Prenatally detected copy number variants in a national cohort: A postnatal follow-up study

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What is already known about this topic?

- In 5 to 10 % of pregnancies with a fetal structural anomaly and in 0.5-2% of pregnancies without ultrasound anomalies, chromosomal microarrays (CMA) reveal cryptic, clinically relevant copy number variants (CNVs).
- Belgian genetic centers established a national prenatal database, gathering data on all prenatal CMAs performed since the switch from conventional karyotyping to CMA in 2013.

What does this study add?

- This study describes a national follow-up project to look at postnatal development in 3-year old children who were diagnosed prenatally with a non-benign CNV.
- To the best of our knowledge, this is the first nationwide project initialized to follow up children with prenatally detected CNVs.
- A significant difference in communicative and personal-social development was found in cases with a reported susceptibility CNV versus cases with an unreported susceptibility CNV and controls. To confirm these findings, a greater number of cases for each CNV category is needed.
- The study design can serve as an example for future postnatal follow up projects.

Abstract (max 200 words)

Objective

Belgian genetic centers established a database containing data on all chromosomal microarrays (CMA) performed in a prenatal context. A study was initiated to evaluate postnatal development in children diagnosed prenatally with a non-benign copy number variant (CNV).

Methods

All children diagnosed with a prenatally detected non-benign CNV in a Belgian genetic center between May 2013 and February 2015 were included in the patient population. The control population consisted of children who had undergone an invasive procedure during pregnancy, with no or only benign CNVs. Child development was evaluated at 36 months using three (3) questionnaires: Ages and Stages Questionnaire Third edition, Ages and Stages Questionnaire Social-Emotional Second Edition and a general questionnaire.

Results

A significant difference in communication and personal-social development was detected between children with a reported susceptibility CNV and both children with an unreported susceptibility CNV and the control population. The outcome of children with a particular CNV is discussed in a case-by-case manner.

Conclusion

Our postnatal follow-up project of children with a prenatally detected non-benign CNV is the first nationwide project of its kind. A higher number of cases for each CNV category is however needed to confirm our findings.

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Main document:

Prenatally detected copy number variants in a national cohort: a postnatal follow-up study.

Introduction

Following the introduction of chromosomal microarray (CMA) in prenatal invasive diagnosis, difficulties arose concerning the interpretation and reporting of prenatally detected copy number variants (CNVs) to future parents [1-7]. Although the added value of using CMA over conventional karyotyping for the analysis of invasively obtained prenatal samples is extensively proven [8-14], the higher resolution of the test not only increases detection of clinically relevant CNVs, but also reveals a higher number of variants of unknown significance (VOUS), incidental findings or variants with a variable expression or incomplete penetrance (susceptibility variants).

Publicly available CNV databases are valuable, but mainly rely on postnatal results and contain cases at the more severe end of the phenotypic spectrum, providing an incomplete characterization of the phenotype associated with a particular CNV, thus complicating the interpretation of prenatally detected CNVs. Additionally, upon reporting a CNV in a prenatal setting, future parents could consider discontinuing the pregnancy, even when only limited information exists on the variant found [1], or they may choose to continue the pregnancy, but remain anxious about the future health of their baby [5, 15].

In Belgium, all genetic centers embarked on a unique national project [7]. They agreed to use CMA for all indications for invasive prenatal testing. As previously published, a uniform national protocol on how to interpret and report variants was developed [3, 7]. (Table S1)

Furthermore, Belgian genetic centers established a shared prenatal database, gathering data on all prenatal CMAs performed since the switch from conventional karyotyping to CMA in

2013. This database facilitates data sharing and communication. In a recent study [3], analysis of the prenatal data gathered over a 3-year period showed pathogenic variants in 1.9% of cases; 71% of these cases were cryptic. The 22q11.2 deletion syndrome was the most frequently found genomic disorder. Of all cases, 1.6% carried a susceptibility CNV of which one-third (33.8%) was reported. The 22q11.2 duplication syndrome was the most frequent reported susceptibility CNV (SR for 'susceptibility reported'), and the 15q11.2 BP1-BP2 duplication the most frequent unreported susceptibility CNV (SNR for 'susceptibility not reported'). VOUS were detected in 5.6% of cases. The overall added value for using CMA instead of conventional karyotyping in all pregnancies where an invasive procedure was performed was 1.8%. The added value increased to 2.7% when anomalies were present in fetal ultrasound.

Since publicly available CNV databases do not provide a complete characterization of the phenotypic spectrum of a CNV, we initiated a national postnatal follow-up project to look at development in children diagnosed prenatally with a non-benign CNV in an unbiased manner. To the best of our knowledge, this is the first nationwide project initialized to follow up on children with prenatally detected CNVs.

Methods

The central ethical committee and the College for Human Genetics of the Federal Ministry of Public Health in Belgium approved this project.

Human reference genome GRCh37 – hg19 was used for indicating start and stop positions of the CNVs.

The patient population was defined as: all children diagnosed in a Belgian genetic center with a prenatally detected pathogenic CNV (including incidental findings, but excluding

aneuploidies and unbalanced translocations), susceptibility CNV (SR or SNR) or VOUS, collectively termed 'non-benign CNVs' between May 2013 and February 2015. The control population consisted of an equal number of children who had undergone an invasive procedure during pregnancy in the same study period, but had only benign CNVs or no benign CNVs. The goal was to create a similar distribution of indications for the invasive procedure compared to the patient population. Unless clear identification of each of the twin members was possible, twin pregnancies were excluded, as well as pregnancies that were known to be discontinued. After parental approval, child development was evaluated using 3 questionnaires when the child reached the age of 36 months.

