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When genotype is not predictive of phenotype: implications for genetic counseling based on 21,594 chromosomal microarray analysis examinations

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Purpose: To compare the frequency of copy-number variants (CNVs) of variable penetrance in low-risk and high-risk prenatal samples and postnatal samples.

Methods: Two cohorts were categorized according to chromosomal microarray analysis (CMA) indication: group I, *low-risk prenatal*—women with uneventful pregnancy (control group); group II, *high-risk prenatal*—women whose fetuses had congenital malformations; and group III, *postnatal*—individuals with unexplained developmental delay/intellectual disability, autism spectrum disorders, or multiple congenital anomalies. CNVs were categorized based on clinical penetrance: (i) high (>40%), (ii) moderate (10–40%), and (iii) low (<10%).

Results: From 2013 to 2016, 21,594 CMAs were performed. The frequency of high-penetrance CNVs was 0.1% (21/15,215) in group I, 0.9% (26/2,791) in group II, and 2.6% (92/3,588) in group III. Moderate-penetrance CNV frequency was 0.3% (47/15,215), 0.6% (19/2,791), and 1.2% (46/3,588), respectively. These differences were statistically significant. The frequency of low-penetrance CNVs was not significantly different among groups: 0.6% (85/15,215), 0.9% (25/2,791), and 1.0% (35/3,588), respectively.

Conclusion: High-penetrance CNVs might be a major factor in the overall heritability of developmental, intellectual, and structural anomalies. Low-penetrance CNV alone does not seem to contribute to these anomalies. These data may assist pre- and posttest CMA counseling.

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Key Words: chromosomal microarray analysis (CMA); copy-number variation (CNV); penetrance; prenatal diagnosis

INTRODUCTION

Chromosomal microarray analysis (CMA) is currently recommended as a first-tier test for individuals with unexplained developmental delay/intellectual disability, autism spectrum disorders, or multiple congenital anomalies.[1] Six percent of pregnancies that resulted in structural malformations and normal karyotype had pathologic copy-number variations (CNVs). Moreover, pregnant women without medical indication for CMA (advanced maternal age) or positive maternal serum screening for Down syndrome who had normal fetal karyotype in prenatal studies were found to have 1.7% residual risk for pathogenic CMA.[2] Nevertheless, identical pathogenic CNVs, even among members of the same family, can have different clinical outcomes.[3–6] This phenomenon can be partially explained by incomplete penetrance. Additional factors might be involved in this process, such as the effect of other CNVs, single-nucleotide variants, and environmental or epigenetic factors.[4,7,8] The fact that many CNVs have incomplete penetrance poses a challenge for genetic counseling.[9] Prenatal counseling in these cases is complicated and knowledge of the penetrance level could help provide more informative counseling. Previous publications estimated the penetrance of common CNVs[5,6,10] and specifically for mental and neurological disorders.[11–13] Since most of the information regarding clinical presentation is from postnatal CMA tests, some of the assumptions and data used were questioned.[14] This study analyzed the penetrance of 20 known CNVs in prenatal and postnatal samples in order to suggest more informative counseling for these complicated cases.

MATERIALS AND METHODS

All CMAs were performed by two of the largest laboratories in Israel, both serving similar population groups. Whole-genome approach CMA, using single-nucleotide polymorphism (SNP)–based array platforms, was performed (Illumina, San Diego, CA) (**Supplementary Materials and Methods** online).

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Cohorts

CMA results from each laboratory were analyzed separately. The two cohorts consisted of 10,184 and 11,410 cases individuals, respectively, recruited from several genetic institutes. However, since the two cohorts were not statistically different from each other, we combine the cohorts for all analyses (**Table 1**). All cases were unrelated. Inclusion criteria were known specific indication and available CMA results. The cases were divided into three groups: group I, *low-risk prenatal*—women with uneventful (low-risk) pregnancy (the control group); group II, *high-risk prenatal*—women whose fetuses had structural malformations detected by prenatal ultrasound scan or magnetic resonance imaging studies (high-risk prenatally); and group III, *postnatal*— patients with intellectual disability, autism, other cognitive impairment and/or congenital malformation.

We focused on 20 recurrent pathogenic autosomal CNVs detected by CMA with documented penetrance. The two cohorts combined were divided into three categories based on the associated penetrance of these CNVs as reported in the literature (**Supplementary Materials and Methods: Tables S1–4**).

