The Egyptian Heart Journal 70 (2018) 267–270

HOSTED BY

Contents lists available at ScienceDirect

The Egyptian Heart Journal

journal homepage: www.elsevier.com/locate/ehj

Original Article

Evaluation of loss of heterozygosity of chromosome 22q11.21 region in patients with congenital heart diseases

Eman G. Behiry [a,*], Azza A. Abo Senna [a], Amr E. Elnagar [b], Magda A. Eshiesh [a]

[a] Clinical & Chemical Pathology, Faculty of Medicine, Benha University, Egypt
[b] Department of Cardiology, Benha University, Egypt

ARTICLE INFO

Article history:
Received 17 April 2018
Accepted 14 July 2018
Available online 30 July 2018

Keywords:
22q11.21 Microdeletion
Congenital heart defects
STR markers

ABSTRACT

The 22q11.21 region is prone to low-copy repeats events that lead to congenital anomaly disorders. We tested genomic DNA of 20 families with non-syndromic CHD patients using a set of three known consecutive high polymorphic short tandem repeat (STR) markers along the 22q11.21 region; D22S941, D22S944 and D22S264 loci. We found loss of heterozygosity (LOH) in D22S941 locus in 2 out of 20 families (10%) with 2 offspring affected by ASD combined with PS and TOF respectively. No LOH found in D22S944 and D22S264 loci either in affected cases or control group and no LOH found in D22S941 in the control group. Also we observed that D22S944 locus prone to be less allele diversity than D22S941 and D22S264 loci.

1. Introduction

The CHDs are present in 75–80% of patients with a Homo sapiens autosomal 22q11 (HSA22q11) deletion.[1] Defects in heart separation, including atrial septal defect (ASD), atrioventricular canal septal defects (AVSD), conotruncal septal defects, and ventricular septal defect (VSD), represent a major cause of congenital heart disease.[2]

The different deletions of the region 22q11.2 are classified as variations of the same clinical spectrum called the 22q11.2 deletion syndrome. The frequency of the 22q11.2 deletion is of approximately 1:3800 livebirths, and presented as sporadic in about 90% of the cases and inherited in 10%.[3]

Patients with 22q11.2 deletion syndrome are reported by either hemizygous deletion of the 3-Mb or 1·5-Mb interval, including 24–30 genes on chromosome 22q11.2. However, the few patients who have different deletions or rearrangements in the region have not been informative in localising disease genes because these arrangements are non-overlapping.[4] Further, rare studies have been described patients with unique nested microdeletion within the large 3-Mb typically deleted region (TDR).[5]

Conotruncal heart defects origin is the highest incidence among del22q11 patients, mainly interrupted aortic arch type B, truncus arteriosus, tetralogy of Fallot (TOF), and pulmonary atresia.[6] There are few studies that spanned 22q11.2 region using consecutive polymorphic short tandem repeats to detect a smaller deletion in non-syndromic CHD patients.

The aims of this study were to determine the frequency of 22q11.21 deletion in non-syndromic CHD patients with emphasis on ASD, VSD and TOF using STR markers, and identify a minimal critical deleted region in these patients.

2. Patients and methods

This research was approved by the Medical Ethical Committee of Benha University according to the "World Medical Association Declaration of Helsinki". A written informed consent was obtained.

Patients; We selected 20 families referred to Cardiology Department, Benha University Hospitals in the period starting from December 2015 to December 2016. Their mean age was 9.6 years, they were 7 males (35%) and 13 females (65%). In addition to 20 healthy control of matched age and gender.

Among them, 2 patients with TOF, 8 patients with ASD, 16 patients with VSD. 15% showed dysmorphic features and 1 cases had MR (5%).

2.1. Inclusion criteria

Congenital heart defects patients with or without extra cardiac anomalies.

Peer review under responsibility of Egyptian Society of Cardiology.
* Corresponding author.
E-mail addresses: Emangamal24@yahoo.com, eman.said@fmed.bu.edu.eg (E.G. Behiry).

https://doi.org/10.1016/j.ehj.2018.07.003
1110-2608/

2.2. Exclusion criteria

1. Patients with known syndromes such as Down and Holt-Oram syndromes except DiGeorge syndrome combined with CHDs.
2. Cardiac cases of non-septal defects as cases of pulmonary stenosis only.
3. Other systemic diseases (e.g. Thalassemia and Diabetes).

