Microdeletion 22q11.2: a reason to feel blue?

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Resumo

Introdução: A microdelecção 22q11.2 é caracterizada por uma grande variabilidade fenotípica, ocorrendo em cerca de 1 em cada 4000 nascimentos.
Objectivo: Este trabalho pretende avaliar a prevalência de cianose em indivíduos com suspeita clínica de microdelecção 22q11.2 e se existe uma correlação entre o fenótipo cianose e a frequência destas alterações estruturais.
Métodos: Foram avaliados 532 pacientes por citogenética convencional e por "fluorescent *in situ* hybridization", recorrendo a sondas específicas para a região crítica 22q11.2 (N25 e TUPLE1).
Resultados: A microdelecção 22q11.2 foi identificada em 23 pacientes com fenótipo variado. A prevalência da delecção no grupo estudado foi de 4,3% e a prevalência de cianose foi de 31,6%. Cerca de 9,5% dos pacientes que apresentavam cianose tinham também microdelecção 22q11.2. Aplicando um teste de "Odds Ratio" obteve-se um valor de 5,368, correspondendo a uma possibilidade cinco vezes maior de um indivíduo com cianose apresentar a microdelecção relativamente a um indivíduo sem cianose.
Conclusão: A cianose poderá ser um indicador para a pesquisa da microdelecção 22q11.2. Testes moleculares de alta resolução – multiplex ligation-dependent probe amplification e array CGH – definirão os pontos de quebra da delecção e permitirão uma melhor correlação genótipo-fenótipo.

Palavras-chave: microdelecção 22q11.2, cianose, cardiopatias congénitas, *shunts* anatómicos, síndrome de DiGeorge, CATCH 22, FISH.

Abstract

Introduction: Microdeletion 22q11.2 is the most common microdeletion syndrome in humans, occurring in 1 in 4,000 live births. It is clinically characterized by a wide phenotypic spectrum.
Objective: This study intended to evaluate the prevalence of cyanosis in patients with clinical suspicion of 22q11.2 microdeletion and whether there is any correlation between the phenotype cyanosis and the presence of the this structural alteration.
Methods: 532 patients with clinical suspicion of 22q11.2 microdeletion were evaluated by conventional cytogenetic studies and fluorescent *in situ* hybridization, using the specific probes for the 22q11.2 critical region N25 and TUPLE1.
Results: 22q11.2 microdeletion was identified in 23 patients with variable phenotypes. The prevalence of the deletion in the group of study is 4,3% and the prevalence of cyanosis is 31,6%. About 9,5% of the patients with cyanosis had also 22q11.2 microdeletion. By an Odds

Ratio test, a value of 5,368 was obtained, meaning that a patient presenting cyanosis has a five more probability of having a 22q11.2 microdeletion than a patient without this clinical feature.

Conclusion: The large phenotypic variability makes more difficult the establishment of a genotype-phenotype correlation. Cyanosis can be an indicator for the analysis of 22q11.2 microdeletion. Future work with high resolution techniques - multiplex ligation-dependent probe amplification and array CGH – will redefine the deletion breakpoints and allow a better phenotype-genotype correlation.

Key-words: microdeletion 22q11.2, cyanosis, congenital heart defects, anatomic shunts, DiGeorge syndrome, CATCH 22, FISH.

1. Introduction

Cyanosis is a bluish discoloration of skin and mucosal membranes that results from an increase of reduced haemoglobin in blood. It can be caused by impaired oxygen perfusion, alveolar hypoperfusion, haemoglobin with low oxygen affinity, metahaemoglobinemia, sulfahaemoglobinemia, carboxyhaemoglobinemia, anatomic shunts, arterial and venous obstruction, redistribution of blood from the extremities and cold exposure (Fauci et al. 2008).

