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ARTICLE

Clinically actionable incidental and secondary parental genomic findings after proband exome sequencing: Yield and dilemmas

Lina Basel-Salmon[1,2,3,4,*], Noa Ruhrman-Shahar[1], Naama Orenstein[2], Michal Levy[1,3], Gabriel A. Lidzbarsky[1], Nurit A. Batzir[2], Marina Lifshitc-Kalis[1], Sarit Farage-Barhom[1], Gali Abel[2], Mayra Petasny[1], Dana Brabbing-Goldstein[1], Avi Fellner[1], Lily Bazak[1]

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ABSTRACT

Purpose: Exome sequencing (ES) could detect pathogenic variants that are unrelated to the test indication, including findings that may have an impact for patients considering conception/reproduction (reproduction-related findings [RRFs]), deliberately searched secondary findings (SFs), and incidental findings (IFs). We aimed to examine the detection rate of clinically actionable findings and to present counseling dilemmas in 840 parents of probands undergoing clinical trio ES testing.

Methods: RRFs/IFs/SFs were actively searched for in the parents as part of ES data analysis. Variants were filtered by frequency, mode of inheritance, ClinVar classification, presence in local pathogenic variant databases, and protein-truncating effect.

Results: In 14 of 420 families (3.3%), 15 RRFs were detected. Shared parental heterozygous status for autosomal recessive disorders was identified in 23.3% of consanguineous and 1.8% of nonconsanguineous couples. SFs were found in 22 of 840 individuals (2.6%), including 15 variants (7 founder variants) in cancer-predisposing genes and 4 in cardiac disease–related genes. IFs were found in 3 individuals without reported symptoms. Overall, variants of potential medical importance were detected in 9.3% of families. Challenges related to the decision whether to report variants included unreported parental phenotype, presymptomatic testing, variable disease expressivity, potential medical implications for children who are already born, and medicolegal aspects.

Conclusion: Active search for RRFs, IFs, and SFs yields a high rate of findings, which may contribute to individual medical care in parents of probands undergoing ES. A structured approach to overcome the challenges associated with reporting these findings should be considered before such an active search can be broadly adopted in clinical genomic data analysis.

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Lina Basel-Salmon and Noa Ruhrman-Shahar contributed equally to this work.

Avi Fellner and Lily Bazak contributed equally to this work.

*Correspondence and requests for materials should be addressed to Lina Basel-Salmon, Rabin Medical Center, Beilinson Campus, Jabotinski st. 39, Petah Tikva, 49100, Israel. *Email address:* basel@tauex.tau.ac.il

Affiliations are at the end of the document.

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Introduction

Exome sequencing (ES) may reveal pathogenic variants in genes associated with conditions unrelated to the indication for testing. These most commonly include the following: (1) secondary findings (SFs), (2) incidental unsolicited findings (incidental findings [IFs]), and (3) findings that may have an impact for patients considering conception/reproduction (reproduction-related findings [RRFs]), such as heterozygous status for severe inherited conditions. Although IFs are unanticipated pathogenic or likely pathogenic (P/LP) variants that are not sought actively and are discovered unintentionally, P/LP SFs are identified when actively searched for in a predefined list of 78 genes that are considered to be actionable as recommended by the American College of Medical Genetics and Genomics (ACMG).[1-3] Practically, RRFs are a subgroup of IFs. Individuals tested can opt out of receiving results of medically actionable findings unrelated to the reason of referral for ES. Recommendations on follow-up actions related to the identification of SFs have been published; they include medical history, physical examination, family history, phenotypic diagnostic testing, and variant correlation.[4] RRFs may be important for family planning and reproduction decisions when both parents carry pathogenic variants in an autosomal recessive gene that is associated with a severe pediatric-onset disorder, or in females who carry such variants in X-linked (XL) recessive genes. Moreover, in some cases, this information may optimize medical care of an offspring with a disease caused by these variants. It should be noted that because of potential ascertainment bias, pathogenic variant penetrance that is derived from family-based studies might be inflated compared with the penetrance estimation found in population-based studies.[5] Therefore, informing individuals of a potentially actionable variant might entail a negative impact with no associated benefit, both from the individual and from the health economics perspectives.

In practice, even if only a predefined gene list is recommended to be included in the analysis, it is not unusual that a presumably disease-related genomic finding is unintentionally found in the variant interpretation process. Specifically, artificial intelligence–based variant filtration programs might highlight such variants as strong candidates, particularly because phenotypic overlap between different conditions is common. The same situation might occur if one of the variant interpretation steps includes applying a filter to identify P/LP variants based on automatic classification by the bioinformatics platform. Therefore, variant interpretation teams commonly face the decision whether to report this type of IFs based on individual parameters in each case, especially if the team includes a clinical geneticist. It is not always possible to simply follow the existing guidelines regarding recommended gene lists because many variants, genes, and conditions have not been evaluated by expert committees yet. Once clinicians in the variant interpretation team are informed about a variant with potential medical importance, it is difficult for them to make a decision to withhold medical information of potential importance from the individual tested, especially when the gene is not included in the SFs gene list that is recommended by the ACMG. Subsequently, ethical and professional dilemmas about whether to report RRFs and IFs may arise.

The purpose of this study was to examine the detection rate of all types of clinically actionable findings in parents of probands who underwent clinical ES because of a suspected monogenic disorder. In addition, we discuss the dilemmas that are related to communicating certain types of findings to presumably asymptomatic individuals.

Materials and Methods

Study setting and participants

The cohort investigated in this study consisted of 840 parents in 420 consecutive clinical ES trios (proband and both parents) performed and analyzed at a tertiary referral center during the period between October 2019 and August 2022. Individuals presenting with multiple congenital abnormalities, dysmorphic features, global developmental delay/intellectual disability, or abnormalities of other organ systems with possible heterogeneous etiology were referred to ES by clinical geneticists after an initial genetic workup, which included a normal chromosomal microarray result or nondiagnostic single-gene or gene panel testing, if such type of testing was considered to be indicated by the referring clinician before performing ES. Before referral to ES testing, probands and their parents were evaluated by a clinical geneticist. Phenotypic information required for ES data analysis was collected by an in-house variant interpretation team from referral letters and photographs of patients and their parents.