The first questionnaire was the "Ages and Stages Questionnaire: a Parent-Completed Child Monitoring System, Third edition (ASQ-3)" [16]. This questionnaire contains 30 developmental items, organized in five areas: communication, gross motor, fine motor, problem solving and personal-social development and one overall section that focuses on general parental concerns. The scores are compared to the mean for each area of development, based on more than 18000 completed questionnaires. Children who score 1-2 standard deviations (SD) below the mean are considered to be in the monitoring zone and require close attention, specialized activities and/or repeat screening. If a child scores $\geq$ 2 SD below the mean, further diagnostic assessment is recommended for that specific area. Inclusion was allowed between 34 months 16 days and 38 months 30 days.

The second survey used was the "Ages and Stages Questionnaire: Social-Emotional Second Edition (ASQ-SE2)" [17], developed to complement the ASQ-3 and which focuses exclusively on the child's social-emotional behavior. If the child scores within the monitoring zone (close to the referral cutoff point), follow-up actions for items of concern are required. Children scoring below the referral cutoff point are identified as needing further attention. The ASQ-SE2 has a permitted age range between 33 months 0 days and 41 months 30 days.

The last survey was a general questionnaire, enquiring about parental age, parental education, ethnicity, course of pregnancy, delivery etc. This questionnaire was composed in collaboration with the Children's Neurodevelopmental Unit of the University Hospital in Antwerp, Belgium.

Patient and control samples were encoded. In each genetic center, only one researcher was granted authority to decode the center's samples and contact the parents. Only the encoded data were used for all further data processing.

Statistics

To test if cases versus controls and responders versus non-responders differed with regard to the indications, a Monte Carlo Chi-square test was carried out. The association between variant type and ASQ-3 and ASQ-SE2 results was tested using a one-way ANOVA, followed by a Posthoc analysis with Tukey correction for multiple testing. The effect of covariates on ASQ-3 and ASQ-SE2 scores was studied using multiple linear regression models with the scores as dependent variables, and parental level of education, multiple languages, surgical interventions, pre-term birth and age of the father as covariates. The model was simplified using stepwise backward elimination. Lastly, the association between pregnancy termination and variant type was investigated using a Monte-Carlo Chi-square test.

Statistical analyses were carried out using SPSS 24 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY.) and R, version 3.5.1 [18].

Results

A non-benign CNV was detected in 757 cases. These children are referred to as the patient group. The control population was composed of 793 random samples. Indications for

performing an invasive procedure on these samples are described in Table 1. There was no statistical difference in indications between both groups (p=0.23).

After excluding unidentifiable members of twin pregnancies, known discontinued pregnancies and patients whose addresses were unavailable, 616 and 719 questionnaires were sent to patients and controls, respectively. Ninety-three parents (93/616, 15.1%) from the patient population and one hundred and thirty-eight parents (138/719, 19.2%) from the control population participated in the study (Figure 1). A statistical difference between indications for performing an invasive procedure between responders and non-responders could be detected (p=0.026). Parents were more likely to participate if the indication for the invasive procedure was 'advanced maternal age' or an 'abnormal result for the Non-Invasive Prenatal Test (NIPT)'. Parents were less likely to participate if the indication for the invasive procedure was 'other indications', which mainly encompasses parental anxiety.

In the patient population, eight parents (8/93) indicated that the pregnancy was terminated: four because of the genetic result, as these fetuses carried a pathogenic variant, and four because of an ultrasound anomaly. None of the 93 patient responders indicated a neonatal death. Of the responders in the control population, thirteen parents (13/138) indicated that the pregnancy was discontinued because of an ultrasound anomaly. Two responders in the control population indicated their child died after birth; in both cases, the child had severe anomalies.

In total, questionnaires were completed for 208 children (85 patients and 123 controls) (Table S2). However, since not all parents completed the questionnaires at the required age, only 125 children were scored for ASQ-3 (41 patients and 84 controls) and 184 children for ASQ-SE2 (75 patients and 109 controls) (Figure 1). Characteristics of these two groups are summarized in Table S3a+b.

To determine the relationship between the genetic result and postnatal clinical and neurological development, the association between variant type and ASQ-3/ASQ-SE2 results was analyzed. Boxplots of ASQ-3 subcategories versus variant group are shown in Figure 2a. Note that there were no children of appropriate age for the ASQ-3 who had a pathogenic CNV. Also, since our reporting policy states not to communicate VOUS, inheritance was known in less than half of the cases (45.1%), and the VOUS population was excluded from all statistical analysis. To study the association between variant type and the five subcategories of the ASQ-3, a one-way ANOVA comparing children with an SR, an SNR and the control population was carried out. Significant differences between the groups were detected for communication and personal-social skills: a one-way ANOVA followed by a posthoc analysis showed that for both outcomes, children with an SR scored worse compared to the control and SNR categories, whereas there was no significant difference in mean outcome between the SNR and control groups. P-values for the different tests and differences in mean outcomes between the groups are shown in table 2. Multiple linear regression analysis showed that the covariates parental level of education, multiple languages, neonatal surgery, pre-term birth and age of the father had no significant effect on the ASQ-3 sub-scores. However, given the small inclusion numbers, no conclusions could be made regarding the covariables tested.

