The BElgian PREnatal MicroArray (BEMAPRE) database : a systematic nationwide repository of fetal genomic aberrations

Title:

The BElgian PREnatal MicroArray (BEMAPRE) database: A systematic nationwide repository of fetal genomic aberrations

Running title:

Prenatal database for chromosomal microarray results

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What's 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, CMA reveals cryptic, clinically relevant CNVs.

What does this study add?

1. This manuscript describes the establishment of a national database for prenatal microarray results in Belgium.
2. This database, which is one of the largest currently available, allows:
   a. calculation of added values of microarray over karyotyping for different categories of indications.
   b. determination of the most common syndromes, incidental findings and susceptibility CNVs in the Belgian prenatal population.
   c. evaluation of our national reporting policy.
   d. reflection on the effect of the implementation of NIPT.

Abstract

Objective

With the replacement of karyotyping by chromosomal microarray (CMA) in invasive prenatal diagnosis, new challenges have arisen. By building a national database, we standardize the classification and reporting of prenatally detected copy number variants (CNVs) across Belgian genetic centers. This database, which will link genetic and ultrasound findings with postnatal development, forms a unique resource to investigate the pathogenicity of variants of uncertain significance and to refine the phenotypic spectrum of pathogenic and susceptibility CNVs.

Methods

The BElgian PREnatal MicroArray (BEMAPRE) consortium is a collaboration of all genetic centers in Belgium. We collected data from all invasive prenatal procedures performed between May 2013 and July 2016.

Results

In this three-year period, 13266 prenatal CMAs were performed. By national agreement, a limited number of susceptibility CNVs and no variants of uncertain significance were reported. Added values for using CMA versus conventional karyotyping were 1.8% in the general invasive population and 2.7% in cases with an ultrasound anomaly. Of the reported CNVs 31.5% would have remained undetected with NIPT as the first-tier test.

Conclusion

The establishment of a national database for prenatal CNV data allows for a uniform reporting policy and the investigation of the prenatal and postnatal genotype-phenotype correlation.

Main text

Introduction

Chromosomal microarray analysis (CMA) scans for the genome-wide presence of microdeletions and microduplications or copy number variants (CNVs). Recent years have seen a steady rise of CMA at the expense of karyotyping in the analysis of invasively obtained prenatal samples (amniotic fluid or chorion villi). With the use of CMA, the requirement for cell culturing, which is a lengthy and failure-prone process, is overcome. Moreover, current array designs allow for a higher resolution than conventional karyotyping (100-400 kb versus 5-10 Mb), enabling the detection of smaller CNVs.[1] In 5 to 10% of pregnancies with a fetal structural anomaly and in 0.5-2% of pregnancies without, CMA reveals cryptic, clinically relevant CNVs. [2, 3, 4, 5, 6, 7]

With the introduction of this new technique, new challenges arose. Due to the higher resolution, genetic variants causing late-onset disorders (e.g., Charcot-Marie-Tooth disease), variants with a reduced penetrance/variable expression (susceptibility CNVs), and variants for which there is no information on possible consequences (Variants Of Unknown Significance (VOUS)) can be detected. [8] There is no international consensus on policy for the reporting of these findings to future parents. Reporting a CNV in a prenatal setting is ethically very different from the postnatal setting: future parents may decide to discontinue the pregnancy, even without 'hard' evidence that the child will be affected; alternatively, when continuing the pregnancy, they may remain anxious about the child's development. In addition, parents may obtain knowledge about their own personal health.

In Belgium, all samples for prenatal genetic diagnosis have been analyzed by CMA since 2013.[9] Despite the use of different types of genomic array platforms (SNP array and array CGH) in the eight genetic centers, a cut-off resolution of 400 kb for both deletions and

duplications was agreed upon in order to maximize the detection of pathogenic variants while minimizing the number of VOUS. In the case that the genomic platform allowed for detection of clearly pathogenic CNVs smaller than 400 kb, these variants were of course reported as well.

CNVs are classified as benign, pathogenic, susceptibility or VOUS. Benign CNVs are repeatedly found in the normal population and are not associated with pathological phenotypes; they are never reported. Pathogenic CNVs are recurrent genomic rearrangements with a well-defined congenital phenotype or aberrations resulting in a known effect on the function of a gene that correlates with a known phenotype (e.g., haploinsufficiency). These CNVs are generally reported. When the finding is unrelated to the indication of the CMA (incidental finding) [10], the following reporting policy is applied: dominant late-onset diseases with clinical utility (therapeutic options, preventive measures, termination of pregnancy) are reported to future parents; carriership for autosomal recessive diseases is reported if the carrier frequency is >1/50; and X-linked carrier status is always reported.

Susceptibility CNVs are genetic risk factors with reduced penetrance and/or variable expression, often associated with a highly unpredictable phenotype that does not present prenatally (e.g., intellectual disability, autism spectrum disorder, epilepsy, psychiatric disorder). A limited number of susceptibility CNVs are reported in the prenatal setting. This list (Table S1), which was composed by geneticists from all of the Belgian genetic centers, takes into account penetrance and severity [11, 12, 13, 14] and is updated on a yearly basis. All CNVs that cannot be classified as benign, pathogenic or susceptibility are designated VOUS.

