Table 4 Pairwise linkage disequilibrium (D' above diagonal) and statistical significance (*P*-value below diagonal) for the four SNPs in the human *MEI1* gene among 26 azoospermic patients (13 European Americans and 13 Israelis). *P*-values based on χ^2 distribution

	SNP1	SNP2	SNP3	SNP4
SNP1		−1	0.4003	0.1539
SNP2	0.5008		1	1
SNP3	0.3857	< 0.05*		1
SNP4	0.921	< 0.05*	< 0.05*	

*Significant at $P = 0.05$

Table 5 Estimated haplotype frequencies of SNP3 and SNP4 between 13 European American azoospermic patients and 61 controls, and between 13 Israeli patients and 60 controls. *P*-values based on χ^2 distribution

Haplotype	European American		Israeli	
	Patients ($n = 13$; %)	Control ($n = 61$; %)	Patients ($n = 13$; %)	Control ($n = 60$; %)
SNP3–SNP4				
C–C	81.9	100	88.8	98.7
C–T	6.8*	0	3.6	0.8
A–C	10.6*	0	7.4	0.8
A–T	8.9*	0	0.2*	0.1

*Significant at $P = 0.05$

longest ORF of the mouse *Mei1* comprises 2,685 bp and is predicted to encode a protein of 894 amino acids. Two alternative *MEI1* transcripts consisting of 2,714 and 2,609 bp encode proteins of 642 and 607 amino acids, respectively, as in the mouse *Mei1* gene (Libby et al. 2003). The human *MEI1* is not assigned to the Y chromosome but is located to 22q13.2. The predominant expression of *MEI1* in the testis is consistent with its putative role in spermatogenesis, as seen in the mouse *Mei1* gene (Libby et al. 2003). Positional cloning showed that mouse *Mei1* is responsible for the mutant mei1 phenotype. The mei1 mice lack the first 58 bp in exon 12 or entirely skip exon 12 of *Mei1*, resulting in a frameshift leading to a predicted truncated Mei1 protein. Male mice with such a mutated *Mei1* show spermatocytes arrested at meiosis, and the RAD51 protein does not load onto chromosomes bearing mutated *Mei1*, suggesting that there is a defect either in recombinational repair or in the production of double-strand breaks (DSBs) that require such repair (Libby et al. 2003). Recent studies on meiosis in ($Mei1^{-/-}$) and ($Dmc1^{-/-}$) mice of both sexes have demonstrated that their phenotypes are identical to those of $Mei1^{-/-}$ mice (Reinholdt and Schimenti 2005). Therefore, *Mei1* can be positioned upstream of *Dmc1* in the genetic pathway that operates during mouse meiosis. Further analysis is needed to determine the relationship between *MEI1* and *DMC1* in man.

We have identified four novel cSNPs in the *MEI1* gene. The present association study has revealed that the genotype distributions for SNP3 (A/C) and SNP4 (T/C) are significantly different between the European American azoospermic patients and their controls: 0.154/0.846 vs 0.000/1.000 for AC heterozygotes/CC homozygotes at the SNP3 site; and 0.154/0.846 vs 0.000/1.000 for TC heterozygotes/CC homozygotes at the SNP4 site, respectively ($P < 0.05$). Likewise, the frequencies of alleles A/C at SNP3 were 0.077/0.923 and 0.00/1.000; and those of alleles T/C at SNP4 were 0.077/0.923 vs 0.000/1.000 in the patients and controls, respectively ($P < 0.05$). These findings suggest that allele C at nucleotide 1,791 in exon 9 and allele C at nucleotide 2,397 in exon 14, or their flanking regions, may play a role in the disruption of spermatogenesis in the European American patients, although the number of patients analyzed was not large enough to allow a definitive conclusion to be drawn; no such association was found in Israeli patients.

HWE tests performed for the four cSNPs in European American and Israeli patients ruled out the equilibrium of SNP2 by its *P* value. This deviation is most likely due to the small sample size in the present study. We performed haplotype analysis on the synonymous SNPs identified as well as their combinations, and found no significant difference between the haplotype frequencies estimated for all four polymorphisms between the patient and control groups. We then performed LD analysis using pairs of SNPs. Consequently, we detected three pairs of SNPs (SNP2–SNP3, SNP2–SNP4 and SNP3–SNP4) with LD values of 1.00 and with *P* values of < 0.05 by χ^2 tests. We carried out haplotype analysis on the SNP3–SNP4 pair containing statistical differences in genotype and allelic levels. All analyses were performed both in European American and Israeli patients. Haplotype analysis demonstrated that three haplotypes, SNP3–SNP4 (C–T), SNP3–SNP4 (A–C) and SNP3–SNP4 (A–T), were markedly associated with azoospermia among the European American patients. Furthermore, such an association of a SNP3–SNP4 (A–T) haplotype was also found among the Israeli patients.

In vitro fertilization (IVF) has been proven to be an efficient way to resolve infertility due to female factors

(Edwards et al. 1980), but it has not been so effective for problems due to severe oligospermia in the male partner (Devroey and Van Steirteghem 2004). Although testicular sperm extraction (TESE)–intracytoplasmic sperm injection (ICSI) is now available for patients with azoospermia, it cannot help patients lacking spermatozoa in their testes due to a complete failure in spermatogenesis. Therefore, treatment for infertility due to non-obstructive azoospermia is an important immediate goal in assisted reproductive technology (ART).

In conclusion, this is the first report showing that *MEI1* SNPs may predispose men to a defect in spermatogenesis, although the mechanism by which these SNPs result in azoospermia remains uncertain. Our results may also advance a better understanding of the molecular basis of early meiotic arrest as a cause of non-obstructive azoospermia. It remains to be confirmed whether the association is seen in larger sample numbers and in similar patients from other ethnic groups, although men with azoospermia caused by meiotic arrest are very rare.

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Comprehensive Genetic Analysis of Relevant Four Genes in 49 Patients With Marfan Syndrome or Marfan-Related Phenotypes

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In order to evaluate the contribution of *FBN1, FBN2, TGFBR1*, and *TGFBR2* mutations to the Marfan syndrome (MFS) phenotype, the four genes were analyzed by direct sequencing in 49 patients with MFS or suspected MFS as a cohort study. A total of 27 *FBN1* mutations (22 novel) in 27 patients (55%, 27/49), 1 novel *TGFBR1* mutation in 1 (2%, 1/49), and 2 recurrent *TGFBR2* mutations in 2 (4%, 2/49) were identified. No *FBN2* mutation was found. Three patients with either *TGFBR1* or *TGFBR2* abnormality did not fulfill the Ghent criteria, but expressed some overlapping features of

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MFS and Loeys–Dietz syndrome (LDS). In the remaining 19 patients, either of the genes did not show any abnormalities. This study indicated that *FBN1* mutations were predominant in MFS but *TGFBRs* defects may account for approximately 5–10% of patients with the syndrome.

Key words: FBN1; FBN2; TGFBR1; TGFBR2; Marfan syndrome

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INTRODUCTION

Marfan syndrome (MFS, OMIM #154700) is an autosomal dominant connective tissue disorder primarily involving skeletal, ocular, and cardiovascular systems. The incidence is estimated to be 1 in 5,000–10,000 individuals [Dietz and Pyeritz, 1995; Gray and Davies, 1996]. MFS was caused by mutations of the fibrillin-1 gene (*FBN1*) at 15q21.1 [Dietz et al., 1991] encoding the large, cysteine rich, extracellular matrix glycoprotein which is the major component of microfibrils. *FBN1* spans a 230-kb genomic region with 65 exons and at least 664 reported *FBN1* mutations spread throughout the gene and are mostly private in each affected family [UMD-FBN1 Mutation Database (http://www.umd.be:2030/)] [Collod-Beroud et al., 2003]. Recently, we found that the transforming growth factor (TGF)-β receptor II gene (*TGFBR2*) was mutated in patients with Marfan syndrome not linked to *FBN1* aberrations [Mizuguchi et al., 2004]. This gave the first genetic evidence of a direct link between abnormal TGF-β signaling and a human connective tissue disorder. Subsequently, Loeys et al. [2005] reported a new dysmorphic syndrome with mutations in either *TGFBR2* or TGF-β receptor I (*TGFBR1*) (Loeys–Dietz syndrome, LDS, OMIM #609192). Several symptoms are overlapped in MFS and LDS, including aortic root aneurysm and other skeletal abnormalities. Furthermore, *TGFBR2* mutations were also detected in patients with familial thoracic aoritic aneurysms and dissections (TAAD) [Pannu et al., 2005].

