GAVIN PUBLISHERS

Biomarkers & Applications

Pérez SP, et al. Biomark Applic: BMAP-108.
DOI: 10.29011/BMAP-108. 100108

Research Article

Contribution of Chromosomal Microarray Analysis to the Prenatal Diagnosis of Fetal Craniofacial Malformation

Silvia Pina Pérez*, Judith Jurado Seguer, Amparo Ramirez Pujadas, Laura Serra Azuara, Judith Lleberia Juanos, Montserrat Mestre Costa

Department of Obstetrics and Gynecology, University Hospital Parc Taulí, Sabadell, Barcelona, Spain

***Corresponding author:** Silvia Pina Pérez, Department of Obstetrics and Gynecology, University Hospital Parc Taulí, Sabadell, Barcelona, Spain. Tel: +34646180299; Email: spina@tauli.cat

Citation: Pérez SP, Seguer JJ, Pujadas AR, Azuara LS, Juanos JL, et al. (2017) Contribution of Chromosomal Microarray Analysis to the Prenatal Diagnosis of Fetal Craniofacial Malformation. Biomark Applic: BMAP-108. DOI: 10.29011/BMAP-108. 100108

Received Date: 22 July, 2017; **Accepted Date:** 2 August, 2017; **Published Date:** 9 August, 2017

Abstract

Objective: To determine the relationship between chromosomal abnormalities and craniofacial malformations (CFMs) in the prenatal stage.

Methods: We included in this retrospective observational study 26 cases of CFMs diagnosed in our hospital between 2010 and 2016 based. We performed a univariate descriptive analysis of the mean, the interval and the standard deviation for continuous variables and an analysis of absolute frequency and percentages for the categorical variables.

Results: The incidence of CFMs in our group was 0,17%. Or official clefts represent 50% of CFMs (N=13). We also found 8 cases of craniofacial dysplasia; 3 cases of craniofronto nasal dysplasia and hypertelorism syndrome (11.5%), 3 cases of maxilla nasal dysplasia (11.5%) and 2 cases of maxillo-mandibular dysplasia (7.7%). Two facial tumors (7.7%) and one case of craniosynostoses (3.8%) were diagnosed. One case of Otocephalia Complex (3.8%) and another of popliteal pterygium syndrome (3.8%) were also described. Fetal karyotype was performed in 81% of the fetuses detecting two cases of trisomy 13. Microarrays were conducted in 27% of the cases and we did not detect any significant change in the number of chromosomal abnormalities.

Conclusions: The incidence of CFMs in our population was 0.17% and the most common CFMs were isolated cleft lip. The 7.7% of the cases of CFMs presented a chromosomal abnormality. Microarrays did not detect any significant change in the number of chromosomal abnormalities, probably due to the small number of cases. Our study demonstrates that an early detection of CFMs at the first trimester morphological ultrasound is possible.

Keywords: Craniofacial Dysplasia; Craniofacial Malformation; Chromosomal Microarray; Dysmorphology; Lip Cleft Palate; Prenatal Diagnosis

Introduction

The conventional karyotype analysis of chromosomes has been the standard method for the detection of chromosomal abnormalities for decades. However, this technique is limited by the detection of chromosomal alterations that are bigger than 5-10 Mb. Sub-microscopic deletions and duplications, which are often associated with mental retardation and fetal malformations, are not detected by conventional Karyotyping [1]. The Chromosomal Microarray Analysis (CMA) allows to identify micro deletions and micro duplications that are not usually detected with the karyotype. It is possible to evaluate the entire DNA genome and detect Copy Number Variations (CNVs), smaller than 50 Kb, which equals an increase of 100 times the resolution compared with the conventional karyotype. Recent studies have focused specifically on the use of CMA in prenatal diagnosis of fetuses with abnormal ultrasound findings. When we reviewed the literature to evaluate the increasing diagnosis with arrays in prenatal samples, we found that CMA detects a 5.2% (1.9-13.9%) more cases of fetuses with structural malformation than the analysis with karyotype [2,3].

