Reproductive Sciences
https://doi.org/10.1007/s43032-020-00419-9

GENETICS: ORIGINAL ARTICLE

Clinical Application of Easychip 8x15K Platform in 4106 Pregnancies Without Ultrasound Anomalies

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Received: 19 August 2020 / Accepted: 29 November 2020

Abstract

Clinical utility of Array-CGH Easychip 8x15K platform can be assessed by testing its ability to detect the occurrence of pathogenic copy number variants (CNVs), and occurrence of variants of uncertain significance (VoUS) in pregnancies without structural fetal malformations. The demand of chromosomal microarray analysis in prenatal diagnosis is progressively increasing in uneventful pregnancies. However, depending on such platform resolution, a genome-wide approach also provides a high risk of detecting VoUS and incidental finding (IF) also defined as "toxic findings." In this context, novel alternative strategies in probe design and data filtering are required to balance the detection of disease causing CNVs and the occurrence of unwanted findings. In a cohort of consecutive pregnancies without ultrasound anomalies, a total of 4106 DNA samples from cultured and uncultured amniotic fluid or chorionic villi were collected and analyzed by a previously designed Array-CGH mixed-resolution custom platform, which is able to detect pathogenic CNVs and structural imbalanced rearrangements limiting the identification of VoUS and IF. Pathogenic CNVs were identified in 88 samples (2.1%), 19 of which (0.5%) were undetectable by standard karyotype. VoUS accounted for 0.6% of cases. Our data confirm that a mixed-resolution and targeted array CGH platform, as Easychip 8x15K, yields a similar detection rate of higher resolution CMA platforms and reduces the occurrence of "toxic findings," hence making it eligible for a first-tier genetic test in pregnancies without ultrasound anomalies.

Keywords Array CGH · Pregnancy without ultrasound anomalies · General cytogenetics · CNV · VOUS · Genetic counseling

Introduction

Chromosome microarray analysis (CMA) has an increasing pivotal role in prenatal diagnosis. Compared with standard karyotype, its advantages include the following: a greater sensitivity in detecting cryptic unbalanced rearrangements (deletions and duplications < 10 Mb, tight characterization of chromosomal aberrations in terms of breakpoints and gene content); the availability of DNA directly extracted from uncultured fetal samples (chorionic villi, amniotic fluid, and fetal tissues) reducing reporting time and culture bias; the possibility to customize the used platforms, focusing on areas of interest. [1]. On the other hand, CMA disadvantages comprise the inability to identify balanced rearrangements and low level of mosaicisms; the risk of detecting variants of uncertain significance (VoUS), incidental findings (IFs), susceptibility loci (SL), or late-onset disease-related copy number variants (CNVs) [1, 2].

Italian and international guidelines [3, 4] recommend CMA in high-risk pregnancies (fetuses with ultrasound anomalies or/and chromosomal de novo rearrangements identified by karyotype) due to the high detection rate of pathogenic CNVs over standard karyotype, ranging 4.1–6.8% [5–8].

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Published online: 06 January 2021

In uneventful pregnancies, also referring to pregnancies without ultrasound anomalies, CMA utilization is still debated [9] despite its ability to detect clinically relevant CNVs in 0.37–0.9% of pregnancies, regardless of any a priori risks (advanced maternal age, altered results at biochemical first trimester screening tests, family history) [5, 10–16]. Controversial opinions are due to its high risk of identifying VoUS, IF, and SL, which have been reported to not have any evaluable impact on fetal prognosis, and are often referred as "toxic information," increasing parental anxiety, and complicating pregnancy management and resulting in greater need for careful pre- and post-test counseling [2]. Furthermore, limited data available on fetal phenotypes (especially in neurocognitive disorders) leads to additional difficulties in data interpretation as well as in genotype-phenotype correlations. To date, clinical utility of CMA in uneventful pregnancies however has not been well established by previous studies, due to small cohort of patients and technical and analytical heterogeneity of available data, including different resolution of array platforms, variability in clinical indications, and operator-dependent parameters for inclusion/exclusion of CNVs.