Congenital contractural arachnodactyly (CCA, OMIM#121050), a similar connective tissue disorder to MFS, is caused by mutations in the fibrillin 2 gene (*FBN2*) homologous to *FBN1* [Putnam et al., 1995; Robinson and Godfrey, 2000; Gupta et al., 2002]. It is often difficult to differentiate clinically between the two syndromes because of their phenotypic similarities in skeletal complications including arachnodactyly, dolichostenomelia, pectus deformities, and kyphoscoliosis. CCA usually presents with multiple joint contracture and crumpled ear helix and very occasionally with aortic root dilatation and eye involvement which may prevent clear distinction of the two syndromes [Viljoen, 1994; Pyeritz, 2000; Gupta et al., 2002; Gupta et al., 2004].

Four genes, *FBN1*, *FBN2*, *TGFBR1*, and *TGFBR2*, were analyzed by direct sequencing in a total of 49 patients with MFS or suspected MFS as a cohort study. Contribution of mutations of each gene and corresponding phenotypes will be discussed.

MATERIALS AND METHODS

Subjects

The clinical features of the probands are given in Table I. Among the 49 probands, 38 were reasonably evaluated according to the Ghent criteria, 14 of whom fulfilled the MFS criteria [De Paepe et al., 1996]. After written informed consent, genomic DNA of blood leukocytes was isolated using DNA isolation systems [Quick Gene-800 (Fujifilm, Tokyo, Japan) and/or NA-3000 (Kurabo, Osaka, Japan)]. Hair and nails were obtained if necessary and their DNAs were extracted using Isohair (Nippon Gene, Tokyo, Japan), respectively. Experimental protocols were approved by the Committee for Ethical Issues at Yokohama City University School of Medicine.

Sequence Analysis

FBN1, *FBN2*, *TGFBR1*, and *TGFBR2*, were analyzed. *FBN1* and *TGFBR2* were sequenced in all patients. In whom both genes were normal, *TGFBR1* and subsequently *FBN2* were analyzed. Primer sequences for *FBN1* and *TGFBR2* were basically described elsewhere [Korkko et al., 2002; Mizuguchi et al., 2004]. Those for exon 7 of *FBN1*, exon 1 of *TGFBR2*, all exons of *TGFBR1* and *FBN2* were newly designed in this study (available on request). All coding exons of *FBN1* (65 exons), *FBN2* (65 exons), *TGFBR1* (9 exons), and *TGFBR2* (7 exons), and their flanking intronic regions were amplified by PCR. In one patient (MFS31), sufficient amount of DNA was not available, thus *FBN2* could not be analyzed. PCR products were purified with ExoSAP-IT (USB, Cleveland, OH) and sequenced by a standard protocol using BigDye terminator (Applied Biosystems, Foster

TABLE I. List of the Clinical and Molecular Data

MFS ID	Age (year)	Sex	Height (cm)	Skeletal	Ocular	Cardiovascular	Pulmonary	Skin	Dura	Familial Hx	Ghent criteria	Gene	Exon	Nucleotide change	Amino acid change	Nature	Novelty
37	10	Female	153	m	M	-	-	-	-	-	Not fulfilling	*FBN1*	2	211T > C	W71R	De novo	Novel
59	39	Female		m		-	m	-	-	+	Not fulfilling	*FBN1*	4	400T > G	C134G		Novel
19												*FBN1*	7	772C > T	Q258X		Novel
10a												*FBN1*	8	937delT	C313A Fs	Familial	Novel
52	35	Male	192	m	-	M	m	m	-	-	Not fulfilling	*FBN1*	10	1285C > T	R429X		Matyas et al. [2002]
42												*FBN1*	15	1904A > G	Y635C		UMD-*FBN1* database
7	13	Male	124	m	-	M	-	-		-	Not fulfilling	*FBN1*	16	2097T > A	C699X	De novo	Novel
39a	38	Female	185	M	M		-	m		M	MFS	*FBN1*	24	2942G > C	C981S	Familial	Novel
40												*FBN1*	24	3043G > A	A1015T		Novel
26	2	Male	101	M	-	M	-	-		-	Not fulfilling	*FBN1*	25	3125G > A	G1042D		Novel
25	16	Female	174	M	M	M	-	-		-	MFS	*FBN1*	33	4099T > C	C1367R		Novel
43a												*FBN1*	34	4283-4284insG	R1428R Fs	Familial	Novel
2	10	Female	148	M	M	M	-	-		-	MFS	*FBN1*	34	4285T > A	C1429S	De novo	Collod-Beroud et al. [1998]
5												*FBN1*	35	4405delC	R1469A Fs		Novel
41a		Female		M	M					M	MFS	*FBN1*	36	4495A > T	S1499C	Familial	Novel
41b		Female		M	M					M	MFS	*FBN1*	36	4495A > T	S1499C	Familial	Novel
38	35	Female	169	m	M	-	-	-	-	-	Not fulfilling	*FBN1*	38	4781G > T	G1594V		Novel
44	34	Female	176	m	M	m				M	MFS	*FBN1*	40	4988G > T	C1663F	Familial	Novel
49	9	Male	143	m	-	-	-	-	-	-	Not fulfilling	*FBN1*	43	5368C > T	R1790X		Novel
57a	40	Female	175	m	-	M	-	m	-	M	MFS	*FBN1*	43	5404A > T	K1802X	Familial	Novel
57b	13	Female	183	M	-	M	-	-	-	M	MFS	*FBN1*	43	5404A > T	K1802X	Familial	Novel
33a	4	Male	111	m	M	-	-	-		M	MFS	*FBN1*	44	5539T > C	C1847R	Familial	Novel
33b	31	Male	198	m	M	M	-	-		M	MFS	*FBN1*	44	5539T > C	C1847R	Familial	Novel
20												*FBN1*	46	IVS46 + 5G > A			Nijbroek et al. [1995]
56		Male	184	M	M	M	-	m		-	MFS	*FBN1*	46	IVS46 + 5G > A		De novo	Nijbroek et al. [1995]
50	49	Male	178	-	-	M	-	-	-	-	Not fulfilling	*FBN1*	50	6236C > G	S2079C		Novel
1a	6	Female	129	-	-	M	-	-		M	Not fulfilling	*FBN1*	58	7241G > A	R2414Q	Familial	Novel
1b	32	Female	171	m	-	M	m	-		M	Not fulfilling	*FBN1*	58	7241G > A	R2414Q	Familial	Novel
54	13	Male	150	M		m	-			-	Not fulfilling	*FBN1*	59	7342T > C	C2448R		Novel
9	3	Male	107	m	-	-	M	-	-	M	MFS	*FBN1*	59	7399C > T	Q2467X	Familial	Halliday et al. [2002]
3a		Female	174	m	-	M	-			M	MFS	*FBN1*	59	7409G > A	C2470Y	Familial	Novel
3b	36	Female	170	m	-					M		*FBN1*	59	7409G > A	C2470Y	Familial	Novel
3c	9	Male	121	M	-	M	-	-		M	MFS	*FBN1*	59	7409G > A	C2470Y	Familial	Novel
3d	7	Female	134	m	-	M	-	-		M	MFS	*FBN1*	59	7409G > A	C2470Y	Familial	Novel
41a		Female		M	M					M	MFS	*FBN1*	63	7978A > C	S2660R	Familial	Novel
41b		Female		M	M					M	MFS	*FBN1*	63	7978A > C	S2660R	Familial	Novel
34	16	Female	159	m	-	-	-	-	-	-	Not fulfilling	*TGFBR2*	4	1067G > C	R356P	De novo	Ki et al. [2005]
55	1	Male	96	m	-	M	-	-		-	Not fulfilling	*TGFBR2*	5	1336G > A	D446N		Disabella et al. [2006]
60b	58	Female	162	m	-	m	-	-		M	Not fulfilling	*TGFBR1*	7	1135A > G	M379V		Novel

M, major criteria is satisfied; m, minor criteria is satisfied; -, no symptom.

