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Easychip 8x15k: A New Tool for Detecting Chromosome Anomalies in Low Risk Pregnancies, Supporting and Integrating Standard Karyotype

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Abstract

Over last decade chromosome microarray analysis has become a routine test, but its use as first tier in prenatal diagnosis still raises disputes specially when applied to low risk pregnancies. In order to limit the identification of incidental findings (IF) and variants of unknown significance (VOUS) we designed EasyChip, a low-resolution oligonucleotide array CGH platform with a functional resolution of 3 Mb in genomic backbone, 300 Kb in sub-telomeric regions, and 150 Kb in 43 regions associated with syndromic disorders, selected considering morbidity, penetrance, and etiological mechanisms. After an "in silico" evaluation, which showed that Easychip would not uncover most of VOUS (24% vs 3%) and any IF, we have validated EasyChip on 169 patients samples, 57 retrospective samples with known imbalances and 112 prospective samples as part of the prenatal diagnosis process. All the known rearrangements were detected and 7 further pathogenic imbalances were detected on the still undiagnosed cohort. To evaluate false positive/negative rate, thirty-eight out of the 112 prospective samples were also processed on an high resolution array CGH, allowing comparing the results in term of diagnostic utility and impact on detection rate. Two positive and pathogenic results were detected by both platforms. EasyChip did not detect 10 of the 11 VOUS nor 2 IF discovered by the high-resolution platform. In conjunction with karyotype, EasyChip is a useful tool in prenatal diagnosis for screening purposes on low risk pregnancies, it enables the detection of cryptic imbalanced subtelomeric rearrangements, microdeletions/duplications within 43 syndromic regions and supports standard cytogenetic analysis at whole genome level. Finally, this tool, differently from higher resolution platforms, significantly reduces the detection rate of VOUS and IF, which represent a major drawback during genetic counselling specially for low risk pregnancies, significantly reduces the time to spend on analysis and limit the need of additional confirmation.

Keywords: Prenatal diagnosis; CMA; CGH; Low risk pregnancies; CNV; VOUS; Deletion duplication syndromes

Introduction

The request for prenatal in-depth analyses over standard karyotype has dramatically increased in recent years. Chromosome Microarray Analysis (CMA) provides information on small rearrangements often overlooked by banded metaphases that can be significant for fetal prognosis, leading to distinct genomic disorders.

CMA for the detection of genomic imbalances (Copy Number Variations, CNVs) is widely used in prenatal diagnosis, in particular after an abnormal ultrasound result and for a deeper characterization of chromosomal anomalies. In this cases it results in a detection rate of pathogenic CNVs significantly high (4.1-6.5%). Conversely, when applied to low risk pregnancies (without sonographic or chromosomal anomalies, i.e. prenatal diagnosis performed for parental anxiety, advanced maternal age, positive 1st trimester screening but normal karyotype) the rate is about 1.1-1.7%, which also deserves consideration. In any case, the major challenge of prenatal CMA is data interpretation, since the clinical significance of many CNVs is still unclear [1-8].

In low risk pregnancies VOUS detection and reporting becomes a major issue because of the absence of an evaluable phenotype to conclude on the pathogenicity of a given CNV. A general strategy to improve the understanding of the prognostic role of a given CNV is to test the parents' DNA, which increases both the cost of analysis and parental anxiety, without reaching conclusive results in many instances [12]. Performing CMA on parental DNA can rise additional ethical dilemmas, when CNVs not present in the fetus are found in the parents or when Incidental Findings (IF) are disclosed (e.g., susceptibility loci for psychiatric illness, tumor predisposition CNVs or late onset pathologies), eventually predicting a clinical effect in the individual, in future pregnancies and in other family members.

The detection rate should not be the only spur for performing a genetic test, which is appropriate when the pieces of information provided are pertinent with the reasons the test was performed for, presenting a positive balance between aim-related advantages and unavoidable disadvantages [13,14]. Ethical aspects and familiar consequences should also be taken into account.

