Absence of GATA4 Mutations in Moroccan Patients with Atrial Septal Defect (ASD) Provides Further Evidence of Limited Involvement of GATA4 in Major Congenital Heart Defects

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ABSTRACT

Objective: Atrial septal defect (ASD) is one of the most common types of congenital heart disease (CHD). It is mainly caused by mutations of NK2 homeobox 5, GATA binding protein 4 (GATA4), and myosin heavy chain 6 in non-syndromic cases. This study aims to carry out, for the first time, the GATA4 mutation screening in a Moroccan population affected by ASD and compare the obtained mutation rate across populations.

Materials and Methods: A total of 33 patients were enrolled in this study. DNAs were extracted from peripheral blood samples, and we performed PCR-sequencing for GATA4 coding regions. Sequences were analyzed by sequence alignment and functional impact prediction tools. Mutation rate comparisons were performed by R software using the appropriate statistical tests.

Results: We detected 7 variants, but no pathogenic mutation was revealed, except for Asn352= that was assessed by human splicing finder algorithms to have a potential impairing effect on the splicing mechanism. Until proven by in vitro functional studies, the current pathogenic mutation rate in our cohort seems to be 0%. Statistical comparison with previous studies from all over the world shows no significant difference. Seemingly, comparison of previous GATA4 mutation rates among tetralogy of Fallot (TOF) populations shows no significant difference.

Conclusion: The low rates of GATA4 mutations observed throughout ASD and TOF international populations may suggest a limited causality of GATA4 mutations in the main CHDs, which further confirms the co-involvement of additional genetic and/or environmental factors in the manifestation of these phenotypes.

Keywords: Atrial Septal Defect, DNA mutational analysis, genetic testing, mutation rate, tetralogy of fallot

Introduction

Congenital heart disease (CHD) is the most common malformation observed at birth with an estimated prevalence of 1% of live births and is responsible for up to 15% of spontaneously aborted fetuses [1, 2]. Atrial septal defect (ASD), which is a left-to-right shunt disorder, is one of the most frequent types of CHD, accounting for 10% of all the CHDs [2]. ASD is caused by deficiency in the atrial septum structure that could be isolated or associated with other syndromic malformations. The syndromes mostly associated with ASD are Holt–Oram syndrome caused mainly by T-box transcription factor (TBX5) mutations and Ellis-van Creveld (EVC) syndrome caused by EVC mutations [3, 4].

Non-syndromic ASD occurs most often sporadically, whereas the familial cases are inherited in an autosomal dominant manner. Non-syndromic ASD is caused by mutations in NK2 homeobox 5 (NKX2-5), GATA binding protein 4 (GATA4), and myosin heavy chain 6 [4–8].

GATA4 is a member of the GATA transcription factor family that is widely expressed in a developing heart. GATA4 is encoded at region 8p23.1 and comprises of 6 coding exons. This zinc-finger transcription factor binds specifically to (T/A)GATA(A/G) motif in downstream targets and interacts with other transcription factors, such as NKX2-5 and TBX5, to regulate the cardiogenesis process [9, 10].

In several studies, GATA4 mutations were associated with CHDs, especially with ASD [7, 11–14]; although in some populations, no GATA4 mutations were found in patients with ASD, notably in Chinese populations [15, 16].

Thus, in this study, we carried out mutational screening of GATA4 coding exons in a cohort of 33 patients with non-syndromic ASD and compared the GATA4 mutation rate across populations.

Materials and Methods

Study Cohort

The 36 unrelated patients recruited in this study were confirmed to have ASD with interatrial communication diameter larger than 5 mm. Diagnosis by electrocardiography and color Doppler echocardiography was performed in the Medicosurgical Unit of Cardiopediatrics. Patients included 21 women and 15 men who underwent further physical examination to identify any additional syndrome traits and were interviewed to evaluate the individual and familial disease histories; 3 patients with syndromic traits were excluded. The present work was approved by Ethics Committee of Hassan II University Hospital and Faculty of Medicine and Pharmacy of Fez under the reference ID: Ref. 06/14.

Mutational Analysis

After obtaining an informed consent, the peripheral venous blood specimens were obtained from the 33 non-syndromic patients with ASD. The genomic DNA was extracted from blood lymphocytes using salting-out method [17].