3. Methods

1. ***Clinical evaluation;*** the clinical evaluation was done through taking history of three-generation pedigree construction, pregnancy and delivery histories, exposure to drugs, fever, trauma, irradiation or any maternal chronic illness. Parental ages at birth of the child, family history and developmental milestones were also recorded. Detailed history and examination of cardiac symptoms were done.
2. **Polymorphic DNA markers mapping**
   Three ml. of the peripheral blood samples was collected from the patients using K_2EDTA as anticoagulant inside vacutainer sterile tubes. Genomic DNA was isolated from peripheral blood leukocytes by QIAamp DNAMini Kit (50 preps), catalog number 51304, Germany (https://www.qiagen.com/eg/). The PCR-STR technique was performed using specific primers for microsatellite genotyping that were directly retrieved from Electronic PCR (http:// www.ncbi.nlm.nih.gov/sutils/e-pcr/; NCBI, USA). Three consecutive polymorphic loci were used and located in the usually 3-Mb deleted region: D22S941, D22S944 and D22S264. The PCR products were tested on 4% agarose gel to investigate the presence of amplification and measure the allelic polymorphisms by Gel Doc™ EZ imager, BIO-RAD.

4. Results

Using triplicate reactions of three polymorphic DNA markers on 22q11.21 region, we found 2 out of 20 families (10%) with 2 offspring affected by ASD combined with PS and TOF respectively with LOH in D22S941 locus Table 1. On the other hand, no LOH was detected in healthy individuals Table 2 & Fig. 1.

Chi-square analysis of the polymorphic percentage of the three used markers showed that polymorphic diversity of the D22S944 is significant less than D22S941 and D22S264 Fig. 2.

5. Discussion

The data obtained in this study suggest that the 22q11.2 deletion is more frequent in patients with congenital heart defect, in particular septal defects patients. Using triplicate reactions of three polymorphic DNA markers (highly polymorphic markers D22S264, D22S941, D22S944) on 22q11.2 region, our study found 2 out of 20

Table 1
Comparison of clinical data according to D22S941 LOH in all studied cases.

	No LOH N = 18		LOH N = 2		*p*
	N	%	N	%	
TOF	1	5.6	1	50	.195
TGA	2	11.1	0	0	.619
Large VSD	8	44.4	0	0	.495
Small VSD	8	44.4	0	0	.495
ASD	7	38.9	1	50	.761
PS	2	11.1	1	50	.284
FTT	1	5.6	0	0	.732
Dysmorphic features	2	11.1	1	50	.284
MR	1	5.6	0	0	.732

Table 2
LOH of 22q11.21 STR loci.

Families	22q11.21 STR loci		
	D22S264	D22S941	D22S944
F1			
Proband	228	267	195
Father	228	267	195
Mother	228/211	267	195
F2			201
Proband	228/211	267/248	201
Father	228/211	267	201
Mother	211	290/267/248	
F3			
Proband	223	290/267/261	210/200
Father	223	290/267/261	210/200
Mother	223/207	267	210
F4			
Proband	223	267	201
Father	223	281/267/251	201
Mother	223/207	284/267	201
F5			
Proband	223/202	282/263	210
Father	223/216	282/263	210
Mother	202	282/263	202/210
F6			
Proband	202	278/261/248	200
Father	223/202	278/261/248	200
Mother	216/202	278/261/233	200
F7			
Proband	214/202	292/278/264	205
Father	202	292/278/264	205
Mother	214/202	278	205
F8			
Proband	214	289/278/251	195/205
Father	214/202	289/278/251	205
Mother	214/202	278	195/210
F9			
Proband	214/202	256/267/242	205
Father	214/202	281/256/242	205/211
Mother	214	267	200/205
F10			
Proband	218	256/239/228	195
Father	218	228	195
Mother	218	256/239/228	195
F11			
Proband	233	233	195
Father	233	233	195
Mother	233	256/239/233	195
F12			
Proband	214/202	284/256	201
Father	214/202	284/256	201
Mother	214	284	201
F13			
Proband	223/202	284	201
Father	223/216	284	201
Mother	202	284	201
F14			
Proband	220/202	290	215
Father	220/216	290/262	215
Mother	202	290	215
F15			
Proband	220	**304** [LOH]	215
Father	220	304	215
Mother	220	291/271	215
F16			
Proband	223/200	304/268	215
Father	223	304/268	215
Mother	200/216	304/268	215
F17			
Proband	218	290	205
Father	218	290/262	205/211
Mother	218	290	200/205

Table 2 (*continued*)

