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The prevalence of prenatal sonographic findings in postnatal diagnostic exome sequencing performed for neurocognitive phenotypes: A cohort study

Rivka Sukenik-Halevy[1,2] | Sharon Perlman[2,3] | Noa Ruhrman-Shahar[1] | Offra Engel[4] | Naama Orenstein[5] | Claudia Gonzaga-Jauregui[6] | Alan R. Shuldiner[6] | Regeneron Genetics Center[6] | Nurit Magal[1] | Ofir Hagari[1] | Noy Azulay[1] | Gabriel Arie Lidzbarsky[1] | Lily Bazak[1] | Lina Basel-Salmon[1,2,5,7]

[1]Raphael Recanati Genetic Institute, Rabin Medical Center – Beilinson Hospital, Petah Tikva, Israel

[2]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

[3]Ultrasound Unit, Helen Schneider Women's Hospital, Rabin Medical Center, Petah Tikva, Israel

[4]Department of Obstetrics and Gynecology, Meir Medical Center, Kfar Saba, Israel

[5]Pediatric Genetics Clinic, Schneider Children's Medical Center of Israel, Petah Tikva, Israel

[6]Regeneron Genetics Center, Tarrytown, New York, USA

[7]Felsenstein Medical Research Center, Petah Tikva, Israel

Correspondence
Rivka Sukenik-Halevy, Genetics Institute, Meir Medical Center, 59 Tschernihovsky St. Kfar Saba 44281 Israel.
Email: Riki.sukenik@gmail.com

Abstract

Objective: Prenatal exome sequencing (ES) is currently indicated for fetal malformations. Some neurocognitive genetic disorders may not have a prenatal phenotype. We assessed the prevalence of prenatally detectable phenotypes among patients with neurocognitive syndromes diagnosed postnatally by ES.

Methods: The medical files of a cohort of 138 patients diagnosed postnatally with a neurocognitive disorder using ES were reviewed for prenatal sonographic data. The Online Mendelian Inheritance in Man (OMIM) database was searched for prenatally detectable phenotypes for all genes identified.

Results: Prenatal imaging data were available for 122 cases. Of these, 29 (23.75%) had fetal structural abnormalities and another 29 had other ultrasound abnormalities (fetal growth restriction, polyhydramnios, elevated nuchal translucency). In 30 patients, structural aberrations that were not diagnosed prenatally were detected at birth; in 21 (17.2%), the abnormalities could theoretically be detected prenatally by third-trimester/targeted scans. According to OMIM, 55.9% of the diagnosed genes were not associated with structural anomalies.

Conclusions: Most patients (52.5%) with postnatally diagnosed neurocognitive disorders did not have prenatal sonographic findings indicating prenatal ES should be considered. The prevalence of specific prenatal phenotypes such as fetal growth restriction and polyhydramnios in our cohort suggests that additional prenatal findings should be assessed as possible indications for prenatal ES.

Key points

What's already known about this topic?

- Prenatal exome sequencing (ES) is currently indicated for fetal malformations.
- Some neurocognitive genetic disorders may not have prenatal phenotypes.

What does this study add?

- We assessed the prevalence of prenatally detectable phenotypes among 138 patients with neurocognitive syndromes diagnosed postnatally by ES.
- Fetal structural abnormalities were present in 23.75%.
- Other ultrasound abnormalities (such as fetal growth restriction, polyhydramnios) were reported in 23.75%.
- Most patients diagnosed with neurocognitive disorders did not have an indication for prenatal ES.