Despite these guidelines, ambiguous situations still occur, which are tackled by a committee of experts. To guide their decisions, an appropriate database relating prenatal genetic and ultrasound findings to postnatal clinical and neurodevelopmental data had to be built. Here

we report on the resulting BElgian PREnatal MicroArray (BEMAPRE) database, which contains the data from all Belgian invasive tests performed in a three-year period (May 2013–July 2016). This database allows the identification of the most frequent pathogenic CNVs, susceptibility CNVs and VOUS in Belgium and to calculate added values for the use of CMA versus karyotyping. To the best of our knowledge, this is the first nation-wide database collecting prenatal genetic results and structural findings on ultrasound as the basis for longitudinal studies of the developmental effect of CNVs. The database, furthermore, ensures unanimous reporting and counseling policy.

Methods

Study Conduct

The BEMAPRE consortium is a collaboration of clinical and laboratory geneticists from every genetic center in Belgium (http://www.beshg.be/index.php?page=centers). It aims to collect data on all invasive procedures performed in Belgium. Approval of the central ethical committee and of the College for Human Genetics of the Federal Ministry of Public Health in Belgium has been granted for this project. Data are stored in a coded manner in the Bench Lab CNV 5.0 platform provided by Agilent Technologies (Cartagenia NV). Agilent Technologies was not involved in this research in any other way.

Data collection

We collected data from invasive prenatal procedures performed between May 2013 and July 2016. The centers provided the indications for the invasive tests and the CMA results obtained. These indications comprised: an aberrant Down syndrome screening test; advanced maternal age; a structural fetal abnormality on ultrasound (including increased nuchal translucency); a familial genetic disorder; an abnormal result for a Non-Invasive Prenatal

Test (NIPT); other (including maternal seroconversion for Cytomegalovirus (CMV) or Toxoplasmosis and anxiety).

Possible CMA outcomes were: no or only benign CNV(s); aneuploidy; pathogenic CNV; VOUS; susceptibility CNV reported; susceptibility CNV not reported. Note that pathogenic CNVs also include incidental findings, because a syndromic genomic disorder can be viewed as such a finding if the reason for the CMA did not relate to the syndrome. To determine the added value of CMA over karyotyping, CNVs were grouped on the basis of their size (larger/smaller than 10 Mb). All VOUS were reanalyzed in September 2017 for a possible class-change to benign or pathogenic, based on recent literature and information in publicly available CNV databases.

For all prenatal cases with a non-benign CNV (pathogenic CNV, susceptibility CNV or VOUS, with the exclusion of aneuploidies and unbalanced translocations), the following information was obtained: chromosome number, start and stop position of the CNV (hg19), size of the CNV, copy number, class, gender, clinical information (Human Phenotype Ontology (HPO)) and (whenever available) mode of inheritance. For 6,660 of 13,266 cases (50.2%), information on the indication for the invasive procedure was acquired.

Data analysis

Recurrence of the following CNVs was evaluated: VOUS deletion, VOUS duplication, pathogenic deletion and pathogenic duplication. CNVs were labeled recurrent if appearing at least five times in our population and when presenting with a smallest overlapping region of at least 80% to account for platform-specific differences. The percentage overlap takes into account the size of the regions and is calculated as follows: 2 times the overlap between 2 CNVs divided by the sum of lengths of both CNVs.

Statistical analysis

Descriptive statistics were used to describe population, patient and CNV characteristics. SPSS 24 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.) was applied to analyze data. Frequency tables describing the association between indication and mutation type were visualized using correspondence analysis. The correspondence plots were generated using the ca package from the software package R, version 3.1.2. [15, 16]

Results

Between May 2013 and July 2016, 13,266 prenatal CMAs were performed in Belgium. The principal indications were a structural fetal abnormality (including increased nuchal translucency) (30.2%) and an aberrant Down syndrome screening test (30.4%). Further indications included advanced maternal age (13.1%), familial genetic disorder (10.8%), positive NIPT (2.0%), and other (13.5%).

1,347 of the 13,266 cases (10.2%) carried an aneuploidy. In 54% of these, at least one structural abnormality was visible on ultrasound investigation. Conversely, in the presence of ultrasound anomalies, 18.1% of cases demonstrated aneuploidy or an unbalanced translocation. As expected, aneuploidies were particularly common in the positive NIPT group (69.6%) (Figure 1).

In 1.9% of cases (246/13,266), a pathogenic CNV was detected; 175 of those (71.1%) had a CNV smaller than 10 Mb that presumably would have escaped detection by karyotyping. (Table S2). More than half of the fetuses (63.0% or 155/246) with a pathogenic CNV had a structural abnormality on ultrasound investigation; 39 (25.2%) of those carried multiple structural anomalies. Figure 2 shows the distribution of CNV classes in cases with ultrasound anomalies. In the category of 'Positive NIPT', a pathogenic CNV was detected in five (2.9%)

cases (Figure 1), four of which were larger than 10 Mb (2.2%). Correspondence plots did not show an association between the indication for the invasive procedure and finding a pathogenic CNV (data not shown).

Table 1 lists the most frequent syndromic genomic disorders in our population. The 22q11.2 deletion syndrome (OMIM #188400) is by far the most common: we detected 41 cases, accounting for 0.31% of all invasive samples. The most common incidental findings were X-Linked Ichtyosis (OMIM #308100; 13 cases, 6 female and 7 male), Hereditary Neuropathy with liability to Pressure Palsies (OMIM #162500; 6 cases) and Charcot-Marie-Tooth type 1A (OMIM #118200; 5 cases) (Table 1 and Table S3).