We carried out PCR to amplify GATA4 coding exons and their flanking introns using the primers derived from published data [18].

PCR was performed in a final volume of 25 mL, containing 10 pmol of each primer, 40 ng of genomic DNA, 1X PCR buffer (Invitrogen, California, USA), 25 mM $MgCl_2$, 10 mM dNTP, and 1 U of Taq (Invitrogen, California, USA). PCR cycling conditions were performed in the Veriti 96-well Thermal Cycler 9902 (Applied Biosystems, Massachusetts, USA) using the following program: 94°C for 5 min; 35 cycles of 94°C for 45 s, 59°C-62°C for 40 s, and 72°C for 45 s; and 72°C for 7 min.

Direct sequencing of the purified PCR products was performed using BigDye Terminator V1.1 Cycle Sequencing Kit (ABI Prism, Applied Biosystems, Massachusetts, USA) and run on an 3500Dx Genetic Analyzer (Applied Biosystems, Massachusetts, USA).

In Silico Analysis

We analyzed the sequences with different bioinformatics analysis tools, in particular sequencing analysis software SeqA v.5.4 (Applied Biosystems, Massachusetts, USA) for chromatogram analysis; nucleotide blast program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (NCBI, Maryland, USA) for pairwise alignment; and PROVEAN (http://provean.jcvi.org/index.php) (J. Craig Venter Institute, Maryland, USA), PolyPhen2 (http://genetics.bwh.harvard.edu/pph2) (Harvard Medical School, Massachusetts, USA), and human splicing finder (HSF) (http://www.umd.be/HSF/) (INSERM, Marseille, France) algorithms for the assessment of variant functional impact.

Statistical Analysis

Comparisons between the study cohorts were carried out using chi-square tests for large cohorts (>5) and Fisher's exact test for smaller cohorts. Tests were performed using R software (R Foundation for Statistical Computing, Vienna, Austria).

Results

In this study, 33 patients with ASD were screened for GATA4 variants. Results show detection of 7 variants: 2 missense and 1 synonymous variants in exon 5 and 4 non-coding variants spread over introns 1 and 4. Among the intronic variants, rs804280 was very recurrent in our ASD popula-

Table 1. Features of GATA4 variants identified in our ASD population

Variants ID	Nucleotide variants	Amino acid variants	Exon/Intron	Substitution type	Affected patients
rs10503425	c.617-64G>C	-	Intron 1	Non-coding	P15, P23, P25
rs76808439	c.997+23A>T	-	Intron 4	Non-coding	P30
rs804280	c.997+56C>A	-	Intron 4	Non-coding	25 patients
rs1462767403	c.998-21G>A	-	Intron 4	Non-coding	P28
rs3729855	c.1056C>T	Asn352=	Exon 5	Synonymous	P11
rs3729856	c.1129A>G	Ser377Gly	Exon 5	Missense	P4, P15, P23, P25, P32
rs114868912	c.1138G>A	Val380Met	Exon 5	Missense	P17, P20