A boxplot of ASQ SE-2 results versus variant group is shown in Figure 2b. A one-way ANOVA comparing the pathogenic, SR, SNR and control groups was performed. No differences in social-emotional development were observed between variant groups ($p=0.069$). Although the boxplot suggests a worse outcome for children diagnosed with a pathogenic CNV, the results were not statistically significant, due to the small study population and the large standard deviation within groups (group means: control 45.825 ± 2.481; pathogenic 78.333 ± 14.956; SNR 43.75 ± 12.952; SR 64.167 ± 10.575). The

association between the ASQ-SE2 and the variant type was not influenced by accounting for the covariates parental level of education, multiple languages, possible operations, pre-term birth or age of the father. In addition, none of these covariates showed a significant effect on the ASQ-SE2. However, given the small inclusion numbers, no conclusions could be made regarding the covariables tested.

In conclusion, we found a statistical difference in performance between children with an SR and children with an SNR or the control population in the categories of communication skills and personal-social skills.

A second aspect that this dataset allows to investigate is the development of children with a specific CNV (susceptibility CNV, VOUS or pathogenic variant).

Susceptibility CNVs

Susceptibility CNVs have a highly unpredictable and often prenatally undetectable phenotype. Following the Belgian reporting system, only a defined number of susceptibility CNVs are reported. Postnatal follow-up of all children with a susceptibility CNV, either reported or not, provides an unbiased insight into the development of these children and helps us to assess the value of our policy. Table 3a+b shows the outcome of children with a reported versus not reported susceptibility CNV, provided that the questionnaire was completed within the correct age range.

a. Reported susceptibility CNVs

1q21.1 duplication syndrome

The chromosome 1q21.1 duplication syndrome, including *GJA5* (OMIM 612475), is a genetic risk factor for intellectual disability, developmental delay, autism spectrum disorder, schizophrenia, macrocephaly and coronary heart disease [19, 20]. The ClinGen dosage sensitivity

score for this duplication is 3. This score (https://www.ncbi.nlm.nih.gov/projects/dbvar/clingen/) refers to the evidence for pathogenicity for a haploinsufficiency phenotype (deletion) or a triplosensitive phenotype (duplication), ranging from 3 (sufficient evidence) to 0 (no evidence). Mean verbal and nonverbal IQ scores are in the low average range and motor function is nearly 2 S.D below age norms [20]. Another study showed that microduplications of 1q21.1 cause a range of developmental delays, neuropsychiatric abnormalities, dysmorphic features and a variety of other congenital anomalies [21]. The phenotype seems to be subject to incomplete penetrance, as Coe et al. reported 48/29085 cases with developmental delay and 5/19584 healthy controls, resulting in a likelihood ratio of 6.46 [22]. Because of the severity of the phenotype and the relatively high likelihood ratio, this duplication is reported in the Belgian prenatal setting. Invasive testing for case 207 in our study was performed because of toxoplasmosis seroconversion, but the fetus was not affected. A 1q21.1 duplication (chr1:145.899.339-147.887.735) was reported. The parents reported a normal pregnancy and delivery, no intervention of the pediatrician and a normal birthweight. However, the child failed all five subcategories of the ASQ-3 as well as the ASQ-SE2.

1q21.1 deletion syndrome

Individuals with the chromosome 1q21.1 deletion syndrome, including *GJA5* (OMIM 612474, Clingen score 3), are susceptible to intellectual disability, developmental delay, autism spectrum disorder, schizophrenia, facial dysmorphism, microcephaly, coronary heart disease, renal and urinary tract anomalies [20]. Coe et al. recorded a likelihood ratio of 7.63 for developmental delay in patients carrying this variant [22]. Two children with a 1q21.1 deletion syndrome participated in our study cohort. In the first case, case 29, the indication for the invasive procedure was intra-uterine growth restriction (IUGR). IUGR is not typically associated with deletion of this region [21]. The child passed all tests provided, although the

child was one month too old for the ASQ-3 survey. The second case, case 47, had an uneventful pregnancy and delivery and passed all tests as well. In conclusion, the two cases with a 1q21.1 deletion in our study performed within the normal range for all developmental areas tested.

15q26 deletion

Coe detected the 15q26 deletion syndrome (chr15:99.360.000-102.520.000), containing *IGFR1* (Clingen score 3), in 11/29085 pediatric patients with intellectual disability, developmental delay or autism spectrum disorder versus 1/19584 healthy controls, resulting in a likelihood ratio of 7.41 and a penetrance of 28.6% [22]. These findings, together with the importance of prenatal ultrasonographic follow-up, justify reporting this variant in a prenatal setting. Case 86 underwent invasive testing because of advanced maternal age and showed an intragenic 15q26 deletion in the *IGF1R* gene (chr.15:99396694-99465285). Veenma described a similar deletion of the *IGFR1* gene in a Dutch family [23] with pre- and postnatal growth retardation, mild to moderate small head circumference, minor facial dysmorphia and mild skeletal anomalies, but without mental retardation. The child in our study was carried to term and had a birth weight of 2630gr (5^{th} percentile). Pregnancy and delivery were uneventful, and the child passed all tests.