Since congenital heart defects can be caused by anatomic shunts, *per se* originating a right-left shunt or a reduction in pulmonary blood flow, they are a major cause of cyanosis. Examples of anatomic shunts causing cyanosis are pulmonary stenosis, Fallot tetralogy, pulmonary atresia with ventricular sept defects or with integral septum, tricuspid atresia and Ebstein's malformation. The conjoined manifestation of cyanosis and congestive heart failure can be due to great arteries transposition alone, with ventricular sept defects or with pulmonary stenosis (Fauci et al. 2008).

The congenital heart defects are frequent disorders found by electrocardiograph examination (Wong et al. 2009), with a prevalence that ranges from 3,7 to 17,5 per 1000 live births (Kapoor and Gupta 2007). They can be part of the clinical features of various syndromes, including the DiGeorge syndrome (DGS) that is the most common chromosomal alteration after Down syndrome (Dempsey et al. 2007, Cyriac et al. 2008 and Goldmunz et al. 2009).

Hemizygous deletions on the 22q11.2 region result in the 22q11.2 microdeletion syndrome (Bittel et al. 2009). These rearrangements lead to recognizable clinical phenotypes such as the Cat Eye syndrome (duplication) and the DiGeorge / velocardiofacial syndrome (DGS/VCFS) (deletion). The DGS occurs in 1 in 4,000 live births. This syndrome is characterized by several clinical features resumed in the acronym CATCH22: congenital heart defects (50%), facial abnormalities, thymic hypoplasia, cleft palate (69%) and hypocalcaemia (50%) (Dempsey et al. 2007). It can also be associated with psychiatric and learning disorders (70-90%) (Weksberg et al. 2006 and Dempsey et al. 2007).

Four different homologous blocks of low copy repeats sequences (LCRs) flank the 22q11.2 region. Mispairing of LCRs during meiosis with unequal crossing over is assumed to cause recurrent deletions (Bittel et al. 2009). The proximal region of the long arm of

chromosome 22 has eight LCR sequences. The four LCRs that mediate the more common deletions are LCR22-2 (A), LCR22-3a (B), LCR22-3b (C) and LCR22-4 (D) (Torres-Juan et al. 2007). 22q11.2 microdeletion occurs in the majority of cases in a typically deleted region (TDR), flanked by LCR A and D (3Mb), being a smaller one (1,5 – 2Mb), mediated by LCR A and C, in 7% of cases (Shaikh et al. 2000).

Microdeletion 22q11.2 is occurs generally as a *de novo* mutation being inherited in 6 to 25% of the cases (Kyburz et al. 2007; Ben-Shachar et al. 2008 and Emanuel 2008).

It is of utmost importance the diagnostic of a congenital heart defect *in utero*, for it can be tested for its association with chromosomal alterations, particularly with microdeletions and microduplications. In those cases when the structural alteration is detected it is important to evaluate its origin. On those cases when the karyotype is normal and the pregnancy is taken to term, surgical procedures can be planned to guarantee a better quality of life for the affected patients.

In this work will be evaluated whether there is any correlation between cyanosis and the prevalence of the microdeletion 22q11.2 in a group of patients followed on the Paediatric Cardiology Clinic in Hospital Pediátrico do Centro Hospitalar de Coimbra (HP-CHC) and referred for laboratory tests in the Cytogenetic Laboratory of the Faculty of Medicine of Coimbra.

2. Material and methods:

2.1 Clinical Selection: 532 patients referred from HP-CHC were grouped according to the following clinical features: A-cyanosis, B-congestive heart failure, C-cyanosis and congestive heart failure and D-other manifestations (hypocalcaemia, cleft palate, facial abnormalities, thymic hypoplasia and mental retardation). All of them were referred for high resolution karyotype analysis and evaluation for detection of microrearrangements in the 22q11.2 region.

Exclusion criteria included all patients with non-anatomic shunt causes of cyanosis - impaired oxygen perfusion, alveolar hypoperfusion, haemoglobin with low oxygen affinity, metahaemoglobinemia, sulfahaemoglobinemia, carboxyhaemoglobinemia, arterial and venous obstruction, redistribution of blood from the extremities and cold exposure.