1 | INTRODUCTION

Exome sequencing (ES) is performed for a wide range of indications including intellectual disability, neurological phenotypes, and congenital anomalies. In the prenatal setting, the only current suggested indication for ES is abnormal fetal ultrasound findings, specifically, structural malformations.[1,2]

In postnatal cohorts, ES has been found to be diagnostic in 25%–31% of cases.[3-7] Detection rates for neurodevelopmental disorders, especially if presenting with additional phenotypes, are even higher, at 27%–60%.[5-8] In a recent meta-analysis, the reported yield of ES was 36% for all neurocognitive phenotypes, 31% for isolated neurodevelopmental disorders, and 53% for neurodevelopmental disorders together with associated conditions.[5]

Small early cohort studies of prenatal ES reported a diagnostic yield of 10%–57%.[9] In the two largest studies of fetuses with structural anomalies, prenatal ES detected pathogenic (P) variants in 8.5%–10% of cases; the rate rose to up to 30% when potential/likely pathogenic variants (LP) were included.[10,11] Hence, the detection rate of prenatal ES is somewhat different than that of postnatal ES in cases of neurocognitive phenotypes. The reported diagnostic yield in very small cohorts of fetuses diagnosed with brain anomalies is around 50%,[12-14] however, neurocognitive syndromes may present with normal fetal brain imaging. Prenatal ES has the potential to detect genetic disorders with a wide range of phenotypes, including neurocognitive phenotypes, that are commonly undetectable during pregnancy. While data analysis of postnatal ES is phenotype-driven, in the prenatal setting, phenotype-driven ES is applicable when the suspected disorder has associated prenatally detectable phenotypes such as fetal structural abnormalities, growth restriction, or polyhydramnios. However, if neurocognitive abnormality is the only phenotype characterizing a certain disorder, variants in genes related to such disorders will be considered an incidental finding. Information regarding the rate of incidental findings in prenatal ES is limited since many studies do not include incidental findings in their report.

The aim of this study was to assess the prevalence of prenatally detectable phenotypes among patients in whom neurocognitive phenotypes or severe childhood-onset neuromuscular disorders were diagnosed postnatally by ES. Information regarding prenatal phenotypes related to such disorders can aid in establishing novel correlations between neurocognitive genes and their related prenatal phenotypes and highlight specific prenatal findings that should be considered as possible indications for prenatal ES. In addition, we sought to determine how many of these disorders could have been detected prenatally based on current indications for ES.

2 | MATERIALS AND METHODS

2.1 | Study setting and participants

During 2015–2020, 601 postnatal ES tests were analyzed at the Raphael Recanati Genetic Institute of Rabin Medical Center. The indication for ES was suspicion of an undiagnosed or heterogenous monogenic disorder by a clinical geneticist following normal chromosomal microarray analysis and, in appropriate cases, single gene or gene panel testing. ES resulted in a diagnosis in 207 patients, including 138 in whom a variant related to a neurocognitive phenotypes or severe childhood-onset neuromuscular disorders was identified; these patients formed the study group (Table 1). ES was performed on a clinical (76.8%) or research (23.2%) basis on the proband-parent trio in 84.1% of cases, on the proband and one parent (with or without additional siblings) in 13% of cases, and on only the proband in 2.9% of cases. All parents/probands received genetic counseling.

Data regarding the study cohort including the age, gender and proband's phenotype provided by the clinician in HPO terms is presented in Supplementary Table S1.

The study was approved by the institutional Research Ethics Committee.

2.2 | Exome sequencing

Of the 601 ES tests, 162 were performed at 1 of 5 accredited laboratories and the data were interpreted by both the external laboratories team and departmental team. Part of this group has been previously described.[15] An additional 127 tests were performed as part of the research collaboration between the Raphael Recanati Genetic Institute and the Regeneron Genetics Center (RGC), as described previously[15] and the remaining 312 tests were done by CeGat laboratory (CeGaT GmbH, Tübingen, Germany). Targeted capture of protein-coding regions was performed using one of the following kits: SureSelectXT Exome V6 (Agilent Technologies, Santa Clara, CA, USA), Twist Human Core Exome or Twist Human Core Exome Plus Kit (Twist Bioscience, San Francisco, CA, USA).

