Congenital Heart Disease as a Warning Sign for the Diagnosis of the 22q11.2 Deletion

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Abstract

Background: To alert for the diagnosis of the 22q11.2 deletion syndrome (22q11.2DS) in patients with congenital heart disease (CHD).

Objective: To describe the main CHDs, as well as phenotypic, metabolic and immunological findings in a series of 60 patients diagnosed with 22q11.2DS.

Methods: The study included 60 patients with 22q11.2DS evaluated between 2007 and 2013 (M:F = 1.3, age range 14 days to 20 years and 3 months) at a pediatric reference center for primary immunodeficiencies. The diagnosis was established by detection of the 22q11.2 microdeletion using FISH (n = 18) and/or MLPA (n = 42), in association with clinical and laboratory information. Associated CHDs, progression of phenotypic facial features, hypocalcemia and immunological changes were analyzed.

Results: CHDs were detected in 77% of the patients and the most frequent type was tetralogy of Fallot (38.3%). Surgical correction of CHD was performed in 34 patients. Craniofacial dysmorphisms were detected in 41 patients: elongated face (60%) and/or elongated nose (53.3%), narrow palpebral fissure (50%), dysplastic, overfolded ears (48.3%), thin lips (41.6%), elongated fingers (38.3%) and short stature (36.6%). Hypocalcemia was detected in 64.2% and decreased parathyroid hormone (PTH) level in 25.9%. Decrease in total lymphocytes, CD4 and CD8 counts were present in 40%, 53.3% and 33.3%, respectively. Hypogammaglobulinemia was detected in one patient and decreased concentrations of immunoglobulin M (IgM) in two other patients.

Conclusion: Suspicion for 22q11.2DS should be raised in all patients with CHD associated with hypocalcemia and/or facial dysmorphisms, considering that many of these changes may evolve with age. The 22q11.2 microdeletion should be confirmed by molecular testing in all patients. (Arq Bras Cardiol. 2014; 103(5):382-390)

Keywords: DiGeorge Syndrome; Crromosome Delection; Heart Defects, Congenital; Hypocalcemia; Chromosomes, Human.

Introduction

The 22q11.2 deletion syndrome (22q11.2DS) is considered the most frequent chromosomal microdeletion syndrome in humans, with an incidence of 1:4,000-5,000 live births[1,2]. This syndrome is currently recognized as occurring with a higher frequency than previously estimated, although precise information about its incidence in our country are unknown.

Microdeletions in the 22q11.2 region can occur in various syndromes such as the DiGeorge (DGS), velocardiofacial and the conotruncal anomaly face syndromes. These diseases represent different phenotypes of the same chromosomal abnormality, which are currently grouped and titled 22q11.2DS[1,3]. This microdeletion is not detected on G-banding karyotyping, a routine cytogenetic exam. The molecular diagnosis of the syndrome is rather established by other techniques such as fluorescence *in situ* hybridization (FISH) and/or quantitative genomic analysis by multiplex ligation-dependent probe amplification (MLPA)[4,5].

Congenital heart diseases (CHDs) are one of the most frequent malformations, with an incidence that ranges from 8 to 10 per 1,000 live births, representing an important cause of morbimortality in the first year of life[6]. The etiology of cardiopathies associated with other types of congenital malformations can be monogenic, as in Holt-Oram, Marfan, Fanconi and Noonan syndromes, or chromosomal, as in Down, 22q1.2DS, 18 trisomy (Edwards) and 13 trisomy (Patau) syndromes[7]. CHDs with conotruncal defects represent an important characteristic in several genetic syndromes, in particular in 22q11.2DS[2]. It is estimated that 5% of the patients with cardiopathy have DGS, which is considered the second most common primary immunodeficiency[8-11].

Clinical manifestations that should arise suspicion for 22q11.2SD are CHD (75%), abnormal psychomotor development (68%), hypocalcemia-associated seizure

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(60%), velopharyngeal insufficiency with nasal voice (46%), genitourinary abnormalities (36%), skeletal abnormalities (17%) and facial dysmorphisms (11-17%)[2,12-15]. The immunological changes associated with 22q11.2DS are variable and secondary to thymic hypoplasia or agenesis, classically named DGS by immunologists[1,5,14-17].

The aim of this study is to describe the main cardiopathies, as well as phenotypic, metabolic and immunologic abnormalities in a series of 60 patients with 22q11.2DS.

Methods

This is a descriptive, transversal, retrospective and prospective study evaluating all patients with 22q11.2DS followed at the Allergy and Immunology Unit and Genetic Unit at *Instituto da Criança* of HC-FMUSP between June 2007 until December 2013. Some of the patients were referred from the Pediatric Cardiology Unit of *Instituto do Coração* (INCOR) – HC-FMUSP after active search at this institution. The sample was composed of 60 children and adolescentes (34 males), with age range from 14 days to 20 years and 3 months (mean 114.2 months, standard deviation 83 months). All the individuals belonged to Brazilian families, and there was no predominance in the cohort of European, African or Oriental descent. The diagnostic criteria adopted were those proposed by the International Union of Immunological Societies-IUIS[18], which included compatible clinical signs and presence of the 22q11.2 microdeletion. All patients had normal karyotype by G-banding. Information about patient's identification, clinical history, physical examinatin and results from laboratory and cytogenomic analyses were collected in a protocol form.