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City, CA) on an ABI PRISM 3100 Genetic analyzer (Applied Biosystems). The nucleotide substitution was confirmed using the SeqScape software ver. 2.0 (Applied Biosystems). If nucleotide changes were identified in patients whose parental samples were unavailable, at least 50 normal controls were additionally screened to confirm whether they were polymorphisms or not.

RESULTS

A total of 27 mutations in *FBN1*, 2 in *TGFBR2*, and 1 in *TGFBR1* were detected in 30 patients in this study (Table I). No mutation in either of four genes was found in the remaining 19 patients. Interestingly, 4495A > T (S1499C) and 7978A > C (S2660R) in *FBN1* were identified in one family [MFS41a (mother) and MFS41b (daughter)], suggesting that both of the mutations resided on one allele as they were of maternal origin. Five *FBN1* mutations, 1285C > T (R429X), 1904A > G (Y635C), 4285T > A (C1429S), 7399C > T (Q2467X), and IVS46+5G > A and two *TGFBR2* mutations, 1067G > C (R356P) and 1336G > A (D446N), were described previously [Nijbroek et al., 1995; Collod-Beroud et al., 1998; Halliday et al., 2002; Matyas et al., 2002; Ki et al., 2005; Disabella et al., 2006]; UMD-FBN1 Mutation Database http://www.umd.be:2030/]. *FBN1* mutations comprised of 17 missense mutations, 6 nonsense mutations, 3 frameshift mutations, and a splice site mutation. The splice site mutation, IVS46 + 5G > A, was found in two independent probands (MFS20 and MFS56). Eleven of 17 missense mutations were occurred at cb-EGF module (calcium-binding epidermal growth factor like module). A total of 13 missense mutations created (15%; 4/27) at or substituted (33%; 9/27) to a cysteine residue in fibrillin-1. Two recurrent missense mutations in *TGFBR2* in two and one novel missense mutation in *TGFBR1*, 1135A > G (M379V) in one were identified. The *TGFBR1* mutation occurred at a well-conserved amino acid of the kinase domain (Fig. 1). No *FBN2* mutation was found. Furthermore polymorphisms of *FBN1*, *FBN2*, and *TGFBR2* found in this study were listed in Table III. We regarded a nucleotide change as a polymorphism if it was identified in either of a patient's healthy parents or normal controls, or was registered as a SNP in the databases.

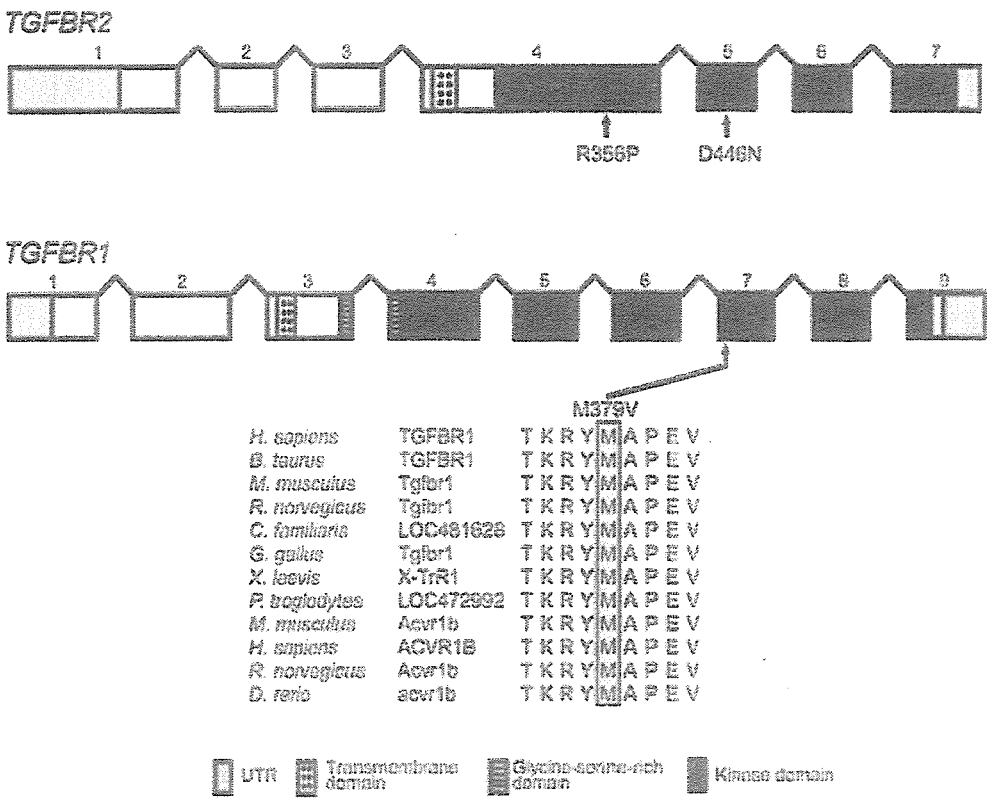

FIG. 1. Genomic structure of *TGFBR2* and *TGFBR1* and mutations found in this study. *TGFBR2* and *TGFBR1* consist of 7 and 9 exons, respectively. Square indicates exon. A transmembrane domain, a glycine-serine-rich domain, a kinase domain, and UTR are shown as a dotted box, a striped box, a black box, and a gray box, respectively. Two missense mutations in *TGFBR2*, 1067G > C (R356P) and 1336G > A (D446N), are found in MSF34 and MFS55, respectively. One *TGFBR1* mutation in MFS60b was also identified. Two *TGFBR2* mutations were previously described [Ki et al., 2005; Disabella et al., 2006]. Multiple sequence alignment using the web-based software, CLUSTALW (http://clustalw.genome.ad.jp/), clearly demonstrated the *TGFBR1* mutation occurred at an evolutionally conserved amino acid of a kinase domain.

TABLE II. Loeys–Dietz Syndrome Features in Patients With *TGFBR2* or *TGFBR1* Abnormality

Symptom	MFS34	MFS55	MFS60b	Frequency (%)[a]
Hypertelorism	+	+	+	93
Cleft palate/bifid uvula	−	+	−	100
Aortic root aneurysm		−	−	100
Arterial tortuosity		−	−	100
Aneurysm of other vessels		−	+	92
Craniosynostosis		−	−	36
Malar hypoplasia	+	+	−	85
Blue sclerae	−	+	−	62
Ectopia lentis	−	−	−	0
Arachnodactyly	+	−	+	57
Dolichostenomelia	+	−	+	29
Pectus deformity	+	−	−	64
Scoliosis	+	+	−	71
Talipes varus	−	+	−	29
Camptodactyly	−	+	+	43
Joint laxity	+	+	+	86
Patent ductus arteriosus	−	−	−	54
Atrial septal defect	−	−	−	31
Chiari type I		−	−	20
Developmental delay	−	−	−	21
Hydrocephalus	−	−	−	15
Others		VSD bicuspid aortic valve aortic root dilatation bil. strabismus umbilical hernia		

[a]Reported by Loeys et al. [2005].

Clinical information based on the Ghent criteria was reasonably available from a total of 38 probands. Among 14 patients fulfilling the criteria, 9 possessed *FBN1* mutations (64%). Among 24 patients not fulfilling the criteria, 10 had *FBN1* abnormalities (42%), 2 *TGFBR2* mutations (8%), and 1 *TGFBR1* mutation (4%). Two patients with *TGFBR2* mutations (MFS34 and MFS55) presented with a similar face found in LDS and some skeletal abnormalities (Table II). The 58-year-old patient with *TGFBR1* abnormality (MFS60b) does not show a typical LDS face but presented with DeBakey IIIb type ascending aortic dissection which was operated at her 51 years (Table II and Fig. 2).