2.2 Conventional cytogenetics: cytogenetic studies were performed on high resolution GTG banded chromosomes obtained from synchronized cultures of peripheral blood lymphocytes according to standard techniques (Rooney and Czepulkowski 1992).

2.3 FISH analysis: Fluorescent *in situ* hybridization (FISH) analyses were performed on metaphases and interphase cells using unique sequence probes N25 (Q-Biogene, Illkirch, France) and TUPLE1 (Vysis, Downers Grove, IL) specific for the *loci* of the DG/VCFS critical region. For each probe, at least 10 metaphase cells and 10 interphase nuclei were observed. FISH images were captured on a Nikon Eclipse E400 Fluorescent microscope and analyzed with Applied Imaging software (Cytovision).

2.4 Statistical analysis: It was performed using SPSS v.17.0. Frequency and percentage of clinical features, such as: cyanosis; congestive heart failure; cyanosis associated with congestive heart failure and other manifestations (hypocalcaemia, cleft palate, facial abnormalities, thymic hypoplasia, mental retardation) were evaluated. Comparison of the presence of microdeletions between the two groups of patients with or without cyanosis was made using χ^2-square test and odds ratio was determined.

3. Results

3.1 Clinical evaluation

After clinical evaluation of the 532 patients they were distributed in four groups: A) 108 patients having cyanosis (Fig.1), B) 45 having congestive heart failure, C) 60 having both cyanosis and congestive heart failure and D) 319 having other non cardiac clinical features - hypocalcaemia, cleft palate, facial abnormalities, thymic hypoplasia and mental retardation (Table I).

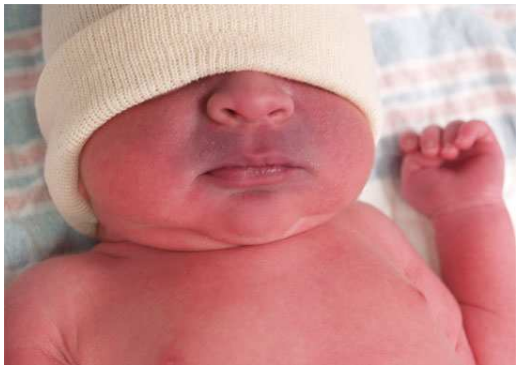

Figure 1: An infant with cyanosis.
Source: http://newborns.stanford.edu

Table I: Distribution of the patients and their frequencies through the different clinical features.

Clinical feature	Number of cases	Frequency (%)
Group A - Cyanosis	108	20,30%
Group B - Congestive heart failure	45	8,46%
Group C - Cyanosis associated with congestive heart failure	60	11,28%
Group D - Other manifestations	319	59,96%
Total	532	100,00%

According to Table I, the D group is the one with more number of cases. This fact can be explained by the fact that the other manifestations included all the non-cardiac manifestations suggestive of DGS (hypocalcaemia, cleft palate, facial abnormalities, thymic hypoplasia and mental retardation). The cardiac phenotype is referred in the other three groups.

There are 168 patients with cyanosis (alone or associated with congestive heart failure) and 105 patients without cyanosis, but with congenital heart defects.

3.2 Conventional cytogenetics and FISH evaluation

Cytogenetic studies ruled out, in all cases, aneuploidies and structural rearrangements particularly of chromosome 22. The evaluation by FISH of the critical region of chromosome 22 in the 532 cases showed a microdeletion (Fig.2) in 23 (4,3%) of them (Table II and III). All of them were *de novo* microdeletions, being the study of both parents normal.