Craniofacial malformations (CFMs), occurring in 1 out of 1000 live births, are serious health conditions that cause a large number of Comorbidities affecting the future patient´s life. Cran-

iofacial conditions as or official clefts, craniosynostoses, mandibulofacial dysostoses and tumors are the most frequent. Many of these conditions which have a genetic etiology (chromosomal, gene disorders or epigenetic mutation), may have a teratogenic cause, be part of a genetic syndrome and also be associated to other organs and systems disorders. The physio pathological classification of the CFMs makes differences between: malformations caused by an early closure of sutures of the craniofacial skeleton and those produced by an intrinsic alteration in the process of development of the different facial structures affecting the first and second bronchial arch [2-5]. We consider that the exploration of the fetal face is becoming increasingly important in the first trimester of gestation. Therefore, to establish the association with fetal chromosomal disorder is important for the prenatal diagnosis and subsequent advice of the parents.

Methods

This was a retrospective observational study that included all CFMs diagnosed in our hospital between 2010 and 2016. We analyzed the CFM type, the weeks of gestation when they were diagnosed, the use of additional diagnostic tests, the fetal karyotype (conventional karyotype and chromosomal microarrays) and the ending of the pregnancy. The inclusion criteria were: fetus with prenatal CFM diagnosis. The exclusion criteria were: loss of gestational follow-up. This study was approved by the Institutional Ethics Committee of our hospital. All the ultrasound examinations were carried out by the authors using an Acuson Antares machine with a 2-3MHz convex transducer. The data has been analyzed with SPSS 17.0. A univariate descriptive analysis of the mean, the interval and the standard deviation for continuous variables, and of absolute frequency and percentages for the categorical variables has been performed.

Results

A total of 26 CFMs were detected, corresponding to an incidence of 0.17%. We distinguished two groups: those caused by an early closure of the sutures of the craniofacial skeleton, called craniosynostoses and faciocranioynostosis, and those that are considered neural crest anomalies, like the first and second brachial arch syndromes, cleft lip and cleft palate. The mean age of the patients studied was 29.1 years and the mean gestational age at the moment of the examination was 12+6 weeks in the patients examined at the first trimester and 21+2 weeks in the patients examined at the second trimester. According to the physiopathological classification, we distinguished those cases caused by an early closure of the sutures of the craniofacial skeleton, called craniosynostoses and faciocranioynostosis, and those that are considered neural crest anomalies, like the first and second brachial arch syndromes, cleft lip and cleft palate. CFMs correspond to 3% of all the malformations diagnosed in our center. (Figure 1) Our results were: 50% (13) of CFMs were or facial clefts. We also found 8 cases of craniofacial dysplasia: 3 cases of craniofrontonasal dysplasia and hypertelorism syndrome (11.5%), 3 cases of maxilla nasal dysplasia (11.5%) and 2 cases of maxilla mandibular dysplasia (7.8%). Two cases of facial tumors (7.8%) and one case of craniosynostoses (3.8%) were diagnosed. One case of otocephaly complex (3.8%) and one case of Popliteal Pterygium Syndrome (3.8%) were described. Malformations in the central nervous system and the skeletal system were the most common anomalies associated (30%, 8 cases) (Figure 2).