22q11.2 duplication

The proximal (A-B) 22q11.2 duplication syndrome is the most frequently reported susceptibility CNV in our Belgian prenatal population [3]. It has a Clingen score of 3 and is a susceptibility factor for developmental delay, epilepsy and dysmorphic features and can also cause microcephaly and coronary heart disease [22]. Although in the majority of cases the duplication is inherited from a normal parent [24], this susceptibility CNV is nevertheless reported prenatally in Belgium because of its possible association with fetal structural

anomalies and the importance of ultrasonographic follow-up. Two cases with such a CNV participated in the study. Case 15 had an uneventful pregnancy and delivery and passed all tests. Case 52 had an increased nuchal translucency at prenatal ultrasound and was born after an instrumental delivery. Parents completed the questionnaire at 40 weeks, hence too late for the ASQ-3. The child passed all tests, including the ASQ-SE2, but scored within the monitoring zone for communications skills. Since the child was too old for the test, the results suggest communicative development is delayed. Speech delay is described in children diagnosed with a 22q11.2 duplication [24-26].

b. Not-reported Susceptibility CNVs

15q11.2 BP1-BP2 duplication

The 15q11.2 BP1-BP2 duplication syndrome, containing *NIPA1* (chr.15: 22.832.519-23.090.897), is the most frequently found SNR in the Belgian prenatal population. It is described as a susceptibility factor for developmental delay, motor delay, speech delay and autism spectrum disorder [27], but its Clingen score is "unlikely", indicating that its pathogenicity is at present doubtful. The highly variable, often mild phenotype and the low penetrance and likelihood ratio justify our decision not to report this CNV [22, 28, 29]. In our study cohort, case 131 with this duplication (chr15:22.652.047-23.300.313) passed all tests provided.

16p13.11 deletion

Deletion of 16p13.11 containing *MYH11* is a susceptibility factor for intellectual disability, developmental delay, autism spectrum disorder, epilepsy and microcephaly [30, 31]. Coe et al. detected 36/29085 cases and 7/19584 controls with this deletion, resulting in a likelihood ratio of 3.45 and a penetrance of 15.7% [22]. The deletion has a Clingen score of 3, signifying its likelihood for pathogenicity, which is confirmed in a more recent study (odds ratio 9.85)

[32]. This deletion is currently not reported in the Belgian prenatal setting since in the majority of cases, it is inherited from an unaffected parent. Case 162, who carries the deletion, failed the ASQ-3 gross motor skills test, scored in the monitoring zone for fine motor skills and passed all other tests; no epilepsy, intellectual disability or autism spectrum disorder was reported. Invasive diagnosis in this twin pregnancy was performed because of increased nuchal translucency in the other member. Delivery was 4 weeks early, but birthweight was within the normal range. The head circumference at birth is unknown; however, prenatal ultrasound indicated a head circumference at percentile 50. No non-benign CNVs were detected in the sibling (case 163), who scored within the monitoring zone for gross motor development, suggesting that other factors may have influenced the children's development. Case 199 was delivered at 36 weeks after a normal pregnancy. Head circumference at birth was within normal range (percentile 50). The child passed all ASQ-3 subcategories and scored within the monitoring zone for the ASQ-SE2. Based on literature [30, 33, 34] and current Clingen scores, reevaluation of the 16p13.11 deletion in our Belgian reporting system is required.

22q11.2 distal duplication

The 22q11.2 distal duplication syndrome (distal type I, D-E/F) has a Clingen score of 3 and is a susceptibility factor for developmental delay, epilepsy and dysmorphic features [35-37], but is not reported in the Belgian prenatal setting. Case 197 was diagnosed with a 22q11.2 distal duplication LCR E-H (Chr22:22.998.284-24.988.402); this particular duplication is awaiting Clingen review. A study in 2011 describes six out of 10 patients with an LCR E/F–H duplication with speech delay and seven with mild to moderate developmental delay [37]. The child in our study underwent invasive testing because of a cleft lip. The mother indicated a delay in speech, but the child passed all tests provided, including the communication test.

Variants of Unknown Significance

VOUS are variants with hitherto unknown clinical significance. Inheritance status is one of the predictors for pathogenicity of a VOUS. However, since VOUS are not reported in the Belgian prenatal setting, inheritance was tested for in less than half of the cases (45.1%). Therefore, we decided to omit children with a VOUS from all statistical analyses. Still, investigation of children with a VOUS, regardless of inheritance, can help to reclassify them as (possibly) pathogenic or (possibly) benign.

Recurrent Variants of Unknown Significance

We detected 7 recurrent VOUS in 44 children [3]. In this postnatal follow-up study, 8 children participated (cases 23, 62, 73, 129, 155, 181, 184, 191) (Table 4). Six of them (62, 73, 129, 155, 184, 191) passed all tests, indicating that these VOUS probably do not affect the developmental domains tested by our surveys.

For case 23, who carries the most frequently found recurrent VOUS (a duplication on 6q22.31 (123.539.625-124.166.602)), the questionnaire was completed at the age of 40 months. Amniocentesis was performed because of Toxoplasmosis seroconversion, but the fetus tested negative. The child inherited the VOUS from the healthy mother, negating its pathogenicity. Although one month too old for the ASQ-3, the child scored within the monitoring zone for fine motor skills and problem-solving skills. The child passed the ASQ-SE2. As described by Srebniak et al., this CNV may represent a variant that is benign when present alone, but acts as a second hit in carriers of an additional VOUS [38]. The VOUS was an isolated finding in this particular child.

Despite being one month too old for the ASQ-3 (40 months), case 181, carrying the recurrent 10q23.31 deletion (chr10:91.626.482-92.035.457), failed the gross motor skills subcategory,

as well as the ASQ-SE2. The VOUS was maternally inherited, negating its pathogenicity, although reduced penetrance and variable expressivity cannot be excluded. Amniocentesis was performed because of Toxoplasmosis seroconversion, but the fetus was not affected. No ultrasound anomalies were detected, and pregnancy and delivery were uneventful. The deleted region encompasses only one pseudogene.

Non-recurrent Variants of Unknown Significance

We identified 30 children with a non-recurrent VOUS and of appropriate age for ASQ-3, and 54 children for ASQ-SE2. Four children with a non-recurrent VOUS failed one or more tests (Table S2). However, for none of the cases, the information was sufficient to reclassify the VOUS as either benign of pathogenic.