The molecular analysis of the microdeletion was conducted at the Cytogenomics Laboratory of the Department of Pathology, with fluorescent *in situ* hybridization (FISH) using a specific probe for the 22q11.2 region. Commercial probes of unique sequences were used for the specific region in 22q11.2 (probes DGS/VCFS, TUPLE1 and N25 D22S75, Cytocell, Cambridge, UK)[4,19] and/or MLPA using various kits (P036-E1, P070-B2, P064-B3, MRC-Holland, Amsterdam, Netherlands – www.mlpa.com). Generated data were analyzed with the software GeneMarker® (*Softgenetics, LLC, State College, PA, USA – www.softgenetics.com*). These techniques are demonstrated in Figure 1.

All patients underwent clinical and imaging evaluations at the Pediatric Cardiology Unit.

Immunocompetence was evaluated with complete blood count, measurement of serum immunoglobulin levels (IgG, IgM and IgA) by nephelometry, and determination of lymphocyte subpopulations in peripheral blood (flow cytometry - BD FACSCalibur) at the laboratory of the Central Institute of HC-FMUSP, using as comparison reference values already described[20,21].

Other performed tests included serum measurement of parathyroid hormone level (PTH, automated chemiluminescent enzyme immunoassay), ionic calcium, total calcium and phosphorus (automated colorimetric method), triiodothyronine (T3), thyroxine (T4), free thyroxine (free T4) and TSH (automated immunoassay), as well as determination of antithyroglobulin and antithyroperoxidase antibodies (indirect immunofluorescence).

Statistical tests were performed with the software MedCalc 10.2 (MedCalc Software, 2009).

The study was approved by the Ethics Committee for Analysis of Research Project of *Hospital das Clínicas* – CAPPesq, registration number 0911/11.

Results

The presence of CHDs was identified in 47 patients (77%) and surgical correction was performed in 34 of these, the most frequent of which were tetralogy of Fallot, ventricular septal defect and pulmonary artery atresy, as described in Table 1 and Graph 1.

Other important phenotypic features found in patients from the overall cohort with 22q11.2DS are shown in Table 2. Facial features, as well as morphology of the ears, mouth, nose and eyes of some patients are shown in Figures 2 and 3.

The presence of facial dysmorphisms was not recognized during the neonatal period in many patients, becoming more evident with increasing age, as observed in Figure 4.

Hypocalcemia was diagnosed in 27 of the 42 patients (64.2%) in whom ionic calcium was measured, 16 (59.3%) of which occurred in the neonatal period. Of these, PTH was measured in 27 patients and was found to be reduced in 7 patients (25.9%). Seizures occurred in 6 patients.

During follow-up of 20 patients with 22q11.2DS through school years and adolescence, we observed that 11 progressed with behavioral and psychiatric abnormalities. The most common finding was attention deficit hyperactivity disorder (ADHD) in 6 patients, 4 of which had the hyperactive/impulsive type and 2 had the predominantly innatentive type. Other findings included learning disability (15%), anxiety (10%) and mental retardation (5%). Until present, none of the patients has progressed with compulsive-obsessive, schizoaffective or psychotic disorders.

In this study, low total lymphocyte count was found in 40% of the patients (18/45 cases), whereas low $CD4^+$ lymphocytes occurred in 53.3% (16/30) and low $CD8^+$ in 33.3% (10/30), as demonstrated in Graph 2. The evaluation of humoral immunity detected 2 patients with reduced IgM concentrations (29.6 mg/dL and 17.6 mg/dL) and one 9-month-old patient with IgG of 328 mg/dL, currently receiving replacement therapy with intravenous gamma globulin.

Discussion

Even though 22q11.2SD is considered in the literature to be a relatively frequent chromosomal abnormality, pediatricians, neonatologists and cardiologists in our country have not been recognizing it sistematically, and the publications with significant number of patients, particularly in the first year of life, are scarce[1,2]. In a study conducted at *Hospital das Clínicas* of HC-FMUSP with 1,008 patients with primary immunodeficiency, only 32 patients with 22q11.2DS were