In order to reach a balance between high rate of detection of clinically relevant CNVs and a low number of VoUS, an appropriate probe design and data filtering strategy is recommendable, especially when monitoring pregnancies without ultrasound anomalies. To address these goals, a custom low-resolution oligonucleotide array CGH platform has previously been designed [16].

Here we report the results of a study carried out on a cohort of 4106 pregnancies without ultrasound anomalies, all analyzed with the customized oligonucleotide-based microarray platform (custom Easychip15K; Agilent Technologies, USA) [16], with the aim to evaluate its clinical utility in pregnancies with no ultrasound anomalies.

Methods

Patients

In a cohort of consecutive pregnancies without ultrasound anomalies, a total of 4106 prenatal samples were analyzed by Array-CGH Easychip 8x15K platform. DNA was isolated from amniocytes or chorionic villi, either cultured or uncultured. The samples were collected in two centers (Bambino Gesù Children's Hospital, Rome, Italy, and San Pietro Fatebenefratelli Hospital, Rome, Italy) from May 2015 to May 2019. At time of chorionic villus sampling or amniocentesis, no ultrasound anomalies were documented and the only clinical indications were advanced maternal age, parental anxiety, and positive first trimester screening tests (biochemical test or high risk for only trisomy 13, 18, 21 at Non Invasive Prenatal Testing - NIPT). Informed consent, advising about risks, advantages, and limits of this procedure, was obtained from each woman admitted to an invasive prenatal testing procedure. Out of 4106 analyses, 863 were performed on DNA extracted from choric villus samples, and 3243 from amniocytes; 16 samples were discharged due to maternal contamination.

CMA Techniques

Array-CGH Easychip 8x15K can be performed with a minimal quantity of DNA (200 ng), providing rapid results. DNA was obtained from uncultured amniotic fluid (6–8 ml), chorionic villi (2–4 mg), or cell cultures by using QIAamp® Blood Mini Kit (QIAGEN, Germany). DNA quality and quantification were assessed using the NanoDrop® ND-8000 spectrophotometer (Thermo Scientific, USA). The analyses were carried out following the Agilent microarray protocol recommendations (Agilent Technologies, USA).

Array Platform

Array-CGH Easychip 8x15K (Agilent Technologies, USA) is designed to assess on 43 genomic regions associated with clinical severe microdeletion/microduplication disorders, with a technical resolution of 125 Kb. All the selected regions are reported in OMIM Database (Online Mendelian Inheritance in Man) in association with a well-defined and high penetrant (> 70%) pathological phenotype (Table 1) [16]. Deletions/duplications reported as disease susceptibility loci or associated with late-onset disorders have not been included because their detection was considered not suitable for a prenatal screening purpose. In order to identify structural rearrangements, such as unbalanced translocations or recombinant chromosomes, the oligonucleotide probes cover subtelomeric regions with an average resolution of 250 Kb. In the remaining genome (backbone), the average technical resolution is limited to 2.5 Mb to reduce the probability of VoUS and/or IF identification. This genome-wide coverage allows detecting pathogenic unbalances at a higher resolution than standard karyotype, while providing a tight characterization of breakpoints and gene content.

In Silico Analysis

Resulting data were analyzed using CytoGenomics 4.0 Software Analysis (Agilent Technologies, USA), setting a minimum of 5 consecutive probes presenting with an over-threshold log2ratio as an analytical parameter for CNVs detection. Some post-analysis filters were also applied before reporting: unknown CNVs were considered only if larger of 3 Mb in the backbone, 300 Kb in subtelomeric regions, and 200 Kb in the 43 disease-causing loci. Gene content, familiar

Table 1 Syndromic regions encompassing the critical genes covered by Easychip [16]