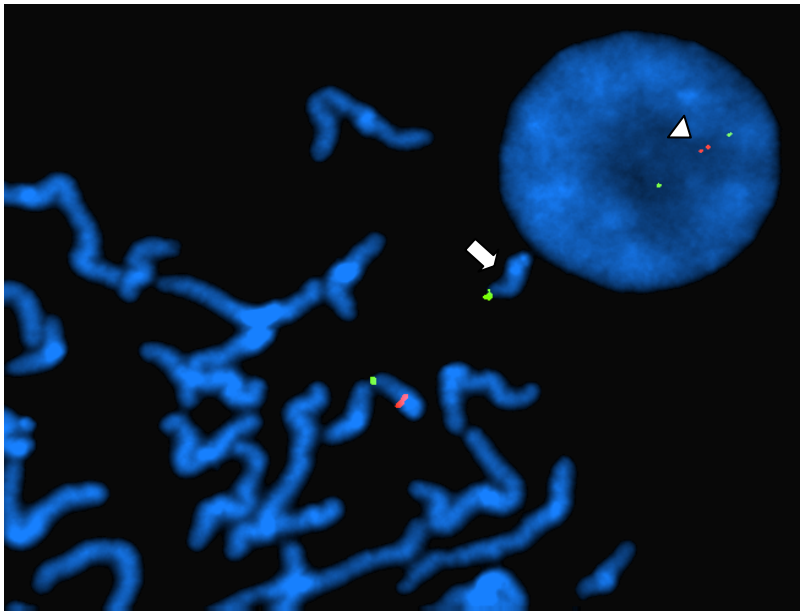

Figure 2: FISH analysis with N25 probe showing 22q11.2 microdeletion (arrow) in a metaphase spread. Also, only one red spot signal was observed in the interphase nuclei (arrow head).

Table II: Number of cases with microdeletion 22q11.2 detected by FISH in the 532 cases studied.

		Number of cases
22q11.2 microdeletion	Absent	509 (95,70%)
	Present	23 (4,30%)
	Total	532 (100,00%)

Table III, shows the correlation of cases with or without 22q11.2 microdeletion with the cases having and not having clinical features of cyanosis on the study group.

Table III: Distribution of cyanosis and deletion amongst the study

		Cyanosis		
		Absent	**Present**	**Total**
Deletion	**Absent**	**357** *(98,08%)* **(67,10%)**	**152** *(90,50%)* **(28,57%)**	**509** **(95,70%)**
	Present	**7** *(1,92%)* **(1,32%)**	**16** *(9,50%)* **(3,00%)**	**23** **(4,30%)**
	Total	**364** *(100,00%)* **(68,42%)**	**168** *(100,00%)* **(31,58%)**	**532** **(100,00%)**

Of the 168 patients having clinical features of cyanosis, 16 (9,5%) have also the 22q11.2 microdeletion. Of the remaining 364 cases without cyanosis, 7 patients (1,39%) also had the microdeletion.

3.3 Statistical analysis

In order to evaluate if there is significant statistical difference in the presence of microdeletions between the two groups studied (patients with or without cyanosis), a χ^2-square test was performed, because the two variables studied were nominal. An Odds Ratio test was also applied (Table IV).

Table IV: Statistic studies performed

	Value
χ^2-square test	$p = 6,156 \times 10^{-5}$
Odds Ratio for deletions (Absent / Present)	5,368 Confidence interval (95%): [2,165 – 13,313]

As shown on Table IV, the χ^2-square test value $p=6,156 \times 10^{-5}$ shows that there is statistical difference between the two groups analysed. The Odds Ratio has a value of 5,368, with a confidence interval of 95%, which means that there is a positive correlation between the presence of cyanosis and 22q11.2 microdeletion.

4. Discussion

The DiGeorge critical region (DGCR) contains 30 to 40 genes, many of which have not been well characterized and are currently under investigation. The DGS is characterized by a 3 million base pairs (3Mbp) deletion of chromosome 22. The deletion occurs as an error during egg or sperm cells formation. It means that one copy of several genes in 22q11.2 is not present in the DGS. It is unclear why there is such variation in symptoms between different deleted individuals. It does not appear to be related to the differences in the amount of genetic material lost, but it seems to be also a result from the interaction between genetic, epigenetic and environmental factors (Bittel et al. 2009 and Fernández et al. 2009).