ES and bioinformatic analysis

Until June 2020, samples were sequenced by CeGat laboratory (CeGaT GmbH). Targeted capture of protein-coding regions was performed using Twist Human Core Exome or Twist Human Core Exome Plus Kits (Twist Bioscience). Starting from June 2020, ES was performed at the Tel Aviv Sourasky Medical Center. Targeted capture of protein-coding regions was performed using IDT Exome Research Panel xGENv2. Paired-end libraries were sequenced on Illumina NovaSeq 6000 (Illumina). At least 97% of target bases were covered at 20× or greater (95% at >100×). The FASTQ files and phenotypic information using Human Phenotype Ontology terms were uploaded to Emedgene's platform (Emedgene Technologies). Bioinformatic data analysis aimed to find the cause for the proband's disorder was performed as described previously by Basel-Salmon et al.[6] Three clinical geneticists participated in the variant analysis along with laboratory personnel, bioinformaticians,

and variant analysts. In addition to the manual variant analysis, automated analysis identified and presented approximately 10 variants likely to solve each case. Clinically actionable findings (RRFs, IFs, and SFs) were actively searched for in the parents as part of the ES trio interpretation process. Lists of SF-related genes changed according to the most recent ACMG guidelines at the time of data analysis. In parallel to data analysis aimed at identification of a potentially causative variant in the proband, variants that are important for parental health and reproductive decisions were actively searched for in the parents using the following filters: variant quality (mapping quality ≥ 45 and depth ≥ 10), frequency $< 5\%$, mode of inheritance, classification as P/LP in ClinVar ($\geq$2 stars), known founder variants (a national genetic database and PubMed), and protein-truncating variants in autosomal dominant, autosomal recessive (AR), and XL genes for which loss of function is a proven disease-causing mechanism (nonsense, frameshift, canonical splicing donor/acceptor variants). Parents were analyzed as mate pairs to identify cases in which both parents were heterozygotes for the same AR disorder. Variants were classified according to the ACMG criteria[1-3] and ClinGen[7] Sequence Variant Interpretation recommendations and were considered for reporting only if classified as LP/P. Copy number variants and mitochondrial genome variants were excluded from the study because detection of these types of findings was not considered satisfactory in ES data. Manual inspection and evaluation of the variant quality, repetitive sequence regions, and homologous sequences was performed for RRFs, IFs, and SFs. If needed, Sanger sequencing was used for confirmation. An internal discussion regarding reasoning for reporting was held in every case. In the parents, only P/LP variants considered not to be related to the cause of proband's referral or to the reported parental phenotype before referral for ES were counted for the purposes of this study. Variants in low- and moderate-penetrance cancer-related genes, low-penetrance variants in the *APC* gene (ie, NM_000038.6:c.3920T>A NP_000029.2:p.(Ile1307Lys)), monoallelic *MUTYH* variants, variants related to thrombophilia, *MEFV* variants in couples with heterozygous pathogenic variants, and mosaic variants in genes that are known to show clonal hematopoietic expansion were excluded from the results of this study. Clinically actionable variants were communicated back to the parents only if the tested individuals gave an a priori consent to be informed of this type of results. The variants in this study are reported in genome build GRCh37/hg19.

Results

From October 2019 to August 2022, clinical ES was performed in 453 probands. A molecular diagnosis explaining the proband's phenotype was identified in 158 families (34.9%). These diagnoses included 94 autosomal dominant disorders, 40 XL disorders, and 24 AR disorders. In 420 of 453 families, both parents were included in ES testing and were therefore included in this study (840 individuals). The probands (167 females; 39.8%) in these 420 families included 51 (12.1%) adults (>18 years of age) and 369 (87.9%) pediatric patients. Of 840 parents, 220 (26.2%) were of Arab origin, and the rest were of Jewish origin. Of 620 Jewish parents, Ashkenazi Jewish origin (full or partial) was reported in 310 parents (50.0%), and Ethiopian Jewish origin was reported in 25 parents (4.0%). Ethnicity was reported by the probands or the parents as part of the routine information collected in each genetic counseling session. Consanguinity was reported in 30 of 420 (7.1%) of the families.

RRFs were defined as P/LP variants in the same AR gene in both parents or in XL gene in a heterozygous female and considered by the variant interpretation team to be of potential importance either for reproductive decisions or for medical care of an already born offspring. These were detected in 15 genes in 14 of 420 couples (3.3%); in 7 of 14 of these couples, the parents reported consanguinity (Table 1). Most variants (11 of 15, 73.3%) were found in genes related to profound, severe, or moderate AR disorders according to the severity classification by Lazarin, and the rest of the variants were related to mild disorders (Table 1).[9] In the whole cohort, RRFs were detected in 7 of 30 (23.3%) of consanguineous couples and 7 of 390 (1.8%) of nonconsanguineous couples. Although a heterozygous status of the same AR disorder in both parents was known before ES testing in 3 of 420 couples, in 11 of 420 couples (2.6%), ES revealed a previously unknown mutual parental heterozygous status of potential medical importance. In addition, 1 heterozygous female for an XL disorder was identified (Table 1). Pediatric-onset disorders of moderate to profound severity were detected in 6 of 30 (20.0%) consanguineous couples and 4 of 390 (1.0%) nonconsanguineous couples. Ten of 16 variants identified (62.5%) were included in a national genetic database as per September 2022, and 7 of them (43.8%) were included in a variant-based expanded genetic screening assay that is widely used locally for reproductive screening.[10]

SFs identified in parents without a compatible phenotype, as reported at the time of referral for ES, are listed in Table 2. In total, 22 of 840 (2.6%) individuals were found to carry variants in genes recommended for reporting by the ACMG. In 2 individuals (cases 11 and 21), the variant identified was found in a mosaic state. In 4 cases (18.2%), the finding was known previously. Of 22 SFs, 14 variants were found in genes commonly associated with cancer-predisposing genetic syndromes, and 4 variants affected genes that are related to cardiac disorders (cardiomyopathy, arrythmia, and sudden cardiac death). In 7 of 22 individuals, known founder variants were detected (31.8%) (Table 2).

Additional types of findings identified were IFs in genes that were not defined by the ACMG as the ones for which SFs should be reported. These were P/LP variants in genes associated with highly penetrant disorders in individuals who were not reported by the referring clinical geneticist as

Table 1 RRFs-P/LP variants considered potentially important for reproductive counseling or for medical care of the offspring