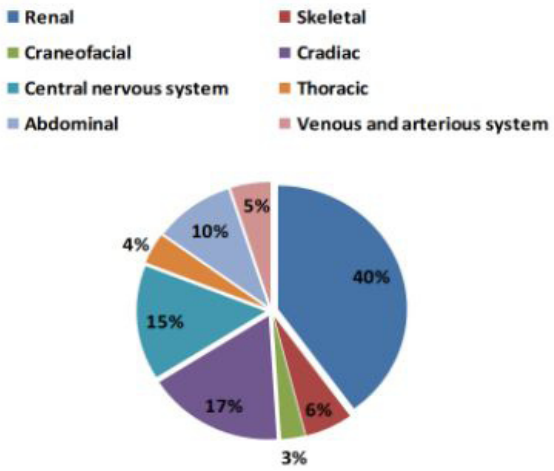

Figure1: Classification of malformations

Syndrome	Inheritance	Genetic or chromosomal anomalies	Incidence	CFM associated
Trisomy 13	Sporadic or translocation	47 XX / 47 XY +13	1/12.000	Holoprosencephaly, cleft lip with or without cleft palate and cardiac malformations
Craniosynostosis	Autosomal dominant or sporadic	FGFR1, FGFR2, FGFR3 and TWIST genes	1/2000	Digital malformations, skeletal defects, cardiac defect, or other organ anomalies
Binder's syndrome	Sporadic	Unclear	Rare	Maxillar and nasal complex malformation.
Pierre–Robin	Sporadic	Changes in the DNA near the SOX9 gene	1/10 000	Micrognathia, glossoptosis
Otocephaly complex	Sporadic	OTX2 and PRRX1 genes	1 /70 000	Microstomia, mandibular hypoplasia or agnathia
Pterygium popliteal syndrome	Autosomal dominant	Mutations in the IRF6 gene	1/300 000	Orofacial anomalies, genital and musculoskeletal

Figure 2: Genetic and chromosomal associations with CFMs.

The fetal karyotype was performed in 81% of fetuses, detecting two cases of trisomy 13. Microarrays were conducted in 27% of the cases and we did not detect any significant change in the number of chromosomal abnormalities. The mutation c.250C>T (p.Arg84Cys) associated with the pterygium popliteal syndrome was located in the IRF6 gene. We detected 19% of the CFMs during the first trimester by early ultrasound and 73% of the CFMs

during the second trimester ultrasound with a false negative rate of 7.6%, because two cases of cleft palate were not detected.

Fetal MRI was performed in 58% of cases and it confirmed the monographic diagnosis in all the cases. Legal interruption of pregnancy was requested by 54% of the pregnant women. We had one case of antenatal mortality (3.8%) and 11 births were attended, which corresponded to 7 cases of or facial cleft, 2 cases of nasal bone hyperplasia and 2 cases of facial tumor. All of them had a good neonatal development, and surgical repair of the CFMs was conducted in 9 of the 11 cases.

Discussion

The face of a fetus represents one of the most interesting anatomical regions to explore prenatally. CFMs had an incidence of 0.17% in our population and the most common CFM was isolated cleft lip, which is consistent with what has been published in the literature so far [6,7]. We also agree that or facial clefts are the most prevalent of the CFMs. The diagnosis rate in our study was acceptable and higher than what has been previously reported by some groups, especially regarding the second trimester of gestation. A study from Southern Sweden [8] about cleft detection reflected the contemporary practice in the period 2006-2010, and it had a detection rate of 31% (isolated midline clefts were excluded). This detection rate compares unfavorably with a study that most closely resembles UK [9] practice, which reported a 64% rate of prenatal detection for cleft lip, palate or both. However, it seems that the use of higher resolution ultrasound equipment and the incorporation of protocols for examining the fetal face can improve sensitivity to as high as 75% [10]. The diagnosis rate in our study (73%) is consistent with what is published in the literature, as we said. Based on the fact that we have performed a detailed and protocol zed fetal ultrasound examination in the first trimester, we have been able, in some cases, to advance the prenatal diagnosis of CFMs.

Over the last few years we have seen improvements in two-dimensional imaging and the arrival of three- and four-dimensional ultrasound technology, coupled with different techniques for visualizing the fetal face, the hard and the soft palates [11,12]. A routine screening for other malformations, especially skeletal, central nervous system and cardiac defects, may need to be considered. Furthermore, genetic counseling may be warranted in the majority of these cases [13]. We have diagnosed eight cases of craniofacial dysplasia: one case of Pierre Robin sequence [14,15] and two cases of Binder syndrome [16]. Interesting by its rarity, otocephaly complex [17] was also diagnosed (Figure 3).

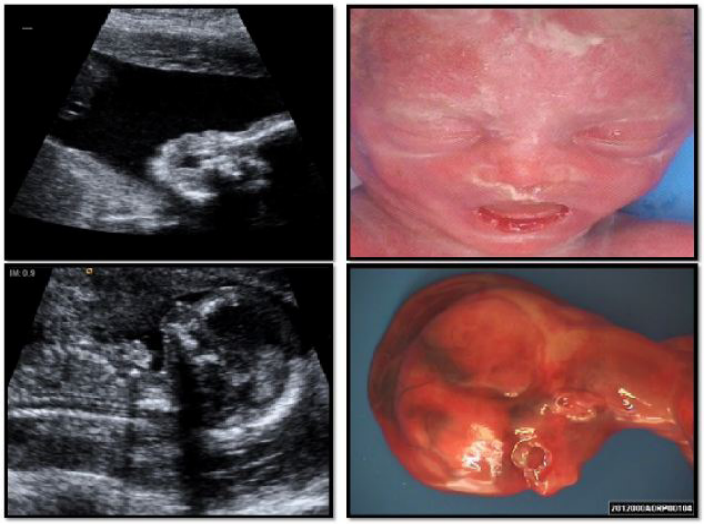

Figure 3: The photos above correspond to a Binder syndrome and below to an Otocephalia complex.