With the purpose of obtaining manageable diagnostic results in low risk pregnancies, specially reducing the VOUS and IF rate, we designed EasyChip, a 15K oligonucleotide- array (oligo array), suitable for both high resolution investigation of specific target regions and with a

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functional resolution of 3 Mb at the genome level. This tool was created to be used in parallel with standard karyotype with the clear attend to generate undisputed results and to avoid any delay in providing diagnostic to patient.

Platform Description

Since the beginning, Easychip Design was devised to be integrated with and not to replace standard karyotype method. For low risk pregnancies, we think that the best is a concomitant use. Easychip is an 8x15K oligonucleotide array design which allows high-resolution investigation of 43 genomic regions detailed in Table 1. Those regions are related to highly penetrant (>70%) morbid conditions, due to deletions/duplications as main etiological mechanism [15,16]. All selected disorders are clinically recognizable, with an OMIM (Online Mendelian Inheritance in Man) entry and literature references, and their associated genomic imbalances can be evidenced with an average resolution of 150 Kb. In addition, EasyChip includes probes covering the subtelomeric regions, at an average functional resolution of 300 Kb. Although not all of them have been associated with distinct conditions, subtelomeric regions have been included in the chip because of their high recombination rate, liability to imbalances and association with intellectual disability in most of the cases [17-19]. EasyChip also enables a 3 Mb functional resolution at the genomic backbone level in order to integrate karyotype analysis and therefore, to streamline cytogenetic workflow, mainly when the quality of metaphases is poor. As other array-CGH platforms, EasyChip does not detect balanced

Syndromic regions	incidence	critical genes
1p36 deletion syndrome	1/5,000	/
1q41q42 microdeletion syndrome	unknown	DISP1
2p15-16.1 microdeletion syndrome	unknown	BCL11A
2q23.1 microdeletion syndrome	unknown	MBD5, EPC2
2q33.1 deletion (Glass syndrome)	unknown	STAB2
2q37 deletion syndrome	<1/10,000	HDAC4
3pter-p25 deletion syndrome	unknown	CNTN4, ITPR1, SRGAP3, VHL
3q29 deletion/duplication syndrome	unknown	FBXO45, PAK2, DLG1
4p16.3 deletion syndrome (Wolf-Hirschhorn)	1/20,000 - 1/50,000	LETM1, WHSC1
4q21 deletion syndrome	unknown	PRKG2, RASGEF1B
5p deletion syndrome (Cri du chat)	1/20,000 – 1/50,000	CTNND2, TERT
5q14.3 deletion syndrome	unknown	MEF2C
5q35 deletion syndrome (Sotos)	1-9/100,000	NSD1
6q13-q14 deletion syndrome	unkown	COL12A1
7q11.23 deletion syndrome (Williams-Beuren)	1/10,000	ELN
8p23.1 deletion syndrome	unknown	GATA4
8q21.11 Microdeletion Syndrome	unknown	ZFHX4, PEX2
8q24.1 deletion syndrome (Langer-Giedion)	unknown	TRPS1, EXT1
9q34.3 deletion syndrome (Kleefstra)	unknown	EHMT1
10p14p13 deletion syndrome (DiGeorge type 2)	unknown	GATA3
11p13 deletion syndrome (WAGR)	unknown	PAX6, WT1
11p11.2 deletion syndrome (Potocki-Shaffer)	unknown	ALX4
11q deletion syndrome (Jacobsen)	1/100,000	/
14q12 microdeletion syndrome	unknown	FOXG1
15q11q13 deletion syndrome (Prader-Willi)	1/25,000	SNRPN
15q11q13 deletion syndrome (Angelman)	1/10,000 - 1/20,000	UBE3A
15q24 deletion/duplication syndrome	unknown	/
16p deletion syndrome (ATR-16)	unknown	HBA1, HBA2
16q24.1 micorodeletion syndrome	unknown	FOXF1, FOXC2
17p13.3 deletion syndrome (Miller dieker)	unknown	PAFAH1B1, YWHAE
17p11.2 deletion syndrome (Smith-Magenis)	1/25,000	RAI1
17p11.2 duplication syndrome (Potocki Lupski)	unkown	RAI1
17q11.2deletion/duplication syndrome	unkown	NF1, SUZ12
17q21.31 deletion syndrome (Koolen-De Vries)	1/16,000	KANSL1
17q23.1-q23.2 deletion syndrome	unknown	TBX2, TBX4
19q13.11 deletion syndrome	unknown	LSM14A, UBA2
Down Sndrome critical region (21q22.12q22.2)	1/650 - 1,000	/
22 partial tetrasomy (Cat eye)	1/50,000 - 1/150,000	/
22q11.2 deletion syndrome (DiGeorge)	1/2,000 – 1/4,000	HIRA, TBX1
22q11.2 distal deletion syndrome	unknown	MAPK1
Xp11.3 deletion syndrome	unknown	RP2
Xp11.22 microduplication syndrome	unknown	HUWE1
Xq12 deletion/duplication (OPHN1)	unknown	OPHN1
Xq22.3 deletion syndrome (AMME COMPLEX)	unknown	COL4A5, ACS4
Xq28 duplication syndrome	unknown	MECP2