Syndromic regions	Critical genes
1p36 deletion syndrome	/
1q41q42 microdeletion syndrome	*DISP1*
2p15-16.1 microdeletion syndrome	*BCL11A*
2q23.1 microdeletion syndrome	*MBD5*, *EPC2*
2q33.1 deletion (Glass syndrome)	*STAB2*
2q37 deletion syndrome	*HDAC4*
3pter-p25 deletion syndrome	*CNTN4*, *ITPR1*, *SRGAP3*, *VHL*
3q29 deletion syndrome	*FBXO45*, *PAK2*, *DLG1*
3q29 duplication syndrome	*FBXO45*, *PAK2*, *DLG1*
4p16.3 deletion syndrome (Wolf-Hirschhorn)	*LETM1*, *WHSC1*
4q21 deletion syndrome	*PRKG2*, *RASGEF1B*
5p deletion syndrome (Cri du chat)	*CTNND2*, *TERT*
5q14.3 deletion syndrome	*MEF2C*
5q35 deletion syndrome (Sotos)	*NSD1*
6q13-q14 deletion syndrome	*COL12A1*
7q11.23 deletion syndrome (Williams-Beuren)	*ELN*
7q11.23 duplication syndrome	/
8p23.1 deletion syndrome	*GATA4*
8p23.1 deletion syndrome	*GATA4*
8q21.11 microdeletion syndrome	*ZFHX4*, *PEX2*
8q24.1 deletion syndrome (Langer-Giedion)	*TRPS1*, *EXT1*
9q34.3 deletion syndrome (Kleefstra)	*EHMT1*
10p14p13 deletion syndrome (DiGeorge type 2)	*GATA3*
11p13 deletion syndrome (WAGR)	*PAX6*, *WT1*
11p11.2 deletion syndrome (Potocki-Shaffer)	*ALX4*
11q deletion syndrome (Jacobsen)	/
14q12 microdeletion syndrome	*FOXG1*
15q11q13 deletion syndrome (Prader-Willi)	*SNRPN*
15q11q13 deletion syndrome (Angelman)	*UBE3A*
15q24 deletion syndrome	/
15q24 duplication syndrome	/
16p deletion syndrome (ATR-16)	*HBA1*, *HBA2*
16q24.1 microdeletion syndrome	*FOXF1*, *FOXC2*
17p13.3 deletion syndrome (Miller-Dieker)	*PAFAH1B1*, *YWHAE*
17p11.2 deletion syndrome (Smith-Magenis)	*RAI1*
17p11.2 duplication syndrome (Potocki-Lupski)	*RAI1*
17q11.2 deletion syndrome	*NF1*, *SUZ12*
17q11.2 duplication syndrome	*NF1*, *SUZ12*
17q21.31 deletion syndrome (Koolen-De Vries)	*KANSL1*
17q23.1-q23.2 deletion syndrome	*TBX2*, *TBX4*
19q13.11 deletion syndrome	*LSM14A*, *UBA2*
Down syndrome critical region (21q22.12q22.2)	/
22 partial tetrasomy (Cat-eye syndrome)	/
22q11.2 deletion syndrome (DiGeorge)	*HIRA*, *TBX1*
22q11.2 duplication syndrome	*HIRA*, *TBX1*
22q11.2 distal deletion syndrome	*MAPK1*
Xp11.3 deletion syndrome	*RP2*
Xp11.22 microduplication syndrome	*HUWE1*
Xq12 deletion syndrome	*OPHN1*

Table 1 (continued)

Syndromic regions	Critical genes
Xq22.3 deletion syndrome (AMME COMPLEX)	*COL4A5*, *ACS4*
Xq28 duplication syndrome	*MECP2*

anamnesis, and further acquired information about the ongoing pregnancy were also carefully evaluated before deciding not to report a CNV basing on its size. We excluded gene desert regions and CNVs reported in more than three different population studies available in Database of Genomic Variant (DGV, http://dgv.tcag.ca/dgv/app/home) thus considered likely benign. Resulting CNVs interpretation was carried out according to the ACMG guidelines [17]. Moreover, a subgroup of 483 cases was reanalyzed without applying any reporting and operator-dependent filters in order to evaluate an unbiased VoUS estimation of our platform. These are consecutive and unbiased cases, arrived during the last collecting year, whose raw data were still available at Bambino Gesu' Children's Hospital.

