

## ONLINE MUTATION REPORT

# A novel *GATA4* mutation completely segregated with atrial septal defect in a large Japanese family

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**A**trial septal defect (ASD) is characterised by left-to-right shunting and increased right ventricular output.<sup>1</sup> Approximately 5–10% of congenital heart diseases (CHD) are due to ASD which is one of the most frequent CHD found in human adults.<sup>2</sup> ASD has been presumed to be caused by genetic factors.<sup>3–4</sup> Recently, a few genes have been implicated in syndromic and non-syndromic ASD. Mutations in *T-BOX5* at 12q24.1,<sup>5–6</sup> *NKX2.5* at 5q34,<sup>7–8</sup> *EVC* at 4p16.1,<sup>9</sup> and *GATA4* at 8p23.1–p22<sup>10</sup> cause Holt-Oram syndrome with ASD or ventricular septal defect (VSD), non-syndromic CHD including ASD and atrioventricular conduction abnormalities, Ellis-van Creveld syndrome with ASD, and a familial isolated ASD, respectively.

We encountered a large family of four generations where 11 members were affected with ASD, and where disease transmission was consistent with an autosomal dominant mode of inheritance. Here we report a novel mutation of *GATA4* in this family.

## METHODS

### Subjects

A large Japanese family composed of a total of 29 members across four generations contained 11 members with ASD (I-1, II-2, II-6, II-7, III-1, III-2, III-4, IV-1, IV-2, IV-5, and IV-6; fig 1A). ASD in five patients (II-7, III-1, III-2, IV-1, and IV-2) was surgically repaired, and two patients (II-2 and IV-1) also had pulmonary stenosis (PS). The heart defects in eight subjects (II-2, II-7, III-1, III-2, IV-1, and IV-2) including two diseased subjects (I-1 and II-6) had been clinically diagnosed by one of co-authors (BK) on the basis of their past histories, operation records, 12-lead electrocardiograms, and echocardiograms with colour Doppler apparatus (fig 1A), while those in three other subjects (III-4, IV-5, and IV-6) were retrospectively found using information provided by the mutation analysis described below. None of the 11 affected members had any other abnormalities in the cardiac conduction system or other organs. After informed consent was obtained, DNA was extracted from peripheral blood leukocytes of 22 family members.

### Linkage and mutation analyses

Linkage analysis was performed in 22 family members at 11 microsatellite marker loci around *GATA4* at 8p23 (D8S561, D8S503, D8S1721, D8S550, D8S265, D8S552, D8S1790, D8S549, D8S254, D8S258, and D8S1771), and two-point linkage analysis was carried out using the computer program MLINK (FASTLINK software, version 4.1P), as described previously.<sup>11</sup> III-4, IV-5, and IV-6 were regarded as unaffected at this initial linkage analysis.

All exons in the 22 members covering the coding region of *GATA4* were then amplified by PCR. PCR and sequencing primers are shown in table 1. PCR was cycled 35 times at 98°C for 30 s, 55–58°C for 30 s, 72°C for 1 min in a 25 µl mixture containing 1×ThermalAce Buffer (or 1×Ex Taq

## Key points

- Linkage analysis in a large Japanese family with atrial septal defect (ASD) suggested *GATA4* as a possible candidate gene for heart disease.
- Mutation analysis of *GATA4* identified a novel 1 bp deletion (c.1074delC) at exon 6 in all nine family members with ASD, but not in the family members without the disease.
- This is the second report linking *GATA4* mutation with an isolated congenital heart defect.