TABLE III. Polymorphisms Found in This Study

Gene	Exon	Polymorphism	SNP ID	Allele frequency in normal control
FBN1	1	79G > A (A27T)	rs25397	
FBN1	4	396T > C (D132D)		1/106
FBN1	11	1415G > A (C472Y)	rs4775765	
FBN1	15	1875T > C (N625N)	rs8033037	
FBN1	22	IVS21-12T > C		Found in a healthy parent
FBN1	27	3442C > G (P1148A)	rs7175654/rs140598	
FBN1	27	IVS27 + 3A > G		Found in a healthy parent
FBN1	42	IVS42 + 14 G > A	rs140650	
FBN1	55	6855T > C (D2285D)	rs363836	
FBN1	56	6888G > A (Q2296Q)	rs363830	
FBN2	6	728T > C (I243T)	NM_001999	
FBN2	12	1643a > c (D548A)		1/106
FBN2	23	2893G > A (V965I)	rs154001	
FBN2	27	3518C > G (T1173S)		1/46
FBN2	29	3762C > T (D1274D)	rs2279582	
FBN2	46	5823T > C (H1941H)	NM_001999	3/40
FBN2	54	6833C > T (T2278M)	rs2307109	
FBN2	54	IVS54 + 28G > A		2/102
FBN2	55	6931A > G (M2311V)	rs32209	
FBN2	57	7200T > C (S2400S)	rs190450	
FBN2	61	7739C > T (S2580L)	NM_001999	3/40
TGFBR2	4	1167C > T (N389N)	rs2228048	

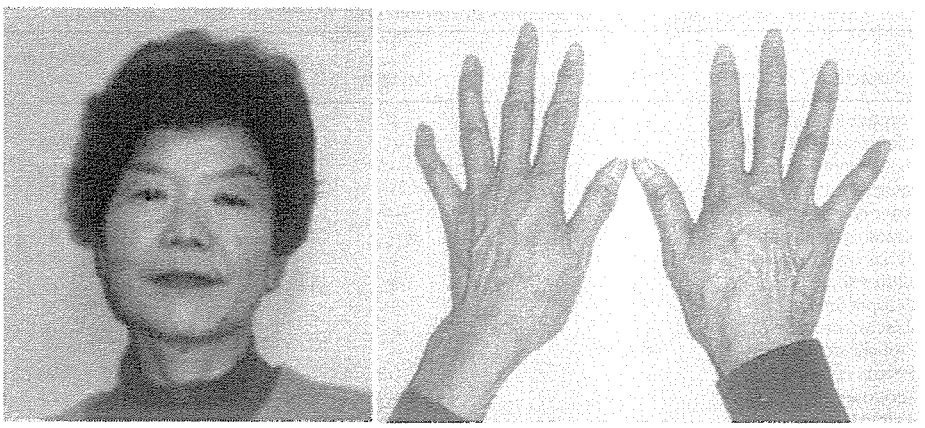

FIG. 2. Photographs of the 58-year-old patient with a *TGFBR1* mutation. Abnormal face implying Loeys–Diets syndrome or arachnodactyly was not identified.

DISCUSSION

The overall detection rate of *FBN1* mutations in this study was 55% (27 out of 49 cases). The rate goes up to 64% in patients fulfilling the criteria and down to 42% in those not fulfilling MFS, being similar to the previous study by Halliday et al. [2002] (77% vs. 60%). According to the UBM-*FBN1* database [Collod-Beroud et al., 2003], *FBN1* mutations can be divided into two classes, a protein truncation type (38.6% of the 562 mutations) and a missense type (60.3%). The latter mostly occurs in cb-EGF-like domains (78%). In this study, the former type (six nonsense, three frameshift mutations, and a splice site mutation) represent 37%, and the latter (17 missense mutations) 63%, all of which were found at a well-conserved amino acid through human, cow, pig, dog, rat, mouse, and chicken Fbn1 homologs (data not shown). Among missense mutations found, 11 are located in cb-EGF-like domains (65%), fitting to the database. Eight missense mutations in cb-EGF-like domains either introducing (2) or substituting (6) cysteine residues may result in abnormal disulfide bonding and misfolding as previously described [Collod-Beroud et al., 2003]. It is also noteworthy that seven out of eight families presenting with major eye involvement showed either missense mutations involving cysteine residues or a splice site mutation as previously suggested [Rommel et al., 2005] (Table I).

Two recurrent missense mutations of *TGFBR2*, R356P and D446N, have been identified in 2 (MFS34 and MFS55) out of 22 patients (9%) with MFS or suspected MFS unlinked to *FBN1* aberrations. The former was previously found in a Korean LDS patient [Ki et al., 2005] and the latter also in a patient with severe cardiovascular disease and skeletal involvement but not compatible with either MFS or LDS [Disabella et al., 2006]. Previously, we identified three *TGFBR2* mutations in 4 of 19 *FBN1*-unrelated MFS patients (21%) [Mizuguchi et al., 2004]. Three *TGFBR2* mutations were reported in two full MFS probands and one MFS-suspected patient unassociated with any *FBN1* mutations [Disabella et al., 2006]. Instead, no *TGFBR2* mutations were found in 30 classic MFS patients, although some of them may have *FBN1* aberration [Ki et al., 2005]. It is obvious that clinical spectrum of *TGFBR2* mutations includes MFS (see also a supportive study by Rommel et al. [2005]), but incidence of *TGFBR2* mutations would be approximately 5% of MFS or suspected MFS, much less than that of *FBN1* mutations (30–90%) (Fig. 1).

The new syndrome, LDS, with either *TGFBR2* or *TGFBR1* mutations is characterized by hypertelorism, bifid uvula, cleft palate, generalized arterial tortuosity, ascending aortic aneurysm, and dissection [Loeys et al., 2005]. None of the LDS patients did meet the Ghent criteria for MFS, but the two syndromes share clinical phenotypes including aortic aneurysms and other skeletal abnormalities [Gibson, 2005; Loeys et al., 2005]. All *TGFBR2* missense mutations identified in LDS were also located at well-conserved amino acids of the kinase domain [Ki et al., 2005; Loeys et al., 2005]. The recent report of a pure type of familial TAAD associated with *TGFBR2* mutations [Pannu et al., 2005] strongly indicated that *TGFBR2* mutations result in severe cardiovascular consequences. It is remarkable that affected family members of the TAAD also had descending aortic disease and aneurysms of other arteries. The two patients (MFS34 and MFS55) with *TGFBR2* mutations in this report did not meet the MFS criteria and presented with some facial characteristics of LDS and a few skeletal abnormalities. However, MFS55 did not show any aneurysm of aortic root and other vessels, and information of cardiovascular phenotypes was not available in MFS34, thus it remains inconclusive whether these patients are classified to LDS (Table II) or MFS. Similarly MFS60b with a *TGFBR1* mutation did not satisfy the Ghent criteria or fit to a LDS phenotype, but her aortic phenotype was severe. No *FBN2* mutation was found in this series,

supporting that it is unnecessary to investigate *FBN2* in MFS.

In conclusion, we have found 27 *FBN1* mutations in 27, 2 *TGFBR2* mutations in 2, and 1 *TGFBR1* mutation in 1 out of 49 patients with MFS or MFS-related disorders. *TGFBRs* screening should be considered in MFS or suspected MFS unlinked to *FBN1* abnormalities, although their abnormalities are occasional, and more information of clinical phenotypes caused by *TGFBRs* mutations is absolutely necessary. No mutation in either of four genes was found in the 19 patients. It is possible that there is an additional causative gene(s) for MFS-related phenotypes. Accumulation of such 19 patients with so far unknown genetic origin will be useful as a resource for finding new MFS-related genes.