Results

Number Anomalies

Out of 4106 samples analyzed by Easychip 8x15K, 88 (2.1%) were identified to harbor pathogenic and likely pathogenic CNVs, and chromosome aneuploidy, some of which could be recognized by standard karyotype. For this reason, our results were grouped into two major classes:

1. Karyotype detectable findings: 69 cases (Table 2), including 63 aneuploidies (1.5%) and 6 CNVs larger than 10 Mb in size (0.2%) (Table 2). In detail, we found twenty-eight trisomy 21, seven 47,XYY, six 47,XXY, six 45,X, four trisomy/tetrasomy 9p, and three 47,XXX. Trisomies involving chromosomes 2, 13, 15, 16, 17, 18, 20, and 22 (some of which are mosaic aneuploidies confined to the placenta, not confirmed by consecutive amniocentesis) were also detected in 9 samples. All these findings were also confirmed by standard chromosome analyses. Within these 69 cases, six had a structural rearrangement: two samples presented with two large duplications each (9p24.3q21.13/20p13p12.2 and 1q21.1q44/18p11.32p11.21, respectively), two harbored a large deletion on X chromosome (at Xq26.1q28, and Xp22.33p11.22, respectively), and two more an unbalanced translocation (9p24.3p22.3 deletion/18q22.1q23 duplication and 7p22.33 deletion/12p13.33p12.3 duplication, respectively) (Table 2).
2. Karyotype undetectable findings: pathogenic or likely pathogenic submicroscopic CNVs were detected in 19 samples (0.5%) (Table 3). Sixteen of them were classified as known CNVs disorders annotated in OMIM (Online Mendelian Inheritance in Man); 3 additional CNVs were classified as likely pathogenic based on segregation pattern and gene content. The detected microdeletion syndromes included the following: DiGeorge (OMIM #188400, 4 cases), Prader-Willi/Angelman (OMIM # 176270/105830, 3 cases), 22q11.2 duplication (OMIM #608363, 2 cases), 7q11.23 duplication (OMIM #609757, 1 case), 15q11q13 duplication (OMIM #608636, 1 case), Miller-Dieker lissencephaly (OMIM #247200, 1 case), 17q11.2 deletion (OMIM #613675 1 case), Koolen-de Vries (OMIM #610443, 1 case), Xp11.22 duplication (OMIM #300705, 1 case). In one sample, the detected microduplication overlapped two different distinct syndromic regions: Lubs X-linked mental retardation syndrome (OMIM #300260) and Xq28 duplication syndrome (OMIM #300815). Three microdeletions were classified as likely pathogenic as they encompassed a dominant-acting disease gene. They included a 6.8 Mb deletion at 18q22.2q23 comprising *TSHZ1* (OMIM * 614427) gene, a 1.8 Mb deletion at 19q13 containing *KMT2B* (OMIM * 606834) gene, and a 437Kb deletion at Xq28 involving *F8* (OMIM* 300841), *RAB39B* (OMIM* 300774), and *CLIC2* (OMIM* 300138) genes. The latter deletion was not considered clinically relevant since it was found in a female fetus, but it was important for assessing the reproductive risk of the parents.

Variant of Uncertain Significance

Out of 4106 samples analyzed, 24 VoUS (0.6%) were reported in this study. Segregation analyses were performed and all of them resulted to be inherited from a healthy parent. Gene content was carefully studied and, when appropriate, a specific ultrasound examination was suggested. In addition, in order to obtain an unbiased estimation of our platform in VoUS detection, a subgroup involving 483 consecutive samples was reanalyzed without applying any reporting and operator-dependent filters. Using these criteria, the final VoUS rate increased to 2.5% (12/483), reflecting only the platform

Table 2 Samples with karyotype detectable aneuploidies and CNVs larger than 10 Mb in size, in a cohort of 4106 samples in uneventful pregnancies. All genomic coordinates used are matching the Human Reference Genome assembly GRCh37/hg19