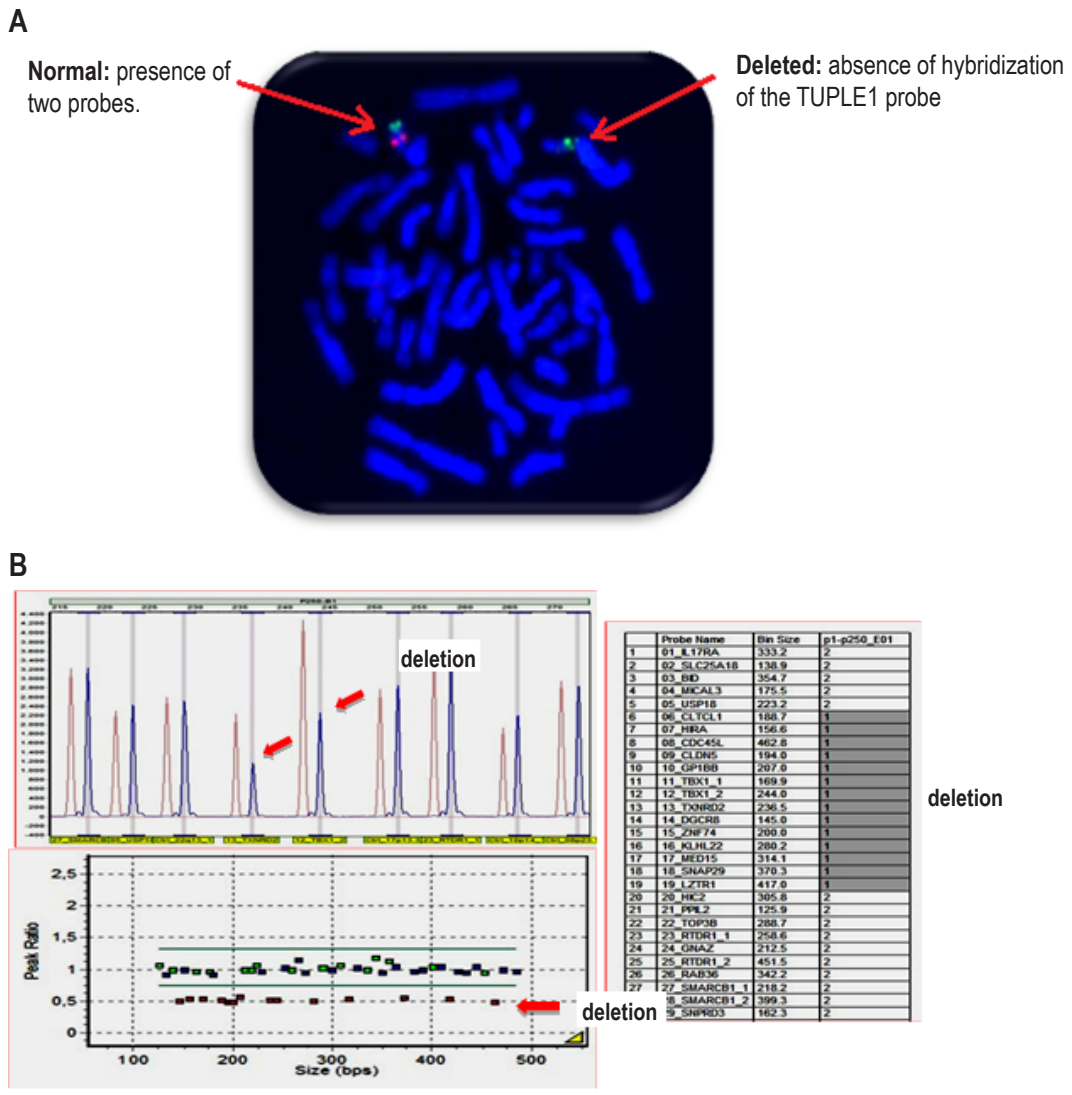
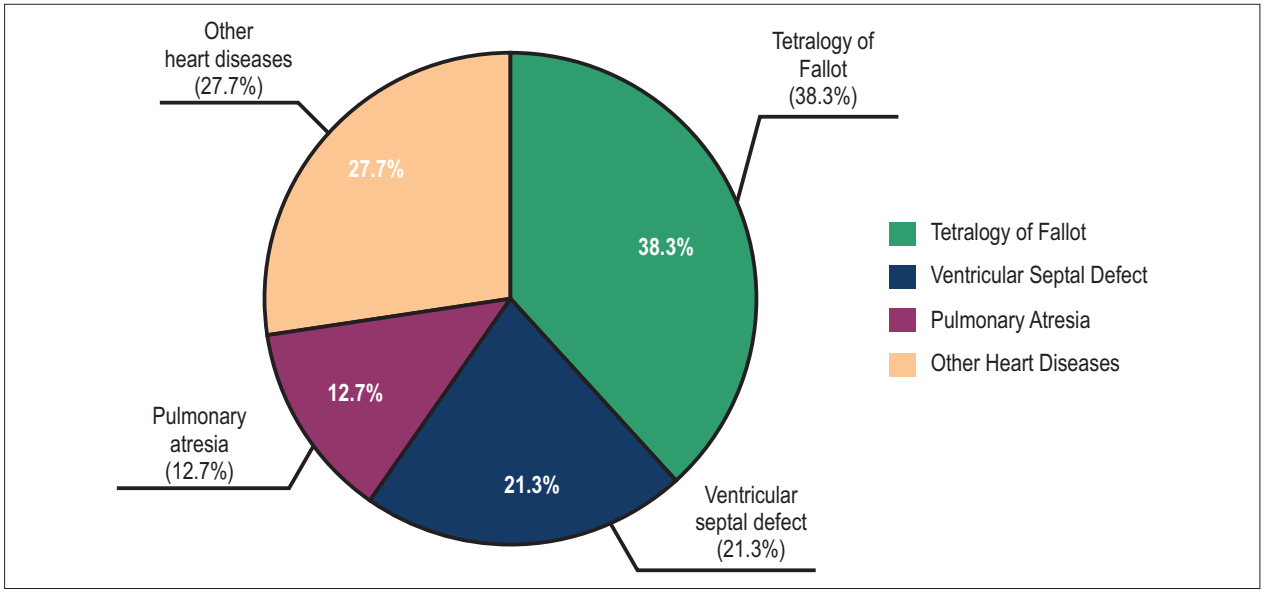