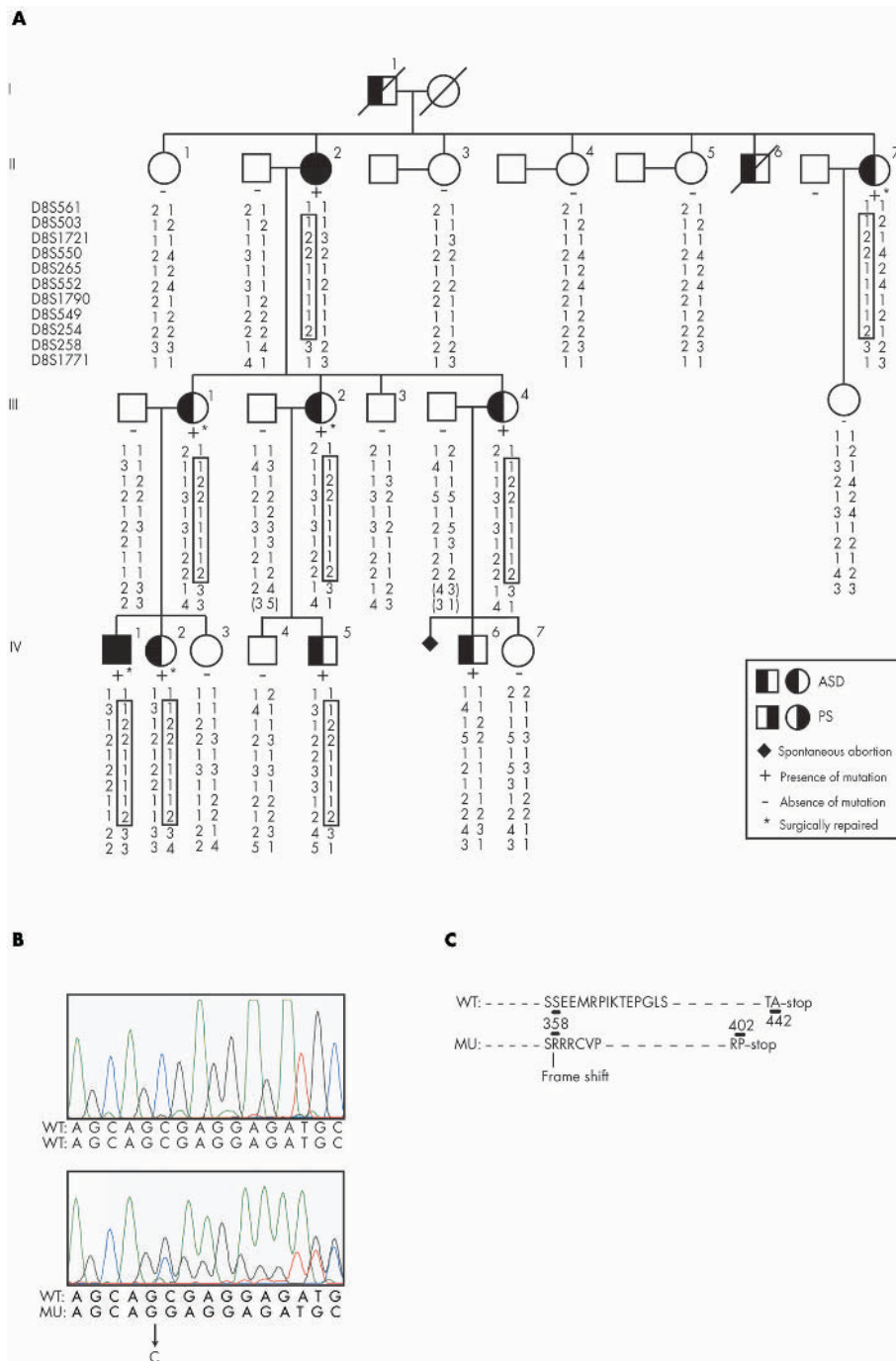
Buffer (Takara, Ohtsu, Japan)), 0.2 mM each dNTP, 0.5 µM each primer, and 1 U ThermalAce DNA polymerase (Invitrogen, Carlsbad, USA) (or 0.5 U TaKaRa Ex Taq™ DNA polymerase (TaKaRa)). PCR products were purified using ExoSap-IT (USB, OH, USA) and sequenced by a standard protocol using BigDye Terminator (Applied Biosystems, Foster City, CA, USA) on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). *GATA4* sequences of affected members were compared with those of normal controls using Autoassembler 2.1 (Applied Biosystems).

## RESULTS AND DISCUSSION

Linkage analysis gave a maximum LOD score of 1.61 (recombination fraction (θ) = 0, penetrance = 0.65) at D8S552 on 8p23.1–p22, the findings suggesting *GATA4* as a possible candidate gene for ASD in this family. The sequence analysis revealed that nine (II-2, II-7, III-1, III-2, III-4, IV-1, IV-2, IV-5, and IV-6) of the 22 members had a heterozygous 1 bp deletion (c.1074delC) at exon 6 of *GATA4* (fig 1B). The 1 bp deletion results in a frame shift and is predicted to create a premature stop codon at amino acid 403, leading to protein truncation (fig 1C). The mutation was never observed in 13 unaffected members of the family or in 100 normal controls.

Early and precise diagnosis of ASD is clinically important, because leaving it uncorrected until adulthood may result in right heart failure, although some patients with minor ASD can often lead a healthy life. As the mortality rate of ASD operations is very low in patients without cardiac failure under the age of 45,<sup>1</sup> early surgical intervention is feasible. Clinical re-evaluation of three members (III-4, IV-5, and IV-6) who were previously thought to be unaffected confirmed their disease. Thus, penetrance in the pedigree we describe is actually complete. This information will be very

**Abbreviations:** ASD, atrial septal defect; CHD, congenital heart diseases; PS, pulmonary stenosis; TOF, tetralogy of Fallot; VSD, ventricular septal defect