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Array Comparative Genomic Hybridization Analysis in First-Trimester Spontaneous Abortions With 'Normal' Karyotypes

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Array comparative genomic hybridization (array CGH) analysis was conducted in chorionic villous samples from 20 first-trimester spontaneous abortions with G-banding normal chromosomes. A microarray, containing 2,173 BAC clones and covering the whole genome with a 1.5-Mb resolution, was constructed and used in the analysis. Two deletions were identified: a 1.4-Mb deletion at 3p26.2-p26.3 and a 13.7-Mb deletion at 13q32.3-qter. Reexamination of chromosome preparations from the sample with the 13.7-Mb deletion documented a mixture of cells with the 13q- chromosome and those with 46,XX chromosomes, the latter of which are likely to have been derived from contaminating decidual cells. This left the 1.4-Mb 3p deletion as the only instance with submicroscopic imbalance detected, giving a frequency of 1 in 19 (5%) G-banding normal abortions.

Key words: spontaneous abortion; chromosomal aberrations; microarray comparative genomic hybridization; array CGH

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INTRODUCTION

Approximately 15% of clinically recognizable pregnancies result in spontaneous abortions, most of which occur in the first trimester. Around half of the first-trimester losses are caused by chromosomal abnormalities, the great majority of which are numerical aberrations. Array comparative genomic hybridization (array CGH) is able to overcome the drawbacks of tissue culturing and chromosome analysis of spontaneous abortions, such as tissue culture failures, overgrowth of maternal cells over fetal cells, suboptimal quality of chromosome preparation, and the limit of resolution in conventional chromosomal banding.

To our knowledge, two studies have been reported using array CGH in the analysis of chromosome abnormalities in spontaneous abortions. Schaeffer et al. [2004] analyzed spontaneous abortions with both chromosomal G-banding and GenoSensor Array 300 (Vysis/Abott, Abott Park, IL), an array CGH kit with 287 targets including telomeres, microdeletions, oncogenes, and tumor suppressor genes. Of 41 spontaneous abortions analyzed, 4 showed abnormalities undetectable with G-banding analysis: one trisomy 21 was also mosaic for trisomy 20, one trisomy 13 had an interstitial deletion of chromosome 9p, one trisomy 16 had an interstitial duplication of the Prader–Willi/Angelman syndrome region of chromosome 15, and one 46,XX had a duplication of the 10q terminal region. Among 25 G-banding normal abortions analyzed, the one with the 10q terminal duplication had a submicroscopic aberration, giving a frequency of 4%. Benkhalifa et al. [2005] studied 26 spontaneous abortions

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that failed to grow in culture, using human genomic microarrays containing 2,600 BAC/PAC clones (Human BAC Containing Array-1Mb System, Spectrum Genetics, Inc., Houston, TX). In addition to 12 cases of numerical chromosome abnormalities including autosomal monosomies and multiple trisomies, 3 cases of possible submicroscopic abnormalities were detected: a case of monosomy 21 also had two amplified clones on Xp, a case contained a gain of one clone in 1pter, and another case showed deletion of a clone in the 22q13 region. These gains and deletions, however, were not supported by FISH analysis. Therefore, 2 out of 13 cases without numerical chromosome abnormalities had possible submicroscopic abnormalities, giving a frequency of 15%.

We here describe array CGH analysis of spontaneous abortions with apparently normal G-banded chromosomes. A microarray with a 1.5-Mb resolution was constructed and used in the study.

MATERIALS AND METHODS

Sample

Chorionic villous samples were collected from spontaneous abortions ranging from 5 to 12 weeks in gestation, following the protocols approved by the institutional review board. A small piece of chorionic villi was washed thoroughly in PBS, separated from maternal decidual tissues by use of a dissecting microscope, dissociated using collagenase, and cultured in Amnio Max™ (GIBCO, Carlsbad, CA) and ES media (Nissui, Tokyo, Japan) supplemented with 20% fetal bovine serum. Cells were harvested and analyzed at the 400-band level using a standard G-banding protocol. DNA was extracted from frozen tissue samples of those judged normal on G-banding chromosome analysis.

Array CGH

Array comparative genomic hybridization analysis was performed using arrays we have developed in which 2,173 Fished BAC clones were spotted [Miyake et al., 2006a]. After complete digestion using *Eco*RI, subject's DNA was labeled with Cy-3 dCTP (GE Healthcare Bio-Sciences, Piscataway, NJ) and reference DNA was labeled with Cy-5 dCTP (GE Healthcare Bio-Sciences) using DNA random primer Kit (Invitrogen, Carlsbad, CA) (CGH1). Dyes were swapped in CGH2 (subject DNA with Cy5 and reference DNA with Cy3) to confirm that the signal patterns were reversed. Prehybridization and hybridization procedures were carried out as described previously [Harada et al., 2004]. Arrays thus processed were scanned with GenePix 4000B (Axon Instruments, Union City, CA) and analyzed using GenePix Pro 4.0 (Axon Instruments). Signal intensity ratios between subject's and control DNA were calculated from the data of the single slide experiment, using the ratio of means formula (F635 Mean−B635Median /F532 Mean−B532 Median) according to GenePix Pro. 4.0. The standard deviation of each clone was calculated. The signal ratio was regarded as "abnormal" where it was outside the ±3SD range in both CGH1 and CGH2.

Copy number polymorphisms (CNPs) found in this study are shown in Figure 1. They were either the same as those found in previous studies [Miyake et al., 2006a,b] or registered in database (Database of Genomic Variants: http://projects.tcag.ca/variation/).

FISH Analysis

BAC/PAC DNA was labeled with SpectrumGreen™-11-dUTP or SpectrumOrange™-11-dUTP (Vysis/Abott) by nick translation, and denatured at 70°C for 10 min. Probe-hybridization mixtures (15 μl) were applied on chromosomes, incubated at 37°C for

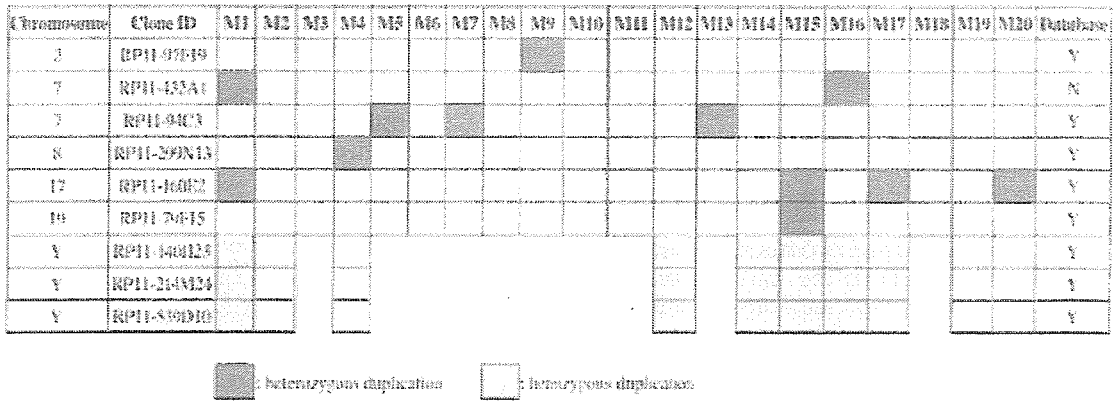

FIG. 1. Copy number polymorphisms (CNPs) encountered in the present study. Ys in the right column stand for those registered in the Database of Genomic Variants (http://projects.tcag.ca/variation/), while Ns indicate those found in our previous studies [Miyake et al., 2006a,b]. Gray box indicates CNP duplication.

16–72 hr, then washed and mounted in antifade solution (Vector, Burlingame, CA) containing DAPI. Fluorescence photomicroscopy was performed as described previously [Miyake et al., 2003].