Case Number	Consanguinity Yes/No	Variant(s) Classification/Known Before ES (Yes/No)/Presence in IMGD	ACMG Criteria (Total Score)	Disorder/Mode of Inheritance/Severity Accordingly to Lazarin et al[8]
1	Yes	*DST* NC_000006.11:g.56392359C>A NM_001374736.1:c.17854G>T NP_001361665.1:p.(Glu5952Ter) LP/No/IMGD	PVS1, PM2_supporting (9)	#614653 Neuropathy, hereditary sensory and autonomic, type VI/#615425 Epidermolysis bullosa simplex 3, localized or generalized intermediate, with bp230 deficiency (isoform dependent)/AR/profound
2	No	*HBB* NC_000011.9:g.5248232T>A NM_000518.5:c.20A>T NP_000509.1:p.(Glu7Val) LP/Yes/IMGD	PS3, PS4, PM2_supporting (9)	#603903 Sickle cell anemia/AR/moderate
3	Yes	*DNAAF4* NC_000015.9:g.55783447_55783448del NM_130810.4:c.278_279del NP_570722.2:p.(Lys93ArgfsTer7) LP/No	PVS1, PM2_supporting (9)	#615482 Ciliary dyskinesia, primary, 25/AR/mild
4	Yes	*CRB1* NC_000001.10:g.197297979_197297987del NM_201253.3:c.498_506del NP_957705.1:p.(Ile167_Gly169del) LP/No/IMGD	PS4, PM1, PM2_supporting, PM4 (9)	#600105 Retinitis pigmentosa-12 a range of phenotypes including early-onset retinitis pigmentosa, Stargardt macular dystrophy, isolated maculopathy, macular dystrophy, and retinal dystrophy/AR/moderate
5	Yes	*HEXA* NC_000015.9:g.72640475C>G NM_000520.6:c.987G>C NP_000511.2:p.(Trp329Cys) LP/Yes/IMGD	PS4[a], PM2_supporting, PP3 (6)	#272800 Tay-Sachs disease/AR/profound
		CYP1B1 NC_000002.11:g.38302350C>T NM_000104.4:c.182G>A NP_000095.2:p.(Gly61Glu) LP/No/IMGD	PS4, PM2_supporting, PM5, PP3 (8)	#617315 Anterior segment dysgenesis 6, multiple subtypes/AR/moderate
6	No	*BRIP1* NC_000017.10:g.59926570G>A NM_032043.3:c.427C>T NP_114432.2:p.(Gln143Ter) LP/No	PVS1, PM2_supporting (9)	#609054 Fanconi anemia, complementation group J/AR/severe
7	Yes	*CFTR* NC_000007.13:g.117199646_117199648del NM_000492.4:c.1521_1523del NP_000483.3:p.(Phe508del) P/Yes/IMGD	PS3, PS4, PM3, PM4 (12)	#219700 Cystic fibrosis/AR/moderate
8	No	*PIGL* NC_000017.10:g.16216917T>G NM_004278.4:c.483T>G NP_004269.1:p.(Tyr161Ter) LP/No	PVS1, PM2_supporting (9)	#280000 CHIME syndrome/AR/profound

(continued)

Table 1 Continued

Case Number	Consanguinity Yes/No	Variant(s) Classification/Known Before ES (Yes/No)/Presence in IMGD	ACMG Criteria (Total Score)	Disorder/Mode of Inheritance/Severity Accordingly to Lazarin et al[8]
9	No	*SYCE1* NC_000010.10:g.135369210G>A NM_001143764.3:c.721C>T NP_001137236.1:p.(Gln241Ter) P/No/IMGD	PVS1, PS4_moderate, PM2_support (11)	#616947 Premature ovarian failure 12?/AR/mild
10	No	*GJB2* NC_000013.10:g.20763612C>T NM_004004.6:c.109G>A NP_003995.2:p.(Val37Ile) P/No/IMGD	PS4, PM1, PM2_supporting, PM5, PP3 (10)	#220290 Deafness, autosomal recessive 1A/AR/mild
11	Yes	*CFTR* NC_000007.13:g.117254753G>C NM_000492.4:c.3454G>C NP_000483.3:p.(Asp1152His) LP/No	PS4, PM2_supporting, PP3 (6)	#219700 Cystic fibrosis/AR/moderate
		NC_000007.13:g.117282620G>A NM_000492.4:c.3846G>A NP_000483.3:p.(Trp1282Ter) P/No/IMGD	PVS1, PS4, PM2_supporting (13)	
12	Yes	*ACAD8* NC_000011.9:g.134131650G>A NM_014384.3:c.958G>A NP_055199.1:p.(Ala320Thr) LP/No	PS4, PM2_supporting, PP3 (6)	#611283 Isobutyryl-CoA dehydrogenase deficiency/AR/moderate
13	No	*GBA* NC_000001.10:g.155205634T>C NM_000157.4:c.1226A>G NP_000148.2:p.(Asn409Ser) P/No/IMGD	PS3, PS4, PM1, PM2_supporting, PP3 (11)	#230800 Gaucher disease, type I/AR/mild
14	No	*UPF3B* NC_000023.10:g.118971928_118971929del NM_080632.3:c.1093_1094del NP_542199.1:p.(Arg365AlafsTer10) LP (heterozygous female)/No	PVS1, PM2_supporting (9)	#00676 Intellectual developmental disorder, X-linked syndromic 14/XL/profound

ACMG, American College of Medical Genetics and Genomics; *AR*, autosomal recessive; *IMGD*, Israeli Medical Genetic Database; *LP*, likely pathogenic; *P*, pathogenic; *XL*, X-linked.

[a]PS4 criterion was used because there is an unpublished Tay-Sachs patient in this family with this variant in both alleles.

Table 2 SFs in genes defined as clinically actionable by the American College of Medical Genetics and Genomics

Case Number/Sex	Gene, Variant/Zygosity (het, hom)/Classification/Founder Variant (Yes/No)/Known Before ES (Yes/No)	ACMG Criteria (Total Score)	Disorder
1/F	*MSH6* NC_000002.11:g.48028028A>G NM_000179.3:c.2906A>G NP_000170.1:p.(Tyr969Cys) het/LP/No/Yes	PS4, PM2_supporting, PP3 (6)	#614350 Colorectal cancer, hereditary nonpolyposis, type 5
2/F	*BRCA1* NC_000017.10:g.41276045_41276046del NM_007294.4:c.68_69del NP_009225.1:p.(Glu23ValfsTer17) het/P/Yes/Yes	PVS1, PS4, PM2_supporting (13)	#604370 Breast-ovarian cancer, familial, 1
3/M	*BRCA2* NC_000013.10:g.32945138_32945139del NM_000059.4:c.8537_8538del NP_000050.3:p.(Glu2846GlyfsTer22) het/P/Yes/No	PVS1, PS4, PM2_supporting (13)	#605724 Breast-ovarian cancer, familial, 2
4/F	*PALB2* NC_000016.9:g.23647033del NM_024675.4:c.839del NP_078951.2:p.(Asn280ThrfsTer8) het/P/No/No	PVS1, PS4_moderate, PM2_supporting (11)	#114480 (Breast cancer, susceptibility to)
5/F	*TTN* NC_000002.11:g.179647599G>A NM_001267550.2:c.3034C>T NP_001254479.2:p.(Arg1012Ter) het/LP/No/No	PVS1, PM2_supporting (9)	#604145 Cardiomyopathy, dilated, 1G; # 613765 Cardiomyopathy, familial hypertrophic, 9
6/M	*BRCA1* NC_000017.10:g.41276045_41276046del NM_007294.4:c.68_69del NP_009225.1:p.(Glu23ValfsTer17) het/P/Yes/No	PVS1, PS4, PM2_supporting (13)	#604370 Breast-ovarian cancer, familial, 1
7/F	*BRCA2* NC_000013.10:g.32914438del NM_000059.4:c.5946del NP_000050.3:p.(Ser1982ArgfsTer22) het/P/Yes/No	PVS1, PS4, PM2_supporting (13)	#605724 Breast-ovarian cancer, familial, 2
8/F	*TTR* NC_000018.9:g.29178618G>A NM_000371.4:c.424G>A NP_000362.1:p.(Val142Ile) het/P/No/No	PS3, PS4, PM2_supporting, PP3 (10)	#105210 Familial amyloid neuropathy
9/F	*PKP2* NC_000012.11:g.:33031164_33031165del NM_001005242.3:c.649_650del NP_001005242.2:p.(Tyr217ProfsTer10) het/LP/No/No	PVS1, PM2_supporting (9)	#609040 Arrhythmogenic right ventricular dysplasia 9
10/F	*MSH6* NC_000002.11:g.48028135C>T NM_000179.3:c.3013C>T NP_000170.1:p.(Arg1005Ter) het/P/No/No	PVS1, PS4, PM2_supporting (13)	#614350 Colorectal cancer, hereditary nonpolyposis, type 5