Figure 1 – *Demonstration of the deletion in region 22q11.2. A. FISH technique. B. MLPA technique.*

Table 1 – Congenital heart diseases in 47 patients with the 22q11.2 deletion syndrome and the surgical corrections performed

Heart diseases	N (%)	Surgical corrections N (%)
Tetralogy of Fallot	18 (38.3)	16 (88.8)
Ventricular septal defect	10 (21.3)	2 (20.0)
Pulmonary atresia	6 (12.7)	6 (100.0)
Truncus arteriosus	4 (8.5)	4 (100.0)
Interrupted aortic arch	4 (8.5)	4 (100.0)
Atrial septal defect	3 (6.4)	1 (33.3)
Transposition of the great arteries	1 (2.1)	1 (100.0)
Anomalous systemic venous drainage	1 (2.1)	0 (0)
Total	47 (100)	34 (72.3)



Graph 1 – *Main heart diseases affecting 47 patients with the 22q11.2 deletion syndrome.*

Table 2 – Phenotypic characteristics of 60 patients with the 22q11.2 deletion syndrome

Phenotypic characteristics	N (%)
Elongated face	36 (60.0)
Elongated nose	32 (53.3)
Narrow palpebral fissure	30 (50.0)
Dysplasic overfolded ears	29 (48.3)
Thin lips	25 (41.6)
Elongated fingers	23 (38.3)
Short stature	22 (36.6)
Palatal abnormalities	15 (25.0)
Dental abnormalities	13 (21.6)
Strabismus	10 (16.6)
Clubfoot	8 (13.3)

identified over 33 years[10]. These data led us to establish an active search of the microdeletion at the Pediatric Cardiology Unit of INCOR, resulting in a duplication of the number of cases over the past two years.

Cardiac malformations, observed in 77% of our cases, are the most critical manifestation of 22q11.2DS and affect between 49% and 95% of the patients according to the literature[1,22]. A point to be highlighted in this cohort was the higher frequency of tetralogy of Fallot (38.3%) when compared with the literature in which frequencies between 17.6% and 20% have been described[22-24]. Another point to be emphasized in our study was the occurrence of ventricular septal defect associated with 22q11.2DS; this was the second most frequent CHD, which contrasts with the literature which shows that conotruncal heart defects are the most prevalent.

The recommendation for 22q11.2DS screening highlights that tests for the 22q11.2 chromosome microdeletion should be conducted in all newborns or children with tetralogy of Fallot, truncus arteriosus, interrupted aortic arch, isolated anomalies of the aortic arch and perimembranous ventricular septal defect with aortic arch anomaly. In all other patients with perimembranous ventricular septal defect without aortic arch anomaly or with any other type of CHD in association with characteristic phenotypic manifestations, clinical suspicion for 22q11.2DS should be raised and screening for the microdeletion should be conducted[3,6,22,25].

Another fact that draws attention for the importance of early diagnosis of 22q11.2DS, ideally before surgery for correction of the heart defect, is the observation that these patients present increased risk of post-operative complications, even though their mortality is comparable to that in patients with similar cardiopathies but without 22q11.2DS[26].

In association with the CHDs mentioned above, the phenotypes found in this study, in particular the peculiar craniofacial dysmorphisms and the hypocalcemia in the neonatal period, are essential to raise diagnostic suspicion of 22q11.2DS. It should be mentioned that most of the clinical features of 22q11.2DS have been described in Caucasoid patients and for the first time, this study describes in details the facial dysmorphisms of the syndrome in the trihybrid Brazilian population, which since the XVI century has been formed by a mixture of Indians, Africans and Portugueses[27].

Another interesting point addressed in this study and not yet presented in other descriptions was the evolving aspect of the facies and other dysmorphisms. Even though

