Clinica Chimica Acta 412 (2011) 2232–2236

Contents lists available at SciVerse ScienceDirect

Clinica Chimica Acta

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

Mutational analysis of JAG1 gene in non-syndromic Tetralogy of Fallot children ☆

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ARTICLE INFO

Article history:
Received 13 October 2010
Received in revised form 8 August 2011
Accepted 10 August 2011
Available online 27 August 2011

Keywords:
JAG1
Tetralogy of Fallot (TOF)
Polymerase chain reaction (PCR)
Novel mutations
DNA sequencing

ABSTRACT

Background: JAG1 is an evolutionarily conserved ligand for Notch receptor and functions in the cell fate decisions, cell–cell interactions throughout the development of heart especially right heart development. Tetralogy of Fallot (TOF) is essentially a right sided heart disease with characteristic features of ventricular septal defect, right ventricular outflow tract obstruction, aortic dextroposition and right ventricular hypertrophy. Hence, the present study was investigated to identify mutations of JAG1 gene in an Indian cohort of patients with TOF.
Methods: The clinical data and blood samples from 84 unrelated subjects with TOF were collected and evaluated in comparison with 87 healthy individuals. PCR based single strand conformation polymorphism analysis and subsequent bidirectional DNA sequencing of conformers was carried in the exon 6 of JAG1 gene.
Results: The DNA sequences aligned with NCBI-BLAST led to the identification of four novel variations including one nonsense 765 C>A, two missense 814 G>T, 834 G>T; and one silent alteration 816 G>T in TOF patients. The protein structure of JAG1 predicts that these variations effect first and second epidermal growth factor like repeat and might disturb ligand-receptor binding ability. The presence of similar variations was not observed in healthy controls. The software CLUSTAL-W showed the inter species conservation of altered amino acids in missense mutations.
Conclusion: Disease-associating novel JAG1 gene variations were found in TOF patients, and seem to play an important role in the causation of the disease.

1. Introduction

The establishment of normal cardiovascular system is dependent on the interplay between genetic and environmental factors and any malformation in either or both of these factors results in abnormal cardiogenesis. Tetralogy of Fallot (TOF: MIM #187500), is a common, congenital heart defect (CHD), characterized by a gross structural abnormality of heart with functional significance [1] and complicates with ventricular septal defects, atrial septal defect or abnormalities in the branching pattern of coronary arteries, obstruction to right ventricular outflow tract (RVOT), aortic dextroposition (AD) and right ventricular hypertrophy (RVH). The abnormality of TOF starts during the first eight weeks of fetal growth and affects approximately 1 in 3000 live newborn [2]. Clinical symptoms include cyanosis/clubbing, hypoxia, breathlessness, refusal to feed, failure to gain weight and severe congenital heart malformation.

Classic TOF and its variants have been observed as part of heritable syndromes such as the Alagille syndrome, Di Georges Syndrome etc. Prenatal infections, exposure to teratogens, maternal illness and folate deficiency are few known causes. However, 70% of TOF cases also occur sporadically, without any other anomaly, and from unknown cause. Recently, we have reported the role of hypoxia induced oxidative DNA damage in children with TOF [3]. Mutations in multiple genes NKX2.4, GATA, and others, environmental risk factors and/or interaction of both contribute to the development of the disease pathogenesis. Haploinsufficiency due to dominant mutations in some of cardiac transcription factor genes can cause TOF. It has been hypothesized that *de novo* mutations in the transmembrane receptors, NOTCH1 and NOTCH2 and their ligand JAG1 and other genes involved in cardiac development might account for isolated TOF [4]. At least 10% of sporadic, non-syndromic TOF reflects *de novo* copy number variants and implicates mutations within these loci as etiologic in other cases of TOF [5].

JAG1 gene encodes an evolutionarily conserved ligand and is essential for various stages of development [6]. JAG1 mediated Notch receptor functions in a core signaling pathway and controls cell fate decisions and cell–cell interactions during early cardiac embryogenesis [7,8]. Using positional cloning, JAG1 gene was mapped to chromosome 20p12. It consists of 26 exons and 25 introns [9]. All exons are

☆ Note: Nucleotide sequence data reported are available in the GenBank databases under the accession numbers FJ439572, FJ439571, FJ439570, FJ013354.

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doi:10.1016/j.cca.2011.08.017

coded and transcribed to generate a 5.5 kb mRNA which contains 5942 bp of cDNA. The protein Jagged1 is a glycosylated transmembrane protein of 180 kDa and 1218 amino acids long. The protein contains several important functional domains including a signal peptide; delta, serrate, lag-2 domain; 16-epidermal growth factor (EGF) like repeats; cysteine rich region; transmembrane domain and a small intracellular region [10].

JAG1 is known to be a candidate gene for Alagille syndrome (AGS) an autosomal dominant disease, characterized by cholestasis, vertebral deformity, right sided heart defects, ocular defects and peculiar facial appearance [11]. However, mutations in JAG1 gene were also found to be associated with few familial and sporadic cases of isolated congenital heart defects [12], right heart obstructive disease including TOF [13,14], deafness resulting from errors in development of the inner ear [15] and both CHD and deafness [16]. Conversely, there is no correlation between the type or location of mutation and the frequency or type of cardiovascular anomaly [17]. The exon 6 of JAG1 codes for EGF-2 and C-terminal part of EGF-1 which are in association with DSL domain and are crucial for ligand binding to the Notch receptor [18,19]. Detection of mutations that alter binding capacity in the ligand binding region of JAG1 is important in order to find out the disease pathogenesis.