TABLE 1 Characteristics of 138 probands with neurocognitive phenotypes diagnosed postnatally by ES

Characteristics	
Age (yr), mean + SD	10.2 ± 9.9
Gender, n (%)	
Male	82 (59.4)
Female	56 (40.6)
Parental consanguinity, n (%)	
Yes	16 (11.6)
No	122 (88.4)
Exome setting, n (%)	
Clinical	106 (76.8)
Research	32 (23.2)
Number of tested individuals, n (%)	
Single	4 (2.9)
Proband and parents	116 (84.1)
Other (additional family members or only one parent)	18 (13.0)
Mode of inheritance of diagnosed disorders, n (%)	
Autosomal dominant	87 (63.0)
De novo	71 (81.6)
Inherited	10 (11.5)
Unknown	6 (6.9)
Autosomal recessive	34 (24.6)
Homozygous	26 (76.5)
Compound heterozygous	8 (23.5)
X-linked (dominant, recessive, both)	17 (12.3)
De novo	10 (58.8)
Inherited	5 (29.4)
Unknown	2 (11.8)

Abbreviations: ES, exome sequencing; SD, standard deviation.

Paired-end libraries were prepared from captured fragments and sequenced on the Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA). The desired coverage level was 20X or greater for at least 97% of target bases (95% at >100x). The study Bioinformatics team reviewed the FASTQ files and performed analysis for all cases (clinical ES tests and research ES tests).

2.3 | Bioinformatics analysis

FASTQ files, along with information on phenotypes using human phenotype ontology (HPO) terms and family structure, were uploaded into Emedgene's HIPAA-compliant platform (Emedgene Technologies, Ltd, Mazor) and analyzed as described previously.[12] Briefly, parameters used for variant interpretation included mapping quality ≥45 and depth ≥10, population frequency (1% or 5% for dominant or recessive inheritance, respectively), and variant severity. Analysis of copy number variants was not performed. Variants were classified according to the criteria of the American College of Medical Genetics (ACMG).[16] If segregation in additional family members was needed, amplicons containing variants of interest were analyzed by Sanger sequencing. If a single mutation in an autosomal recessive gene related to a very specific phenotype was detected, deletions in the second copy of the gene were searched by additional methods.

We selected all cases in which P/LP variants in genes related to neurocognitive phenotypes or severe childhood-onset neuromuscular disorders were identified on ES.

2.4 | Clinical data collection

To determine the number of subjects with a prenatal phenotype, we searched the medical records for all data pertaining to the prenatal sonographic evaluation as well as abnormal findings detected at birth. We excluded cases for which we did not have information regarding prenatal work-up, in addition to cases in which the subject was more than 25 years old (as access to their prenatal data was limited). In total 16 cases were excluded.

In Israel, the Society for Maternal-Fetal Medicine and the Society for Ultrasound in Obstetrics and Gynecology currently recommend performing a nuchal translucency (NT) ultrasound scan at 11–14 weeks, and a routine anatomical between 20 and 25 weeks of gestation. If fetal structural abnormalities are detected, targeted anatomical scans and follow-up are performed according to local and international guidelines (Society for Maternal-Fetal Medicine [SMFM], International Society for Ultrasound in Ob GYN [ISUOG]).

We calculated the number of cases in which fetal anomalies were detected prenatally out of the total number of cases with neurocognitive syndromes diagnosed postnatally using ES for which sufficient data regarding prenatal anatomical scans were available. In cases where a structural malformation was diagnosed postnatally, we assessed it according to the professional committee guidelines and classified it as detectable by routine anatomical scans/detectable by targeted scans/not-detectable prenatally.

In addition, we searched the Online Mendelian Inheritance in Man (OMIM: www.omim.org; Clinical synopsis) for prenatally detectable phenotypes for all identified diagnostic genes.