(continued)

Table 2 Continued

Case Number/Sex	Gene, Variant/Zygosity (het, hom)/Classification/Founder Variant (Yes/No)/Known Before ES (Yes/No)	ACMG Criteria (Total Score)	Disorder
11/M	*RB1* NC_000013.10:g.49027156C>T NM_000321.3:c.1723C>T NP_000312.2:p.(Gln575Ter) het/P/No/No	PVS1, PS4, PM2_supporting (13)	#180200 Retinoblastoma
12/F	*BRCA2* NC_000013.10:g.32912046_32912047del NM_000059.4:c.3554_3555del NP_000050.3:p.(Thr1185SerfsTer2) het/P/No/No	PVS1, PS4, PM2_supporting (13)	#612555 Breast-ovarian cancer, familial, 2
13/F	*PMS2* NC_000007.13:g.6026429dup NM_000535.7:c.1970dup NP_000526.2:p.(Asn657LysfsTer7) het/P/No/No	PVS1, PS4, PM2_supporting (13)	#614337 Colorectal cancer, hereditary nonpolyposis, type 4
14/M	*TTR* NC_000018.9:g.29178618G>A NM_000371.4:c.424G>A NP_000362.1:p.(Val142Ile) het/P/No/No	PS3, PS4, PM2_supporting, PP3 (10)	#105210 Amyloidosis, hereditary, transthyretin related
15/F	*MSH6* NC_000002.11:g.48028028A>G NM_000179.23:c.2906A>G NP_000170.1:p.(Tyr969Cys) het/LP/No/No	PS4, PM2_supporting, PP3 (6)	#614350 Colorectal cancer, hereditary nonpolyposis, type 5
16/M	*BRCA1* NC_000017.10:g.41276045_41276046del NM_007294.4:c.68_69del NP_009225.1:p.(Glu23ValfsTer17) het/P/Yes/No	PVS1, PS4, PM2_supporting (13)	#604370 Breast-ovarian cancer, familial, 1
17/F	*TTN* NC_000002.11:g.179439983C>A NM_001267550.2:c.70876G>T NP_001254479.2:p.(Glu23626Ter) het/LP/No/No	PVS1, PM2_supporting (9)	#604145 Cardiomyopathy, dilated, 1G; # 613765 Cardiomyopathy, familial hypertrophic, 9
18/M	*CACNA1S* NC_000001.10:g.201009474del NM_000069.3:c.5254del NP_000060.2:p.(Glu1752ArgfsTer36) het/LP/No/No	PVS1, PM2_supporting (9)	#170400 Hypokalemic periodic paralysis, type 1; # 601887 (malignant hyperthermia susceptibility 5)
19/M	*BRCA2* NC_000013.10:g.32914438del NM_000059.4 c.5946del NP_000050.3:p.(Ser1982ArgfsTer22) het/P/Yes/Yes	PVS1, PS4, PM2_supporting (13)	#612555 Breast-ovarian cancer, familial, 2
20/F	*BRCA1* NC_000017.10:g.41209082dup NM_007294.4:c.5266dup NP_009225.1:p.(Gln1756ProfsTer74) het/P/Yes/Yes	PVS1, PS4, PM2_supporting (13)	#604370 Breast-ovarian cancer, familial, 1

(continued)

Table 2 Continued

Case Number/Sex	Gene, Variant/Zygosity (het, hom)/Classification/Founder Variant (Yes/No)/Known Before ES (Yes/No)	ACMG Criteria (Total Score)	Disorder
21/F	*MSH6* NC_000002.11:g.48033441_48033450del NM_000179.3:c.3747_3756del NP_000170.1:p.(Tyr1249Ter) het/LP/No/No	PVS1, PM2_supporting (9)	#614350 Colorectal cancer, hereditary nonpolyposis, type 5
22/F	*TTN* NC_000002.11:g.179398466A>T NM_001267550.2:c.102876T>A NP_001254479.2:p.(Tyr34292Ter) het/LP/No/No	PVS1, PM2_supporting (9)	#604145 Cardiomyopathy, dilated, 1G; # 613765 Cardiomyopathy, familial hypertrophic, 9

ACMG, American College of Medical Genetics and Genomics; *ES*, exome sequencing; *F*, female; *het*, heterozygote; *hom*, homozygote; *M*, male; *LP*, likely pathogenic.

having phenotypic abnormalities related to the variants that were discovered but considered by the variant interpretation team as being potentially important to report, either for reproductive or health-related purposes (Table 3, cases 1-3). In case 1 (*TRPS1* gene), the disorder was recognized through reverse phenotyping by the referring clinician based on mild but characteristic dysmorphic features. In case 2 (*HIVEP2* gene), no abnormal parental phenotype was reported by the referring clinical geneticist before ES or after the communication of the result. In this family, several relatives with developmental abnormalities in both paternal and maternal families were reported, but no further details were available, and a variant segregation study was not possible. In case 3 (*SGCE* gene), absence of phenotype may be explained by the fact that this gene is an imprinted gene and the disorder is phenotypically expressed when a pathogenic variant is inherited on the paternal allele.

In total, findings considered by clinical geneticists as potentially important for the health of a parent carrying the variant, reproduction decisions, or the health of an offspring were detected in 9.3% of the families.