No of cases	aCGH output (ISCN 2016)	Size	Syndromes (OMIM #) / disease genes	Note
28	arr(21)x3		Down syndrome # 190685	1/28 in mosaic 1/28 plus trisomy 3 in mosaic
6	arr(X)x2,(Y)x1		Klinefelter syndrome	1/6 in mosaic
7	arr(X)x1,(Y)x2		47,XYY	1/7 in mosaic
3	arr(X)x3		47,XXX	1/3 in mosaic
6	arr (1–22)x2,(X)x1		Turner syndrome	1/6 in mosaic
4	arr(9p)x4 / arr(9p)x3		Tetrasomy 9p / Trisomy 9p	3/4 tetrasomy 9p 1/4 trisomy 9p
1	arr(13)x3		Trisomy 13	
1	arr(16)x3		Trisomy 16	
2	arr(18)x3		Trisomy 18	
1	arr(20)x3		Trisomy 20	
1	arr(22)x3		Trisomy 22	
1	arr(2)x2~3		Trisomy 2 mosaicism	
1	arr(15)x2~3		Trisomy 15 mosaicism	
1	arr(17)x2~3		Trisomy 17 mosaicism	
1	arr[GRCh37] 9p24.3q21.13(371798_74974102)x3, 20p13p12.2(80198_11178673)x3	75 Mb/11 Mb	Duplication syndrome	
1	arr[GRCh37] 1q21.1q44(144009907_249197762)x3, 18p11.32p11.21(64847_14915809)x4	105 Mb/15 Mb	Duplication syndrome	
1	arr[GRCh37] Xq26.1q28(128891217_155232877)x1	26 Mb	Deletion syndrome	
1	arr[GRCh37] Xp22.33p11.22(318707_50371486)x1	50 Mb	Deletion syndrome	
1	arr[GRCh37] 9p24.3p22.3(371798_14811201)x1, 18q22.1q23(62749488_78010032)x3	14.4 Mb/15.3 Mb	Unbalanced translocation	
1	arr[GRCh37] 7p22.3(65558_1087076)x1~2, 12p13.33p12.3(192511_16072872)x2~3	1 Mb/16 Mb	Unbalanced translocation in mosaic	

resolution. Unknown CNVs were predominantly located within the highest resolution regions: six were detected within the 43 syndromic regions, without harboring the syndrome-associated critical genes; five were detected in the subtelomeric regions and were not associated with structural unbalanced rearrangements. Only one VoUS mapped into the backbone, where the lower resolution did not allow detecting imbalances smaller than 2.5 Mb.

Discussion

As results of our previously study, Easychip 8x15K platform had proposed for screening purposes in pregnancies without ultrasound anomalies, in association with standard karyotype [16]. It takes advantages by requiring low amount of DNA (only 200 ng), which can be easily obtained from 8 to 10 ml of uncultured amniotic fluid or 2–4 mg chorionic villi, and

Table 3 Samples with submicroscopic CNVs less than 10 Mb not detectable by standard karyotype in uneventful pregnancies. Each case is identified by the OMIM ID number. All genomic coordinates used are matching the Human Reference Genome assembly GRCh37/hg19