Experimental studies show that the foetal heart defects can be associated with loss of expression of T-box gene (Tbx-1). Heterozygote mice for this gene develop cardiac malformations such as persistent truncus arteriosus and vessel abnormalities including a double aortic arch, which can be included in the congenital heart defects observed in DGS (Jerome et al. 2001). So, with this association of haploinsufiency/ absence of expression of Tbx-1 gene and congenital heart defects, a possible relation between this gene and the cardiac phenotype could eventually be made.

Perhaps this gene may be the key-gene to unlock the congenital heart defects association with the presence/absence of cyanosis in patients with DGS.

With the finding of a gene responsible for the cardiac and other features of CATCH22, a directed analysis could be made, giving the clinicians a new tool for diagnosing the cause of the underlying heart defect, which, in a certain period of time, will be surgically corrected. With surgical intervention quality of life of these patients could be remarkably improved or be cured.

In this work FISH studies were used for identifying the presence of microrearrangements in the 22q11.2 region. This technique is useful to identify 22q11.2 deletions but can show some limitations in the detection of duplications. Currently in our cytogenetic laboratory, a wide range of molecular diagnostic techniques are being made such as multiplex ligation-dependent probe amplification (MLPA). This has been proven to be a very effective mean of detection of both deletions and duplications at 22q11.2, as well as in the determination of the extension of the rearrangements (Stachon et al. 2007 and Yu et al. 2008).

In this work, of the 532 cases analysed, the percentage of the 22q11.2 microdeletion is in agreement with those published that set prevalence of microdeletion 22q11.2 in a range from 4-21% (Table III) (Koshiyama et al. 2009).

It was interesting to evaluate whether the presence of the clinical feature cyanosis, in patients having an anatomic shunt was related to the presence of the 22q11.2 deletion. In our study group, 168 patients were found to have the clinical features of cyanosis and 105 having clinical features of congestive heart failure, as evidenced on Table III. The prevalence cyanosis in this group was 31,6%, being 9,5% of these patients confirmed by FISH studies as having the 22q11.2 microdeletion.

χ^2-square test compared the presence of microdeletions between the groups of patients with and without cyanosis. The p value < 0,001 is indicative that there is statistically significant differences between the two groups considered.

Although having statistical significance it does not mean that the results obtained have clinical significance. A way to access this is to perform an OR test, which showed a value of 5,368, with a confidence interval of 95% established between the values of [2,165 – 13,313]. This result means that a patient presenting cyanosis has a possibility five times higher of having a 22q11.2 microdeletion than a patient without cyanosis. This finding is important, because a clinical symptom could be suggest this laboratory test.

It can be concluded that a clinical feature of cyanosis can have a genetic background. This can be related with several genes in 22q11.2 or in other chromosomes, such as 10p (p13pter) microdeletion, which has been associated with DiGeorge second *loci* (DGS2) (Chao et al. 2009). This could suggest that a patient with cyanosis should have a karyotype done as well as the 22q11.2 region evaluated by FISH studies.

From these results several questions could be raised: 1- Is there any correlation between the size of the microdeletion in 22q11.2 with or without cyanosis? 2- Are the results consistent with the clinical evaluation? 3- How can the size be precisely evaluated?

This can be done using the recent advances in molecular biology and cytogenomic techniques that allow more in depth studies, namely MLPA and “array comparative genome hybridization” (aCGH). The former is currently being performed in our cytogenetic laboratory. Most of the patients evaluated in this project are being clinically re-evaluated and blood collected for DNA extraction. Preliminary results have already detected one microduplication in a case previously reported as normal by FISH analysis.

“Array CGH” can be used to make a whole genome approach. “High resolution oligonucleotide-band microarray” aCGH will be used to ascertain the breakpoints of the cohort of the 23 patients with 22q11.2 microdeletion, evaluate other target regions to establish a better genotype-phenotype correlation.

5. Conclusion

As one can conclude, a patient having cyanosis caused by an anatomic shunt, in the study group, has a five times more probability of having 22q11.2 microdeletion than a patient that does not present this clinical feature

Cyanosis could be an indicator for the investigation of microrearrangements in 22q11.2 region.

So, “blue” is a reason for the investigation of the 22q11.2 microdeletion.

6. References

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