

Original article

Benefits of cytogenetic testing in diagnosis of plurimalformative syndromes with congenital heart defects

Beneficiile testării citogenetice în diagnosticul sindroamelor plurimalformative cu afecțiuni cardiace congenitale

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Abstract

Congenital heart defects (CHD) are among the most common types of major birth defects; the common causes of CHD are chromosomal abnormalities when CHD associate multiple congenital anomalies (MCA). We performed a retrospective study, with the purpose of establishing the benefits of genetic tests in etiologic diagnosis and to estimate the frequency and the types of chromosomal abnormalities, in 1123 patients with MCA who were clinically and cytogenetically evaluated during the period of 2000-2010 in Iași Medical Genetics Center. CHD were present in 232 (30.49%) out of 761 patients with chromosomal abnormalities; CHD were more frequent in 22q11.2 microdeletion (6/7 cases or 85.71%), 18 trisomy (9/15 cases or 60%), 21 trisomy (177/558 cases or 31.72%) and X monosomy (11/74 cases or 14.86%). We detected chromosomal abnormalities in 232 (72.04%) out of 322 cases with CHD. Septal defects and patent ductus arteriosus (PDA), of all types of CHD, were more frequently associated with a chromosomal abnormality. Our study proved the benefits of cytogenetic testing in diagnosis of CHD and MCA cases. When “standard” chromosome analysis shows a normal karyotype, molecular cytogenetic techniques are useful to detect submicroscopic chromosomal abnormalities.

Keywords: congenital heart defects, chromosomal abnormalities, cytogenetic testing

Rezumat

Malformațiile congenitale de cord (MCC) reprezintă unele dintre cele mai frecvente anomalii congenitale (AC); atunci când asociază anomalii congenitale multiple (ACM) sunt identificate frecvent anomalii cromozomiale. Scopul acestui studiu retrospectiv a fost stabilirea utilității testelor genetice în diagnosticul etiologic al MCC asociate cu ACM și estimarea frecvenței și a tipului de anomalii cromozomiale, în cazul a 1123 pacienți cu

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ACM, care au fost evaluați clinic și citogenetic (2000-2010) în cadrul Centrului de Genetică Medicală Iași. Din 761 pacienți cu anomalii cromozomiale 232 (30,49%) au prezentat MCC; MCC au fost mai frecvente în microdeleția 22q21 (6/7 cazuri sau 85,71%), trisomia 18 (9/15 cazuri sau 60%), în trisomia 21 (177/558 cazuri sau 31,72%) și monosomia X (11/74 cazuri sau 14,86%). Din 322 cazuri cu MCC au fost identificate anomalii cromozomiale în 232 cazuri (72,04%). Dintre toate tipurile de MCC defectele septale și persistența canalului arterial (PCA) au fost asociate cu un procent ridicat de anomalii cromozomiale. Studiul curent a dovedit utilitatea testelor citogenetice în diagnosticul cazurilor cu MCC și ACM. Atunci când analiza cromozomială "clasică" evidențiază un cariotip normal este utilă folosirea unor tehnici de citogenetică moleculară pentru evaluarea existenței unei anomalii cromozomiale submicroscopice.

Cuvinte cheie: defecte cardiace congenitale, anomalii cromozomiale, teste citogenetice

Introduction

Congenital heart defects (CHD) are among the most common type of major birth defects with an estimated prevalence of 14 cases per 1000 live births (1). Congenital heart defects may be isolated or associated with multiple congenital anomalies (MCA), mental retardation (MR), craniofacial dysmorphism or growth abnormalities (2, 3). According to the Baltimore-Washington Infant Study (BWIS), the overall frequency of associated extracardiac anomalies in newborns with CHD is 20% with the chromosomal abnormalities being the common cause (4). Standard chromosomal analysis and molecular techniques, like fluorescence in situ hybridization (FISH), revealed chromosomal abnormalities in 12.3% of cases with CHD and MCA; the most common observed were: trisomy 21 (52.8%), trisomy 18 (12.8%), 22q11.2 deletion (12.2%) and trisomy 13 (5.7%) (5-7). According to Hartman's study, certain types of CHD are more frequently associated with a chromosomal abnormality: interrupted aortic arch (type B and not otherwise specified 69.2%), atrioventricular septal defect (67.2%), and double-outlet right ventricle (33.3%) (5, 8-10). Determining the genetic etiology of MCC and ACM is important for a proper genetic counseling in families with affected members.

