracellular acidification. This gene, was initially reported in 2017 in one family. This report strengthens gene - disease association prompting its incorporation into current diagnostic panels