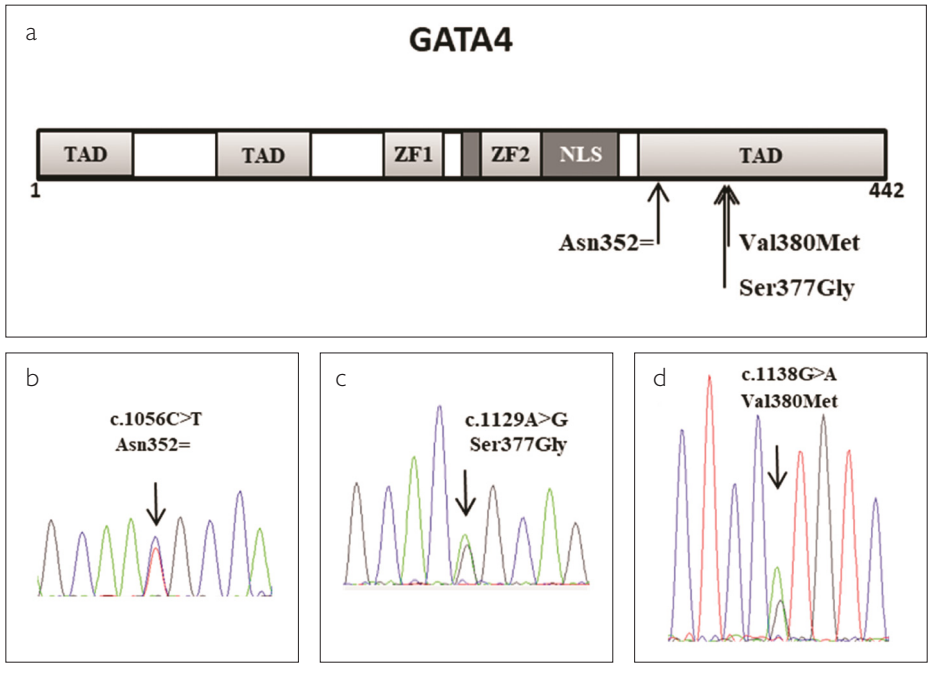


Figure 1 a-d. (a) GATA4 domains with sites of exonic variants and (b-d) Sequencing chromatograms of detected GATA4 variants

Main Points

- Screening of a Moroccan Atrial Septal defect (ASD) population for GATA4 mutations reveals absence of pathogenic mutations.
- Comparison of GATA4 mutation rates throughout worldwide ASD and Tetralogy of Fallot (TOF) populations shows no significant differences.
- The noticed worldwide limited involvement of GATA4 mutations in major Congenital Heart Defects (CHDs) strongly suggests co-involvement of additional genetic and/or environmental factors.

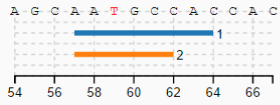
Predicted signal	Prediction algorithm	cDNA Position	Interpretation
New ESS Site	1 - Sironi et al. - Motif 3		Creation of an exonic ESS site. Potential alteration of splicing.
	2 - ESR Sequences from Goren et al.		

Figure 2. Output of HSF algorithms prediction for Asn352= impact on splicing process

Table 2. PROVEAN and PolyPhen2 prediction results for identified missense variants

Variant	PROVEAN score	Prediction	PolyPhen2 score	Prediction
Ser377Gly	0.149	Neutral	0.00	Benign
Val380Met	0.082	Neutral	0.002	Benign

Table 3. Comparison of GATA4 mutation rates between different ASD populations

Region	ASD Population	Pathogenic Mutation rate of *GATA4* (%)	p*	Reference
Africa	Egypt	0/8 (0)	1	[29]
Australia America	Australian	1/157 (0.6)	1	[19]
	American	0/14 (0)	1	[21]
	American	3/122 (2.5)	1	[13]
Asia	Chinese	0/19 (0)	1	[16]
	Chinese	0/48 (0)	1	[22]
	Chinese	0/37 (0)	1	[15]
	Japanese	1/76 (1.3)	1	[23]
	Japanese	2/16 (12.5)	0.1	[7]
Our study	Moroccan	0/33 (0)	-	-

* Compared to our study. ASD: atrial septal defect

Table 4. GATA4 mutation rates in tetralogy of Fallot (TOF) throughout the world

Region	TOF Population	Pathogenic Mutation rate of *GATA4* (%)	Reference
Africa	Moroccan	0/31 (0)	[24]
	Egypt	0/10 (0)	[29]
Europe	British	2/93 (2.1)	[25]
America	American	1/201 (0.5)	[13]
Asia	Chinese	2/64 (3.1)	[15]
	Japanese	1/125 (0.8)	[26]
	Japanese	0/28 (0)	[23]

TOF: Tetralogy of Fallot

tion. Table 1 and Figure 1 give more details about the features of the detected variants.

Putative functional impact of the identified variants was assessed using *in silico* predictive tools. We used PROVEAN and PolyPhen2 for missense variants and HSF in case of silent variants to assess their particular impact on splicing process. No pathogenic impact was noticed for the missense or intronic variants. However, in case of the synonymous exonic variant Asn352=, HSF algorithms detected a potential altering impact on splicing through creation of an exonic splicing silencer (ESS) site. Table 2 and Figure 2 report more details about the prediction results.