Studies on the association between CHD and chromosomal abnormalities show conflicting data at global level, while in Romania they are lacking. In this context, we aimed to analyze

retrospectively the patients from Iasi Genetics Medical Centre (GMC), in order to evaluate the benefits of genetic tests for etiologic diagnostic of cases with CHD and MCA.

Materials and methods

Between 1st January 2000 and 31st December 2010, 1123 patients with MCA±MR were evaluated at clinical, paraclinical and cytogenetic level in Iași GMC. The age ranged from 3 days to 29 years old.

The clinical examination included anthropometric measurements (weight, height and cranial perimeter), evaluation of CA, craniofacial dysmorphism and psychomotor development. Of these, 321 patients had CHD. CHD type and severity were established by 3D echocardiography. The rigorous selection for cytogenetic evaluation was based on clinical guidelines from literature and GMC Iasi clinical scores (2, 11-17).

Patients were divided, based on 550 bands chromosomal analysis results, into group A (749 cases) with unbalanced chromosomal abnormalities and group B (374 cases) with normal karyotype. Group A was subdivided into subgroup A1 (699 cases), including patients with suggestive phenotype for known chromosomal syndromes, which were cytogenetically confirmed and subgroup A2 (50 cases), patients with ACM ± MR, in which cytogenetic analysis revealed unbalanced chromosomal abnormalities. Group B, with normal karyotype, was divided, based on phenotype, into subgroup B1 (34 cases) with suggestive phenotype for microdeletion

Table 1. Chromosomal analysis results and CHD frequency

Phenotype	Karyotype result		CHD	
	Abnormality type	No. cases	No. cases	Total
MCA ± MR Chromosomal disease phenotype (subgroup A1)	21 trisomy	558	177 (31.72%)	
	18 trisomy	15	9 (60%)	
	13 trisomy	12	4	
	X monosomy	74	11 (14.86%)	206
	XXY trisomy	34	1 (2.94%)	(29.47%)
	Deletion 4p	2	1	
	Deletion 5p	3	2	
	Deletion 7q11.2	1	1	
MCA ± MR (sub- group A2)	Add	22	7/22	
	Del	16	7/16	
	Ins	11	2/11	17
	r	1	1/1	(34%)
			749	223 (29.77%)
MCA ± MR (group B)	Normal	374	374	99 (26.47%)
TOTAL			1123	322 (28.67%)

syndromes (22q11.2, 7q11.23, 4p16), and subgroup B2 (340 cases), with MCA±MR, without presumptive clinical diagnosis.

For subgroup B.1 patients, we applied fluorescent *in situ* hybridization technique (FISH) with specific probes to confirm clinical diagnosis. For subgroup B2 patients we would attempt to apply pangenomic technique (array CGH) or other molecular tests for detection of a possible monogenic mutation. For patients in whom standard and molecular cytogenetic analysis confirmed the clinical diagnosis (subgroups A1 and B1) we analyzed the frequency of different types of CHD corroborated with chromosomal abnormality type.

Chromosomal analysis was based on a short-term culture of activated T- lymphocytes stimulated with phytohemagglutinin (Moorhead method, improved in our laboratory) (2, 18-19). The slides were stained and examined on an optical microscope, directly and after application of G- banding (after trypsin treatment). For

each case a minimum of 32 cells were analyzed, thus enabling the identification of chromosomal mosaics in more than 90% of cases. When we detected more than 2 cell lines, the number of analyzed cells was increased to 64 or 96.