Families	22q11.21 STR loci		
	D22S264	D22S941	D22S944
F18			
Proband; AVSD	220/202	281/250	215
Father	220/216	281/250	215
Mother	202	281/263/250	215
F19			
Proband	223/215	**299** [LOH]	205
Father	218/215	299	205
Mother	223	290/271	205
F20			
Proband	228	223	195
Father	228	223	195
Mother	228	223/207	195

TOF: fallot tetralogy, **TGA**: transposition of great arteries, **PS:** pulmonary stenosis, **FTT:** failure to thrive, **MR:** mitral regurge.

families (10%) with 2 offspring affected by ASD combined with PS and TOF respectively with LOH in D22S941 locus. On the other hand, no LOH was detected in control group, In case number15 we found that it has just one allele 304 of D22S941 marker from the father and the other allele from the mother is deleted. The same is in case number 19 as the proband has just one allele 299 of D22S941 marker from the father and other allele from the mother is deleted while the remaining18 proband are having alleles both from the mother and the father and showing no deletion

McElhinney et al.,[1] detected chromosome 22q11 deletion in 12 (10%) of 125 patients (age ranged from15 to 28 years) with a conoventricular (n 100) posterior malalignment (n 14) or conoseptal hypoplasia (n 11) VSD by FISH examination. This is the same percentage as our study.

Beauchesne et al.,[7] Results of study done by **Beauchesne et al., (2005)** found that 103 Patients underwent testing with FISH analysis. The intracardiac diagnoses consisted of TOF in 77

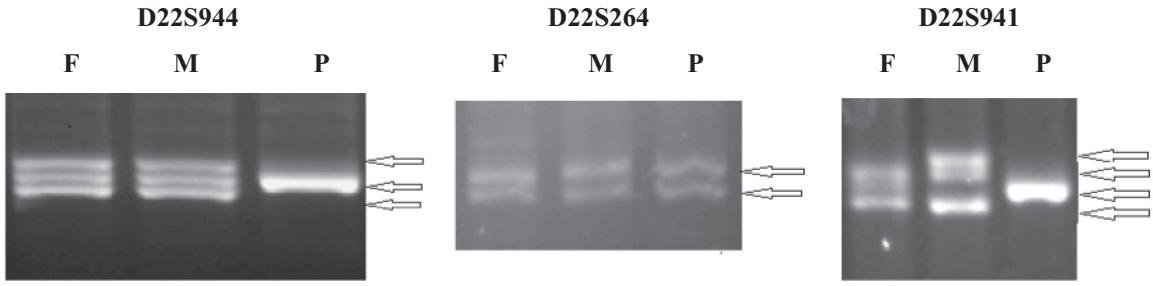

Fig. 1. Microsatellite segregation analysis shows the deletion at marker D22S941 in the affected patient, in contrast, loci D22S944 and D22S264 shows no deletion. F: father; M: mother; P: patient.