According to these findings, GATA4 mutation rate in our ASD cohort seems to be 0%. We compared this with the corresponding mutational rates of different ASD populations all over the world, and Table 3 shows the results of this comparison.

Discussion

GATA4 is a zinc-finger transcription factor involved in heart development. It controls transcription of target genes through binding to its corresponding consensus site.

In the first part of this study, we reported the molecular findings of GATA4 mutation screening in a Moroccan population suffering from ASD, a condition that was often associated with deficiency of transcription factors involved in cardiogenesis. We found three exonic variants including one synonymous substitution and four intronic variants.

To assess the potential effect of the identified variants on GATA4 function, we used different prediction algorithms, notably PolyPhen2 and PROVEAN for missense variants and HSF in case of synonymous or intronic variants.

Missense variants Ser377Gly (c.1129A>G) and Val380Met (c.1138G>A) found in 5 and 2 patients, respectively, were predicted to be neutral or benign. These 2 variants, located in transcription activity domain, were proven to have no effect on the secondary structure of GATA4 protein and seem to be quite frequent in both healthy and affected populations [14, 19, 20], which further confirms that these variants are most probably benign with no pathogenic effect on the GATA4 activity.

Regarding the silent variants, the intronic variants were predicted to have no pathogenic effect on the splicing mechanism, including both variants found in 1 patient, such as c.997+23A>T and c.998-21G>A, and recurrent variants found in up to 25 patients, such as c.997+56C>A.

However, according to the HSF algorithms, synonymous variant Asn352= (c.1056C>T) seems to have a potential altering effect on the splicing process, notably by creating an ESS site that may lead to splicing out the concerned exon, resulting in an inoperative truncated protein. This particular finding should be further confirmed by functional studies.

According to these data, mutation rate of GATA4 in our population seems to be 0%. In the second part of this study, we attempted to compare our GATA4 mutation rate with those of different ASD populations. Results in Table 3 show no significant difference between our

study and previous studies. Moreover, comparison of mutation rates among the studied populations does not reveal any significant difference.

These findings allowed us to conclude, on one hand, that our mutation rate was consistent with that of the previous studies, and on the other hand, that GATA4, although seen as the second most common cause of septal defects after NKX2.5, seems to be responsible for only a few ASD cases as reported by different populations (Table 3).

The same conclusion could be drawn in case of conotruncal defects. For instance, tetralogy of Fallot (TOF) cases caused by GATA4 mutations are quite limited as well, and this could be observed in different populations around the world regardless of their origins, as illustrated in Table 4.

These conclusions are consistent with recent studies that performed targeted next generation sequencing (NGS) or whole exome sequencing (WES). In the first study [27], among a cohort of 68 patients with CHD, targeted NGS detected 20 pathogenic mutations spread over 9 genes. Among them, only 1 mutation was detected in GATA4 in a patient with ASD and pulmonary stenosis. In the second study [28], among 9 familial CHD probands, WES identified 3 pathogenic mutations, 1 of which was found in GATA4 in a patient with ASD. In the latter study, the relatively high rate of GATA4 mutations is attributed to the familial context, which commonly exhibits further association with genetic etiology.

It is worth mentioning that there are some previous studies reporting higher GATA4 mutation rates, such as studies of Hirayama-Yamada et al. [7] (12.5%) and Dinesh et al. [20] (16.6%). These high rates may be owing to the reduced number of patients they worked on (16 and 12 patients, respectively).

Taken together, these findings suggest that, among the genetic factors that lead to CHD manifestation, GATA4 mutations, although considered as the second most common cause after NKX2-5 thus far, are indeed responsible for only a small part of ASD and TOF phenotypes, which strongly implies the potential co-involvement of additional genetic and/or environmental factors. Finally, this work is the first study in Morocco about GATA4 mutational profile in an ASD group, and it would be pertinent to confirm these preliminary findings using a larger Moroccan population. Further investigations in different additional world populations would be of great interest as well.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Hassan II University Hospital and Faculty of Medicine and Pharmacy of Fez (Ref. 06/14).

Informed Consent: Written informed consent was obtained from patients participating in the present study.

Peer-review: Externally peer-reviewed.

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Conflict of Interest: Authors have no conflicts of interest to declare.

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