Susceptibility CNVs were diagnosed in 1.6% (210/13266) of our population; based on our national guidelines (see Vanakker et al. and Discussion), one third of those (71/210 or 33.8%; 0.5% of the total population) were reported (Table S1). In cases with an ultrasound anomaly, 0.7% carried a reported susceptibility CNV; this was not significantly different compared to the prevalence in the entire prenatal population, in accordance with the fact that susceptibility CNVs are rarely associated with ultrasound anomalies. Table 1 shows the most frequent susceptibility CNVs: the 22q11.2 duplication syndrome (OMIM #608363; 24 cases) and the 15q11.2 BP1-BP2 duplication [17] (32 cases) are respectively the most common reported and unreported susceptibility CNV. Susceptibility CNVs were all cryptic.

The overall added diagnostic value of using CMA compared to karyotyping was 1.8%. Added values were calculated by taking into account all reported CNVs (pathogenic CNVs and reported susceptibility CNVs). Table 2 shows the added diagnostic value of CMA per indication. In cases with versus without an ultrasound anomaly, CMA had an added diagnostic value of respectively 2.7% and 1.5%.

Of all the cases, 5.6% (746/13,266) carried a VOUS: a deletion in 23.6% of the cases (176/746), a duplication in 72.9% (544/746), and both in 3.5% (26/746) (Table S2). In 38.5% (287/746) of these, structural fetal abnormalities were present on ultrasound; this percentage increased to 46.8% in cases with more than one VOUS. VOUS were distributed evenly among the different indications, as revealed by correspondence analysis (data not shown).

Seven recurrent VOUS were detected in our population, one deletion and six duplications (Table 1). The most frequent recurrent VOUS was a duplication on chromosome 6q22.31 (ten cases). The common region (chr6:123.539.625-124.328.531; 789 kb) contains the genes *TRDN* (Triadin) and *NKAIN2* (NA+/K+ Transporting ATPase-interacting 2). As described by Srebniak et al., this may represent a private variant that is benign when present alone, but may act as a second hit in carriers of an additional VOUS.[18] In all our cases, this was an isolated finding. Moreover, indications for invasive testing and fetal phenotype were different, supporting Srebniak's conclusion that this variant is benign when occurring privately, although a common postnatal phenotype cannot be excluded. The only recurrent deletion is located on chromosome 10q23.31 and was diagnosed in six cases (common region: chr10: 91.626.482-92.035.457; 409 kb). This region encompasses only one pseudogene. In three cases, the deletion was inherited from a phenotypically normal parent, arguing against its pathogenicity.

To explore the effect of CNV load, we examined the phenotype of children with more than one reported CNV (excluding cases with an aneuploidy or unbalanced translocation) or with one reported CNV and one VOUS, versus those with an isolated reported CNV. Of 317 cases with a reported CNV (246 with a pathogenic CNV and 71 with a susceptibility CNV), 33 cases (10.4%) had more than one reported CNV. Of those, 20 (60.6%) had structural abnormalities. Another 27 of 317 cases (8.5%) had both a reported CNV and a VOUS. Of those, 18 (66.7%) had structural abnormalities. Of the remaining 257 cases with a reported

CNV, structural abnormalities were found in 143 cases (55.6%). There was no significant difference in the presence of ultrasound anomalies between groups (p = 0.497).

With the implementation of NIPT, invasive prenatal testing will increasingly become restricted to pregnancies with ultrasound anomalies and those with a known genetic defect in the family. If NIPT becomes the first-tier test for all other indications, subchromosomal aberrations will be missed. Presuming a NIPT technology that can detect all aneuploidies, this would account for 31.5% (100/317) of reported CNVs in our study population. . This percentage decreases slightly to 26.2% (83/317) in case of "genome-wide NIPT" (detecting all aberrations above 10Mb) (Table S4). For the added values of using CMA versus karyotyping and NIPT, see Table 2.

Discussion

In Belgium, approximately 125,000 children are born every year. Over a three-year period (May 2013-July 2016), 13 266 invasive prenatal procedures were performed.

The most frequently detected genomic disorder was the 22q11.2 deletion syndrome. We encountered this deletion in 0.31% of our population (41 cases). In their prospective study analyzing 9,500 prenatal samples, Grati et al. found a comparable prevalence (0.3%). [19] The reported postnatal prevalence of the syndrome is much lower: in a large population-based study involving 255,849 babies, 0.017% carried the deletion.[20] We can discern several reasons for this discrepancy. First, the phenotypic spectrum of the 22q11.2 deletion syndrome is broad, causing underdiagnosis of this syndrome in the postnatal setting. Second, prenatal cases with ultrasound anomalies are more likely to be terminated. Finally, 22q11.2 pregnancies are thought to be more prone to end in a miscarriage: in a recent study in which the incidence of 22q11.2 deletions in 26,101 products of conception were examined, [21] 12/9,398 (0.13%) samples which were normal at karyotype resolution had an isolated

22q11.2 deletion, approaching the prevalence in our prenatal population. Of our 41 cases, 53.7% had an ultrasound anomaly that was clearly related to the genetic finding.