Pathogenic CNVs

Three children with a pathogenic variant were included in the statistical analysis of ASQ-SE2 results. Case 30 underwent an invasive procedure because of cardiac anomalies on prenatal ultrasound and carries a 9 Mb duplication on chromosome 16 (chr16:12.061.688-21.301.937). The child was born at 35 weeks with a dysmature birthweight and underwent a cardiac operation after birth. This case scored within the monitoring zone for the ASQ-SE2. Although too old for the ASQ-3 (39 months), the only passed the communication skills test. The CNV has been discussed at a national level and reported as pathogenic because of its size.

Case 169 was diagnosed with the 22q11.2 deletion syndrome (OMIM 188400). Ultrasound investigation indicated multiple anomalies, among which a ventricular septal defect and IUGR. The child passed the ASQ-SE2 test. Despite being too old for the ASQ-3 (41 months),

the child failed the communication, problem-solving and personal-social skills test, confirming developmental delay in these areas.

Case 142 was diagnosed with a small deletion in the *SHOX* gene (chrX:594.241-597.792). Haploinsufficiency of this gene results in a short stature, shortening of the medial segments of the limbs, with a progressive decline in the height SD score from birth onwards. The child scored within the monitoring zone for the ASQ-SE2 test. The child was born at full term with a birthweight of 4200 grams and a birth length of 50 centimeters; current length and weight remain within normal range.

Discussion

The landscape of prenatal diagnosis is changing drastically. Over the last 5 years, CMA has increasingly replaced conventional karyotyping for the analysis of invasively obtained samples. The Non-Invasive Prenatal Test (NIPT) has a very high uptake in both average and low risk pregnancies, and whole exome sequencing is slowly being introduced in the prenatal setting for certain ultrasonographic anomalies [42]. While invasive prenatal testing suffered a steep decline with the introduction of NIPT, the expansion of NIPT to karyotype resolution has partially reversed this trend, as NIPT-positive cases need to obtain a confirmatory invasive test.

In Belgium, approximately 125,000 children are born every year. Over a 22-month period (May 2013-February 2015), circa 9200 invasive procedures were performed nationwide. Of these, 14.5% were invited to participate in this national research project. The overall response rate was 17.3%. Response rates for postal questionnaires vary from 8-9% when there is no reminder; rates increase to 31.1-32.1% after a single reminder and up to 63% after 3 reminders [43]. Although 1 reminder was sent in this study, the response rate was lower than the

expected rate; this could be due to the length of the questionnaire, which had three sections or 20 pages of questions [44]. While a shorter questionnaire might have improved response rates, we opted for a more complete overview of the child's development.

A statistical difference was detected in indications for the invasive procedure between responders and non-responders (p=0.026). Parents were more likely to answer questionnaires if the indication was 'advanced maternal age' or 'an abnormal result for NIPT'. They were less likely to participate if the indication for the procedure was 'other', a category including mostly cases in which the amniocentesis was performed because of parental anxiety (e.g. because of a previous pregnancy with an aberrant genetic result.) There was no statistical difference in response rate for the indication 'fetal ultrasonographic anomaly. It is unclear why the response rate differed for some categories. It also remains uncertain whether a worse outcome of the child influences participation rate of the parent.

We detected a significant difference in the development of children with an SR in the categories of communications skills (p=0.0001) and personal social skills (p=0.003), when compared to children with no non-benign CNV or an SNR. The phenotype of a susceptibility variant is highly unpredictable. Belgian geneticists compiled a limited list of susceptibility loci that should be reported and a non-exhaustive list of those that are not reported (http://www.beshg.be/index.php?page=guidelines) [3, 7]. This list is based on recent literature, describing the clinical spectrum, odds ratios and penetrance values and takes into account the expected severity and the fetal and parental phenotype [3, 7, 22, 29, 45, 46]. The rationale behind this strict reporting policy is to avoid anxiety in and stigmatization of future parents over a CNV for which the outcome is highly uncertain [5, 15]. This approach has been subject to international discussion [47] and concerns have been raised about its legal implications. However, the Belgian genetic centers believe it to be a valuable strategy as it prevents

inconsistencies in reporting between genetic centers [2, 48-52] and reduces parental anxiety and needless terminations of pregnancy [53, 54].

The differences we found between children with an SR versus children with an SNR support our choice of susceptibility CNVs to report. However, due to the low participation grade, there is currently insufficient data to validate our policy to not report other susceptibility CNVs.

We identified one child who failed the survey in the SNR category: a child with a 16p13.11 deletion. The deletion including *MYH11* was assigned a Clingen score of 3. In our study, the child failed the gross motor skills test and scored within the monitoring zone for fine motor skills. The child's twin sibling, who did not have the deletion, also needed close attention for gross motor skills, indicating the possibility that other factors besides the CNV influenced the child's development. A second child with the 16p13.11 deletion succeeded all tests. However, based on current literature, the 16p13.11 deletion will be reevaluated in our Belgian reporting system because current literature and Clingen scores suggest a pathogenic nature.

Postnatal follow-up of children with a prenatally detected non-benign CNV is of importance since it allows complete phenotypical characterization of a particular CNV, as it is not dependent on cases at the more severe end of the phenotypical spectrum. Conversely, since our study population is small, our results do not necessarily reflect the results in the whole SNR population, nor can it be concluded that the developmental issues in the SR population are indeed related to the presence of a particular CNV. Hence, elaborate pre- and post-test counseling remain crucial, just as it is important to continuously review and adapt guidelines based on the most recent literature and current evidence. This is why we recently initiated a

study investigating the opinion of Belgian gynecologists, general practitioners and future parents on the Belgian approach.