The prenatal and postnatal frequencies of CMA findings were compared in each penetrance category. The frequencies of these groups were compared to the low-risk group assuming they all have similar variance. A chi-square test was used for comparisons, and $P < 0.05$ was considered statistically significant (**Table 1**). The study was approved by the institutional review board (Beilinson Medical Center).

RESULTS

From 2013 through 2016, 21,594 CMA tests were performed on 15,215 low-risk pregnancies, 2,791 high-risk pregnancies, and 3,588 postnatal samples. Most of the prenatal CMAs were on DNA extracted from amniotic fluid. Chorionic villus sampling, before anatomic ultrasound of the fetus, was performed in fewer than 10% of the cases and was evenly distributed between groups I and II. All cases of postnatal CMA used DNA that was extracted from blood.

The first cohort consisted of 10,184 cases. Twenty recurrent CNV were detected in 55/5,149 cases (1.1%) in the low-risk prenatal group, in 56/2,447 cases (2.3%) in the high-risk prenatal group, and in 129/2,588 cases (5.0%) postnatally. The second cohort consisted of 11,410 cases. The same 20 recurrent CNVs were detected in 98/10,066 (1.0%) among the low prenatal risk group, in 14/344 (4.1%) in the high prenatal risk group, and in 44/1,000 (4.4%) postnatally. Penetrance category and the indication for CMA group were compared using the total number (**Table 1**).

High-penetrance CNV syndromes

High-penetrance CNVs were detected in 21/15,215 (0.1%) control cases (low-risk prenatal), 26/2,791 (0.9%) high-risk prenatal cases, and 92/3,588 (2.6%) postnatal cases. The difference was statistically significant ($P < 0.005$).

Table 1 The penetrance pattern according to chromosomal microarray analysis indication in each group

Combined cohorts							
Indication/penetrance	I: LR prenatal *N* (%)	II: HR prenatal *N* (%)	III: Postnatal *N* (%)	Total	*P* group I vs. II (95% CI)	*P* group I vs. III (95% CI)	*P* group II vs. III (95% CI)
High	21 (0.001)	26 (0.009)	92 (0.026)	139	<0.0001 (0.0826–0.2616)	<0.0001 (11.8356–30.6302)	<.0001 (1.8057–4.3375)
Moderate	47 (0.003)	19 (0.068)	46 (0.013)	112	<0.01 (0.2832–0.8170)	<0.0001 (0.1698–0.3795)	<.05 (0.3085–0.9028)
Low	85 (0.006)	25 (0.009)	35 (0.010)	145	<0.05 (0.3972–0.9727)	<0.005 (0.3841–0.8468)	NS (0.5479–1.5366)
Total	15,215	2,791	3,588	21,594			

CI, confidence interval; HR, high risk; LR, low risk; NS, not significant.

Medium-penetrance CNV syndromes

Medium-penetrance CNVs were detected in 47/15,215 (0.3%) control cases (low-risk prenatal), 19/2,791 (0.6%) high-risk prenatal cases, and 46/3,588 (1.2%) postnatal cases. The difference was statistically significant ($P < 0.005$).

Low-penetrance CNV syndromes

Low-penetrance CNVs were detected in 85/15,215 (0.6%) control cases (low-risk prenatal), 25/2,791 (0.9%) high-risk prenatal cases, and 35/3,588 (1.0%) in postnatal cases. The difference was not statistically significant ($P = 0.57$).

Sex bias

High-penetrance CNVs (all autosomes) were found in 139 cases (79 males and 60 females). However, the ratio of males to females was significantly higher only in the postnatal group (58/79 (73%) versus 34/60 (56%); $P < 0.05$).

DISCUSSION

The current study focused on the frequency of 20 recurrent CNV syndromes.[10,13] We analyzed CMA results from two laboratories that performed 21,594 CMA tests in similar populations. These CNVs were detected in 399 cases (1.8%): 1.0% (153/15,215) in group I (low-risk prenatally that served as a control group), 2.5% (70/2,791) in group II (high-risk prenatally due to malformations detected with ultrasonography or magnetic resonance imaging) and 4.8% (173/3,588) in group III (CMA performed postnatally).