Fluorescence in situ Hybridization (FISH) was performed on metaphase chromosome spreads using the Kreatech Diagnostics cell samples (Amsterdam, Netherlands) and the Vysis FISH microdeletion probes kit (Illinois, USA) (2, 18). DNA probes used were specific for microdeletions: velo-cardio-facial syndrome (22q11/22q13), Williams-Beuren syndrome (7q11/7q22), Wolf-Hirschhorn (4p16/SE 4) and Cri du chat (5p15/5q31).

Results

Of 1123 patients cytogenetically tested, 749 (66.7%) cases had a chromosomal abnormality and they formed group A (Table 1). Of

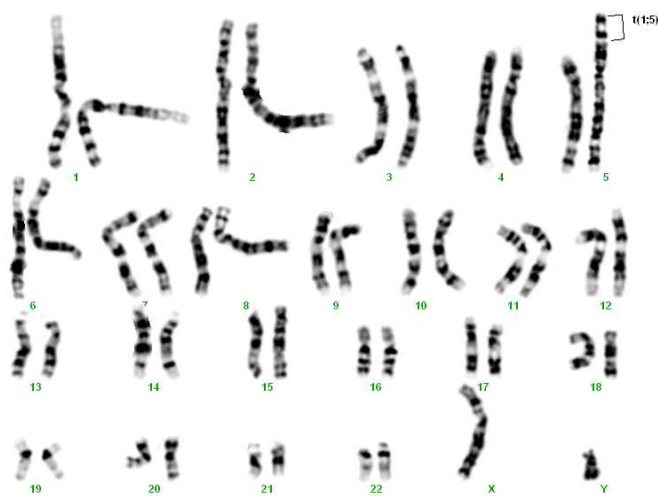


Figure 1. Karyotype 46,XY,t(1;5)(q32;p15.3)

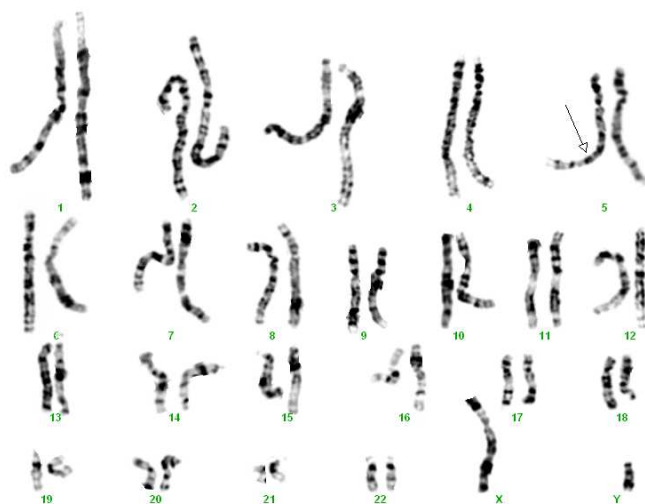


Figure 2. Karyotype 46,XY,del(5)(q22.2;q31.22)

these, 699 (93.33%) had a suggestive phenotype for a chromosomal abnormality and karyotype confirmed clinical diagnosis – subgroup A1, and 50 (6.67%) had an abnormal phenotype (MCA ± MR) without a specific diagnosis – subgroup A2, and chromosomal analysis identified unbalanced chromosomal abnormalities: derivative chromosomes with addition material of unknown origin (add), deletions (del), insertions (ins), ring chromosomes (r) (Figures 1 and 2).

223/749 (29.77%) patients from group A (abnormal karyotype) had a CHD: 206/699 (29.47%) from the cases with syndromic phenotype (subgroup A1) and 17/50 (34%) from the cases with MCA ± MR (subgroup A2). In subgroup A1, patients with clinical and cytogenetic diagnosis of chromosomal conditions, CHD were more frequent in: 18 trisomy (9/15 cases or 60%), 21 trisomy (177/558 cases or 31.72%) and X monosomy (11/74 cases or 14.86%). In subgroup A2, from 50 patients with MCA ± MR and chromosomal abnormalities, 17 cases (34%) had CHD. The CHD percentage is significantly higher in group A2 than in group B2 (340 cases) – patients with MCA ± MR and normal karyotype, in which CHD were identified in 74/340 cases or 21.76% ($\chi^2 = 6.371$; $p < 0.01$; $p < 0.05$) (Table 1).