Conclusion

In this paper, we reported a national postnatal follow-up project, initiated to determine the relationship between the prenatal genetic results, prenatal phenotypic findings and postnatal clinical and neurological development. This study is, to the best of our knowledge, the first of its kind. Postnatal follow-up of children with a prenatally detected non-benign CNV is of great value in determining the full phenotypic spectrum of CNVs. Despite the small inclusion numbers, we could detect a significant difference in communicative and personal-social development between cases with a reported susceptibility CNV and cases with an unreported susceptibility CNV. However, a higher number of cases for each CNV category is needed to confirm our findings and we hope our study will be followed by many others worldwide.

Data sharing statement:

The data that support the findings of this study are available in the supplementary data and on request from the corresponding author.

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Legend:

Tables

Table 1: Indications for invasive procedure in the patient population versus control group.

Indications include: a fetal abnormality, including increased nuchal translucency; an aberrant Down syndrome screening test: first trimester combined test [ultrasound measurement of nuchal translucency + pregnancy-associated plasma protein A (PAPP-A) + free beta human chorionic gonadotrophin (hCG)] or second trimester triple test [alpha-fetoprotein (AFP) + hCG (total or free-β) + unconjugated estriol]; advanced maternal age: 35 years or older at the time of conception; a familial genetic disorder: known cytogenetic or molecular aberration for which a prenatal test is warranted; toxoplasmosis or CMV† (cytomegalovirus) seroconversion; an abnormal result for NIPT‡ (non invasive prenatal test); other indications: this includes e.g. parental anxiety; unknown indication. There is no statistical difference in indications between both groups. CMV†: cytomegalovirus; NIPT‡: non invasive prenatal test.

Table 2: Statistical analysis of ASQ-3 results

This table describes the statistical analysis of ASQ-3 results, subdivided in the five different categories: communication skills, gross motor development, fine motor development, problem-solving skills and personal-social development. Significant differences between the groups were detected for two developmental areas: children with an SR scored worse in the categories communications skills (p=0.0001) and personal-social skills (p=0.003), compared to children in the control population and children with a SNR. CI[†]: Confidence Interval; SNR[‡]: unreported susceptibility CNV; SR[¶]: reported susceptibility CNV.

Table 3. A: Outcome of the ASQ-3 and ASQ-SE2 questionnaires in children with a reported susceptibility CNV (SR); B: Outcome of children with an unreported susceptibility CNV (SNR).
Abbreviations: ID[†]: intellectual disability; DD[‡]: Developmental disorder; ASD[§]: autism spectrum disorder; SZ[¶]: schizophrenia

Table 4: Outcome of the ASQ-3 and ASQ-SE2 questionnaires in children with a recurrent Variant Of Unknown Significance (VOUS)

Table S1: Belgian reporting policy for prenatally detected CNVs

This table describes the uniform national protocol on how to interpret and report variants detected by chromosomal microarray in samples obtained by amniocentesis or chorion villus biopsy.

Table S2: Results of all study participants.
Overview of all children from the patient and control groups that participated in the study. The table provides information on the type of CNV (if any), the prenatal phenotype, neonatal parameters and the outcome of the questionnaires. In total, questionnaires were completed for 208 children (85 patients and 123 controls).
Abbreviations: VOUS[†]: Variant Of Unknown Significance; SR[‡]: reported susceptibility CNV; Path[§]: pathogenic CNV; SNR[¶]: unreported susceptibility CNV; del/dup[††]: deletion/duplication.

Table S3. A: Patient description for ASQ-3 participants per CNV type; B: Patient description for ASQ-SE-2 participants per CNV type. The table provides information about characteristics of 125 children, scored for ASQ-3 (41 patients and 84 controls) and of 184 children, scored for ASQ-SE2 (75 patients and 109 controls).
VOUS[†]: Variant of Unknown Significance (VOUS); SR[‡]: reported susceptiblity CNV; SNR[§]: unreported susceptibility CNV; Path[¶]: Pathogenic CNV; Control: no or only benign CNVs.

Figures

Figure 1: Inclusion flow chart of the patient and control groups.

Inclusions and exclusions in the patient population and control group. Abbreviations: CNV: Copy Number Variant; VOUS: Variant Of Unknown Significance; SNR: unreported susceptibility CNV; SR: reeported susceptibility CNV; Path: Pathogenic CNV.

Figure 2. A: Boxplots of ASQ-3 subcategories versus variant group; B: Boxplots of ASQ-SE2 versus variant group. Children with an SR scored worse in the categories communications skills and personal-social skills in comparison to children in the control population and children with an SNR. ASQ-3: Ages and Stages Questionnaire: a Parent-Completed Child Monitoring System, Third edition; ASQ-SE2: Ages and Stages Questionnaire: Social-Emotional Second Edition; VOUS: Variant Of Unknown Significance; SNR: unreported susceptibility CNV; SR: reported susceptibility CNV; Path: Pathogenic CNV.