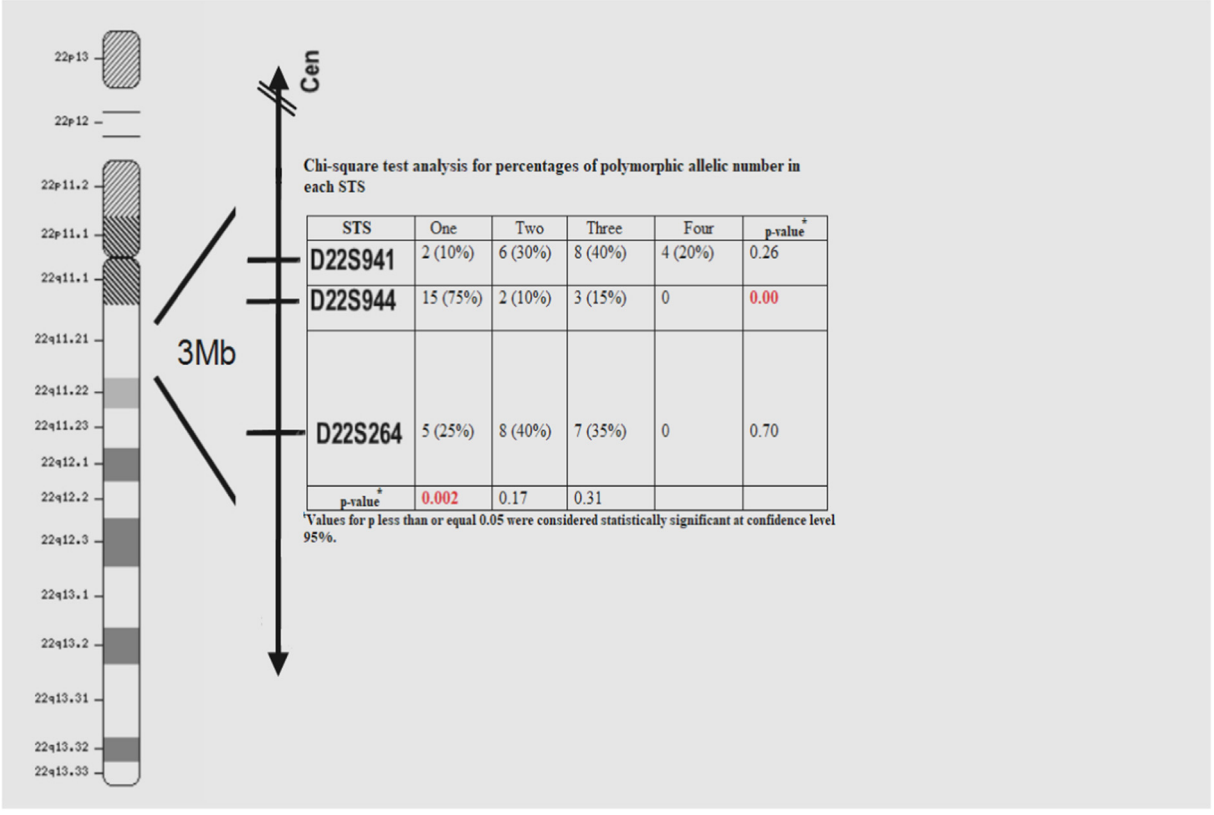

Fig. 2. Diagrammatic shape for 22q11.21 region shows the used three STS loci, and chi-square analysis of the polymorphic allelic number percentage.

patients, Pulmonary atresia (PA)/VSD in 23, and Truncus arteriosus (TA) in 3. They identified 6 patients (5.8%) to have 22q11 deletions (3 TOF, 2 PA/VSD, and 1 TA) using Fluorescence in situ hybridization (FISH) for the DiGeorge/ velocardiofacial syndrome chromosome region. Our results reported higher percentage (10%) of CHD patients with HSA22q11 deletions and this due to rare studies that accomplished on non syndromic CHD patients using STR markers on 22q11.2 locus.

The reported incidence of deletion varies between studies and is apparently higher among TOF patients who are syndromic for DGS or VCFS.[8] This is partially agree with our results, where in the current study the higher percentage of the deletion was found in TOF patients with 50% out total TOF patients.

Our results suggest a distinct segment of 22q11.21 as a TOF and ASD susceptibility region around D22S941 marker, therefore, these results indicate the clinical significance of screening 22q11.21 markers for proband for the early diagnoses and design of the treatment strategies.

Few studies have used polymorphic microsatellites that span the HSA22q11 region to detect micro deletion.[9] So the detection of minimal nested micro deletion on 22q11.2 region was rare. Therefore, we recommend usage of STR-based PCR assay in 22q11.2 genotyping, because it provides a higher resolution than traditional FISH analysis. Also, we observed the marked variability between the different studies that may be due to differences in selection of patients, their number, and the method of analysis.

In contrast to our research **Fernandez et al.,** compared the results of three techniques MLPA, FISH and STR in a group of 30 patients affected with 22q11.2 deletion syndrome and found that MLPA correctly called all patients who had been previously diagnosed by FISH and STR Furthermore, this technique resolved seven cases that were undetermined by STR analysis. These results confirm the efficiency of MLPA as a rapid, reliable, economical, high-throughput method for the diagnosis of 22q11.2 deletion syndrome rather than STR.[10]

Using MLPA **Monteiro et al.,** found that the deletion frequency was 33.3 %of 194 cardiac patients.[11] In contrast to our study, Cytogenetic analysis of 78 cardiac patients (age ranged from 1 day to 37 years) revealed a normal G-banded karyotpic pattern with no additional chromosome defects at 550 band stages However, FISH analysis of the same 78 patients showed 68 had a normal FISH pattern while only 10 patient showed del22q11.2 (12.8%).[12]

Goldmuntz et al., in a study designed to determine the frequency of the 22q11 deletion in a large, prospectively ascertained sample of 251 patients (age ranged from 2 to10 years) with conotruncal defects, identified the deletion in 17.9% of the patients. The deletion frequency varied with the primary diagnosis and was highest in patients with interrupted aortic arch and TA (50 and 34.5%, respectively) in contrast to our research where the deletion is presented among patients with PS, TOF and ASD.[8]