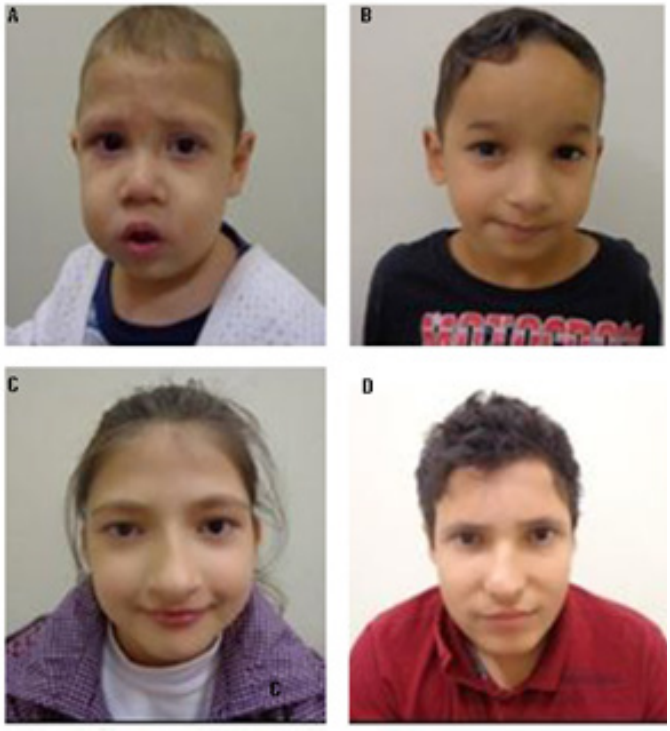


Figure 2 – *A) Preschooler with elongated nose. B) Preschooler with narrow palpebral fissure and thin lips. C) School-age child with elongated face and nose. D) Typical facies with narrow palpebral fissure, prominent nose, and mouth with thin lips.*

the dysmorphisms of 22q11.2DS recognized later in life (narrow palpebral fissures, elongated nose, overfolded ears) are already present at birth, the facies of the newborn and young infant draws less attention as a "syndromic facies" for the general pediatrician and/or cardiologist in this phase of life.

Palatal abnormalities occur in 9–16% of the patients with 22q11.2DS and are associated with a high morbidity. It is, therefore, fundamental to examine the palate carefully and include a search for bifid uvula which may suggest the presence of submucosal cleft palate[2,14]. In this series, we observed that this finding was more frequent than that described in the literature. Goldmuntz et al in 2005 demonstrated that in addition to cleft palate, about 80% of the patients also present velopharyngeal insufficiency manifesting as nasal voice and compensatory articulation disorders[3]. Nasal voice is an underappreciated sign for suspicion of 22q11.2DS, probably because it is observed later in life, but it should be considered a warning sign for the occurrence of the syndrome.

Parathyroid hypoplasia or aplasia is very common in 22q11.2DS due to involvement of the third and fourth pharyngeal arches during embryogenesis. In 49% to 60% of the newborns affected by the syndrome, transient hypocalcemia may be present leading to tetany and seizures of difficult control[22], data that was similar in the patients in this study. Pediatricians and cardiologists should be attentive during the neonatal period to the presence of hypocalcemia without other apparent physiopathological cause, a manifestation that is highly suggestive of the syndrome.

Another variable manifestation in 22q11.2DS is immunodeficiency, considered to be due to abnormal development of the thymus, denominated DGS in these cases. Around 80% of the patients with DGS have immune system abnormalities. Even though the thymus is hypoplasic or absent in great part of the patients, most present mild to moderate immunodeficiency, independent of other clinical features[28,29]. A study conducted by Patel et al[29] showed that a minority of the patients have low immunoglobulin levels and, in general, between 2% and 3% need immunoglobulin replacement therapy[29]. In our study, 3 patients had low levels of immunoglobulins and only 1 patient required immunoglobulin replacement therapy.

Since in patients with 22q11.2DS the initial manifestation may be hyperactivity, anxiety and depression, the authors emphasize the importance of an early diagnosis and a multiprofessional follow-up appropriate for these patients. The diagnosis of ADHD, anxiety disorders, mood disorders and disorders of the autism spectrum can occur in one third to half of the children with the deletion. Mood abnormalities and psychotic disorders can increase significantly during early adulthood, it is therefore fundamental to monitor carefully the occurrence of psychiatric symptoms during adolescence and early

Figure 3 – *Main phenotypic characteristics of patients with the 22q11.2 deletion syndrome. A) Narrow palpebral fissure. B) Elongated face and/or nose. C) Thin lips.*

adulthood[30]. These patients may also present poor academic performance, which is an important aspect to be addressed with the educational institutions.

It is imperative to disseminate warning signs for the 22q11.2DS among general pediatricians, neonatologists and pediatric cardiologists. Even though the pioneering proposal of *Instituto da Criança* and adopted by the Ministry of Health for detection of primary immunodeficiencies in the first year of life already includes 4 signs associated with the diagnosis of 22q11.2DS (CHD, in particular anomalies of the great vessels; lymphopenia < 2,500/mm; hypocalcemia with or without seizures; and absence of a thymic image on chest radiograph)[31,32] it would be important to establish specific warning signs for this syndrome, adding phenotypic abnormalities and velopharyngeal insufficiency to the ones described above.

The current study has limitations peculiar to a transversal descriptive study, but highlights the main clinical and laboratorial findings of a highly selected population. The description of this population, however, allows us to identify and understand the demographic features of the studied group and the morbidity profile of the 22q11.2DS, and may offer data for a more adequate health policy for these patients.

The management of children with 22q11.2DS requires interaction of a multidisciplinary team of pediatrician, cardiologist, geneticist, and in some circumstances, endocrinologist, neurologist, plastic surgeon, psychologist and speech therapist.