Despite the limitations of our sample, there are just a few series reported in the literature about the impact of chromosomal abnormalities in the prenatal diagnosis of CFMs, attributable to the low prevalence of these malformations. Karyotype abnormalities were reported in 7,7% of craniofacial malformations: two cases of trisomy 13 which, according to what has been published by other authors, are chromosomal numerical abnormalities that can cause a CFM [18].

It has been discussed that the microarray provides an improvement of the diagnosis between 1.9 -13.9% [3,4] in the prenatal diagnosis, according to different publications. While it is true that our sample is small, we could say that it is one of the few published series that emphasizes the contribution of the microarray in these cases. Microarrays did not detect any significant change in the Copy Number Variants (CNV), presumably due to the small number of cases.

Exonic mutations in Interferon Regulatory Factor 6 (IRF6) was associated with popliteal pterygium syndrome. We performed direct sequence analysis of the interferon regulatory factor 6 exons and we identified the mutation c.250C> T (p.Arg84Cys) in the fetus affected by this syndrome, confirming the diagnosis. This syndrome is a condition inherited in an autosomal dominant manner and it is caused by point mutations in the IRF6, a gene located at 1q32. Specifically, this missense mutation causes a change of an arginine by a cysteine at the position 84 related to or official clefting disorders and has been previously reported [19]. Muta-

tions in the IRF6 are identified in 97% of the patients affected by the popliteal pterygium syndrome.

A genetic contribution to CFMs has been described in many syndromic associations. Some genes have been identified and we know that they play a role in the craniofacial development (1p36, 2p21, 3p11.1, 8q21.3, cleft lip and palate transmembrane protein 1, GAD1, AXIN2, FGFR1, FGFR2, IRF6, PDGFC) [20-22]. Although many clefts occur within the families, in a large number of cases there is no corresponding syndromic appearance identified yet. Moreover, our data about the genetic factors that contribute to the more common cases is still incomplete. The current knowledge of CFMs is partial, but we believe that advances in genetics such as comparative genomic hybridization and whole-exome analysis may reveal new. Early ultrasound detection is essential for the management of this congenital defect and for the counseling of these parents.

Conclusions

A significant number of chromosomal abnormalities and non-chromosomal syndromes have facial alterations associated to them. In our series, CFMs presented a chromosomal abnormality in the 7.7% of the cases. Microarrays did not detect any significant change in the number of chromosomal abnormalities, presumably due to the small number of cases. When we talk about mutations, sequencing analysis can be very useful. Our study demonstrates that an early detection of CFMs by morphological ultrasound at the first trimester is possible. In addition, prognosis can be achieved through a multidisciplinary approach.

References

1. Hillman SC, McMullan DJ, Hall G, Togneri FS, James N, et al. (2013) Use of prenatal chromosomal microarray: prospective cohort study and systematic review and meta-analysis. Ultrasound Obstetric Gynecol 41: 610-620.

2. Tyreman M, Abbott KM, Willatt LR, Nash R, Lees C, et al. (2009) High resolution array analysis: diagnosing pregnancies with abnormal ultrasound findings. J Med Genet 46: 531-541.

3. Wapner, Ronald J, Martin CL, Levy B, Ballif BC, et al. (2012) Chromosomal Microarray versus Karyotyping for Prenatal Diagnosis. N Engl J Med 367: 2175-2184.

4. Brady PD, Delle Chiaie B, Christenhusz G, Dierickx K, Van Den Bogaert K, et al. (2014) A prospective study of the clinical utility of prenatal chromosomal microarray analysis in fetuses with ultrasound abnormalities and an exploration of a framework for reporting unclassified variants and risk factors. Genet Med 16: 469-476.