The decision on whether to report potentially clinically actionable variants found in the probands' parents entailed different types of clinical dilemmas encountered by the clinical geneticists in the variant interpretation team. These included the following types of dilemmas: (1) a variant of uncertain significance that is leaning LP with possible severe consequences regarding reproductive decisions if not reported, (2) one of the parents identified with variant(s) causing a disorder that is still not apparent clinically in the parent, (3) both parents are found to be heterozygous for variants in the same AR gene that is related to a treatable or untreatable disorder, (4) both parents are found to be heterozygous for variants in the same AR gene that is related to a disorder with incomplete penetrance and variable expressivity, (5) a mosaic variant state in the parent with possible implications regarding parental health and reproductive decisions, and (6) dilemma regarding the decision whether additional attempts to explain the consequences of not receiving information on clinically actionable findings should be made in certain cases because of the importance for reproductive decisions. Examples of these dilemmas are shown in Table 4.

Discussion

Broad genomic sequencing is increasingly being used in clinical settings. This has triggered an active debate regarding screening for and reporting of clinically actionable findings that are not associated with the indication for testing. In this study, we found that parental findings unrelated to the ES indication but potentially important for the health of the parents and their offspring as well as for reproduction-related decisions were present in 9.3% of the families of probands who were referred to clinical ES because of a suspected monogenic disorder. We elaborated

Table 3 IFs - P/LP variants in genes for conditions not included in the ACMG SFs list

Case Number/Sex	Variant(s)/Zygosity (het, hom)/Classification	ACMG Criteria (Total Score)	Disorder/Mode of Inheritance	Known Before ES: Yes/No	Reported Phenotype After Additional Evaluation by a Clinical Geneticist in Light of the Variant Identified	Comments
1/M	*TRPS1* NC_000008.10:g.116599690dup NM_014112.5:c.2238dup NP_054831.2:p.(Ser747IlefsTer10) het/LP	PVS1, PM2_ supporting (9)	#190350 Trichorhinophalangeal syndrome, type I/AD	No	Features compatible with the disorder were found through reverse phenotyping	Might be undetected if the presentation is relatively mild. Important for reproductive counseling
2/M	*HIVEP2* NC_000006.11:143089518C>A NM_006734.4:c.5342+1G>T het/LP	PVS1, PM2_ supporting (9)	#616977 Intellectual developmental disorder, autosomal dominant 43/AD	No		No definite explanation for absent symptoms. Interpretation is limited without RNA studies.
3/F	*SGCE* NC_000007.13:g.94285390C>T NM_003919.3:c.21G>A NP_003910.1:p.(Trp7Ter) het/LP	PVS1, PM2_ supporting (9)	#159900 Dystonia-11, myoclonic/AD	No		Suggested potential explanation is that pathogenic *SGCE* variants typically cause disease only if found on the paternally derived (expressed) allele. Alternatively, this might be the case of a late-onset disease. In addition, the transcript used for variant interpretation might be not the main transcript involved in the disease.

ACMG, American College of Medical Genetics and Genomics; *AD*, autosomal dominant; *ES*, exome sequencing; *F*, female; *het*, heterozygote; *hom*, homozygote; *M*, male; *LP*, likely pathogenic.

Table 4 Examples of findings leading to a dilemma whether to report them

Types of Dilemmas	Variant/Zygosity (het, hom)/Classification/ Included in the Report: Yes/No	ACMG Criteria (Total Score)	Disorder	Potential Negative Consequence If Not Reported	Possible Negative Consequence If Reported	Eventually Reported (Yes/No)
A female with a variant of questionable pathogenicity in a severe X-linked disorder. It was classified as LP by an automated variant interpretation platform and therefore automatically presented to a clinical geneticist participating in variant interpretation. The variant was reclassified as VUS by the variant interpretation team.	*OPHN1* NC_000023.10:g.67339179T>C NM_002547.3:c.1277-5A>G/ het/VUS/Yes	PM2_supporting, P13 (2)	#300486 Intellectual developmental disorder, X-linked syndromic, Billuart type	Although classified as VUS, splice AI and VarSEAK programs predict high likelihood for abnormal splicing (splice AI AG 0.99, VarSEAK – 5, use of a de novo splice site). A pathogenic variant is associated with a high risk (50%) of having an affected male. Not reporting this variant will preclude familial segregation studies, which in turn might prevent potential reclassification of the variant's pathogenicity	If no males available for segregation testing in the family or the familial phenotypic data are lacking, then difficulty in reaching a conclusion regarding prenatal or preimplantation testing may be encountered	Yes, to perform segregation studies. It was found in a healthy male in the family
Parental mutual heterozygous state for a condition of moderate severity with variable expressivity	*CRB1* NC_000001.10:g.197297979_ 197297987del NM_201253.3:c.498_506del NP_957705.1:p. (Ile167_Gly169del)/both parents het/P/Yes	PS4, PM1, PM2_supporting, PM3, PM4 (11)	#600105 Retinitis pigmentosa-12 A range of phenotypes including early-onset retinitis pigmentosa, Stargardt macular dystrophy, isolated maculopathy, macular dystrophy, and retinal dystrophy	Parents will not be given a chance to make reproductive decisions (eg, preimplantation genetic testing). No early follow-up will be recommended (50% of patients reported to have a visual acuity of ≤0.3 at the age of 18 years and of ≤0.1 at the age of 35 years[11])	Emotional burden: reporting parental heterozygous status might possibly lead to presymptomatic testing in their children	Yes, because it was considered to be important for reproductive purposes
Parental mutual heterozygous state for a treatable condition	*GBA* NC_000001.10:g.155205634T>C NM_000157.4:c.1226A>G NP_000148.2 p.(Asn409Ser)/both parents het/P/Yes	PS3, PS4, PM2_supporting, PP3 (10)	#230800 Gaucher disease, type I	Delayed treatment, referral to additional genetic testing of the affected children (costs, time) when the diagnosis could have been made earlier based on ES results	Emotional burden: considering reduced penetrance in homozygotes for this variant and treatment availability, it is debatable whether preimplantation/ prenatal actions should be considered	Yes, because of the possibility of early diagnosis and treatment

(continued)