Conclusion

Considering that in our country 2.5 million children are born each year, we estimate that 500 to 750 new cases of 22q11.2DS should be identified yearly, which indicates that the syndrome is currently underdiagnosed. It is therefore crucial to maintain an alert for the occurrence of the syndrome in the presence of CHD associated with hypocalcemia, facial dysmorphisms, velopharyngeal insufficiency, thymic hypoplasia or absence on chest radiograph, and confirm the diagnosis by identification of the microdeletion.

Author contributions

Conception and design of the research and Statistical analysis: Grassi MS, Jacob CMA, Pastorino AC, Carneiro-Sampaio M; Acquisition of data: Grassi MS, Jacob CMA, Pastorino AC, Miura N, Jatene MB, Pegler SP, Carneiro-Sampaio M; Analysis and interpretation of the data: Grassi MS, Jacob

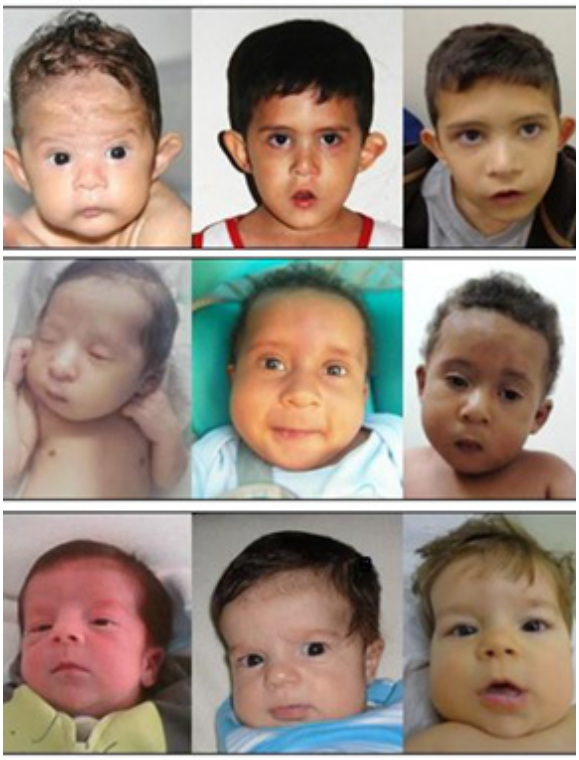


Figure 4 – *Pictures showing evolving features of patients with the 22q11.2 deletion syndrome at different ages. A) Newborn with thin lips and dysplasic ears. These phenotypic features become more characteristic at school age. B) Newborn with facial dysmorphism (elongated face and nose, narrow palpebral fissure, thin lips). C) Infant with elongated face and nose more evident during development.*

CMA, Kulikowski LD, Pastorino AC, Dutra RL, Carneiro-Sampaio M; Obtaining financing: Jacob CMA, Pastorino AC, Carneiro-Sampaio M; Writing of the manuscript: Grassi MS, Jacob CMA, Kulikowski LD, Pastorino AC, Carneiro-Sampaio M; Critical revision of the manuscript for intellectual content: Jacob CMA, Kulikowski LD, Pastorino AC, Kim CA, Carneiro-Sampaio M.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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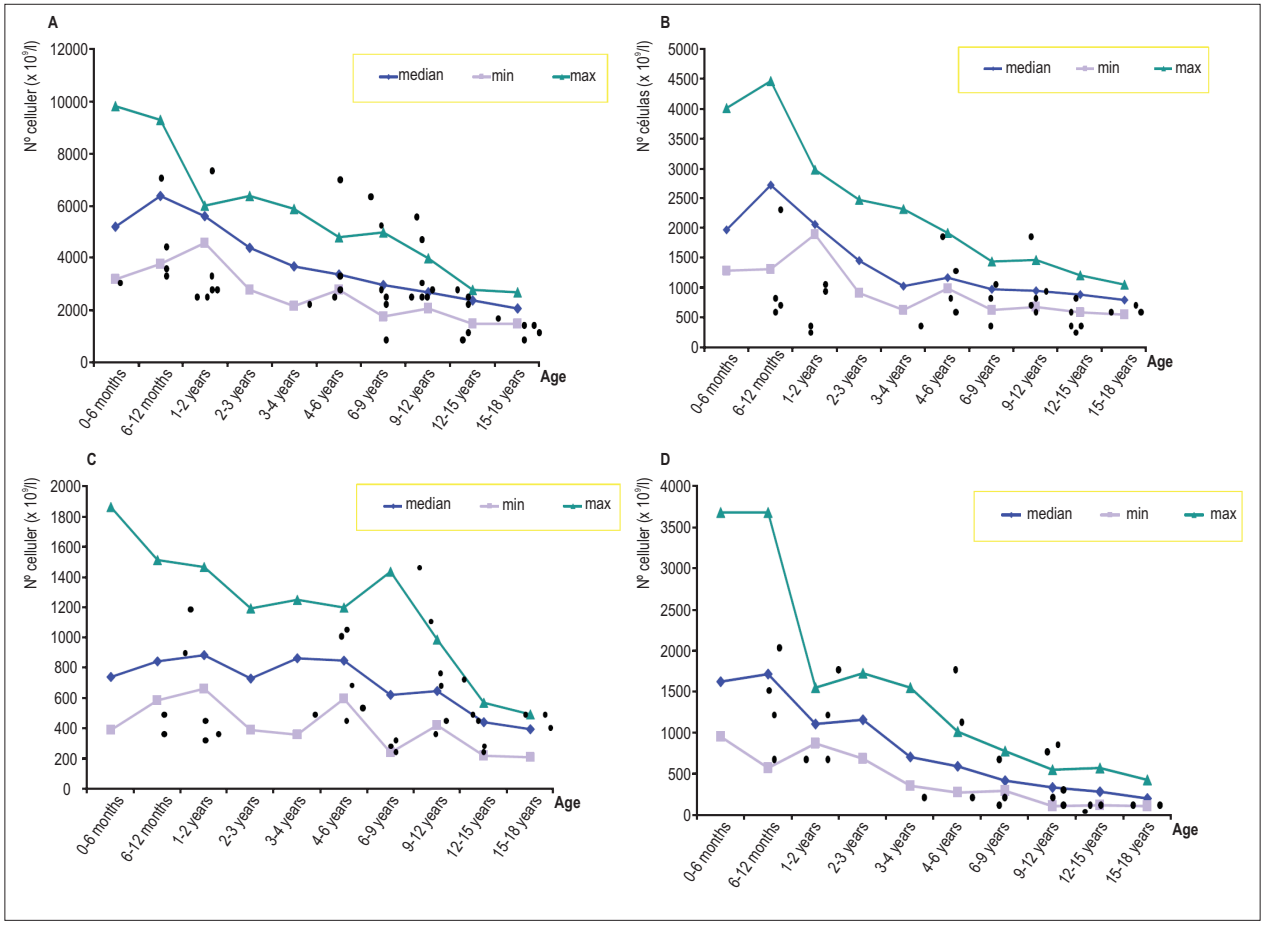
Study Association