Table 1: List of syndromic regions selected to be covered by EasyChip

structural rearrangements (i.e. translocations, inversions or insertions not associated with gain or loss of genetic material) or uniparental disomy.

The EasyChip platform has been developed for monitoring low risk pregnancies, with the aim of improving the detection rate of regions known to be associated with microdeletion/microduplication disorders, and limiting the detection of VOUS and IF.

Methods

Probes selection

Easychip design is composed of three main probes groups: group1 contains probes for the 43 genomic regions from Table1, group 2 probes are for subtelomeric regions and group3 probes, referred as backbone, define the remaining genomic segments not overlaying either with group1 or group2. All genomic coordinates used are matching Human Reference Genome assembly GRCh37/hg19. Easychip design have been created using the CGH advanced mode of SureDesign online design tool (https://earray.chem.agilent.com/suredesign/). This advanced mode give access to a CGH High Definition (HD) Database of 28 millions of predesigned CGH probes (Agilent Technologies, Santa Clara, CA, USA). CGH probes of group1 have been selected from the HD database on all regions (including intra and intergenic regions) following an iterative way. First, using an average probe spacing of 25kb and similarity score filter on. Similarity score of SureDesign CGH HD database is a stringent option filtering out all probes with similarity in multiple genomic locations. Out of the 43 regions, the median probe spacing was 22.7Kb (21.3Kb to 24.4Kb) with a total coverage >98% and >98.5% of high quality probes. In a second step, a new search in the HD database using relax criteria (similarity score filter off) was proceeded on gaps of first results. Most of those gaps were collocated with segmental duplication track of UCSC genome browser http://genome.ucsc.edu/ [20]. This strategy enabled to add probes in most of the gaps. We have verified that those additional probes were evenly distributed along the gaps. As expected the percentage of high quality probes was slightly reduced (96.5%) whereas the total coverage increased to > 99.5% without changing significantly the median probe spacing leading to an average functional resolution of 150 Kb.

For 2q33.1 and 5q14.3 deletion syndromes, whose critical region is too small to be detected by group 1 resolution, responsible genes (SATB2 and MEF2C) were specifically covered, by selecting CGH probes (ten for each gene) directly from one Agilent CGH catalog design (AMADID: 014693).