Table 1

Indication	Patient group (% of n=757)	Control group (% of n=793)
Fetal abnormality	37,6	28,1
An aberrant down syndrome screening test	27,7	29,4
Advanced maternal age	12,2	12,6
A familial genetic disorder	11	8,7
Toxoplasmosis or cmv[†] seroconversion	6,1	6,3
An abnormal result for a NIPT[‡]	0,5	0,5
Other	3,7	3,7
Unknown	1,2	10,7

Table 2

	P-value ANOVA	Compared groups	Difference in mean	95%CI† Lower limit	95%CI Upper limit	P-value Tukey corrected
Communication	0,00012					
		SNR‡ - Control	1.96	**-5.17**	**9.09**	0.85
		SR¶ - Control	-15.79	**-22.92**	**-8.66**	<0.001
		SR – SNR	-17.75	**-27.60**	**-7.90**	0.0019
Gross motor	0,318					
Fine motor	0,586					
Problem-solving	0,2488					
Personal-social	0,003079					
		SNR - Control	-0.27	**-6.11**	**5.56**	0.995
		SR - Control	-10.27	**-16.11**	**-4.44**	0.0022
		SR - SNR	-10	**-18.06**	**-1.94**	0.04

Table 3A: Outcome of children with a reported susceptibility CNV

				ASQ-3 subscale score					AS
CNV	Phenotype of the microdeletion/ microduplication	Case number	Age child (months)	Communication	Gross motor	Fine motor	Problem solving	Personal Social	
1q21.1 dup	ID[†], DD[‡], ASD[§], SZ[¶]	207	36	Fail	Fail	Fail	Fail	Fail	Fail
1q21.1 del	ID, DD, ASD, SZ, facial dysmorphism	29	35	Pass	Pass	Pass	Pass	Pass	Pass
		47	40	-	-	-	-	-	Pass
15q26 del	ID	86	35	Pass	Pass	Pass	Pass	Pass	Pass
22q11 dup	DD, epilepsy, dysmorphic features	15	35	Pass	Pass	Pass	Pass	Pass	Pass
		52	40	-	-	-	-	-	Pass

Table 3B: Outcome of children with a not reported susceptibility CNV

				ASQ-3 subscale score					ASQ-SE2 score
CNV	Phenotype of the microdeletion/ microduplication	Case number	Age child (months)	Communication	Gross motor	Fine motor	Problem solving	Personal Social	
15q11.2 BP1-BP2 dup	DD[‡], motor delay, speech delay, ASD[§]	131	38	Pass	Pass	Pass	Pass	Pass	Pass
16p13.11 del	ID[†], DD, ASD, epilepsy	162	35	Pass	Fail	Monitor	Pass	Pass	Pass
		199	36	Pass	Pass	Pass	Pass	Pass	Monitor
22q11 distal dup	DD, epilepsy, dysmorphic features	197	37	Pass	Pass	Pass	Pass	Pass	Pass

Table 4

					ASQ-3 subscale score					ASQ-SE2 score
CNV	Locus (hg19)	Case number	Mode of inheritance	Age child (months)	Communication	Gross motor	Fine motor	Problem solving	Personal Social	
3p14.2 dup	chr3: 59.666.501 – 60.993.079	62	unknown	39	Pass	Pass	Pass	Pass	Pass	Pass
		191	maternal	38	Pass	Pass	Pass	Pass	Pass	Pass
6q22.31 dup	chr6: 123.539.625 - 124.328.531	23	maternal	39	Pass	Pass	Monitor	Monitor	Pass	Pass
9p23 dup	chr9: 10.164.926 - 11.868.588	129	unknown	34	Pass	Pass	Pass	Pass	Pass	Pass
		155	unknown	38	Pass	Pass	Pass	Pass	Pass	Pass
10q23.31 del	chr10: 91.626.482 - 92.035.457	181	maternal	40	Pass	Fail	-	Pass	Pass	Fail
17p13.3dup	chr17: 148.092 - 597.702	73	maternal	9	Pass	Pass	Pass	Pass	Pass	Pass
22q11.23dup	chr22: 23.720.181 - 24.959.827	184	maternal	35	Pass	Pass	Pass	Pass	Pass	Pass