The 22q11.2 duplication syndrome was the most frequently reported susceptibility CNV in our prenatal population (24 cases or 0.18%). In a control population, the frequency is 0.05%. [12] The variant has a broad phenotypic spectrum. The most common symptoms are intellectual disability/learning difficulties (97%), delayed psychomotor development (67%), growth retardation (63%), muscular hypotonia (43%), and cardiac anomalies (20%). [22, 23] Patients with a 22q11.2 duplication are 4.1 to 10 times more at risk of developing a neurodevelopmental disorder. [24] Although in the majority of cases (69%), the duplication is inherited from a normal parent, [22] this susceptibility CNV is nevertheless reported prenatally because of its possible association with fetal structural anomalies and the importance of ultrasonographic follow-up. In this study, 11/24 (45.8%) of fetuses with a 22q11.2 duplication syndrome had ultrasonographic abnormalities (short femora (2), transposition of the great arteries (1), increased nuchal translucency (4)).

The 15q11.2 duplication (chr15:22800000–23090000, minimal size 290 kb) is the most frequently found unreported susceptibility CNV in our population (32 cases). The phenotypic spectrum of developmental delay is highly variable, from motor coordination problems to autism spectrum disorder and obsessive compulsive disorder. [25] Although initially described as a susceptibility region for neurological dysfunction, [17] several more recent reports failed to show a clear genotype-phenotype association. Cooper described 64/15,767 patients with developmental delay versus 36/8,329 healthy controls (penetrance 0.64), [13] Coe detected the 15q11.2 duplication in 128/29,085 patients with developmental delay versus 60/19,584 healthy controls, resulting in a likelihood ratio of 1.44. [11] In a study of 2,521 autism spectrum disorder families, Chaste found no difference in frequency between patients and healthy

siblings. [26] The highly variable and often mild phenotype and the low penetrance and likelihood ratio justify our reporting policy.

The phenotype resulting from a susceptibility CNV 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 (Table S1 and Table S5), based on the clinical spectrum, expected severity, and published odds ratios or penetrance values. [11, 12, 13, 14] The fetal and parental phenotype is also taken into account. These lists are re-evaluated on a yearly basis. We observe a strong correlation between our reporting policy and the dosage sensitivity score given by ClinGen (https://www.ncbi.nlm.nih.gov/projects/dbvar/clingen/). All reported loci have a score of 3 (sufficient evidence), with the exception of the 16p11.2 distal deletion (score 2; some evidence). Conversely, unreported susceptibility CNVs have a score of 0 (no evidence), 1 (little evidence) or 2. The 2p16.3 deletion has been given a score of 3 by ClinGen; at last evaluation, we concluded that penetrance had not been sufficiently determined for this CNV. 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. [27, 28] Nonetheless, one might still reflect on the ethical consequences of not reporting a variant that unexpectedly does cause disease. Thus, elaborate pretest and posttest genetic counseling remain crucial when using CMA in prenatal diagnosis.

The added value of using CMA rather than conventional karyotyping was 1.8% in the general invasive population and increased to 2.7% in cases with an ultrasound anomaly. Upon inclusion of unreported susceptibility CNVs, the added values rose to 2.5% and 3.7%, respectively. In 2014, De Wit and colleagues performed a systematic review of the added value of prenatal CMA in fetuses with an isolated structural anomaly. [3] They found that in 5.6% of these pregnancies a pathogenic, cryptic CNV could be detected. Discrepancies in added values between different studies, even after homogenizing cohorts, were explained by

small samples sizes, differences in cohort selection and differences between array platforms applied. Our study data show that in addition, the classification and reporting policy of the laboratory strongly affects the added values. In the absence of structural anomalies on ultrasound, the added value of using CMA was 1.5% in our prenatal population; this further decreased to 1.1% when taking only uneventful pregnancies (advanced maternal age or maternal anxiety) into account. In a recent systematic review of the literature and meta-analysis, a similar risk figure of 0.86% for a submicroscopic pathogenic CNV was found for uneventful pregnancies. [29]

CNV load is known to contribute to the severity of neurodevelopmental and psychiatric disorders, but evidence of an association of CNV load and ultrasound anomalies is lacking. [30] In this study, having a higher CNV load (2 vs 1 pathogenic CNV) was not associated with a higher incidence of ultrasound anomalies. (p=0.497)

Knowing the inheritance pattern of a VOUS can be powerful information: a *de novo* VOUS is more likely to be pathogenic than a VOUS inherited from an unaffected parent. As our reporting policy dictates not to communicate VOUS, examining inheritance is not obligatory. Consequently, the inheritance pattern was investigated for only 27.1% of our cases. Of the *de novo* cases (3.9% or 29 cases), 65.5% had ultrasound anomalies versus 30.6% in cases with an parentally inherited VOUS (173 cases or 23.2% of the population). We acknowledge that knowledge on the inheritance mode of all VOUS would have strengthened the paper and will reconsider our policy for future cases.

Worldwide, the number of invasive procedures is declining rapidly with the growing implementation of NIPT. [31] As of July 1, 2017, Belgium became the first country in the world to fully reimburse NIPT for all pregnancies, resulting in an even steeper increase in NIPT uptake. Our study population (invasive prenatal testing between May 2013 and July 2016)

was given the opportunity for a non-reimbursed NIPT for all indications. In the case of ultrasound anomalies, we observed a 4% difference (18.1% versus 22.1%) in the diagnostic yield of NIPT versus CMA (Table 2), clearly demonstrating that NIPT cannot replace CMA for this indication. With respect to the implementation of NIPT for pregnancies without ultrasound anomalies, concerns have also been raised, as subchromosomal pathogenic CNVs will be missed. [32, 33] In our population, 26.2% (83/317) of reported CNVs below 10 Mb were found in cases with the indication 'an aberrant Down syndrome screening test', 'advanced maternal age' or 'other indications', all of which would have remained undetected with NIPT as the first-tier test, even when assuming a resolution similar to that of karyotyping. Extensive pretest counseling is and will remain absolutely crucial to inform patients about the pros and cons of NIPT versus invasive prenatal testing and to help them choose the prenatal test that is most appropriate for their situation.