For group 2, similarly to group 1, the probes selection within the SureDesign CGH HD database was done using an average probe spacing of 50Kb and similarity score filter on, then turning similarity score filter off on gaps. For this subtelomeric regions group the median probe spacing was 44.9Kb with a total coverage of 98.8% and of 94.9% of high quality probes enabling a functional resolution of 300 Kb for all subtelomeric regions. Finally for the backbone (group3), in order to get an average functional resolution of 3 Mb all along the genome, all probes were selected using an average probe spacing of 500Kb and similarity score filter on. The median probe spacing was 453.5Kb with a total coverage >99.8% and >99.5% of high quality probes.

SRY and Xist genes were also covered, because of their relevance in fetal sexual determination and in chromosome X inactivation respectively. Ten CGH probes covering SRY gene were selected directly from one Agilent CGH catalog 4x180K design (AMADID : 029830). For Xist gene, probes were selected on a region of 73Kb subdivided into a 30kb center region of the gene with a median probe spacing of 0.8Kb and from each side of it the regions have the median probe spacing of 2Kb.

Analytical parameters

For data analysis the algorithm of choice was ADM-2 algorithm (threshold 6) from Agilent CytoGenomics 3.0 software (Agilent Technologies, Santa Clara, CA, USA). In order to obtain reliable data, reducing the number of false positive, still maintaining a good performance in detecting rearrangements (including mosaicisms >50%), we did not consider the CNVs whose log2ratio comprise between -0.5<0<0.3 or defined by less than 7 consecutive probes. Using those analysis criteria, we decided to report only imbalances > 3 Mb for the backbone, > 300kb in the subtelomeric regions and > 150kb in the syndromic regions.

"*In Silico*" analysis

Before to run both a retrospective and a prospective study using the EasyChip design, we have decided to evaluate the genomic coverage effectiveness of EasyChip and its potential usefulness in the prenatal setting by doing a large "in silico" analysis of prenatal samples previously analyzed by a high resolution 4x180K oligo array in our Laboratory. The goal was to evaluate the capability of this region dependent resolution platform in terms of unmasking a series of submicroscopic highly penetrant and morbid microdeletion/microduplication syndrome and overcoming some interpretation and reporting problems (VOUS and IF). Two-hundred and ninety prenatal samples were "in silico" re-run considering the EasyChip design and analysis parameters described above, taking into account the average spacing and the number of oligonucleotide probes covering the different genomic region represented into the array. This cohort was split into two subgroups, respectively high- and low-risk pregnancies. Of them, 177 were analyzed by array-CGH because of ultrasound anomalies or presence of some chromosome aberrations (structural rearrangements, marker chromosomes). Other 113 prenatal samples were from low-risk pregnancies, and array-CGH analysis performed for parental anxiety. They included cases with positive screening test results, advanced maternal age with normal karyotype, and psychological indications. At time of the "in silico" analysis, all these samples had a CMA report, based on laboratory analytical filters, including imbalances size, gene content and genotype-phenotype association. In this process, we took into account all the CNVs detected in the primary high-resolution analysis, considering the "calls" of more than five consecutive aberrant oligonucleotide probes, independently from the final choice whether or not to report. We excluded CNVs reported in Database of Genomic Variants (DGV, http://dgv.tcag.ca/dgv/app/home) by at least three different studies, and CNVs not containing RefSeq genes. We did not use any other analytical filter. Then, we compared the overall results to those "potentially" detected by the EasyChip platform according to its resolution, and compared the percentage of VOUS, IFs and pathogenic CNVs deciphered by the two different platforms.

Validation study

Easychip platform was validated in two steps, a retrospective and a prospective study. Experiments were carried out following Agilent Microarray protocols (Agilent Technologies, Santa Clara, CA, USA). Since the design is thought to be used for reaching rapid results in low risk pregnancies, we choose to validate the platform starting from a minimal amount of genomic DNA (200 ng), which can be normally obtained from 10 ml of uncultured amniotic fluid, at 16 week gestation.

The retrospective study was carried out on 57 samples (peripheral blood, amniotic fluid, and chorionic villi samples) harboring known aberrations, supposed to be detected by EasyChip, for evaluating the oligonucleotide probes call in the investigated regions.