In group B, 374 patients with MCA ± MR had a normal karyotype; in 34 cases (subgroup B1) with suggestive phenotype for different microdeletions syndromes (Table 2), FISH test was performed. In 12/34 cases (35, 29%) from subgroup B1 in whom the clinical diagnosis was confirmed, the majority 9/12 had

MCC. In subgroup B2, 340 cases with MCA ± MR and normal karyotype, 74/340 cases (21, 76%) had CHD (Table 2, Figures 3 and 4).

Frequency of chromosomal abnormalities varied by type of CHD and was higher in cases with atrioventricular canal (AVC), persistent ductus arteriosus (PDA), ventricular septal defects (VSD) and atrial septal defects (ASD), than in pulmonary stenosis (PS), aortic stenosis (AS) and Fallot tetralogy (FT) (Table 3).

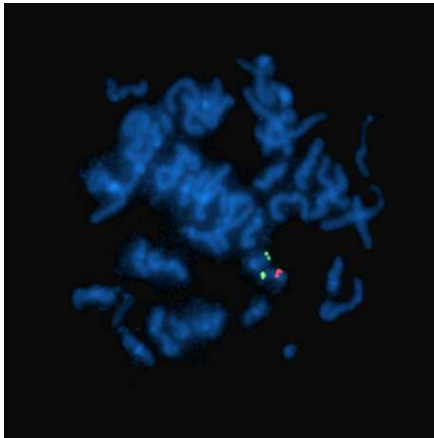


Figure 3. 46,XY.ish del(22q11.2) (TUPLE 1-); probes 22q11.2 LSI N25/ Tuple 1 Spectrum Orange and 22q13(control probe, LSI ARSA) Spectrum Green

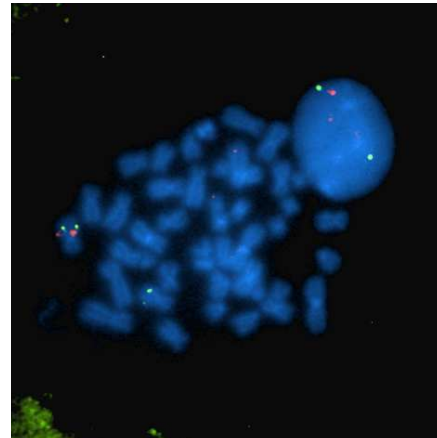


Figure 4. 46,XX.ish del(7)(q11.23)(ELN-); probes 7q11.23 LSI ELN (ELN LIMK1, D7S613 Spectrum Orange and 7q31 (loci D7S486 and D7S522) Spectrum Green

Discussions

Following clinical evaluation at Iasi GMC over the last 10 years, cytogenetic tests were requested for 1123 patients, of whom 322 (28.67%) had CHD. G banding karyotype (550 bands) revealed chromosomal abnormalities in 749 cases (group A), representing 66.7% of all patients tested. This high percentage proves a correct clinical selection of cases for chromosomal analysis, based mainly on association of MCA±MR. The remaining 374 patients (33.3%) had a normal karyotype (group B); this group certainly includes cases with submicroscopic chromosomal abnormalities detectable only by molecular cytogenetic techniques and array CGH. The detection of microdeletions, using FISH test, in 12/34 patients from subgroup B1, with suggestive phenotypes, is an argument for this.