Publicly available CNV databases such as the Database of Genomic Variants, DECIPHER, Ecaruca and The International Collaboration for Clinical Genomics are valuable, but mainly consist of postnatal cases. As a consequence, these databases contain cases at the more severe end of the phenotypic spectrum, providing an incomplete characterization of the phenotype associated with a particular CNV. To increase our knowledge of the phenotypic spectrum of CNVs, we embarked on a postnatal follow-up project, the aim of which is to determine the relationship between the genetic result, prenatal findings and postnatal development to reclassify VOUS and increase our knowledge about both susceptibility and pathogenic CNVs. On January 2017, postnatal clinical and neurodevelopmental follow-up at the age of 3 years for all children included in the BEMAPRE database was launched.

Conclusion

In Belgium, a uniform reporting system facilitates the national registration of all non-benign CNVs. Our prenatal strategy is unique, as we are the only country with a nationwide uniform approach to prenatal CMA analysis, reporting and communal CNV data storage. In this paper, we reported on our national prenatal data. This large and unique dataset provides us with insights into the incidence of CNVs, possible associations with the indication for the invasive procedure and the fetal phenotype. The content of the database is made publicly available to researchers and clinicians worldwide through the website of the Belgian Society of Human Genetics (http://www.beshg.be/index.php?page=guidelines) and will be updated on a regular basis. Postnatal follow-up has been initiated and will be extremely valuable, as it will facilitate the association between prenatally detected CNVs and postnatal phenotypes.

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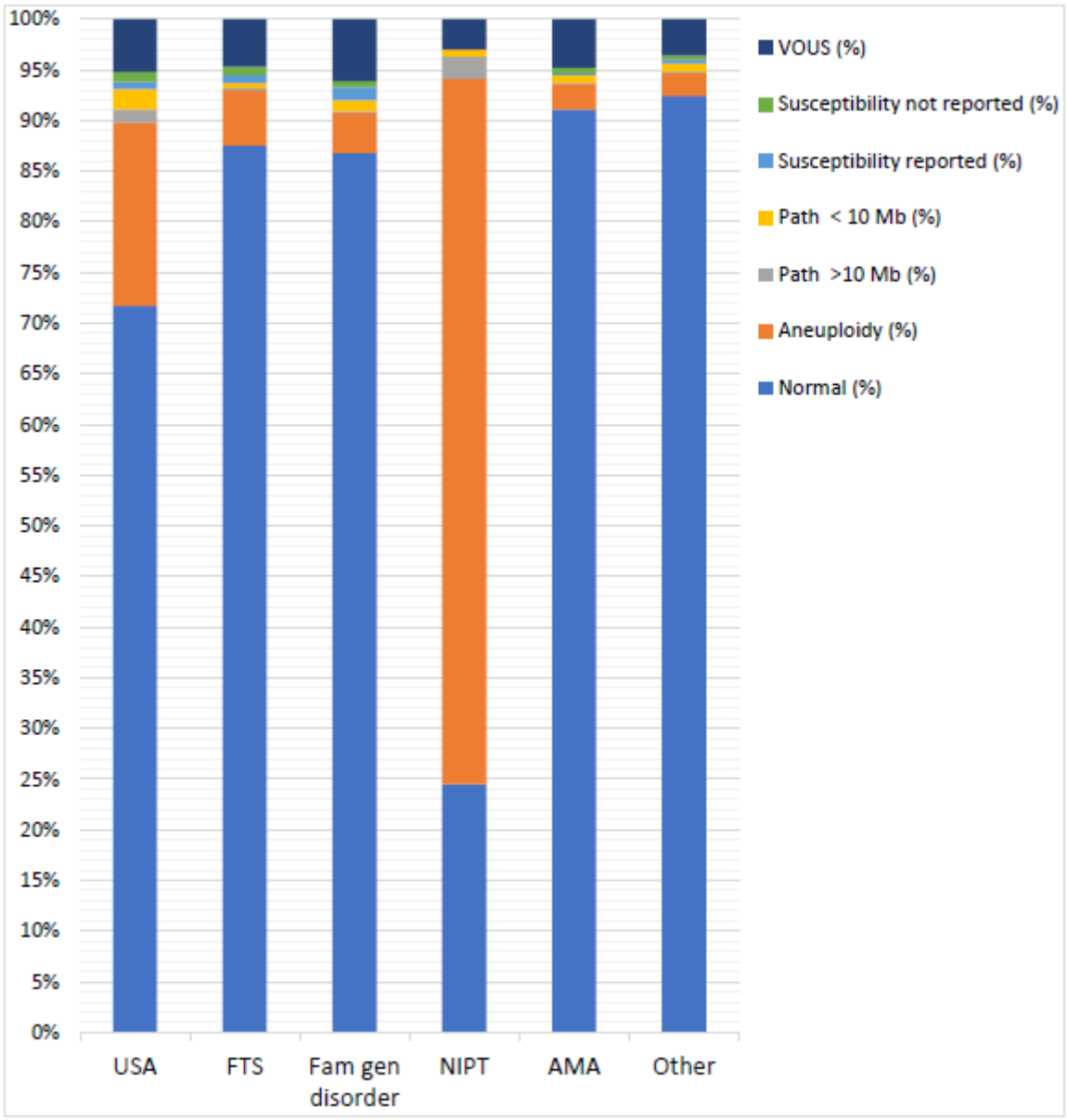