The prospective study was carried out on 112 prenatal samples with EasyChip, 38 of which simultaneously analyzed with a high-resolution platform routinely used in our laboratory (oligo array 180K or 60K). All results were considered in the between platforms comparison, independently from the final report which followed the analytical filters. The only excluded CNVs were those present in the general population according to DGV and gene desert regions. All positive results were confirmed by mean of a different technique (Karyotype, FISH, MLPA, Prenatal BacsOnBeads). The aim of this second step of validation was to evaluate the quality of our platform on real prenatal samples and to assess whether the systematic diagnostic use of EasyChip could offer a real advantage in term of balance between detection rate and undesired findings.

Results and Discussion

"*In silico*" analysis

We included in this analysis a cohort of 290 prenatal samples, 177 from high-risk pregnancies and 113 from low risk pregnancies, previously analyzed in our Laboratory with an oligo-array 4x180K at its maximum resolution level. Under these conditions, a total of 117 CNVs were detected. Of them 82 in 69 samples were considered VOUS, 7 in 7 patients were IF, and 28 in 23 patients were pathogenic, according to test indication.

The proportion of VOUS and IF was quite similar in high- and low-risk pregnancies (respectively 23% vs 25%, and 3% vs 2%), while, as expected, the number of pathogenic variations was significantly overrepresented in high-risk pregnancies (12% vs 2%, Table 2).

Differently from our expectations, the VOUS percentage did not increase in high-risk pregnancies, possibly due to a bias resulting from smallness of our cohorts. Independently from the indication, the number of unknown findings detected by high-resolution platform was consistent. This endorses the use of advanced analytical filters and/or the comparative analysis of parental DNA, which can rise some ethical dilemmas, increases the costs of the diagnostic process often, does not reach decisive conclusions and contribute to increase parental anxiety.

As anticipated by its genomic coverage and probe spacing , the "in silico" analysis of the exact same series of prenatal samples using the EasyChip Design instead of the high resolution 180K design decreases the VOUS figure to 3%, while IF are completely unnoticed.

Using the EasyChip tool on the "in silico" cohort successfully enables the detection of all pathogenic CNVs. They involved syndromic highly covered regions and large backbone regions (> 3 Mb, Table 2).

The results of the "in silico" study indicate the EasyChip design is in line with the goals of our initial strategy and suggest that this platform can be useful to monitor low-risk pregnancies in which the detection of VOUS or IFs should be minimized.

Validation study

Furthermore, EasyChip was assessed in a validation process, including a retrospective and a prospective study. Twenty-five of the 43 critical regions represented on the platform were tested for call, using known DNA samples. Six of them were less than 1 Mb in size (250-859 Kb), 9 were 1-5 Mb in size (1.3-3.7 Mb), 5 in the range of 5-10 Mb (5.2-8.1 Mb), and 5 were visible with standard karyotype (22.9-33 Mb). Twenty-nine subtelomeric rearrangements were also tested, including 6 smaller than 1 Mb (300-696 Kb), 8 ranging in size between 1.4 and 4.5 Mb, 8 between 5.5 and 10 Mb, while 7 were visible with standard karyotype (10.4-40.2 Mb). The backbone response was tested in 9 samples harboring CNVs 3-9 Mb in size.

All known imbalances were correctly detected. This retrospective study was followed by a prospective one in which 112 prenatal samples, from pregnancies with and without sonographic or chromosomal indications, was tested by EasyChip together with standard karyotype, as part of the standard prenatal diagnostic process of the laboratory. Seven of them tested positive (6%). Thirty-eight where simultaneously analyzed also by mean of a high-resolution platform (180K or 60) because of the presence of an ultrasound evidence. The high-resolution platform detected 11 VOUS (29.9%), only one of which, a paternally inherited duplication 22q11.23, 1.2 Mb in size, was included in the report after having applied the analytic filters. Two IF were also detected: a 535 Kb microduplication involving NIPA1 gene (15q11.2), and a 1.8 Mb microduplication involving MYH11 gene (16p13.11). These latter, whose potential pathogenicity is still controversial, were not reported to parents, according to the recently published recommendation of the British Society for Genetic Medicine [21]. Only two clinically relevant results were revealed, a mosaic 45,X/46,X,i(X)(q10) in a fetus presenting with left heart hypoplasia and a mosaic 47,XX,+20/46,XX in a fetus with ventricular septal defect. This result was confirmed by karyotype analysis of amniocytes.