Srichaisawat et al., reported that 24 of 83 (28.9%) patients with cardiovascular malformations were found with 22q11.2 deletion syndrome by FISH examination. 19 of 56 (33.9%) patients with conotruncal heart defects and 5 of 27 (18.5%) patients with non-conotruncal heart defects were found to have the deletion. The 22q11 deletion were found in 14 of 24 (58.3%) patients with TOF (95%), 3 in 12 (25%) patients withASD (95%), 1 in 7 (14.3%) patients with PS (95%), 1 in 4 (25%) patients with TGA and 1 in 4 (25%) patients with VSD.[13]

The reported incidence of deletion varies between studies and is apparently higher among TOF patients who are syndromic for DGS or VCFS.[14] This is partially agree with our results, where in the current study the higher percentage of the deletion was found in TOF patients with 50% out total TOF patients.

5.1. Limitations

Further studies should be done on 22q11.21 in congenital heart disease especially on siblings.

Conflict of interest

No conflict of interest.

Funding

No fund.

Ethical approval

This study protocol was approved by ethical review board of Benha University. Written, informed consent was obtained from each patient included in the study. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

References

1. McElhinney DB, Geiger E, Blinder J, Benson DW, Goldmuntz E. NKX2. 5mutations in patients with congenital heart disease. *J Am Coll Cardiol*. 2003;42:1650–1655.
2. Sayasathid J, Supachokchaipattana P, Pipatvech K, Sukonpan K, Somboonna N, Pannarunothai S. The prevalence of unrecognized congenital heart disease among healthy elementary school students in northern Thailand; 2010.
3. McDonald-McGinn DM, Driscoll DA, Bason L, et al. Autosomal dominant "Opitz" GBBB syndrome due to a 22q11. 2 deletion. *Am J Med Genet Part A*. 1995;59:103–113.
4. Lindsay EA, Vitelli F, Su H, et al. Tbx1 haploinsufficiency in the DiGeorge syndrome region causes aortic arch defects in mice. *Nature*. 2001;410:97.
5. Saitta SC, Harris SE, Gaeth AP, et al. Aberrant interchromosomal exchanges are the predominant cause of the 22q11. 2 deletion. *Human Mol Genet*. 2003;13:417–428.
6. Ryan AK, Goodship JA, Wilson DI, et al. Spectrum of clinical features associated with interstitial chromosome 22q11 deletions: a European collaborative study. *J Med Genet*. 1997;34:798–804.
7. Beauchesne LM, Warnes CA, Connolly HM, et al. Prevalence and clinical manifestations of 22q11. 2 microdeletion in adults with selected conotruncal anomalies. *J Am Coll Cardiol*. 2005;45:595–598.
8. Goldmuntz E, Clark BJ, Mitchell LE, et al. Frequency of 22q11 deletions in patients with conotruncal defects. *J Am Coll Cardiol*. 1998;32:492–498.
9. Shi YR, Hsieh KS, Wu JY, et al. Genetic analysis of chromosome 22q11. 2 markers in congenital heart disease. *J Clin Lab Anal*. 2003;17:28–35.
10. Fernandez L, Lapunzina P, Arjona D, et al. Comparative study of three diagnostic approaches (FISH, STRs and MLPA) in 30 patients with 22q11. 2 deletion syndrome. *Clin Genet*. 2005;68:373–378.
11. Monteiro FP, Vieira TP, Sgardioli IC, et al. Defining new guidelines for screening the 22q11. 2 deletion based on a clinical and dysmorphologic evaluation of 194 individuals and review of the literature. *Eur J Pediat*. 2013;172:927–945.
12. Salil V, Pankaj G. The incidences of 22q11. 2 microdeletion syndrome in congenital heart diseases. *Curr Pediat Res*. 2014;18.
13. Srichaisawat P, Wichajarn K, Chaikitpinyo A, Panamonta M, Kampan J. AB061. Prevalence of 22q11. 2 deletion syndrome in patients with congenital heart diseases in North-eastern Thailand. *Ann Trans Med*. 2017;5(Suppl 2).
14. Maeda J, Yamagishi H, Matsuoka R, et al. Frequent association of 22q11. 2 deletion with tetralogy of Fallot. *Am J Med Genet Part A*. 2000;92:269–272.