3 | RESULTS

3.1 | Genes and phenotypes according to OMIM

Among the 138 patients diagnosed with neurocognitive phenotypes or severe childhood-onset neuromuscular disorders on postnatal ES, variants in a total of 114 genes were considered causative. Variants in 12 genes were identified in two subjects and variants in six genes were diagnosed in three. For three genes, a gene-phenotype

association has been reported at the time the report was issued,[17-19] however the OMIM database has not been updated, hence there was no OMIM phenotype listed. For the other 111 genes, an OMIM phenotype was listed, as shown in Figure 1. In 49 disorders related to these genes (44.1%; diagnosed in 67 cases), congenital malformations were listed in the OMIM clinical synopsis, and for five genes (4.5%) diagnosed in five cases, prenatal phenotypes other than congenital malformations were listed (low birth weight in three and decreased fetal movements defined as a subjective patient report in two). In 10 cases (9%), the gene-related disorder was associated with fetal growth anomalies (fetal growth restriction or overgrowth; Figure 1).

Data regarding the genes and specific variants detected in the probands included in the study cohort is presented in Supplementary Table S1.

3.2 | Prenatally detected anomalies

Of the 138 patients, 16 patients were excluded from the analysis regarding prenatal reported phenotypes because the proband was more than 25 years old and we could not assume a routine anatomical scan was performed (n = 9), prenatal testing data were unavailable (n = 3), or prenatal follow-up did not include an anatomical scan (n = 4; Figure 2). NT measurements were available in 61 cases.

The prenatal ultrasound was defined as abnormal in 58 cases (47.5%), including 17 (29.3%) in which the abnormal findings were identified during the third trimester. Fetal structural abnormalities were detected in 29 cases (23.75%). These included: heart malformations (ventricular septal defect, atrial septal defect), brain anomalies (ventriculomegaly/hydrocephalus, abnormal brain sulcation, polymicrogyria, partial agenesis of the corpus callosum, vermis anomaly, microcephaly, macrocephaly), kidney malformations (horseshoe kidney, hydronephrosis, dysplastic kidneys) gastrointestinal anomalies (absent gallbladder) skeletal anomalies (clubfoot, short long bones, polydactyly), craniofacial anomalies (cleft lip). In some cases, more than one anomaly was detected. In the other 29 (23.75%), there were no prenatally detectable malformations, but other sonographic anomalies were found, namely, fetal growth restriction (below the 3ed percentile for gestational age) in 14 (11.5%; as an isolated finding in 10) polyhydramnios (amniotic fluid index above the 97th percentile for gestational age) in 10 cases (8.2%) (as an isolated finding in 4), elevated NT (above 3 mm) in 7 (5.7%) (as an isolated finding in 3). In four cases isolated "soft" ultrasonographic signs, were reported including intracardiac echogenic foci, Choroid plexus cyst (CPC), a case of CPC and cervical cyst and a case of mild unilateral pyelectasis (Figure 2). Of the cohort of 122 cases, 52.5% has no prenatal sonographic abnormality reported.

Data regarding the prenatally reported phenotypes is presented in Supplementary Table S1.