EasyChip detected a single VOUS, the 22q11.23 duplication, 1.2 Mb in size, since it was located in the distal part of the 22q11.2 deletion syndrome critical region, well represented in the array. Differently, no IFs were detected by EasyChip, while the 45,X/46,X,i(X)(q10) and the 47,XX,+20/46,XX mosaic were correctly resolved. For the remaining 74 samples, only analyzed by karyotype and Easychip, 5 positive results were detected, consisting in: a trisomy 21, a Klinefelter syndrome fetus, a huge supernumerary marker chromosome arisen from chromosome 9, and a DiGeorge syndrome region.

In conclusion, EasyChip is as useful tool for monitoring low risk pregnancies, by providing information on 43 genomic regions whose imbalances are associated with highly penetrant morbid conditions, and on subtelomeric regions, frequently associated with intellectual disability. On the contrary, high resolution platforms should be addressed to high-risk pregnancies, for example in the presence of ultrasound abnormalities, or for in deeper characterization of anomalies detected by standard cytogenetic (i.e. unbalanced or apparently balanced chromosomal rearrangements and extra marker chromosomes). In fact, in these cases the high rate of unknown results could be balanced with the necessity of obtaining the most information available. Several different issues must be considered for choosing the more suitable approach to prenatal diagnosis [7]. While genomic microarrays can detect imbalances at a much higher resolution compared to standard karyotype, they cannot detect some anomalies that should be considered when assessing the fetal risk and the parents' recurrence risk, such as balanced rearrangements, small heterochromatic markers and low level of mosaicism. Additionally, analysis of DNA exctracted from chorionic villi can also provide misleading results due to the presence of aberrations confined to extraembryonic tissues.

In our opinion, considering the technical limits of microarray and resolution limit of standard cytogenetics, the ideal approach to the prenatal screening should be the integration of both of them. The introduction of a targeted microarray with low-resolution genome wide coverage in diagnostic routine supports the information provided