This study is not associated with any thesis or dissertation work.



Graph 2 – *Distribution of values of total lymphocytes, CD4⁺, CD8⁺ and CD19⁺ in patients with the 22q11.2 deletion syndrome. A) Number of total lymphocytes. B) CD4⁺ count. C) CD8⁺ count. D) CD19⁺ count. Each dot (•) corresponds to an individual patient. Max: Maximum; Min: Mimum*

References

1. Gennery AR. Immunological aspects of 22q 11.2 deletion syndrome. Cell Mol Life Sci. 2012;69(1):17-27.
2. Kobrynski L, Sullivan K. Velocardiofacial syndrome, DiGeorge syndrome: the chromosome 22q11.2 deletion syndromes. Lancet. 2007;370(9596):1443- 52.
3. Goldmuntz E. DiGeorge Syndrome: new insights. Clin Perinatol. 2005;32(4):963-78.
4. Pinkel D, Straume T, Gray JW. Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc Natl Acad Sci U S A. 1986;83(9):2934-8
5. Dutra RL, Honjo RS, Kulikowski LD, Fonseca FM, Pieri PC, Jehee FS, et al. Copy number variation in Williams-Beuren syndrome: suitable diagnostic strategy for developing countries. BMC Res Notes. 2012; 5:13.
6. Momma K. Cardiovascular anomalies associated with chromosome 22q11.2 deletion syndrome. Am J Cardiol. 2010;105(11):1617-24.
7. Fahed AC, Gelb BD, Seidman JG, Seidman CE. Genetics of congenital heart disease: the glass half empty. Circ Res. 2013;112(4):707-20.
8. Porto MH, Birroli MI, Duarte AJ, Carneiro-Sampaio MM. Peripheral lymphocyte subpopulations in selective IgA deficiency. Rev Hosp Clin Fac Med São Paulo. 1988;43(6):294-7.
9. Jacob CM, Pastorino AC, Fahl K, Carneiro-Sampaio M, Monteiro RC. Autoimmunity in IgA deficiency: revisiting the role of IgA as a housekeeper. J Clin Immunol. 2008;28(Suppl 1):S56-61.
10. Carneiro-Sampaio MM, Vasconcelos DM, Kokron CM, Jacob CM, Barros MT, Dorna MB, et al. Primary Immunodeficiency Diseases in Diferente Age Groups: A Report on 1008 Cases from a Single Brazilian Reference Center. J Clin Immunol. 2013;33(4):716-24.
11. Fomin AFB, Pastorino AC, Kim CA, Pereira CA, Carneiro-Sampaio M, Abe-Jacob CM. DiGeorge Syndrome: a not so rare disease. Clinics (São Paulo). 2010;65(9):865-9.
12. Karayiorgou M, Simon TJ, Gogos JA. 22q11.2 microdeletions: linking DNA structural variation to brain dysfunction and schizophrenia. Nat Rev Neurosci. 2010;1(6):402-16.
13. De la Morena MT, Eitson JL, Dozmorov IM, Belkaya S, Hoover AR, Anguiano E, et al. Signature MicroRNA expression patterns identified in humans with 22q11.2 deletion/DiGeorge syndrome. Clin Immunol. 2013;147(1):11-22.