Multiple Ligation-Dependent Probe Amplification (MLPA) Analysis

Multiple ligation-dependent probe amplification analysis was performed using SALSA P070 and P036B

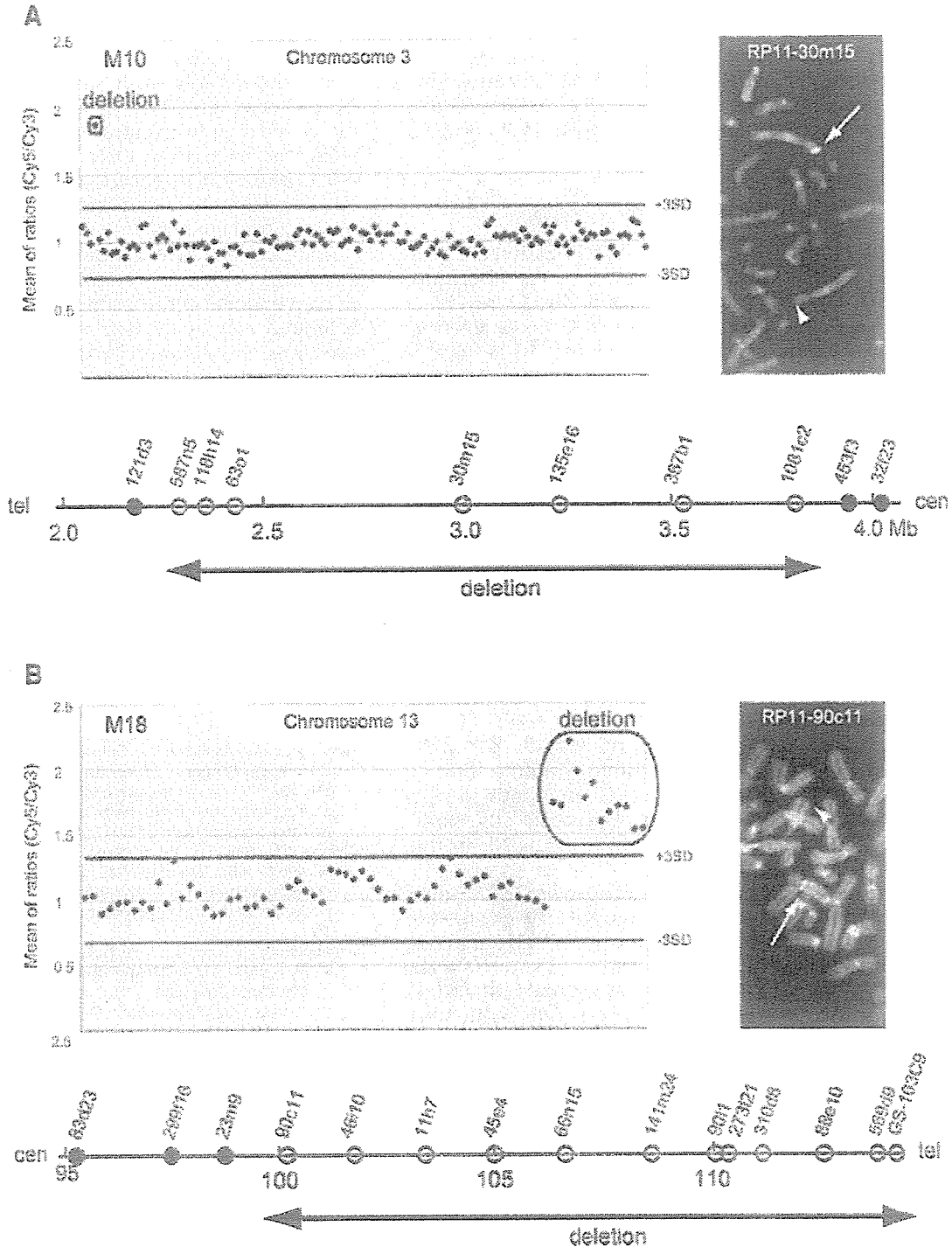

FIG. 2. Array CGH and FISH analyses in M10 (**A**) and M18 (**B**). Hybridization ratios of normal sex-matched control DNA (Cy5) and sample DNA (Cy3) are indicated as a function of Cy5/Cy3 signal-intensity (**left top**). Note that the ratios of deleted clones are above +3SD. FISH analyses (**right top**) show intact (arrows) and deleted (arrowheads) signals. Clones in the summarized scheme (bottom) are from human RPCI-11 library except for GS-163c9 (purchased from Genome Systems, St. Louis, MO).

human telomere kits (MRC-Holland, Amsterdam, The Netherlands) and according to the provider's protocol. The ligation products were amplified by PCR using the common primer set with the 6-FAM label distributed by the supplier. Amplification products were identified and quantified by capillary electorphoresis on an ABI 3100 genetic analyzer, using Gene Mapper software (all from Applied Biosystems, Foster City, CA). The peak areas of the PCR products were determined. The results in the patient were compared with those in a normal control.

RESULTS AND DISCUSSION

Array comparative genomic hybridization analysis of 20 G-banding normal spontaneous abortions (10 cases of 46,XX and 10 cases of 46,XY) revealed deletions in two samples. Sample M13 had a deletion at 3p26.2, corresponding to clone RP11-30m15 (Fig. 2A). FISH analysis indicated the deletion extending 1.4-Mb at 3p26.2-p26.3, from RP11-587n5 to RP11-1081c2 (UCSC coordinates, chromosome 3 nucleotide 2321725-3744837). Its karyotype was therefore 46,XX,del(3)(p26.2p26.3). The deletion overlapped with the 4.5-Mb critical region for 3p deletion syndrome (from D3S3630 to D3S1304, UCSC coordinates, chromosome 32575496-6994583) [Cargile et al., 2002], and contained at least four genes, *CNTN4, IL5RA*, *TRNT1*, and *CRBN*. *CNTN4* disruption by a balanced chromosomal translocation resulted in developmental delay and several anomalies were found in the 3p deletion syndrome [Fernandez et al., 2004]. A 0.2-Mb duplication containing *TRNT1* and *CRBN* (Locus 0327, UCSC coordinates chromosome 3 nucleotide 3134219-3326584) (Database of Genomic Variants: http://projects.tcag.ca/variation/) has been identified as a CNP [Shaw-Smith et al., 2004]. Deletion of the same region has never been registered as a CNP in the database.

Sample M18 had a 13.7-Mb deletion at 13q32.3-qter corresponding to 12 clones from RP11-90c11 to GS-163c9 (Fig. 2B), and confirmed with FISH analysis (UCSC coordinates, chromosome 13 nucleotide 100477931-114103243). Reexamination of G-banded chromosome slides showed two cells with 13q deletion and 18 cells with 46,XX chromosomes. The 46,XX cells were most likely derived from contaminating decidual cells. Duplication of GS-52m11, an 18p subtelomeric clone, was also noted in M18. The duplication, however, was not validated with FISH analysis using GS-52m11 as a probe, possibly because the duplicated FISH signals fused with each other. MLPA analysis also failed to support the duplication, even though the probes used for the analysis (*USP14* and *THOC1*) did not exactly represent the region of GS-52m11 (90–140 kb centromeric).

It would be fair to exclude M18 from our study of chromosomally normal abortions, because it had a G-banding detectable deletion that was masked by the growth of intermingling decidual cells. This will leave M13 as the only submicroscopic imbalance in the present series, giving a frequency of 1 in 19 abortions (5%). This is close to one submicroscopic duplication in 25 chromosomally normal abortions (4%) detected in a study using targeted microarray [Schaeffer et al., 2004]. In an analysis of abortions that failed to grow in culture, Benkhalifa et al. [2005], using the microarray with 1-Mb resolution, found one case each of duplication and deletion of a single clone among 13 abortions without aneuploidies, giving a frequency of 15%.

Array comparative genomic hybridization has an advantage of higher resolution over that (5–10 Mb) in conventional chromosome analysis. Its resolution is limited only by the size of the large-insert clones used and the distance between clones. It must be asked, however, whether the phenotypic effects of dosage difference in a few clones are severe enough to lead to spontaneous abortions.