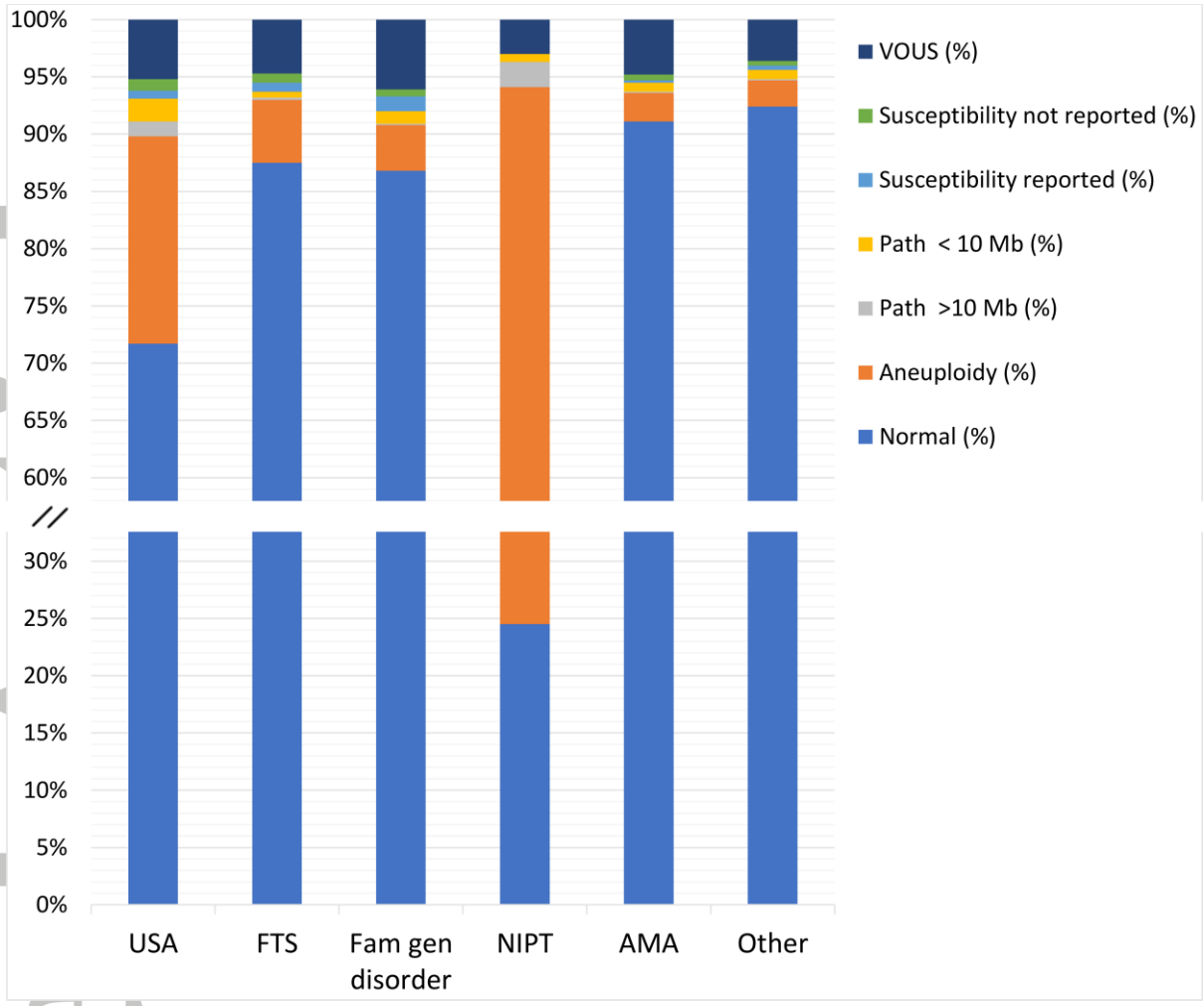

Figure 1. CMA results in prenatal cases subdivided according to indication for invasive prenatal testing . CMA results are classified as normal (no or only benign CNVs), aneuploidy, pathogenic CNV > 10 Mb, pathogenic CNV < 10 Mb, reported susceptibility CNV, unreported susceptibilty CNV and VOUS. 1a. Graphic view. 1b. Table view. Please note that numbers and percentages are based on 6660 cases (50.2% of the population).