A percentage of 29.77% of the patients with chromosomal abnormalities (group A) had CHD. The percentage is slightly higher but not statistically significant compared to group B (patients with MCA±MR and normal karyotype) in which only 26.47% patients had CHD. CHD were present in 232 (30.49%) out of 761

patients with chromosomal abnormalities (749 group A and 12 subgroup B1), a similar result of those found in other studies (5). CHD were more frequent in 22q11.2 microdeletion (6/7 cases or 85.71%), 18 trisomy (9/15 cases or 60%), 21 trisomy (177/558 cases or 31.72%) and X monosomy (11/74 cases or 14.86%). CHD frequencies, in different types of chromosomal abnormalities, in our study, are lower than in other studies (5); a possible explanation is the higher mortality in CHD patients during first year of life. CHD frequency in 21 trisomy (Table 4) is relevant for this; this is at the lower limit of reported frequencies in other studies (20-24); probably due to higher mortality in severe CHD and lack of complete investigations in many cases (Table 4).

We detected chromosomal abnormalities in 232 cases (72,04%) (206 in subgroup A1, 17 in subgroup A2 and 9 in group B1) out of 322 cases with CHD from our study (223 in group A and 99 in group B). This high percentage is due to clinical selection for genetic testing.

Septal defects (AVC, VSD) and patent ductus arteriosus of all types of CHD were more frequently associated with a chromosomal

Table 2. FISH results and CHD frequency in subgroup B1

Suggestive phenotype	No. cases	FISH results	No. cases	CHD
Microdeletion 22q11.2	22	positive	7	6
		negative	15	10
Microdeletion 7q11.23	8	positive	3	2
		negative	5	4
Microdeletion 4p16	4	positive	2	1
		negative	2	2

Tabel 3. Chromosomal abnormalities frequency in different types of CHD

CHD type	AVC	PDA	VSD	ASD	PS	AS	FT
No cases with chrs	34/38	44/50	43/55	82/112	15/23	9/15	5/18
abn/ total cases	(88.23%)	(88%)	(78.18%)	(73.21%)	(65.21%)	(60%)	(27.77%)

AVC - atrioventricular canal, PDA - persistent ductus arteriosus, VSD - ventricular septal defects, ASD - atrial septal defects, PS - pulmonary stenosis, AS - aortic stenosis, FT - Fallot tetralogy

Table 4. CHD frequency in 21 trisomy

	Current study (Iasi)	Khoury and Erickson, 1992 (Atlanta)	Freeman, 1998 (Atlanta)	Stoll, 1990 (France)	Wells, 1994 (Alabama)
Study period	2001-2010	1968-1989	1989-1995	1979-1987	1988-1992
Number of cases	558	532	227	139	118
CHD frequency in 21 trisomy	31.72%	33%	44%	47%	48%

abnormality. AVC association with chromosomal abnormalities, especially 21 trisomy, is reported in other studies, too (3, 20-23). VSD and PDA association with a high percent of chromosomal abnormalities is probably due to participation in complex CHD. Low percentage of chromosomal abnormalities in TF is due to the severity of CHD causing high early mortality and probably the small number of cases studied. The lack of association of FT with microdeletion 22q11.2 is surprising, and this can be the result of a population characteristic.

Genetic investigations, in cases with MCA±MR without chromosomal abnormalities detected by classic karyotype or FISH, will be

completed by high-resolution techniques for detection of small abnormalities or monogenic mutations. Probably some of them have a multifactorial etiology.

Conclusions

Our study showed a high frequency of chromosomal abnormalities among cases with CHD+MCA and proved the benefits of cytogenetic testing in diagnosis of plurimalformative syndromes with CHD. These investigations are necessary especially in certain types of CHD: AVC, VSD, PDA or FT. When “standard” chromosome analysis shows a normal ka-

ryotype, molecular cytogenetic techniques (FISH and array CGH) are useful to detect sub-microscopic chromosomal abnormalities.

Abbreviations

CHD – congenital heart defect
MCA – multiple congenital anomalies
GMC – Genetic Medical Centre
MR – mental retardation
FISH – fluorescence in situ hybridization
AVC – atrioventricular canal
VSD – ventricular septal defects
ASD – atrial septal defects
PS – pulmonary stenosis
AS – aortic stenosis
FT – Fallot tetralogy

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