High-penetrance CNVs occurred at a ratio of 1:40 (2.6%) for postnatal findings compared to 1:111 (0.9%) in high-risk pregnancies and 1:1,000 (0.1%) in low prenatal risk cases ($P < 0.001$). Compared to low-risk pregnancies, the frequencies of high-penetrance CNVs were 25-fold in postnatal testing and 9-fold in high-risk pregnancies. Analyzing moderate-penetrance CNVs showed a similar pattern, although with lower absolute values (1.3% postnatally, 0.7% high-risk, and 0.3% in low-risk prenatal cases; $P < 0.05$). The frequency of low-penetrance CNVs in postnatal testing was 1.0%, in high-risk pregnancies 0.9%, and in low-risk pregnancies 0.6%. Hence, this group did not exhibit the same pattern and no statistical difference was found between groups. This observation suggests that low-penetrance CNVs define a different group and probably do not cause developmental delay/intellectual disability, autism spectrum disorders, or multiple congenital anomalies by themselves. CNV studies are important for several reasons: (i) CNVs have a central role in the etiology of intellectual disability and neurodevelopmental problems,[11,12,15] (ii) CMA is becoming widespread in the prenatal setting,[2] (iii) CNVs might interact with point mutations with compound heterozygous genotypes,[16] and (iv) CNVs might interact with additional CNVs, resulting in a phenotype different than either CNV alone.[4] A "second-hit" model was proposed based on the observation that affected persons with a microdeletion on chromosome 16p12.1 are more likely to have more large CNVs than controls do.[17] Of note is the fact that the segment of chromosome 16p11.2—the region of the gene *SH2B1* that is covered by 57 probes and deletions or duplications—is well detected by our system. One possible explanation for the relatively low numbers detected in two large laboratories compared to other databases[18] might be a different population profile.

Our observations suggest that high-penetrance CNVs that have a major contribution to the overall heritability of developmental disorders are a more frequent cause of postnatal phenotype, and their representation in the prenatal setting is low. Analyzing specific CNVs in the high-penetrance category found a significant difference between group II and group III CNVs 2, 4, and 5, whereas CNVs 6, 7, and 8 did not show such a difference (**Supplementary Table S4**). A plausible explanation may be due to the fact that CNVs 6, 7, and 8 are associated with abnormal findings in imaging of the fetus ultrasound and magnetic resonance imaging. No such association exists in CNVs 2, 4, and 5. Low-penetrance CNVs are found in similar frequencies in all groups and are not overexpressed in males. This observation is in accordance with previous reports that found more boys with genomic disorders. These results led the authors to believe that females are less vulnerable to the effects of large variations than are males. This would explain the observation of an increased prevalence of neurodevelopmental disorders among males compared to females.[4] Thus, we suggest that low-penetrance CNVs detected in low-risk pregnancies should not be considered pathogenic and should not be used as phenotype predictors. They could act in concert with other genetic factors or as part of a multifactorial pattern of inheritance combined with environmental events, but their influence on phenotype cannot be predicted prenatally. Although 15q11.2 deletion is common, we do not think that this CNV has clinical impact that is characterized by cause–result relationship. We feel that this CNV increased selection bias and activates mainly through multifactorial mode of inheritance. Consequently, a prenatal report of a low-penetrance CNVs could create unfounded concerns. A study that found that penetrance may be quite different when looking at the effect of a genetic variation detected as part of a screening test in the general population (in our study represented by low-risk prenatal samples) compared to individuals who were evaluated postnatally due to a personal or strong family history of a neurological disorder[19] supports these conclusions. CNVs with moderate penetrance should be treated as a separate group. Their associated increased frequency in the postnatal group compared to low-penetrance CNV cases needs to be considered in cautious counseling, especially in the prenatal setting.

A limitation of the current study is that the control group comprised low-risk pregnancies that underwent CMA due to maternal request. The outcome of these pregnancies was not known in most cases. In addition, we evaluated only 20 common syndromes in our cohorts and did not analyze the rest.

In conclusion, low-penetrance CNV syndromes are not by themselves a cause of intellectual disability or congenital

anomalies. Thus, we suggest they should be classified as low-penetrance recurrent CNVs of unclear clinical significance, not otherwise specified. We recommend they should not be reported to the parents. CNVs with moderate penetrance constitute a unique group, found with increased frequency among individuals with developmental delay/intellectual disability, autism spectrum disorders, or multiple congenital anomalies, but definitely to a lesser extent than high-penetrance CNVs are. Prenatal counseling in these cases is expected to be complicated. The parents' CNVs status does not provide complete information about the expected postnatal phenotype because these CNVs are associated with syndromes with partial penetrance and variable clinical expression. The population frequency data are more informative. The frequency of pathogenic CNVs with different penetrance levels in pre- and postnatal CMA might assist couples and genetic counselors who are considering this test during pregnancy. Once recurrent CNVs are detected by CMA, the penetrance information and perhaps the fetal sex should be factors in genetic counseling.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

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DISCLOSURE

The authors declare no conflict of interest.

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