Figure 1b

Indications	Total indication (%)	Normal (%)	Aneuploidy (%)	Path[ǂǂ] >10 Mb (%)	Path < 10 Mb (%)	Susceptibility reported (%)	Susceptibility not reported (%)	VOUS[§§] (%)
USA[ł]	2013 (100)	1444 (71,7)	364 (18,1)	26 (1,3)	41 (2)	14 (0,7)	20 (1)	104 (5,2)
FTS[ǂ]	2022 (100)	1770 (87,5)	111 (5,5)	5 (0,2)	9 (0,5)	16 (0,8)	15 (0,8)	96 (4,7)
Fam gen disorder[§]	720 (100)	625 (86,8)	29 (4)	1 (0,1)	8 (1,1)	9 (1,3)	4 (0,6)	44 (6,1)
NIPT [¶]	135 (100)	33 (24,5)	94 (69,6)	3 (2,2)	1 (0,7)	0 (0)	0 (0)	4 (3)
AMA [łł]	874 (100)	796 (91,1)	22 (2,5)	1 (0,1)	7 (0,8)	2 (0,2)	4 (0,5)	42 (4,8)
Other	896 (100)	828 (92,4)	21 (2,3)	1 (0,1)	7 (0,8)	4 (0,4)	3 (0,4)	32 (3,6)
Total	6660 (100)	5496 (82,5)	641 (9,6)	37 (0,6)	73 (1,1)	45 (0,7)	46 (0,7)	322 (4,8)

Abbreviations: ł : USA: Ultrasound anomaly, ǂ: FTS: an abberant Down screening test , §: Fam gen disorder: Known genetic disorder in the family, ¶: NIPT: abnormal result on Non-Invasive Prenatal Test, łł : AMA: Advanced Maternal Age, ǂǂ: Path: Pathogenic CNV, §§: VOUS: Variant of Unknown Siginificance, Other: CMV, toxoplasmosis, anxiety and remaining indications.

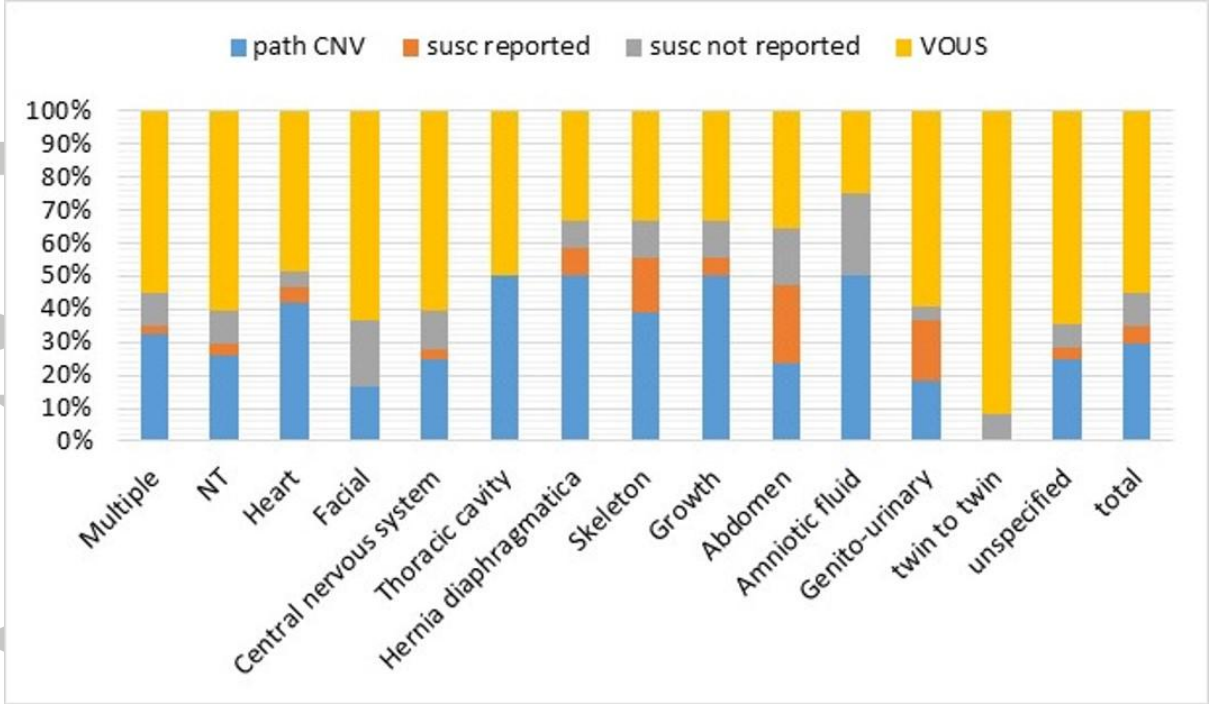

Figure 2. Distribution of CNV classes in cases with ultrasound anomalies, sorted according to the organ system involved. The following subcategories are defined: multiple anomalies, increased nuchal translucency (NT), cardiac anomaly, facial anomaly, anomalies of the nervous system, intrathoracic anomaly, hernia diaphragmatica, skeletal anomaly, growth anomaly, anomalies of the abdomen (including gastroschisis and omphalocoele), anomaly of the amniotic fluid, genito-urinary anomaly, twin-to-twin transfusion syndrome and unknown anomaly. Cases are classified as multiple if more than one compartment is affected. 2a. Graphic view. 2b. Table view.