Current screening techniques allow for the identification of JAG1 mutations in 60–70% of individuals with AGS. Clinical genotype and phenotype correlations are well established in Alagille syndrome [20]. To date studies have documented the frequency of JAG1 mutations, and range of clinical manifestations in probands with TOF and their parents and relatives [12]. In the current study family history of congenital heart defects was seen only in 6% and immediate family members of the proband phenotypically did not qualify for any diagnostic criteria of any congenital heart problems. Further failure of preliminary attempts to recruit the parents/relatives of the proband in the study for genotyping was a limitation and set the design of the study. Hence, and in view of lack of data on JAG1 gene mutations in TOF cases from India, a pilot study was undertaken to document the type and frequency of mutations in exon 6 region of JAG1 gene, mainly in sporadic non-syndromic children only using PCR-SSCP and DNA sequencing.

2. Materials and methods

2.1. Subjects

The present study was carried out at the Department of Environmental Toxicology, Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Hyderabad, Andhra Pradesh, India. The study was designed considering 1. The prevalence of sporadic and non-syndromic TOF children was considered with statistical relevance. 2. Children should be 2 years or older. 3. Many of the patients visiting the hospital were mainly from rural background from lower socio economic status with possible deficiency of folates and other nutrients. 4. Lack of parental consent was a limitation for recruiting the subjects — probands/controls. Hence eighty four sporadic non-syndromic cases of TOF patients in the age group of 2–15 years, selected randomly from 2005 to 2007, formed the study group. Eighty seven age and sex matched healthy children with no family history of CHD and other associated genetic diseases were included in the control group. The study was approved by ethics committee of CARE Hospital, Banjara Hills, Hyderabad, Andhra Pradesh, India. Written, informed and educated consent was obtained from the parents of all children who have participated in the study. Clinical evaluation of TOF was made based on physical examination, chest radiography, electrocardiography and 2D echocardiography. Clinical data of patients, information on genetic diseases running in the family, history of congenital heart defects, maternal infections during pregnancy, consanguinity among parents etc. were recorded using a standard questionnaire.

2.2. Genotyping of JAG1 exon 6

Genomic DNA was isolated from peripheral blood lymphocytes of all subjects (n = 171) as described previously [21]. The coding sequence (198 bp) of exon 6 was amplified with specific oligonucleotide primers [22]; and 100 ng genomic DNA, 25 pmol of each forward and reverse primers, 10 mM dNTP mix, 5× PCR buffer, and 3 U Taq DNA polymerase in 50 μL Millipore water. The amplification was carried in Bio-Rad MJ mini gradient thermal cycler with initial denaturation for 5 min at 95 °C, followed by denaturation for 5 min at 95 °C, annealing at 63 °C for 1 min and extension at 72 °C for 1 min with a total of 35 cycles. Final extension was carried out at 72 °C for 10 min.

2.3. Mutation detection and structure prediction

For identification of mutations, exon 6 of JAG1 was screened using single stranded conformation polymorphism (SSCP) carried [23] with minor modifications. Equal volumes of PCR amplicons and formamide loading buffer were heated to 95 °C for 15 min to denature the DNA, and immediately quenched on ice prior to loading on non-denaturing 10% PAGE. Electrophoresis was performed at 40 °C for 12–14 h at 100 V. The migration of DNA bands was visualized using silver staining. PCR samples of conformers were purified and subjected to direct DNA sequencing on an ABI prism 377 DNA sequencer (Macrogen, Seoul, Korea). BLAST search and blastx in Swissprot were performed to identify homology and sequence conservation across phylogenetic spectrum.

3. Results

The screening of exon 6 of JAG1 gene led to the identification of four novel mutations in four out of eighty four (4.7%) children with TOF. The PCR samples of patients showed a shift in the banding pattern when compared to controls (Fig. 1). DNA sequencing and BLAST analysis revealed four novel C765A; G814T; G834T and G861T variants (Fig. 2) and the location of each variation was represented in Fig. 3. These variations affect second of 16 tandemly repeated EGF like domain in Jagged1 protein and have not been reported so far in the literature. The nucleotide variations, corresponding amino acid substitutions and affected region in the protein were represented in Table 1 with NCBI GenBank accession numbers. None of the 87 control group showed similar variations at these positions.

3.1. Nonsense mutation 765 C→A

A novel nucleotide change was observed at position 765 resulting in a transversion of cytosine to adenine (765 C→A) in a six year old boy. The variation results in the substitution of amino acid tyrosine to stop codon at amino acid residue 255 (Y255X) in the protein

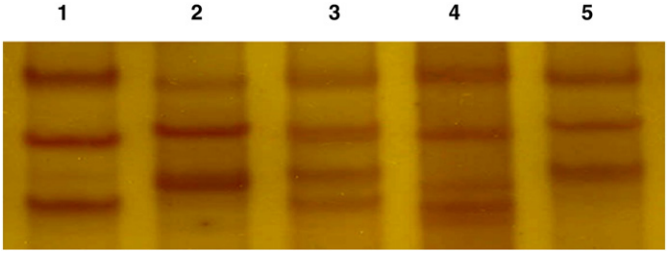

Fig. 1. SSCP conformers in 12% polyacrylamide gel electrophoresis. Lane 1: Control children and Lanes 2–5 show conformation changes of genomic DNA in Tetralogy of Fallot. Lanes 2–5 correspond to: 765 C>A; 814 G>T, 834 G>T and 861 C>T variations respectively.