3.3 | Phenotypes present at birth

An abnormal phenotype was present at birth in 63 cases (51.6%). In 30 cases (24.6%) there were no abnormal findings reported during
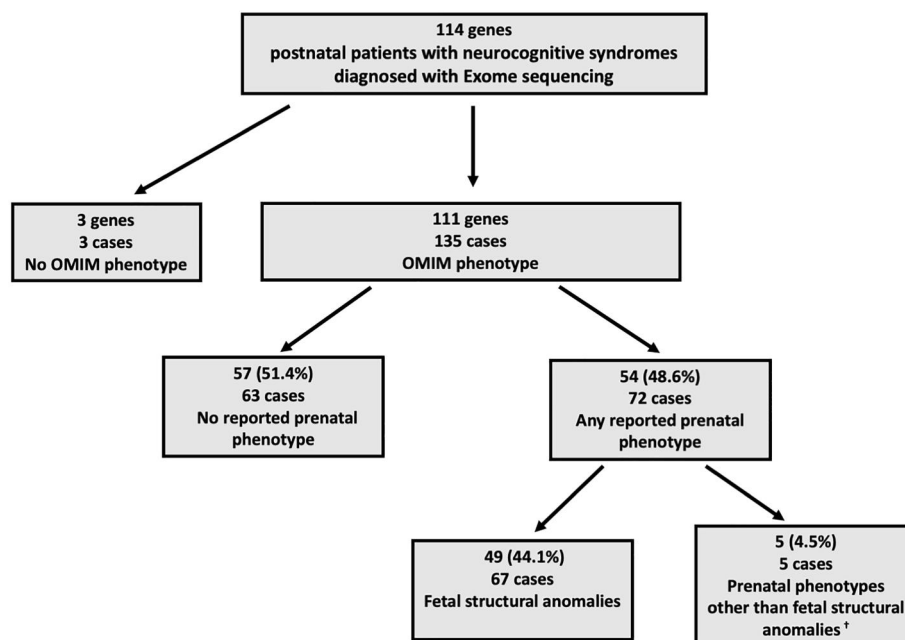
FIGURE 1 Neurocognitive genes and related prenatally reported phenotypes. Prenatal phenotypes of the genes detected in the postnatal cohort were retrieved from Online Mendelian Inheritance in Man (OMIM) clinical synopsis (https://www.ncbi.nlm.nih.gov/omim). [†]Other sonographic anomalies that could suggest an increased likelihood of a genetic syndrome such as fetal growth restriction, polyhydramnios, elevated nuchal translucency
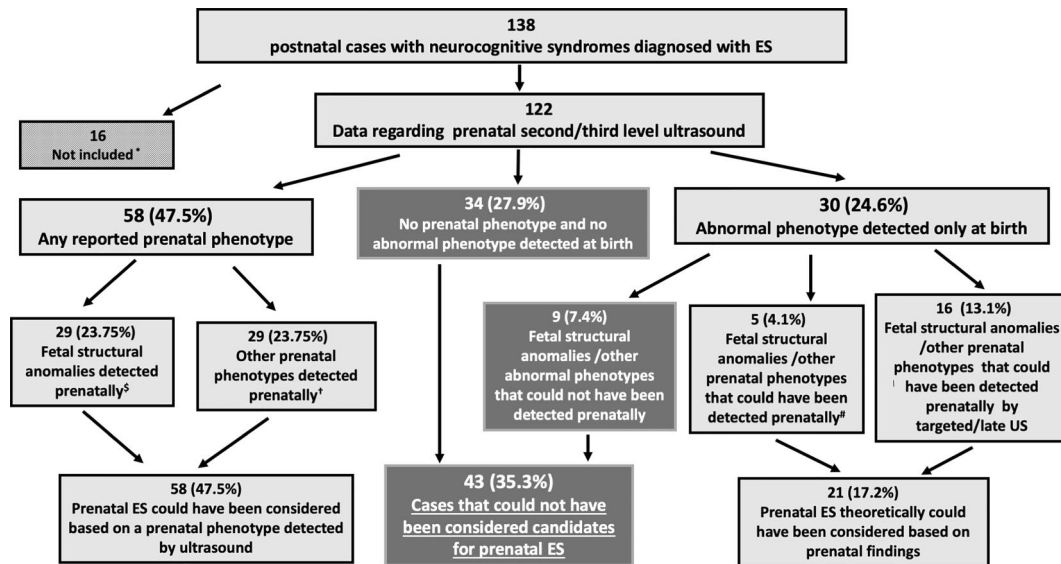
FIGURE 2 Clinical prenatal and postnatal phenotypes in the postnatal cohort diagnosed with Neurocognitive disorders or severe childhood-onset neuromuscular disorders. *Not included: 9-Adults, 3-No data available, 4-Anatomical scans not performed. $Cases where ES is suggested by current guidelines. †Other sonographic anomalies that could suggest an increased likelihood of a genetic syndrome such as; fetal growth restriction (below the third percentile for gestational age), polyhydramnios (above the 97th percentile for gestational age), dysmorphic features, elevated nuchal translucency. # Malformations/other abnormal phenotypes that could have been detected prenatally by routine anatomical scan. ES, exome sequencing; US, ultrasound