Fetal sex	aCGH output (ISCN 2016)	Size	Syndromes (OMIM #) / disease genes (OMIM *)
Female	arr[GRCh37] 7q11.23(72700414_74233342)x3 dn	1.5 Mb	7q11.23 duplication syndrome # 609757
Female	arr[GRCh37] 15q11.2q13.1(24738180_28434569)x1	3.7 Mb	Prader-Willi syndrome # 176270 / Angelman syndrome # 105830
Female	arr[GRCh37] 15q11.2q13.1(24738180_28434569)x1 dn	3.7 Mb	Prader-Willi syndrome # 176270 / Angelman syndrome # 105830
Male	arr[GRCh37] 15q11.2q13.1(24738180_28434569)x1 dn	3.7 Mb	Prader-Willi syndrome # 176270 / Angelman syndrome # 105830
Female	arr[GRCh37] 15q11.2q13.1(24722605_28434569)x3 mat	3.7 Mb	15q11q13 duplication syndrome # 608636
Female	arr[GRCh37] 17p13.3p13.2(148092_6203874)x1 dn	6 Mb	Miller-Dieker lissencephaly syndrome # 247200
Male	arr[GRCh37] 17q11.2(29114365_30342666)x1 dn	1.2 Mb	17q11.2 deletion syndrome # 613675
Male	arr[GRCh37] 17q21.31(43655747_44158721)x1 dn	500 Kb	Koolen-De Vries syndrome # 610443
Male	arr[GRCh37] 18q22.2q23(67270651_74053398)x3 dn	6.8 Mb	*RTTN* (* 610436 AR), *CYB5A* (* 613218 AR), *TSHZ1* (* 614427 AD)
Female	arr[GRCh37] 19q13.12 (36078544_37891229)x1 dn	1.8 Mb	*COXB61* (* 124089 AR), *KMT2B* (* 606834 AD), *PSENEN* (* 607632 AD), *NPHS1* (* 602716 AR), *TYROBP* (* 604142 AR), *SDHAF1* (* 612848 AR), *SYNE4* (* 612848 AR), *WDR62* (* 613583 AR).
Male	arr[GRCh37] 22q11.21(18729944_21463108)x1 dn	2.7 Mb	DiGeorge syndrome # 188400
Male	arr[GRCh37] 22q11.21(18729944_20311762)x1 dn	1.6 Mb	DiGeorge syndrome # 188400
Female	arr[GRCh37] 22q11.21(18847189_21463108)x1 dn	2.6 Mb	DiGeorge syndrome # 188400
Male	arr[GRCh37] 22q11.21(18919942_21440514)x1	2.5 Mb	DiGeorge syndrome # 188400
Male	arr[GRCh37] 22q11.21(18889039_21463108)x4	2.6 Mb	22q11.2 duplication syndrome # 608363
Male	arr[GRCh37] 22q11.21(18919942_20719171)x3 dn	1.8 Mb	22q11.2 duplication syndrome # 608363
Female	arr[GRCh37] Xp11.22(53480069_53707032)x3 mat	227 Kb	Xp11.22 duplication syndrome # 300705
Female	arr[GRCh37] Xq28(154124170_154561274)x1 dn	437 Kb	*F8* (* 300841), *RAB39B* (* 300774), *CLIC2* (* 300138)
Male	arr[GRCh37] Xq28(152788477_153832724)x2 mat	1 Mb	Lubs X-linked mental retardation syndrome (# 300260), Xq28 duplication syndrome (# 300815)

provides results in a few days (3–7 days after amniocentesis/villocentesis). Of note, working directly on uncultured fetal material (amniotic fluid/chorionic villi) allows obtaining results not affected by culture bias, being particularly important in the case of mosaicisms. The platform is designed to cover the whole genome at different resolution levels, with a higher probe coverage in pathogenic genomic areas. Similarly to high-resolution CMA, this platform allows to detect pathogenic CNVs and provides detailed information about chromosome rearrangements in terms of breakpoints and gene content. On the other hand, differently from other CMA platforms commonly used, it provides a minimal possibility of finding VoUS or IF, which are considered toxic knowledge [2]. In some instances, segregation analysis may help in VoUS evaluations by assessing its de novo or inherited occurrence. The trio-extended analyses, however, are time-consuming, increase the risk of identifying IFs in parents, and are not always exhaustive due to reduced penetrance and/or variable expressivity of many disorders.

In this study, we provide data obtained on a large cohort of 4106 unbiased consecutive samples from uneventful pregnancies by using Easychip 8x15K platform.

The included women underwent invasive procedure for advanced maternal age, parental anxiety, and positive first trimester screening tests (biochemical test or Non Invasive Prenatal Testing - NIPT). We recognized clinically relevant chromosomal aberrations with a detection rate of 2.1%.

The occurrence of VoUS in previous CMA-based prenatal analyses is discordant, ranging between 7.3 and 0.4%, due to the different operator-dependent filters and VoUS classification, and the resolution used in each study [14, 18–20]. In our cohort, the platform adopted differential resolutions in backbone versus critical regions, by using the appropriate reporting filters (i.e., gene content, parental inheritance, and family history) and the geneticist's experience, resulting in a VoUS detection rate of 0.6%. Notably, to estimate an unbiased VoUS detection rate, we reanalyzed raw data of a subgroup of 483 samples without applying any reporting filters, thus considering the only probe design. This subgroup was selecting only considering the samples arrived in Bambino Gesù Children's Hospital in 2019, in their consecutive order, without introducing any selection bias. By using this alternative probe setting, the detection rate increased to 2.5%. These data corroborate the present platform may be a versatile tool for screening purposes in prenatal setting in particular when no ultrasound anomalies occur.

