Atrial septal defect (ASD) is characterised by left-to-right shunting and increased right ventricular output. Approximately 5–10% of congenital heart diseases (CHD) are due to ASD which is one of the most frequent CHD found in human adults. ASD has been presumed to be caused by genetic factors. Recently, a few genes have been implicated in syndromic and non-syndromic ASD. Mutations in T-BOX5 at 12q24.1, 10 Nkx2.5 at 5q34, 7, 6 EvC at 4p16.1, 1 and GATA4 at 8p23.1–p22 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, III-1, III-2, 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, D8S590, D8S265, D8S352, 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. 3, 4, 5, 6, 7 Two-point linkage analysis was used to determine the location of the disease gene in the family. In addition, the disease gene was ultimately confirmed to reside on chromosome 8 using Autoassembler 2.1 (Applied Biosystems). 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, IV-1, IV-4, IV-2, IV-4, 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.

RESULTS AND DISCUSSION

Linkage analysis revealed a novel mutation with 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, 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 useful for clinical practice.

Abbreviations: ASD, atrial septal defect; CHD, congenital heart diseases; PS, pulmonary stenosis; TOF, tetralogy of Fallot; VSD, ventricular septal defect.
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 \textit{GATA4} mutations by Garg \textit{et al} \cite{Garg}: 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 \rightarrow A (G296S). The missense mutation, c.886 G \rightarrow A, disturbed the interaction between \textit{GATA4} and TBX5. \cite{Garg}

\textit{TBX5} and \textit{NKX2-5} mutations can cause Holt-Oram syndrome with tetralogy of Fallot (TOF)
and non-syndromic TOF, respectively.\textsuperscript{5,12,13} TOF includes PS. As GATA4 functionally interacts with TBX5 and NKX2-5,\textsuperscript{14,15} the c.1074delC in the family may describe disturbed 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.\textsuperscript{16} In summary, the GATA4 c.1074delC likely results in haploinsufficiency, and causes a cardiac phenotype similar to that described in 8p deletion syndrome.\textsuperscript{17} In addition, a syndromic PS associated with Noonan/LEOPARD syndrome and Alagille syndrome has been shown to be caused by mutations of PTPN11 at 12q22-23\textsuperscript{18} and JAG1 at 20p12.\textsuperscript{19} Respectively, JAG1 mutation can also result in isolated PS.\textsuperscript{20} 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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**REFERENCES**