the pregnancy (in these cases second/third-level ultrasound scan was performed but was reported to be normal). On revision of these cases considering the guidelines for fetal anatomical scans, we concluded that theoretically, in 5/122 (4.1%), fetal structural abnormalities could have been detected by routine anatomical scan. In an additional 16/122 cases (13.1%), the malformations could have been detected only on a targeted scan performed during the third trimester. Thus, even after theoretical manipulation, only 21 (17.2%) of the abnormal phenotypes detected at birth could have been detected by fetal ultrasound.

Thus altogether, in 79 cases (64.7%), abnormal prenatal findings were either diagnosed prenatally by ultrasound (58 cases) or could theoretically have been diagnosed prenatally according to findings detected at birth (21 cases; Figure 2), and hence prenatal ES might have been considered. In 43 cases (35.3%), there were no abnormal sonographic findings that could have been detected during the pregnancy that could have suggested a discussion regarding prenatal ES, even if comprehensive sonographic evaluation and follow-up would have been performed. Data regarding the phenotypes detected after birth is presented in Supplementary Table S1.

4 | DISCUSSION

We assessed the prevalence of prenatally detected fetal structural abnormalities and other sonographic anomalies among patients diagnosed with neurocognitive phenotypes or severe childhood-onset neurological disorders diagnosed postnatally by ES. We found

that in less than half the patients there were structural abnormalities or other anomalies detectable on prenatal ultrasound.

Currently prenatal ES is suggested only in cases of known fetal anomalies,[1,2] and the findings are intended to explain the prenatal phenotype. However, ES analysis may also yield incidental findings, including variants in genes associated with neurocognitive phenotypes and other severe childhood-onset disorders that are not related to the prenatal phenotype. Fu et al.[20] reported incidental findings in 6.1% of 196 cases in which prenatal ES was performed to evaluate fetal malformations, and Petrovski et al.[11] reported that in 4 out of 234 prenatal ES tests, conditions unrelated to the fetal structural anomalies were diagnosed, none of which was related to a neurocognitive phenotype.

The guidelines of the ACMG stipulate that incidental findings on ES should be reported.[2] In some of these disorders, such as those related to neurocognitive phenotypes, the phenotypic spectrum does not always include anatomic malformations or other prenatally recognizable phenotypes. In 51.4% of cases in the present study, the gene related to the diagnosed disorder has not been reported to be related to congenital malformations or other prenatal phenotypes according to the OMIM database. However, this finding should be interpreted with caution because there are currently no dedicated fetal genotype–phenotype correlation databases, and for many disorders, data on prenatal phenotypes are scarce. Therefore, we assumed that retrospective analysis of the prenatal sonographs of individuals with postnatally diagnosed monogenic disorders could shed light on the proportion of cases for which prenatal ES could have been recommended based on the fetal sonographic findings. In addition, such analysis could aid in establishing novel correlation

between genetic syndromes and their prenatal phenotypes and highlight specific prenatal anomalies that should be considered as possible indications for prenatal ES. Looking retrospectively, in our cohort, fetal structural abnormalities were detected prenatally in 23.75% of subjects. Therefore, if prenatal ES is performed solely for the indication of fetal structural abnormalities, many severe monogenic disorders might be missed. In some of the cases included in our study, only a single anomaly was detected, including anomalies commonly associated with multifactorial inheritance (four cases of ventricular septal defect and one case of unilateral hydronephrosis). These cases would probably have been considered as cases without a high likelihood of a monogenic genetic syndrome. Of note, recent data, indicates that the diagnostic yield of prenatal ES in cases of isolated congenital heart defects is 6.5%[21] and the reported yield for isolated septal defects is 7.1%.[22]