**Figure 1** (A) Pedigree of a large Japanese family with heart defects, haplotypes at marker loci on chromosome 8, and *GATA4* mutation. ASD was observed in 11 members and PS in two. A common haplotype associated with ASD at eight marker loci, D8S503, D8S1721, D8S550, D8S265, D8S552, D8S1790, D8S549, and D8S254, was boxed. All members with the heart defects had a *GATA4* mutation, c.1074delC. (B) Electropherogram of an unaffected family member with wild type alleles of *GATA4* (upper), and an affected member heterozygous for a mutated allele, c.1074del, at exon 6 of *GATA4* (lower). (C) Wild type (WT) and mutated (MU) amino acid sequences of *GATA4* protein. The c.1074del causes a frame shift and creates a premature stop codon at amino acid 403.

useful for genetic counselling for this family, because testing for c.1074delC in new family members can be followed by careful medical examination and prophylactic intervention if ASD is found. Revised linkage analysis using information from newly confirmed patients (III-4, IV-5, and IV-6) provided a maximum LOD score of 4.43 (recombination fraction ( $\theta$ ) = 0, penetrance = 0.95) at D8S552.

ASD in two patients with the mutation in our family was associated with PS. A similar finding has been reported in

two families with *GATA4* mutations by Garg *et al*<sup>10</sup>: one family (family B) had isolated ASD and a deletion mutation, c.1075delG, while the other (family A) presented with complex heart defects such as atrioventricular septal defect, PS, aortic regurgitation, patent ductus arteriosus in addition to ASD, and a missense mutation, c.886 G>A (G296S). The missense mutation, c.886 G>A, disturbed the interaction between *GATA4* and *TBX5*.<sup>10</sup> *TBX5* and *NKX2-5* mutations can cause Holt-Oram syndrome with tetralogy of Fallot (TOF)

**Table 1** PCR primer sequences

Exon	Forward (5'-3')	Reverse (5'-3')	Product size (bp)
1	AGACCCAGACCTGGACTT	GCAAAGTCAAAGCAGAGCA	448
2-1	AACGCCTGGACAAAACAAAG	CCAAGCTCTGATACATGGTC	652
2-2	CAGTTCCTCCCACGCATATT	CTTCTGCCTTCCCTAGAAAC	872
3	AAAGGGCATTGTTTCTGTGC	GATGCACACCTCAAGTTC	377
4	GGGCAGTGACACACCTTTTAC	GAGAGATGGGCATCAGAAGG	232
5	GCTTAGGTGTGCTTCTCG	TGCCTAACCCGGAAGATATG	270
6	ACTGTAGCCCTCCGAGATA	TGAGGCCTGGTGCAAGTC	404
7-1	ACATCTGCATAGCAGGGCAC	TGCCATGCCTCAAGCGGAT	755
7-2	CGAGGATCTGAGAACAAGCG	CGTGTGCAGAATCTCTGGC	755
7-3	GCTCAAGCCAGTCTGGCAAG	AGTGGGTCAGTGCTACACG	755

Sequencing primers are the same as PCR primers except for 2-1 forward, 2-1 reverse, 2-2 reverse, and 7-3 reverse. Sequences (5'-3') of newly designed sequencing primers for 2-1 forward, 2-1 reverse, 2-2 reverse, and 7-3 reverse are AGCGTGGAGAAGCAACCAC, GTCTTCTGCCCAATAGGT, GAAGGCGTGGTGAAAAAC, and TTCTCTGTCACATGCTG, respectively.

and non-syndromic TOF, respectively.<sup>5 12 13</sup>. TOF includes PS. As GATA4 functionally interacts with TBX5 and NKX2-5,<sup>10 14 15</sup> the c.1074delC in the family we described may disturb the coordinated interaction between GATA4 and TBX5 or NKX2-5 in cardiogenesis, resulting in PS in the two affected members of our family. It is also noted that the truncated protein generated by the c.1074delC is very similar to that generated by the c.1075delG except for a serine residue. It is likely that the c.1074delC is unable to activate transcription of downstream genes, being similar to the c.1075delG as reported by Garg *et al.*<sup>10</sup> In summary, the GATA4 c.1074delC likely results in haploinsufficiency, and causes a cardiac phenotype similar to that described in 8p deletion syndrome.<sup>16</sup> In addition, a syndromic PS associated with Noonan/LEOPARD syndrome and Alagille syndrome has been shown to be caused by mutations of PTPN11 at 12q22<sup>17 18</sup> and JAG1 at 20p12,<sup>19 20</sup> respectively. JAG1 mutation can also result in isolated PS.<sup>21</sup> It might be interesting to investigate the relationship between GATA4 and PTPN11 or JAG1.

In conclusion, we identified a 1 bp deletion of GATA4 in a large Japanese family with ASD. Segregation of ASD with the GATA4 mutation with complete penetrance in this family suggests that one type of ASD is caused by a single gene defect. It remains to be seen how much GATA4 mutations contribute to the pathogenesis of congenital heart defects in man.

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