14. Ryan AK, Goodship JA, Wilson DI, Philip N, Levy A, Seidel H, et al. Spectrum of clinical features associated with interstitial chromosome 22q11 deletions: a European collaborative study. J Med Genet. 1997;34(10):798-804.

15. Duke SG, McGuirt WF Jr, Jewett T, Fasano MB. Velocardiofacial syndrome: incidence of immune cytopenias. Velocardiofacial syndrome: incidence of immune cytopenias. Arch Otolaryngol Head Neck Surg. 2000;126(9):1141-5.

16. Belangero S, Bellucco F, Kulikowski L, Christofolini D, Cernach M, Melaragno M. 22q11.2 deletion in patients with conotruncal heart defect and del22q syndrome phenotype. Arq Bras Cardiol. 2009;92(4):307-11.

17. Markert ML, Devlin BH, McCarthy EA. Thymus transplantation. Clin Immunol. 2010;135(2):236-46.

18. Al-Herz W, Bousfiha A, Casanova JL, Chapel H, Conley ME, Cunningham-Rundles C, et al. Primary immunodeficiency diseases: an update on the classification from the international union of immunological societies expert committee for primary immunodeficiency. Front Immunol. 2011;2:54.

19. Edelmann L, Hirschhorn K. Clinical utility of array CGH for the detection of chromosomal imbalances associated with mental retardation and multiple congenital anomalies. Ann N Y Acad Sci. 2009;1151:157-66.

20. Aksu G, Genel F, Koturoglu G, Kurugol Z, Kutukçuler N. Serum immunoglobulin (IgG, IgM, IgA) and subclass concentrations in healthy children: a study using nephelometric technique. Turk J Pediatr. 2005;47(1):19-24.

21. van Gent R, van Tilburg CM, Nibbelke EE, Otto SA, Gaiser JF, Jansssens-Korpela PL et al. Refined characterization and reference values oh the pediatric T- and B- cell compartments. Clinical Immunol. 2009;133(1):95-107.

22. Ziolkowska L, Kawalec W, Turska-Kmiec A, Krajewska-Walasek M, Brzezinska-Rajszys G, Daszkowska J, et al. Chromosome 22q11.2 microdeletion in children with conotruncal heart defects: frequency, associated cardiovascular anomalies, and outcome following cardiac surgery. Eur J Pediatr. 2008;167(10):1135-40.

23. McDonald-McGinn DM, Kathleen E. Sullivan KE. Chromosome 22q11.2 Deletion Syndrome (DiGeorge Syndrome/ Velocardiofacial Syndrome). Medicine.(Baltimore). 2011;90(1):1-18.

24. Rosa RFM, Trevisan P, Koshiyama DB, Pillsa CB, Zens PRG, Varella- Garcia M, et al. Síndrome de deleção 22q11 e cardiopatias congênitas complexas. Rev Assoc Med Bras. 2011;57(1):62-5.

25. Rosa RF, Zen PR, Ricachinevsky CP, Pilla CB, Pereira VL, Roman T, et al. 22q11.2 duplication and congenital heart defects. Arq Bras Cardiol. 2009;93(4):e67-9.

26. McDonald R, Dodgen A, Goyal S, Gossett JM, Shinkawa T, Uppu SC, et al. Impact of 22q11.2 deletion on the postoperative course of children after cardiac surgery. Pediatr Cardiol. 2013;34(2):341-7.

27. Pena DJ, Pietro G, Fuchshuber-Moraes M, Genro JP, Hutz MH, Kehdy FSG, et al. The genomic ancestry of individuals from different geographical regions of Brazil is more uniform than expected. PLoS One. 2011;6(2):e17063.

28. Sullivan KE. Immunologic issues in VCFS/ chromosome 22q11.2 deletion syndrome. Prog Pediatr Cardiol. 2002;15(2):103-8.

29. Patel K, Akhter J, Kobrynski L, Benjamin Gathmann MA, Davis O, Sullivan KE, et al. International DiGeorge Syndrome Immunodeficiency Consortium. Immunoglobulin deficiencies: the B-lymphocyte side of DiGeorge Syndrome. J Pediatr. 2012;161(5):950-3.

30. Drew LJ, Crabtree GW, Markx S, Stark KL, Chaverneff F, Xu B, et al. The 22q11.2 microdeletion: fifteen years of insights into the genetic and neural complexity of psychiatric disorders. Int J Dev Neurosci. 2011;29(3):259-81.

31. Carneiro-Sampaio M, Jacob CM, Leone CR. A proposal of warning signs for primary immunodeficiencies in the first year of life. Pediatr Allergy Immunol. 2011;22(3):345-6.

32. Hospital das Clínicas/FMUSP. Instituto da Criança. (São Paulo).12 sinais de alerta para Imunodeficiências Primárias no 1º anos de vida. [Citado em 2013 ago 26]. Disponível em: http://portal.saude.gov.br/portal/saude/visualizar_texto.cfm.dtxt =41993