A major limitation for performing prenatal ES is that there are limited data regarding the prenatal presentation of many genetic syndromes. This creates a significant challenge when analyzing ES results in cases where findings that do not have a known prenatal phenotype are detected (incidental findings). The formation of large and updated databases targeted at providing information regarding specific fetal phenotypes such as elevated NT, abnormal amniotic fluid volume, abnormal prenatal growth and other prenatal phenotypes will help to establish better prenatal genotype/phenotype correlations and to have more confidence in the interpretation of ES findings.

Increased NT is considered a prenatal phenotype for which testing for a monogenic disorder using, for example, a RASopathy gene panel, should be considered. Two large studies of prenatal ES[10,11] included cases of increased NT. Recently, several scholars have assessed the yield of ES in cases of increased nuchal translucency. Sparks el al.[23] reported a diagnostic yield of 31% in a cohort of 29 cases with cystic hygroma or NT above 3.5 mm, however, the diagnostic yield among the 15 isolated cases was only 7%. Yang et al.[24] assessed the yield of a panel of 4200 clinically relevant disease-causing genes in 73 fetuses with increased NT (≥3.5 mm) and found a disease-causing variant in 4 cases. In 3 out of 4 cases structural anomalies on ultrasound were detected at mid pregnancy hence the diagnostic yield of isolated NT was 1.4%. In a larger cohort reported by Mellis et al.[25] the diagnostic yield among 213 fetuses with increased NT ≥ 3.5 recruited to the Prenatal Assessment of Genomes and Exomes (PAGE) and Columbia fetal whole exome sequencing reported diagnostic variants was 22.2% for fetuses presenting with non-isolated increased NT. The yield was higher (32.4%) for fetuses with isolated increased NT in the first trimester and additional abnormalities later in pregnancy, but was only 1.8% in 111 fetuses with no other abnormalities on subsequent scans. The authors concluded that the diagnostic yield of prenatal ES is low for fetuses with isolated increased NT but significantly higher where there are additional structural anomalies. In our cohort, increased NT was present in seven patients, including three in whom it was the only sonographic anomaly and one case where the only other findings was an echogenic focus in fetal heart. Interestingly, none of the genes

implicated in these cases are included in current RASopathy gene panels. This may suggest that ES should be offered as a more comprehensive approach to couples opting to perform additional genetic work-up for pregnancies with elevated NT following normal CMA results.

Performing prenatal ES for indications of fetal growth abnormalities and polyhydramnios, although perhaps considered appropriate by some professionals, is an expansion of the current guidelines. To the best of our knowledge, there are currently no published cohorts reporting prenatal ES results in cases of isolated fetal growth abnormalities and polyhydramnios, hence further studies are needed to shed light on the diagnostic yield in these cases. In our study, including abnormal ultrasonic findings other than congenital fetal structural abnormalities would have increased the proportion of candidates for prenatal ES from 23.75% to 47.5%. Importantly, in 8.2% of these fetuses, the only abnormal sonographic finding was growth restriction.

According to the data presented here, and in line with the current literature, in low-risk, non-referred populations, initial standard sonograms do not identify a substantial proportion of fetal anomalies.[26] In our cohort, second-trimester ultrasound did not detect up to ~25% of structural abnormalities detected at birth. This constitutes a considerable limitation of the practice of performing prenatal ES for the sole indication of sonographically detected anomalies.