Excluding chromosome aneuploidies, which represent the highest genetic risk in this specific cohort of women (especially considering samples collected for advanced maternal age and positive results at first trimester screening tests), we detected a 0.5% of extra clinical relevant imbalances. These latter involve microdeletions and microduplications undetectable by standard karyotype, arisen de novo, and are associated with severe and highly penetrant syndromes. This class of aberrations is not dependent of any a priori risk. Notably, the affected fetuses did not show any sonographic anomalies at the time of sampling which could lead to a selection bias, so the detected percentage (0.5%) could be considered representative for microdeletion/microduplication syndromes incidence in human population. This result is consistent with scientific literature, which documented a detection rate ranging between 0.37 and 0.9% [5, 10, 11, 14, 15].

Basing on previously reported epidemiologic data, the 22q11.21 genomic region is believed to be the higher pathogenic cryptic imbalance (ranging between 0.1 and 0.5% in general population) due to its flanked repetitive elements, which are prone to rearrangements [21]. Similarly, our data report an incidence of the DiGeorge syndrome deletion of approximately 1/1000 (4/4106) and a reciprocal duplication accounting for 1/2000 (2/4106), resulting in an overall incidence of 22q11.21 imbalances in pregnancies without ultrasound anomalies of about 1 in 684 (0.15%). Similar considerations apply with respect to the incidence of 15q11.2 region imbalance, whose deletions are associated with Prader-Willi/Angelman syndrome. Out of 4106 samples, three cases of deletion and one case with the reciprocal duplication were identified, pointing to an overall incidence of 15q11.2 region imbalance of 1 in 1000 in the present series. A larger cohort of clinically unselected pregnancies, however, are needed to assess more accurate figures of incidence of these pathogenic imbalances in the general population.

In conclusion, on the dilemma of the application of CMA in women willing to undergo invasive prenatal testing, regardless of the maternal age, of other a priori risks [5, 12], this study proposes the Easychip 8x15K platform as a valuable tool in the prenatal first-tier genetic test. Our data provide evidence that this platform may be suitable for screening purposes and routine prenatal diagnosis in pregnancies without ultrasound anomalies, allowing advantages in prenatal counseling and pregnancy management.

Acknowledgments The authors would like to acknowledge all the pregnant women participating to this study.

Author Contributions O.V. conceptualization, data collection and analysis, project development, manuscript writing and editing; A.V.: conceptualization, data collection and analysis, manuscript editing, project development; D.G.G: data collection and analysis, methodology, project development; C.M.: data collection and analysis, methodology; C.C.: methodology; N.A.M.: data management; C.G.: methodology; L.M.T.: methodology; S.E.: data collection and analysis; D.T.S.: data collection and analysis; M.G.D.G.: data management, patients counseling; C.F.: data collection; N.M.: manuscript editing; B.G.: project development, data management; D.B.: manuscript editing; N.A.: conceptualization, project development, data management manuscript editing.

Compliance with Ethical Standards

Conflict of Interest The author(s) declared no conflicts of interest.

Ethics Approval Clinical data were obtained in accordance with the ethical standards of the Bambino Gesù Children's Hospital (Rome, Italy) review board (RRC-2018-2365812).

Informed Consent Informed consent was obtained from all individual participants included in the study.