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Clinical Report

Mild Craniosynostosis With 1p36.3 Trisomy and 1p36.3 Deletion Syndrome Caused by Familial Translocation t(Y;1)

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We report on a 20-year-old man and a 16-year-old woman with a chromosomal imbalance derived from a balanced translocation, t(Y;1)(q12;p36.3) of the father. The man had a partial trisomy for 1p36.3-pter [46,X,der(Y)t(Y:1)(q12;p36.3)] and mild craniosynostosis of metopic and sagittal sutures as well as a borderline mental impairment, while the woman with a deletion for 1p36.3-pter [46,XX,der(1)-t(Y;1)(q12;p36.3)] showed dysmorphic face with large anterior fontanel and severe developmental delay. Fluorescence in situ hybridization (FISH) showed that his trisomy spanned the 5.3-Mb region from 1p telomere harboring the critical region for craniosynostosis. To our knowledge, the man is the first case of a pure type of simple 1p36.3 trisomy as the effect of heterochromatic Yq12-qter deletion likely does not affect phenotype. © 2006 Wiley-Liss, Inc.

Key words: 1p36.3; monosomy; trisomy; craniosynostosis; familial translocation

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INTRODUCTION

Subtelomeric chromosomal rearrangements have been widely recognized as one of major causes of unexplained mental retardation [De Vries et al., 2003]. Among these, 1p36.3 deletion syndrome is the most common [Shaffer and Lupski, 2000; Battaglia, 2005], but 1p36.3 duplication is extremely rare. To the best of our knowledge, two cases of 1p36.3 complex rearrangements including deletion, duplication, and triplication were well described [Gajecka et al., 2005] and another of der(10)t(1;10)(pter;qter) was found among 11,688 patients with developmental disabilities and briefly documented [Ravnan et al., 2005].

Here we describe a family of a man having 1p36.3-pter trisomy and his sister with 1p36.3 monosomy due to a paternal balanced chromosomal translocation, t(Y;1). Detailed cytomolecular analysis along with clinical information will be described.

CLINICAL REPORTS

Patient 1

The 20-year-old man was the first child of non-consanguineous healthy parents. The pregnancy was uneventful. He was born at 39 weeks of gestation

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by vaginal delivery. His birth weight was 3,250 g (+0.4 SD), length 51 cm (+0.9 SD), and OFC 33 cm (−0.3 SD). Family history described his atomic-bombed paternal grandmother lost her 1-year-old daughter (the patient's paternal aunt) for an unknown cause. He was referred to us at age 9 months for evaluation of his developmental delay. He could not sit without support. He had a mild slopping forehead with mild bitemporal narrowing and large hands. His weight was 8.0 kg (−0.8 SD) and OFC 43.5 cm (−1.0 SD). He could walk alone at age of 15 months. He spoke one-word sentences at age of 23 months and three-word ones at $3^{2}/_{12}$ years. His DQ at 3 years was scored as 80. He had not attended our hospital since then. At age 20 years, he and his sister returned to our hospital with the parents for evaluation. His height was 172 cm (+0.3 SD), weight 65 kg (+0.8 SD), and OFC 58.5 cm (+1.0 SD). He presented with slopping forehead, bitemporal narrowing, bilateral mild blepharophimosis, left ptosis, and large hands (Table I and Fig. 1A). Brain MRI did not identify any intracranial abnormalities. The 3D-computed tomography showed the mild metopic and sagittal craniosynostosis (Fig. 1A). Mental development was judged as a borderline impairment. He graduated from a regular high school and a technical school afterward with low to lowest scores in all subjects and now stays at home without working. The secondary sexual characteristics were normal.

Patient 2

She was born vaginally at 40 weeks of gestation as a younger sister of the Patient 1 after uneventful pregnancy. Her birth weight was 2,060 g (−2.4 SD), length 48 cm (−0.2 SD), and OFC 32 cm (−0.8 SD). She was referred to us at her age of 16 years for evaluation of her severe developmental delay with self-injurious behaviors. Her weight was 32.4 kg (−2.6 SD), height 132 cm (−5.0 SD), and OFC 51 cm (−2.9 SD). She showed specific clinical features including pre- and post-natal growth delay, post-natal microcephaly, characteristic face, large anterior fontanel by late closing, prominent forehead, deep-set eyes, short and downslant palpebral fissures, flat nasal bridge, cleft palate, midface hypoplasia, pointed chin, thickened ear helices, small hands and feet, scoliosis, and congenital heart defect (ventricular septal defect) as well as severe developmental delay (DQ = 6). Because of these features, 1p36.3 monosomy syndrome was strongly suspected (Fig. 1A).

MOLECULAR CYTOGENETIC ANALYSIS

G-banded chromosomal analysis of peripheral blood lymphocytes from Patient 2 revealed that her karyotype was 46,XX,der(1)t(Y;1)(q12;p36.3) (Fig. 1B). The father and Patient 1 showed 46,X,t(Y;1)(q12;p36.3) and 46,X,der(Y)t(Y;1)(q12;p36.3),

TABLE I. Clinical Features of Patients With 1p36.3 Duplication/Triplication

	Patient 1	Subject 69 Gajecka et al. [2005]	Subject 71[a] Gajecka et al. [2005]	Case 306[b] Ravnan et al. [2005]
Age	20 years	$2^{3}/_{12}$ years		3 years
Sex	Male	Female		Male
IUGR	−	−		
Growth retardation	−	+ (<3 percentile)		
MR	Border line	Moderate ~ mild	Severe	
Craniofacial features	Dysmorphic	Dysmorphic	Dysmorphic	Dysmorphic
pCraniosynostosis	Metopic and sagittal	Metopic (severe)	Sagittal and coronal	
Microcephaly	−	+	+	+
Dilatation of lateral ventricles	−		Mild	
Bitemporal narrowing	+	+		
Slopping forehead	+	+		
Blepharophimosis	+ (Mild, bilateral)	+ (Operated)		
Blepharoptosis	+ (Left)		+ (Bilateral)	
Hypertelorism	−		Mild	
Intermittent exotropia	−		+	
Hypermetropia	−		+	
Upturned nose	−		+	
ASD	−	+	+	
PDA	−	−	+	
Others	Large hands	Rectal stenosis Syndactyly (2–3 toes)	Anterior placement of anus Sacral dimple with tethered cord 13 pairs of ribs Hypotonia at birth Swallowing dysfunction	

[a]19q subtelomeric trisomy was also involved.
[b]1p36.3 trisomy due to der(10)t(1;10)(pter;qter).