ID	Sex	Test indication	Sample type	aCGH result (hg 19)	detectable on EasyChip	Mb	genes	role	References	morphol. US	US 30th GW	F.U. at born
7	M	mos 47,XY,+mar[9]/46,XY[7] .ish (CEPX++)	chorionic villi colture	Xp11.1q13.1(56,556,892-67,950,691) x2 dn	YES	11,4	>10 RefSeq, 4 OMIM MORBID	Pathological		Therapeutic termination of pregnancy		
10	M	47,XY,+mar (mosaicism 60%)	chorionic villi colture	18p11.32p11.21(14,346-14,115,025) x2~3 dn	YES	14.1	>10 RefSeq, 6 OMIM MORBID	Pathological		Therapeutic termination of pregnancy		
12	M	47,XY+mar (mosaicism 30%)	chorionic villi colture	22q11.1q11.21(14,797,037-20,138,979) x2~3 dn	YES	5,3	>10 RefSeq, >10 OMIM MORBID	Pathological	Cat Eye Syndrome (OMIM # 607576), 22q11.2 Duplication syndrome #608363)	Therapeutic termination of pregnancy		
21	M	46,XY,inv(20)(p13q13.3) dn	amniocytes colture	Xq21.3 3q22.1(96,603,211-102,495,361) x2 mat	YES	5,9	>10 RefSeq, 7 OMIM MORBID	Pathological	Cheng et al 2005; Cremers et al 1987	spontaneous abortion		
51	F	growth delay, single umbilical artery, hypoplastic left heart suspect, diaphragmatic hernia, club foot suspect	chorionic villi colture	15q26. 2q26.3(96,853,120-102,465,326)x1 dn	YES	5,6	27 RefSeq, 8 OMIM	pathological	You 2005; Klaassens 2005			
68	F	46,X,der(X)dn	chorionic villi colture	Xp22.3p21.1(61,115-33,465,266)x1 dn	YES	33,4	>10 RefSeq, >10 OMIM MORBID	pathological		Therapeutic termination of pregnancy		
				Xq26.3q28(134,909,242-155,257,104) x3 dn	YES	20,2	>10 RefSeq, >10 OMIM MORBID	pathological				
69	F	46,XX,der(2).ish der(2)(2qter-)dn	chorionic villi	2q37.3(241,560,272-242,968,420) x1 dn	YES	1,4	>10 RefSeq, 4 OMIM MORBID (KIF1A,AGXT,D2HGDH,PDCD1)	pathological		Therapeutic termination of pregnancy		
				16p13.3p13.11(72,798-15,577,010) x3 dn	YES	15,5	>10 RefSeq, >10 OMIM MORBID	pathological				
75	F	47,XX,+mar dn Single umbilical artery, Echogenic intracardiac focus	amniocytes colture	18p11.32p11.21(14,346- 15,380,657) x4 dn	YES	15,4	>10 RefSeq, >10 OMIM MORBID	pathological	Tetrasomy 18p (OMIM#614290)	Therapeutic termination of pregnancy		
79	F	normal cardiac fetal activity but with absence of fetal movements. Bladder agenesis	chorionic villi colture	4q28.3(134,923,047-135,180,140) x1 pat	NO	0,257	1 RefSeq	unknown				
				8q22.2(99,880,457-100,312,677) x3 mat	NO	0,432	1 RefSeq, 1 OMIM MORBID (VPS13B)	unknown				
				22q11.1q11.23(16,054,713-23,654,193) x1~2 dn	YES	7,6	> 10 RefSeq, >10 OMIM MORBID	pathological	DiGeorge Syndrome (OMIM # 188400)			
90	M	47,XY,+mar dn	chorionic villi colture	18p11.32p11.21(14,346- 15,380,657) x4 dn	YES	15,3	>10 RefSeq, >10 OMIM MORBID	pathological	Tetrasomy 18p (OMIM#614290)			
113	M	IUGR	amniocytes colture	21q22.2q22.3(41,683,959-47,508,175) x3 mat	YES	5,8	> 10 RefSeq, >10 OMIM MORBID	unknown		short limbs	short limbs	SHOX deletion phenotype
				Xp22.33(61,115-1,611,364)x1 dn	YES	1,2	> 10 RefSeq, 2 OMIM MORBID (SHOX, CSF2RY)	pathological	Leri-Weill dyschondrosteosis (OMIM ##127300)			
130	F	ultrasound anomalies (note: trisomy 10 mosaicism confined to the placenta)	chorionic villi	10p15.3-10q26.3(116,477-135,506,704)x2~3 dn	YES	135,4	>10 RefSeq, >10 OMIM MORBID	pathological	Mosaic trisomy 10	Therapeutic termination of pregnancy		
147	F	46,XY,der(7) dn Saethre-Chotzen syndrome+ hydrocephalus	amniocytes colture	3q25.1q25.31(152,450,190-155,106,521) x1 pat	NO	2,3	8 RefSeq, 1 OMIM MORBID (MME)	unknown				