Figure 2b

	MULTIPLE	NT[¶]	HEART	FACIAL	CENTRAL NERVOUS SYSTEM	THORACIC CAVITY	HERNIA DIAPHRAGMATICA	SKELETON	GROWTH	ABDOMEN	AMNIOTIC FLUID	GENITO-URINARY	TWIN TO TWIN	UNSPECIFIED	TOTAL
PATH[ŀ] CNV	39	28	25	5	15	2	6	7	9	4	6	4	0	7	155
SUSC[ǂ] REPORTED	3	4	3	0	2	0	1	3	1	4	0	4	0	1	26
SUSC NOT REPORTED	12	11	3	6	7	0	1	2	2	3	3	1	1	2	53
VOUS[§]	67	66	29	19	37	2	4	6	6	6	3	13	11	18	287

Abbreviations: ŀ : Path: Pathogenic, ǂ: Susc: Susceptibility, §: VOUS: Variant of Unknown Significance, ¶: NT: Increased Nuchal Translucency

Table 1. Most frequent syndromic disorders, susceptibility CNVs (reportedand unreported), incidental findings and VOUS in our prenatal population. The table shows the genomic location, the number of cases with this CNV, the frequency in our prenatal population and the percentage and number of cases with an ultrasound anomaly.

Syndromic disorders	**Location**	**n**	**% of total invasive population**	**cases with an ultrasound anomaly % (n)**
22q11 del (OMIM 188400)	22q11	41	0,31	80 (33)
X-Linked Ichtyosis (OMIM #308100)	Xp22.3	13	0,10	23 (3)
Hereditary Neuropathy with liability to Pressure Palsies (OMIM #162500)	17p12	6	0,05	50 (3)
Wolf-Hirschhorn (OMIM 194190)	4p16.3	5	0,04	100 (5)
Charcot-Marie-Tooth type 1A (OMIM #118200)	17p12	5	0,04	40 (2)
Williams Beuren (OMIM 194050)	7q11.23	5	0,04	100 (5)
Susceptibility CNVs (reported)	**Location**	**n**	**% of total invasive population**	**cases with an ultrasound anomaly % (n)**
22q11.2 dup (OMIM 608363)	chr22:19.020.000-20.290.000	24	0,18	44 (11)
GJA5 dup (OMIM 612475)	chr1:146.570.000-147.390.000	14	0,11	21 (3)
CHRNA7 del (OMIM 612001)	chr15:31.130.000-32.480.000	8	0,06	14 (1)
GJA5 del (OMIM 612474)	chr1:146.570.000-147.390.000	7	0,05	50 (4)
TBX6 dup (OMIM 614671)	chr16:29.590.000-30.190.000	5	0,04	40 (2)
TBX6 del (OMIM 611913)	chr16:29.650.000-30.200.000	5	0,04	40 (2)
HNF1B del (OMIM 614527)	chr17:34.820.000-36.210.000	5	0,04	80 (4)
Susceptibility CNVs (unreported)	**Location**	**n**	**% of total invasive population**	**cases with an ultrasound anomaly % (n)**
15q11.2 dup	chr15:22.800.000-23.090.000	32	0,24	34 (11)
15q11.2 del (OMIM 615656)	chr15:22.800.000-23.090.000	25	0,19	56 (14)
CHRNA7 dup	chr15:31.130.000-32.480.000	21	0,16	10 (2)
MYH11 dup	chr16:14.980.000-	16	0,12	56 (9)

	16.480.000			
NPHP1 dup	chr2:110.870.000-110.980.000	13	0,10	46 (6)
HFE2 dup	chr1:144.970.000-146.100.000	10	0,08	20 (2)
MYH11 del	chr16:14.980.000-16.480.000	9	0,07	44 (4)

VOUS	**Location**	**n**	**% of total invasive population**	**cases with an ultrasound anomaly % (n)**
6q22.31 dup	chr6: 123.539.625 - 124.328.531	10	0,07	50 (5)
17p13.3 dup	chr17: 148.092 - 597.702	6	0,05	0 (0)
9p23 dup	chr9: 10.164.926 - 11.868.588	6	0,05	33 (2)
10q23.31 del	chr10: 91.626.482 - 92.035.457	6	0,05	33 (2)
22q11.23 dup	chr22: 23.720.181 - 24.959.827	6	0,05	17 (1)
14q11.2 dup	chr14: 22.323.879 – 22.964.864	5	0.04	40 (2)
3p14.2 dup	chr3: 59.666.501 – 60.993.079	5	0.04	20 (1)

Table 2. Yield of karyotyping, CMA and NIPT in prenatal samples subdivided according to indication. The added value of using CMA vs karyotyping is shown in the last column. Yield is the percentage of diagnoses detected by using a particular test compared to not testing at all.

Indications	Yield karyotyping in %	Yield CMA‡‡ in %	Yield NIPT§§ (all aneuploidies) in %	Added value CMA vs karyotyping in %
USA†	19,4	22,1	18,1	2,7
FTS‡	5,7	7	5,5	1,3
Fam gen disorder§	4,2	6,7	4	2,5
NIPT ¶	71,9	72,6	69,6	0,7
AMA ††	2,6	3,7	2,5	1,1
Other	2,6	4,2	2,3	1,6
Total	**10,1**	**11,9**	**9,6**	**1,8**

Abbreviations: † : USA: Ultrasound anomaly, ‡: FTS: an abberant Down screening test , §: Fam gen disorder: Known genetic disorder in the family, ¶: NIPT: abnormal result on Non-Invasive Prenatal Test, †† : AMA: Advanced Maternal Age, ‡‡: CMA: Chromosomal Microarray Analysis, §§: Non Invasive Prenatal Testing, Other (including CMV, toxoplasmosis, anxiety)