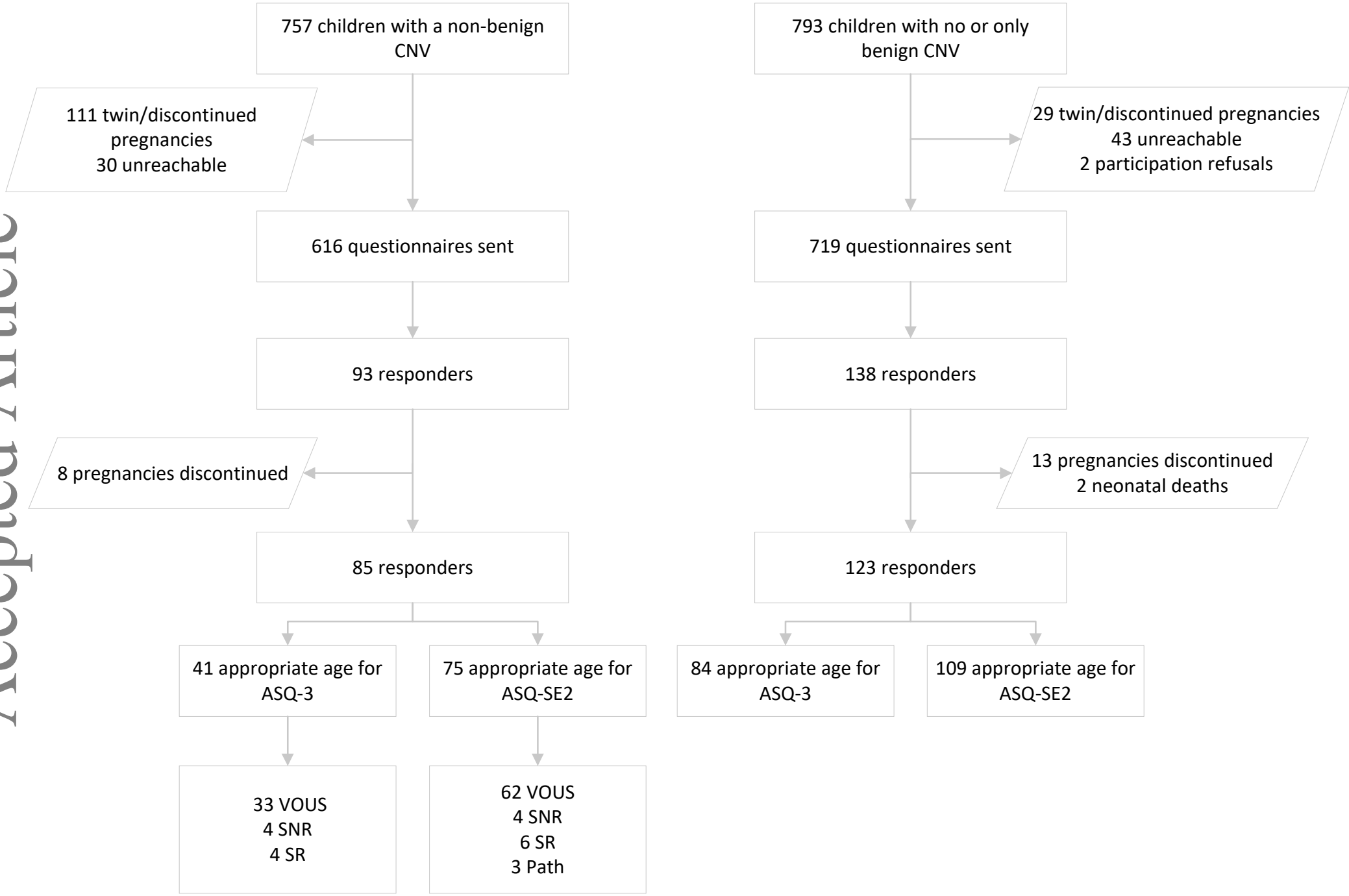

Figure 2a

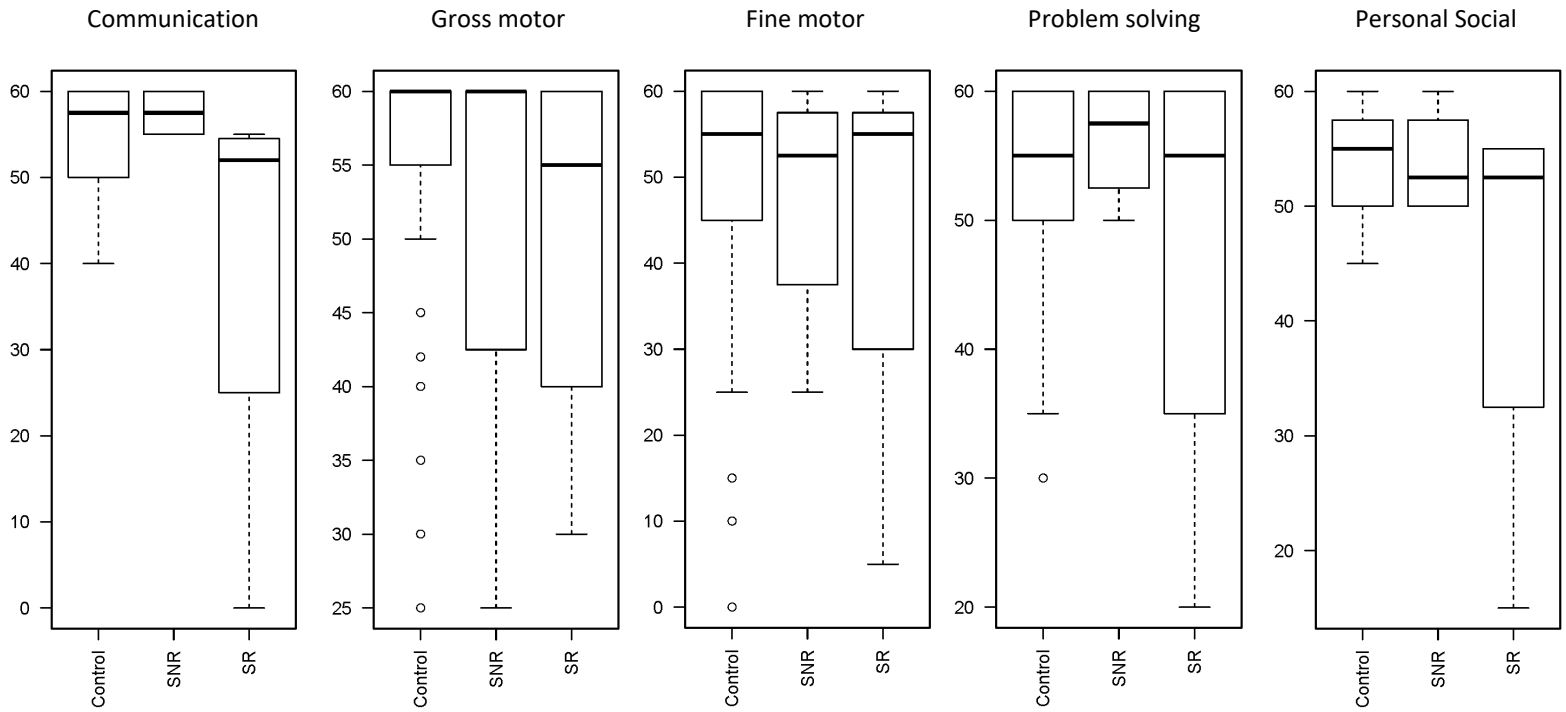

Figure 2b