References

1. South ST, Lee C, Lamb AN, et al. ACMG Standards and Guidelines for constitutional cytogenomic microarray analysis, including postnatal and prenatal applications: revision 2013. Genet Med. 2013;15(11):901–9.
2. Bernhardt BA, Soucier D, Hanson K, et al. Women's experiences receiving abnormal prenatal chromosomal microarray testing results. Genet Med. 2013;15(2):139–45.
3. SIGU, SIEOG (2017) Uso appropriato delle tecniche di CMA (Chromosomal Microarray Analysis) nella diagnosi prenatale. Available from URL: https://www.sieog.it/wp-content/uploads/2017/06/Uso-appropriato-delle-tecniche-di-CMA-Chromosomal-Microarray-Analysis-nella-diagnosi-prenatale.pdf Accessed: May 2017.
4. Silva M, de Leeuw N, Mann K, Schuring-Blom H, Morgan S, Giardino D, et al. European guidelines for constitutional cytogenomic analysis. Eur J Hum Genet. 2019;27(1):1–16.
5. Wapner RJ, Lese Martin C, Levy B, et al. Chromosomal microarray versus karyotyping for prenatal diagnosis. N Engl J Med. 2012;367(23):2175–84.
6. de Wit MC, Srebniak MI, Govaerts LC, et al. Additional value of prenatal genomic array testing in fetuses with (isolated) structural ultrasound abnormalities and a normal karyotype: a systematic review of the literature. Ultrasound Obstet Gynecol. 2014;43:139–46.
7. Shaffer LG, Dabell MP, Fisher AJ, Coppinger J, Bandholz AM, Ellison JW, et al. Experience with microarray-based comparative genomic hybridization for prenatal diagnosis in over 5000 pregnancies. Prenat Diagn. 2012;32:976–85.
8. Hillman SC, McMullan DJ, Hall G, et al. Use of prenatal chromosomal microarray: prospective cohort study and systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2013;41:610–20.
9. Novelli A, Cavalli P, Bernardini L. The future of prenatal diagnosis: karyotype, microarray or both? Technical and ethical considerations. Expert Rev Proteomics. 2013;10(2):131–4.
10. Srebniak MI, Joosten M, Knapen MFCM, Arends LR, Polak M, van Veen S, et al. Frequency of submicroscopic chromosomal aberrations in pregnancies without increased risk for structural chromosomal aberrations: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2018;51:445–52.
11. Lee CN, Lin SY, Lin CH, Shih JC, Lin TH, Su YN. Clinical utility of array comparative genomic hybridisation for prenatal diagnosis: a cohort study of 3171 pregnancies. BJOG. 2012;119(5):614–25.
12. American College of Obstetricians and Gynecologists Committee on Genetics. Committee Opinion No. 581: the use of chromosomal microarray analysis in prenatal diagnosis. Obstet Gynecol. 2013;122(6):1374–7.
13. Hay SB, Sahoo T, Travis MK, Hovanes K, Dzidic N, Doherty C, et al. ACOG and SMFM guidelines for prenatal diagnosis: is karyotyping really sufficient? Prenat Diagn. 2018;38(3):184–9.
14. Papoulidis I, Sotiriadis A, Siomou E, Papageorgiou E, Eleftheriades M, Papadopoulos V, et al. Routine use of array comparative genomic hybridization (aCGH) as standard approach for prenatal diagnosis of chromosomal abnormalities. Clinical experience of 1763 prenatal cases. Prenat Diagn. 2015;35(13):1269–77.
15. Stern S, Hacohen N, Meiner V, Yagel S, Zenvirt S, Shkedi-Rafid S, et al. Universal chromosomal microarray analysis reveals high proportion of copy number variants in low risk pregnancies. Ultrasound Obstet Gynecol. 2020. https://doi.org/10.1002/uog.22026.
16. Alesi V, Bernardini L, Goidin D, et al. Easychip 8x15k: a new tool for detecting chromosome anomalies in low risk pregnancies, supporting and integrating standard karyotype. J Genet Syndr Gene Ther. 2016;7:1.
17. Kearney HM, Thorland EC, Brown KK, Quintero-Rivera F, South ST, Working Group of the American College of Medical Genetics Laboratory Quality Assurance Committee. American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants. Genet Med. 2011;13(7):680–5.
18. Van Opstal D, de Vries F, Govaerts L, et al. Benefits and burdens of using a SNP array in pregnancies at increased risk for the common aneuploidies. Hum Mutat. 2015;36(3):319–26.
19. Levy B, Wapner R. Prenatal diagnosis by chromosomal microarray analysis. Fertil Steril. 2018;109(2):201–12.
20. Sagi-Dain L, Cohen Vig L, Kahana S, et al. Chromosomal microarray vs. NIPS: analysis of 5541 low-risk pregnancies. Genet Med. 2019;21(11):2462–67.
21. Grati FR, Molina Gomes D, Ferreira JC, et al. Prevalence of recurrent pathogenic microdeletions and microduplications in over 9500 pregnancies. Prenat Diagn. 2015;35(8):801–9.