Ultrasound is an operator-dependent imaging modality. Prenatal ultrasound doesn't identify a substantial proportion of postnatally detectable phenotypes, for example, low-set ears, subtle facial dysmorphism, abnormal hairline, simian line and other subtle phenotypic features. In the context of prenatal fetal structural abnormalities, its ability to detect abnormal fetal phenotypes is challenged by maternal habitus, fetal position, amniotic fluid index, and gestational age. Thus, to assess the prevalence of prenatal findings in the postnatal diagnostic exome, we reassessed the cohort and considered if malformations that were detected at birth hypothetically could have been diagnosed prenatally, with inclusion of findings that could only be detected late in pregnancy or by targeted scans. According to our results, even if all congenital malformations diagnosed prenatally, together with sonographic findings other than fetal structural abnormalities are considered and including malformations that hypothetically could have been diagnosed prenatally by targeted scans; more than 35% of our subjects did not have any sonographic anomaly and would not have been referred prenatally for ES.

Detection of sonographic anomalies that can be visible late in the pregnancy and may lead to a diagnosis of a neurocognitive syndrome may aid in perinatal management. This also provides patients valuable information regarding the prognosis and enables them to prepare for the future. In addition, in extremely severe conditions decisions about continuing the pregnancy may be considered in countries where advanced pregnancy interruptions are optional.

Based on our findings, we recommend further discussion on expanding the indications for prenatal ES. In Israel, all couples are informed of the option to perform ES, and utilization of prenatal ES testing in non-malformed fetuses is increasing. In some cases, couples

that have an affected child due to a de novo variant seek unindicated ES in their next pregnancy. Another potential scenario might be advanced parental age. When performing prenatal ES in cases with no abnormal prenatal phenotype, all variants detected are equivalent to incidental findings. A recently published study reported a diagnostic yield of 0.62% for ES performed in 160 pregnancies with no sonographic anomalies, and a diagnostic yield of 10% among 50 cases with minor sonographic anomalies.[27] To the best of our knowledge, there are currently no other data regarding the yield of unindicated prenatal ES.

Performing ES for fetuses without structural abnormalities detected by US has potential risks and disadvantages, specifically when incidental findings are interpreted for disorders presenting with exclusively postnatal phenotypes. The dangers of unindicated fetal ES include making a false-positive diagnosis, identifying a mosaic fetus that might never express the disorder, or diagnosing a condition that might be nonpenetrant. These situations can potentially lead to unnecessary parental anxiety and, in some cases, unnecessary pregnancy termination. The tradeoff with minimizing the possibility of missed diagnoses of severe neurodevelopmental disorders is not easily resolvable.

This study was limited by the retrospective design. It is important to emphasize that the study was not intended to analyze the diagnostic rate of unindicated prenatal ES nor was it designed to report the yield of prenatal ES for specific sonographic anomalies. In addition, the rate of fetal structural abnormalities and other prenatally detected phenotypes may differ between individuals with neurocognitive phenotypes and individuals with other categories of genetic disorders. We selected cases of neurocognitive phenotypes because their prenatal detection may be an accepted approach in some countries. The rate of prenatal sonographic findings in the population of individuals with neurocognitive phenotypes that underwent ES with negative results was not assessed in this study. It would be interesting to analyze the rate of prenatal findings in this cohort in a follow-up study.

To conclude, performing prenatal ES solely for the indication of fetal structural abnormalities and/or additional sonographic anomalies could potentially lead to a significant number of undiagnosed neurocognitive disorders. Additional specific prenatal findings such as fetal growth restriction and polyhydramnios should be assessed as possible indications for prenatal ES.

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CONFLICT OF INTEREST

Alan R. Shuldiner is a full-time employee of the Regeneron Genetics Center from Regeneron Pharmaceuticals Inc. and receive salary and stock options as part of compensation. The remaining authors declare that they have no conflict of interest. Claudia Gonzaga-Jauregui was an employee at the time of the study.

DATA AVAILABILITY STATEMENT

Data available on request from the author.

ORCID

Rivka Sukenik-Halevy https://orcid.org/0000-0003-4418-7551

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.