Table 4 Continued

Types of Dilemmas	Variant/Zygosity (het, hom)/Classification/ Included in the Report: Yes/No	ACMG Criteria (Total Score)	Disorder	Potential Negative Consequence If Not Reported	Possible Negative Consequence If Reported	Eventually Reported (Yes/No)
Adult-onset condition identified in an individual with no reported symptoms	*FAM161A* NC_000002.11:g.62066784_62066785del NM_001201543.2:c.1355_1356del NP_001188472.1:p.(Thr452SerfsTer3)/hom/P/No	PVS1, PS4, PM2_supporting (13)	#606068 Retinitis pigmentosa 28	Delayed diagnosis; possible diagnostic odyssey if molecular diagnosis is not reported (costs, time)	Emotional burden: unintended presymptomatic testing; possibility of nonpenetrance	No, because it was considered as "toxic knowledge" for the parent
Mosaic variant state	*RB1* NC_000013.10:g.49027156C>T NM_000321.3:c.1723C>T NP_000312.2:p.(Gln575Ter)/het/P/Yes	PVS1, PS4, PM2_supporting (13)	#180200 Retinoblastoma	Parents will not be given a chance to make reproductive decisions; no early follow-up will be offered to their children	Although the father is no longer at risk for retinoblastoma, emotional burden related to the possibility of nonocular malignancy may arise	Yes, to check if the offspring inherited the variant and they are at risk for retinoblastoma
Information on parental mutual heterozygous status might be important for the future medical care of the offspring.	*SYCE1* NC_000010.10:g.135369210G>A NM_001143764.3:c.721C>T NP_001137236.1:p.(Gln241Ter)/both parents het/P/Yes	PVS1, PS4, PM2_supporting (13)	#616947 Premature ovarian failure 12? #616950 Spermatogenic failure 15?	In the future, better fertility preservation means may be available	Emotional burden without any medical benefit: currently, it is not clear if offspring's egg/sperm cryopreservation is effective because this rare disorder was first described only recently	Yes, for possible future fertility preservation in the offspring
A monogenic disorder that was not suspected previously is identified through trio ES in a parent who has not consented to receive medically actionable findings unrelated to the proband's disorder. Dilemma whether an additional attempt to explain the consequences of not receiving information on clinically actionable findings should be made	*TRPS1* NC_000008.10:g.116599690dup NM_014112.5:c.2238dup NP_054831.2:p.(Ser747IlefsTer10)/het/LP/No	PVS1, PM2_supporting (9)	#190350 Trichorhinophalangeal syndrome, type I	Individual carrying a variant will not be given a chance to make reproductive decisions	Stigmatization, emotional burden	No, because the individual heterozygous for this variant was not interested in incidental findings

ACMG, American College of Medical Genetics and Genomics; *AD*, autosomal dominant; *ES*, exome sequencing; *F*, female; *het*, heterozygote*; hom*, homozygote; *homo,* homozygous; *M*, male; *P*, pathogenic; *LP*, likely pathogenic*; VUS*, variant of uncertain significance.

on the different types of findings discovered and the various dilemmas associated with informing the parents about these findings.

In this study, we found RRFs in a notable proportion of couples (3.3%). The importance of this finding is highlighted by the previous studies that investigated the prevalence of a heterozygous status of AR disorders among couples who underwent genomic testing. It has been shown that at least 1 P/LP variant in genes associated with AR disorders is found in the vast majority of individuals undergoing ES, with an average of 1.3 P/LP variants associated with a severe AR disorder and 2.2 P/LP variants associated with any AR disorder, and that about 0.8% to 1% of nonconsanguineous European couples are estimated to be at risk for having a child with a severe AR disorder.[12] Importantly, P/LP variants affecting genes associated with AR diseases (excluding unclear or mild phenotypes) are identified in 28% of consanguineous couples.[8] In another study, shared heterozygous status for P/LP variants associated with AR disorders of moderate to profound severity was identified in 9.8% of consanguineous couples.[13] In our study, RRFs related to disorders of moderate to profound severity were detected in 10 of 420 (2.4%) couples, of which 6 of 30 (20.0%) were in consanguineous couples and 4 of 390 (1.0%) in nonconsanguineous couples. However, it should be noted that consanguinity is not always self-reported; therefore, the prevalence of these findings in consanguineous couples found in our study might be underestimated. Interestingly, 62.5% of RRFs identified in this study are included in a national genetic database of known pathogenic variants in all local populations and ethnic groups. Our results demonstrate that although founder variants are frequent and variant-based genetic screening is widely used and funded in the population investigated in this study, 37.5% of families would escape detection of a mutual parental heterozygous state if only a variant-based screening is used.

The prevalence of SFs varies in different study cohorts and populations, with most studies pointing to an estimated prevalence of 2% to 3%.[5,14-17] Different genes and founder variants contribute to the prevalence of SFs in various countries. Our results (2.6%) are compatible with the results of previous studies. Interestingly, despite the high prevalence of founder variants in the population investigated in this study, only 7 of 22 variants detected were founder variants. These variants were known founder variants in the Ashkenazi Jewish population in the *BRCA1* and *BRCA2* genes.[18] Therefore, *BRCA1* and *BRCA2* founder variant–based screening for Ashkenazi Jewish women is expected to leave undetected more than half of the individuals with hereditary cancer predisposition syndromes (8 of 15 individuals with hereditary cancer-related disorders would have been missed).

In some cases, presumably fully penetrant loss-of-function variants in haploinsufficient genes are discovered in healthy adult individuals.[19,20] There are several possible explanations why a presumably pathogenic genomic variant does not lead to an abnormal phenotype: (1) irrelevance of the exon-containing variant to the transcript involved in disease development[21]; (2) generalized or clonal mosaicism (eg, in *ASXL1* or *DNMT3A*)[22]; (3) previously unreported incomplete penetrance and variable expressivity, especially for ultrarare or very recently described disorders; (4) the reported association of a loss-of-function mechanism with a specific phenotype is based on a small number of reported cases with a nonspecific phenotype and therefore questionable; and (5) truncated protein acts via gain of function instead of loss of function.[23] In this study, several IFs were identified in individuals reported by the referring clinical geneticist not to be affected. In an individual with a pathogenic variant in the *TRPS1* gene, the disorder was recognized retrospectively. Underdiagnosed parental phenotype discovered during ES data interpretation and the need for parental reevaluation has been reported previously: as a result of reverse phenotyping, in 12 of 16 (75%) cases, the definition of affected vs unaffected status in one of the family members has changed after detection of a pathogenic variant in the proband.[6] In the adult male with LP variant in the *HIVEP2* gene, nonpenetrance or mosaic state in tissues other than blood could theoretically explain the absence of expected phenotype. Of note, relatively mildly affected individuals with loss-of-function variants in this gene have been described.[24] An alternative explanation is that this variant potentially affecting splicing is not disease causing. In this case, although in silico predictions for this variant are deleterious, its effect on the transcript cannot be determined without RNA studies; therefore, the interpretation of this result is limited. A question on if an IF discovered in ES data in an asymptomatic individual is disease causing cannot be always answered, especially if the variant is de novo or if variant segregation analysis in family members is not possible. In an individual with a variant in the *SGCE* gene, absence of phenotype at the age of 42 could theoretically be explained by maternal inheritance of the variant because this is an imprinted gene. Further testing of the proband's maternal grandparents was not possible; therefore, this explanation could not be validated. Another possible explanation can be related to the age of onset of the disease. Although the onset of *SGCE*-related myoclonic dystonia is typically during the first decades of life, later-onset disease is possible in some cases, and this parent may express disease symptoms later in life. In addition, the transcript used for variant interpretation might not be the main transcript involved in the disease; therefore, the variant identified might not be disease causing.