5. Granström G1, Jacobsson C (1999) First and second bronchial arch syndrome: aspects on the embryogenesis, elucidations, and rehabilitation using the osseointegration concept. Clin Implant Dent Relat Res1: 59-69.

6. Bister D, Set P, Cash C, Coleman N, Fanshawe T (2011) Incidence of facial clefts in Cambridge, United Kingdom. Eur J Orthod 33: 372-376.

7. Clementi M, Tenconi R, Bianchi F, Stoll C (2000) Evaluation of prenatal diagnosis of cleft lip with or without cleft palate and cleft palate by ultrasound: experience from 20 European registries. Prenat Diagn 20: 870-875.

8. Berggren H, Hansson E, Uvemark A, Svensson H, Sladkevicius P, et al. (2012) Prenatal ultrasound detection of cleft lip, or cleft palate, or both, in southern Sweden, 2006-2010. J Plast Surg Hand Surg 46: 69-74.

9. Boyd PA, Tonks AM, Rankin J, Rounding C, Wellesley D, et al. (2011) Monitoring the prenatal detection of structural fetal congenital anomalies in England and Wales: register-based study. J Med Screen 18: 2-7.

10. Hassan WA1, Lees CC (2014) Facial cleft detected: is the palate normal? Best Pract Res Clin Obstet Gynaecol 28: 379-389.

11. Wilhelm L, Borgers H (2010) The 'equals sign': a novel marker in the diagnosis of fetal isolated cleft palate. Ultrasound Obstet Gynecol 36: 439-444.

12. Ghi T, Perolo A, Banzi C, Contratti G, Valeri B, et al. (2002) Two-dimensional ultrasound is accurate in the diagnosis of fetal craniofacial malformation. Ultrasound Obstet Gynecol 19: 543-551.

13. Chmaid R, Pretorius D (2006) prenatal detection of associated anomalies in fetuses diagnosed with cleft lip with or without cleft palate in utero. Ultrasound Obstet Gynecol 27: 173-176.

14. Kaufman MG, Cassady CI, Hyman CH, Lee W, Watcha MF, et al. (2016) Prenatal Identification of Pierre Robin Sequence: A Review of the Literature and Look towards the Future. Fetal Diagn Ther 39: 81-89.

15. Evans KN, Sie KC, Hopper RA, Glass RP, Hing AV, et al. (2011) Robin Sequence: From Diagnosis to Development of an Effective Management Plan. Pediatrics 127: 936-948.

16. Cantarell SM, Azuara LS, Pérez SP, Juanos JL, Navarro FM, et al. (2016) prenatal diagnosis of Binder's syndrome: report of two cases. Clin Exp Obstet Gynecol 43: 279-283.

17. Diep J, Kam D, Munir F, Shulman SM, Atlas G (2016) Otocephaly Complex: Case Report, Literature Review and Ethical Considerations. A & A Case Reports 7: 44-48.

18. Bird LM, Pretorius DH, Mendoza AE, Jones MC (1997) Anencephaly with holoprosencephalic facies due to ring chromosome 18. Clin Dysmorphol 6: 351-358.

19. de Lima RL, Hoper SA, Ghassibe M, Cooper ME, Rorick NK, et al. (2009) Prevalence and nonrandom distribution of exonicmutations in interferon regulatory factor 6 in 307 families with Van der Woude syndrome and 37 families with popliteal pterygium syndrome. Genet Med 11: 241-247.

20. Ludwig KU, Mangold E, Herms S, Nowak S, Reutter H, et al. (2012) Genome-wide meta-analyses of non-syndromic cleft lip with or without cleft palate identifies six new risk loci. Nat Genet 44: 968-971.

21. Shi M, Murray JC, Marazita ML, Munger RG, Ruczinski I, et al. (2012) Genome wide study of maternal and parent-of-origin effects on the etiology of or facial clefts. Am J Med Genet A 158A: 784-794.

22. Beaty TH, Taub MA, Scott AF, Murray JC, Marazita ML, et al. (2013) Confirming genes influencing risk to cleft lip with/without cleft palate in a case-parent trio study. Hum Genet 132: 771-781.