				7p21.3p15.3(7,854,662-21,325,587) x1 dn	YES	13,5	>10 RefSeq, 3 OMIM MORBID (GLCCI1, ISPD, TWIST1)	pathological	Sifakis et al, 2012	IUGR	Therapeutic termination of pregnancy	
166	F	mos 45,X/46,X,der(X)/46,XX	amniocytes colture	12p13.33p11.1(163,618-34,756,180) x2~3 dn	YES	34,6	>10 RefSeq, >10 OMIM MORBID	pathological		Therapeutic termination of pregnancy		
				Xq21.1q28(82,400,679-155,232,885) x1~2 dn	YES	72,8	>10 RefSeq, >10 OMIM MORBID	pathological				
				8q13.2(69,482,639-70,492,700)x1	NO	1	2 RefSeq (C8orf34, LOC100505718, SULF1)	unknown				
239	M	46,XY/47,XY,+mar dn	chorionic villi	2p11.2q11.2(86,336,170-97,678,220) x2~3 dn	YES	11,3	> 10 RefSeq, 7 OMIM MORBID	pathological				
240	M	46,XY/46,XY,der(7)	chorionic villi	7q34q36.3(139, 545,935-159,118,537) x1~2 dn	YES	19,6	> 10 RefSeq, >10 OMIM MORBID	pathological		Therapeutic termination of pregnancy		
				17q24.3q25.3(68,264,322-81,044,524) x2~3 dn	YES	12,8	> 10 RefSeq, >10 OMIM MORBID	pathological				
241	F	46,XY,der(18)dn	amniocytes colture	4p16.3p15.1(56,802-34,053,664)x3 dn	YES	34	> 10 RefSeq, >10 OMIM MORBID	pathological		Therapeutic termination of pregnancy		
				18p11.32(14,346-7,157,933)x1 dn	YES	7,1	> 10 RefSeq, >10 OMIM MORBID	pathological				
				2p15(63,546,581-63,718,034)x1	NO	0,172	1 RefSeq (WDPCP)	unknown				
252	F	47,XX,der(21),+mar[12]/ 46,XX,der(21)[11]	chorionic villi colture	21p11.2q22.2(10,864,216-41,104,250) x2~3 dn	YES	30,2	> 10 RefSeq, >10 OMIM MORBID	pathological				
				21q22.2q22.3(41,127,051-48,090,288) x1~2 dn	YES	7	> 10 RefSeq, >10 OMIM MORBID	pathological				
273	M	cystic hygroma	chorionic villi colture	17q21.31(43,585,247-44,289,232) x1 dn	YES	0,704	9 RefSeq (MAPT)	pathological	Koolen-De Vries syndrome (OMIM #610443)			
				Xp22.2(11,692,320-12,160,711)x2 mat	NO	0,468	1 RefSeq (MSL3)	unknown				
88	M	maternal anxiety	chorionic villi colture	22q11.21(18,894,865-21,561,492) x1 dn	YES	2,7	> 10 RefSeq, >10 OMIM MORBID	pathological	DiGeorge Syndrome (OMIM # 188400)			
261	M	maternal anxiety	amniocytes colture	8p23.1p22(7,169,520-12,503,654) x3 mat	YES	5,3	> 10 RefSeq, 4 OMIM MORBID (MFHAS1, RP1L1, BLK, GATA4)	pathological	Barber Jc et al, 2013			

Table 2: Pathogenic CNVs detected on a cohort of 290 prenatal samples

by standard karyotype analysis facilitating the cytogenetic workflow. In addition, this platform allows the detection of small aberrations, which could have been gone overlooked in low quality chromosome preparations, with a minimal risk of detecting undesired findings. Moreover, the low amount of starting DNA required for testing, allow working on direct tissues, providing further information not biased by culture artifacts.

A 1 year data collection is going on, involving several Italian centers working on prenatal diagnosis and having introduced EasyChip into their laboratory routine. The aim of this further step is to evaluate in deep the value of our platform in prenatal workflow in term of diagnostic and VOUS/IF percentage.

Conflicts of Interest

The author Goidin Didier declare his involvement in a company with a financial interest in the materials discussed in this manuscript (Genomics Group, Agilent Technologies, Les Ulis, Fr)

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