The decision on whether an IF is disease causing can have greater implications for future reproductive planning than for the medical care of the heterozygote individual. Parental age, religion, and ethical views on prenatal and preimplantation testing may play a role in the willingness to be notified of RRFs. Reporting of RRFs may no longer be of practical importance to older couples but could be for future generations. Individual preferences for obtaining information about genetic variation across several different disease

categories were assessed by Thompson et al. The vast majority of their study participants (>90%) chose to receive identified information regarding several recessive diseases (cystic fibrosis, CFTR MIM: 219700; beta-thalassemia, HBB, MIM: 613985; sickle cell disease, HBB, MIM: 603903; and Tay-Sachs disease, HEXA, MIM: 272800).[14]

It should be noted that genomic data interpretation is especially challenging when a possibly pathogenic variant is found in an individual who does not express any relevant associated phenotypic features. A similar challenge may arise when such variants are found in genes associated with late-onset conditions and in genes associated with conditions characterized by incomplete penetrance and/or variable expressivity, in which no familial history of the disease is known. As listed in Table 4, clinical geneticists in the variant interpretation team faced different dilemmas as part of the decision on whether to report a potentially clinically actionable variant. One type of dilemma included situations in which variant discovery entailed presymptomatic testing. Another type of dilemmas concerned the difficulty in reaching a consensus on disease severity when considering reproductive counseling and prenatal or preimplantation testing. Additional challenges arose when a decision was needed on whether to report a heterozygous parental status for a pathogenic variant to provide better medical care to already born and future children. Moreover, clinical geneticists in the variant interpretation team had to also consider medicolegal aspects related to the inability to make reproductive decisions if a variant potentially causing a severe genomic disorder was not reported. Importantly, they had to weigh the potential improvement in medical care against the emotional burden on the individual and the potential economic impact on the health care system.

It is debatable whether certain findings related to a better postnatal medical care of the offspring, but not to parental reproductive decisions, should be reported, for example, parents who are heterozygous for variants with incomplete penetrance, such as the *GJB2* NP_003995.2:p.(Val37Ile) variant, or heterozygous for a severe pathogenic variant and the low-penetrance NP_000483.3:p.(Asp1152His) variant in *CFTR*. Reporting such findings would cause parental anxiety, potentially unjustified requests for prenatal or preimplantation diagnostics, and financial expenditures for possibly unnecessary follow-up visits, laboratory, and imaging investigations. It should be noted that penetrance figures for many disorders in populations without preexisting risk are not available yet. High-quality studies are still lacking because most diagnostic tests historically have been performed in individuals with high pretest likelihood of a genetic etiology. Another question that was raised in certain cases was whether a repeated explanation should be given regarding the possible negative consequences of refusing to be informed of clinically actionable findings. It has been shown that people may not fully understand how genomic information might affect their lives and may change their minds after an additional explanation. In one study, 49.4% of refusers opted to receive SFs results after an additional explanation. Importantly, 75% of the refusers who changed their minds thought they had originally agreed to receive SFs.[25] Reporting parental SFs raises another ethically debated dilemma regarding the need to report these finding in the proband. This issue was highlighted by a recently published study that investigated the parents' approach to SF disclosure in the context of their child and other family members' lives and found that most families desired SF information. The authors argued that SF disclosure should be reconceptualized to reflect the lived experience of those who may receive this information.[26]

Our findings further highlight the importance of pre- and post-test counseling. Pretest counseling should include a discussion regarding the potential detection of SFs, IFs, and RRFs, and it should enable to understand each individual's preferences regarding such findings. Post-test counseling is essential to explain the medical implications of such findings.

Our study has several limitations. One potential limitation is that our cohort comprises parental couples collected via affected probands. This might lead to a selection bias, which results in the overestimation of RRFs prevalence because of a higher rate of consanguinity. However, probands with AR disorders diagnosed by ES in our cohort comprised 15.27% of the diagnostic cases, similar to or even lower than in other published cohorts.[27] In addition, consanguinity in our cohort was reported in only 7.1% of the couples. On the other hand, detection rate of RRFs in this study may be underestimated because in the studied population, according to the current Ministry of Health policy, most couples are tested for common founder variants before planning pregnancy. An additional limitation of this study is that variants in genes with high homology (eg, exon 7 deletion in the *SMN1* gene) and copy number variants (eg, *DMD* deletions/duplications) were not included. It has been shown that ES misses 10.7% of the diagnostic variants later identified through genome sequencing.[28] Therefore, the detection rate of potentially clinically actionable findings in apparently healthy parents of probands might be even higher than the one found in this study. In addition, because of a high prevalence of founder variants in genes associated with both RRFs and SFs in the population investigated in this study, the detection rate found may be higher than in some other populations. An additional point to consider is that the definition of clinically actionable parental genomic findings may change in the course of time. Therefore, variants that are currently not interpreted as clinically actionable may be considered as such in the future. The effect of these potential changes on the yield of parental genomic testing should be investigated by additional studies in the future.

Conclusion

This study demonstrates that an active search for RRFs, SFs, and IFs yields a high rate of clinically significant findings in parents of probands undergoing trio ES. Yet, the decision on

whether to report these findings involves a complex array of considerations and dilemmas. Further studies are needed to improve the prioritization of clinically significant findings unrelated to the original indication for referral. Such studies will hopefully shed light on the decision process regarding the type of findings that should be searched for and for what purpose. A structured approach to overcome the challenges associated with reporting these findings should be considered before an active search for such variants can be broadly adopted in clinical genomic data analysis. Country-specific guidelines for reporting of clinically actionable findings reflecting local pathogenic variant landscape, population structure, and people's views are of the utmost importance in implementing preventional genomic medicine wisely.

Data Availability

The deidentified data used in this study are available to academic investigators upon request from the corresponding author. When using deidentified genomic data, a theoretical possibility of reidentification based on DNA sequence information exists; therefore, parental data will be available upon request if approved by the Rabin Medical Center Institutional Review Board.

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Author Information

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Ethics Declaration

This study was approved by the Rabin Medical Center Institutional Review Board. Participants' informed consent for this study was received as required by the institutional review board and archived.

Conflict of Interest

The authors declare no conflicts of interest.

Affiliations

[1]The Raphael Recanati Genetics Institute, Rabin Medical Center, Petah Tikva, Israel; [2]Pediatric Genetics Unit, Schneider Children's Medical Center of Israel, Petah Tikva, Israel; [3]Sackler Faculty of Medicine, Tel Aviv University, Tel-Aviv, Israel; [4]Felsenstein Medical Research Center, Tel Aviv University, Tel-Aviv, Israel

References

1. Green RC, Berg JS, Grody WW, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genet Med*. 2013;15(7):565-574. http://doi.org/10.1038/gim.2013.73
2. ACMG Board of Directors. ACMG policy statement: updated recommendations regarding analysis and reporting of secondary findings in clinical genome-scale sequencing. *Genet Med*. 2015;17(1):68-69. http://doi.org/10.1038/gim.2014.151
3. Miller DT, Lee K, Abul-Husn NS, et al. ACMG SF v3.1 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2022;24(7):1407-1414. http://doi.org/10.1016/j.gim.2022.04.006
4. Katz AE, Nussbaum RL, Solomon BD, Rehm HL, Williams MS, Biesecker LG. Management of secondary genomic findings. *Am J Hum Genet*. 2020;107(1):3-14. http://doi.org/10.1016/j.ajhg.2020.05.002
5. Forrest IS, Chaudhary K, Vy HMT, et al. Population-based penetrance of deleterious clinical variants. *JAMA*. 2022;327(4):350-359. http://doi.org/10.1001/jama.2021.23686
6. Basel-Salmon L, Ruhrman-Shahar N, Orenstein N, et al. When phenotype does not match genotype: importance of "real-time" refining of phenotypic information for exome data interpretation. *Genet Med*. 2021;23(1):215-221. http://doi.org/10.1038/s41436-020-00938-5
7. Rehm HL, Berg JS, Brooks LD, et al. ClinGen—the clinical genome resource. *N Engl J Med*. 2015;372(23):2235-2242. http://doi.org/10.1056/NEJMsr1406261
8. Sallevelt SCEH, Stegmann APA, de Koning B, et al. Diagnostic exome-based preconception carrier testing in consanguineous couples: results from the first 100 couples in clinical practice. *Genet Med*. 2021;23(6):1125-1136. http://doi.org/10.1038/s41436-021-01116-x
9. Lazarin GA, Hawthorne F, Collins NS, Platt EA, Evans EA, Haque IS. Systematic classification of disease severity for evaluation of expanded carrier screening panels. *PLoS One*. 2014;9(12):e114391. http://doi.org/10.1371/journal.pone.0114391
10. Davidov B, Levon A, Volkov H, et al. Pathogenic variant-based preconception carrier screening in the Israeli Jewish population. *Clin Genet*. 2022;101(5-6):517-529. http://doi.org/10.1111/cge.14131
11. Mathijssen IB, Florijn RJ, van den Born LI, et al. Long-term follow-up of patients with retinitis pigmentosa type 12 caused by CRB1 mutations: a severe phenotype with considerable interindividual variability. *Retina*. 2017;37(1):161-172. http://doi.org/10.1097/IAE.0000000000001127
12. Fridman H, Yntema HG, Mägi R, et al. The landscape of autosomal-recessive pathogenic variants in European populations reveals phenotype-specific effects. *Am J Hum Genet*. 2021;108(4):608-619. http://doi.org/10.1016/j.ajhg.2021.03.004
13. Mor-Shaked H, Rips J, Gershon Naamat S, et al. Parental exome analysis identifies shared carrier status for a second recessive disorder in couples with an affected child. *Eur J Hum Genet*. 2021;29(3):455-462. http://doi.org/10.1038/s41431-020-00756-y
14. Thompson ML, Finnila CR, Bowling KM, et al. Genomic sequencing identifies secondary findings in a cohort of parent study participants.

Genet Med. 2018;20(12):1635-1643. http://doi.org/10.1038/gim.2018.53

15. Schwartz MLB, McCormick CZ, Lazzeri AL, et al. A model for genome-first care: returning secondary genomic findings to participants and their healthcare providers in a large research cohort. *Am J Hum Genet*. 2018;103(3):328-337. http://doi.org/10.1016/j.ajhg.2018.07.009
16. Haer-Wigman L, van der Schoot V, Feenstra I, et al. 1 in 38 individuals at risk of a dominant medically actionable disease. *Eur J Hum Genet*. 2019;27(2):325-330. http://doi.org/10.1038/s41431-018-0284-2
17. Gordon AS, Zouk H, Venner E. Frequency of genomic secondary findings among 21,915 eMERGE network participants. *Genet Med*. 2020;22(9):1470-1477. http://doi.org/10.1038/s41436-020-0810-9
18. Struewing JP, Hartge P, Wacholder S, et al. The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. *N Engl J Med*. 1997;336(20):1401-1408. http://doi.org/10.1056/NEJM199705153362001
19. MacArthur DG, Balasubramanian S, Frankish A, et al. A systematic survey of loss-of-function variants in human protein-coding genes. *Science*. 2012;335(6070):823-828. http://doi.org/10.1126/science.1215040
20. Cummings BB, Karczewski KJ, Kosmicki JA, et al. Transcript expression-aware annotation improves rare variant interpretation. *Nature*. 2020;581(7809):452-458. http://doi.org/10.1038/s41586-020-2329-2
21. Schoch K, Tan QKG, Stong N, et al. Alternative transcripts in variant interpretation: the potential for missed diagnoses and misdiagnoses. *Genet Med*. 2020;22(7):1269-1275. http://doi.org/10.1038/s41436-020-0781-x
22. Carlston CM, O'Donnell-Luria AH, Underhill HR, et al. Pathogenic ASXL1 somatic variants in reference databases complicate germline variant interpretation for Bohring-Opitz syndrome. *Hum Mutat*. 2017;38(5):517-523. http://doi.org/10.1002/humu.23203
23. Coban-Akdemir Z, White JJ, Song X, et al. Identifying genes whose mutant transcripts cause dominant disease traits by potential gain-of-function alleles. *Am J Hum Genet*. 2018;103(2):171-187. http://doi.org/10.1016/j.ajhg.2018.06.009
24. Park J, Colombo R, Schäferhoff K, et al. Novel HIVEP2 variants in patients with intellectual disability. *Mol Syndromol*. 2019;10(4):195-201. http://doi.org/10.1159/000499060
25. Schupmann W, Miner SA, Sullivan HK, et al. Exploring the motivations of research participants who chose not to learn medically actionable secondary genetic findings about themselves. *Genet Med*. 2021;23(12):2281-2288. http://doi.org/10.1038/s41436-021-01271-1
26. Miner SA, Similuk M, Jamal L, Sapp J, Berkman BE. Genomic tools for health: secondary findings as findings to be shared. *Genet Med*. 2022;24(11):2220-2227. http://doi.org/10.1016/j.gim.2022.07.015
27. Quaio CRDC, Moreira CM, Novo-Filho GM, et al. Diagnostic power and clinical impact of exome sequencing in a cohort of 500 patients with rare diseases. *Am J Med Genet C Semin Med Genet*. 2020;184(4):955-964. http://doi.org/10.1002/ajmg.c.31860
28. Halldorsson BV, Eggertsson HP, Moore KHS, et al. The sequences of 150,119 genomes in the UK Biobank. *Nature*. 2022;607(7920):732-740.