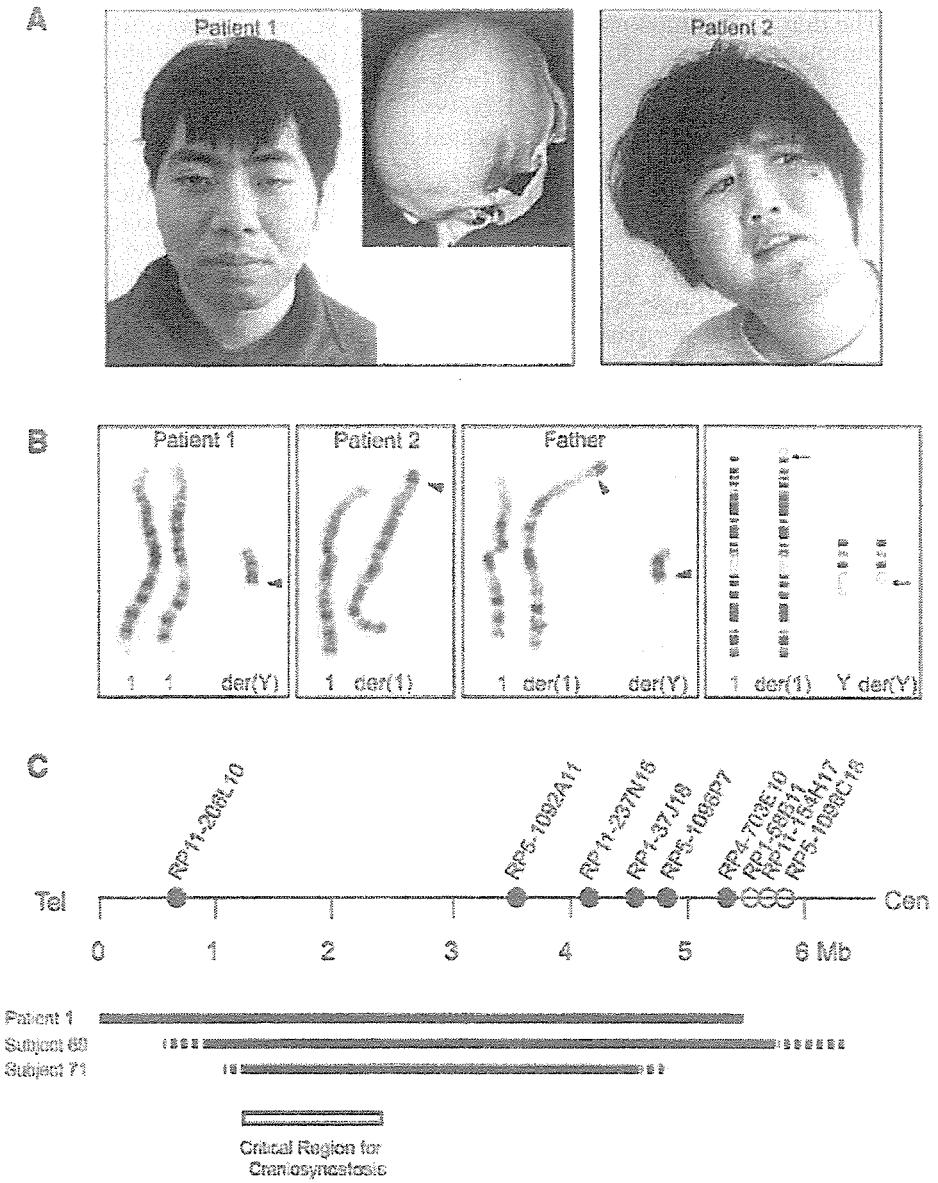

FIG. 1. **A**: Facial appearance of Patients 1 and 2 and 3D-CT image of the cranium of Patient 1, demonstrating mild metopic and sagittal ridges. **B**: Partial karyotype of Patients 1 and 2, and their father as well as ideograms of chromosomes 1, der(1), Y, and der(Y). Arrow (head) indicates a breakpoint. **C**: Schematic presentation of duplicated/triplicated regions of Patient 1, and Subjects 69 and 71 [Gajecka et al., 2005]. Solid line indicates confirmed duplicated/triplicated region, and dotted shows unconfirmed region. White line is a critical region for craniosynostosis [Gajecka et al., 2005].

respectively (Fig. 1B). The mother was 46,XX. Initial chromosomal analysis of the father and Patient 1 strongly suggested that the Yq heterochromatin was translocated to 1p36.3 as the der(Y) possessed little heterochromatin in both of them. Thus unbalanced chromosomal translocations found in Patients 1 and 2 were of paternal origin.

FISH analysis of Patient 1 was done using BAC/PAC clones mapped to 1p36.3 (RP11-206L10, RP5-1092A11, RP11-237N15, RP1-37J18, RP5-1096P7, RP4-703E10, RP1-58B11, RP3-491M17, RP11-154H17, and RP5-1098C18) and Y (RP11-236J18, RP11-263C17, and RP11-945P24) in combination with commercial probes such as D1Z2 (Oncor, Inc., Gaithersburg, MD), DYZ2 (Mitsubishi Kagaku BCL, Inc., Tokyo, Japan), Xq/Yq telomere probe (Vysis, Inc., Downers Grove, IL), and DAZ probe (Mitsubishikagaku BCL, Inc.). The 1p36.3 trisomy spanned the 5.3-Mb region from 1pter to RP4-703E10 (UCSC genome browser coordinate, chromosome 1 nucleotide 0–5319133), including the 1.1-Mb critical region harboring the dosage-sensitive gene(s) for craniosynostosis/large, late-closing anterior fontanel [Gajecka et al., 2005]

(Fig. 1C). The der(Y) chromosome did retain all the three euchromatic probes (RP11-236J18, RP11-263C17, and RP11-945P24) (UCSC genome browser coordinate, chromosome Y nucleotide 0–27068336), thus the breakpoint in der(Y) should be located at Yq12 (Fig. 1B).

DISCUSSION

1p36 monosomy is now a well-recognized syndrome with distinct congenital anomalies and accounts for 0.5–0.7% of idiopathic mental retardation [Giraudeau et al., 2001; Battaglia, 2005]. 1p36.3 deletion variably consists of pure terminal deletions, interstitial deletions, derivative chromosomes, and complex rearrangements basically with no single common breakpoint [Heilstedt et al., 2003], making the molecular mechanisms for generation of 1p36.3 monosomy obscure. As some familial unbalanced translocations were reported to result in 1p36.3 monosomy, a different pattern of chromosomal segregation is supposed to cause 1p36.3 trisomy, but its prevalence is extremely rare. This may be partly explained by mild phenotype of 1p36.3 trisomy like our Patient 1. He had not been followed by us until age 20 years, when his sister with severe developmental delay was referred and diagnosed as 1p36.3 monosomy syndrome. If the duplicated region does not contain the dosage-sensitive craniosynostosis critical region, or if the duplication contains the region but with the incomplete penetrance of the craniosynostosis phenotype, it may be very difficult to positively recognize the 1p36.3 trisomy phenotype unless any overt craniosynostosis is present.

Patient 1 and Subject 69 (which was initially described as a case of a pure 1p36.3 duplication by Heilstedt et al. [1999] but was later re-evaluated by Gajecka et al. [2005]) with similar duplication/triplication region share clinical features including metopic craniosynostosis, bitemporal narrowing, slopping forehead, blepharophimosis, and borderline to mild mental retardation. Subject 71 with a similar duplication/triplication showed more severe MR and sagittal and partial coronal synostosis, not metopic (Fig. 1C). This difference may be partly due to an associated larger 1p36.3 deletion (approximate 1.2 Mb from the 1p telomere) in Subject 71 than that in Subject 69. Case 306 was only described to have microcephaly and dysmorphic features [Ravnan et al., 2006], but microcephaly was also noted in the two patients, subjects 69 and 71 [Gajecka et al., 2005] (Table I). As 1p36.3 monosomy syndrome showed large and late-closing anterior fontanel, it is obvious that the 1p36.3 region may contain the gene(s) responsible for the suture closure of cranial bones in gene-dosage-dependent manner (that is, late closure if deleted and early if duplicated or triplicated). The critical region from RP5-890O3 to RP4-758J18 for the suture closure was determined by combination of duplicated and triplicated regions associated with craniosynostosis [Gajecka et al., 2005], but duplication (not triplication) containing the region in Patient 1 was found to be sufficient at least for the metopic and sagittal synostosis. It is also interesting to note the large hands in Patient 1 and small hands and feet in Patient 2. The duplicated region in Patient 1 may also contain the region affecting the size of limbs with analogy to the gene-dosage-dependent craniosynostosis region.

In conclusion, we found a patient with 1p36.3-pter trisomy and his sister with 1p36.3-pter monosomy due to the paternal chromosomal translocation. It should be noted that mild craniosynostosis and borderline mental retardation in the trisomic patient was overlooked until his sister with evident 1p36.3 monosomy features was properly diagnosed. Trisomy or duplication of chromosomal subtelomeric regions should be kept in mind in patients with the milder clinical phenotypes. Array CGH or other techniques may help determine how such changes contribute human phenotypes.

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Clinical Report

Trigonocephaly in a Boy With Paternally Inherited Deletion 22q11.2 Syndrome

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Deletion 22q11.2 syndrome is a well-known contiguous gene syndrome, for which the list of findings is extensive and varies from patient to patient. We encountered a unique patient who had a familial 3-Mb deletion 22q11.2 associated with trigonocephaly derived from craniosynostosis of the metopic suture. Almost all the symptoms of the patient, including polymicrogyria, microcephaly, facial abnormalities, internal anomalies, seizures, and mental retardation, were compatible with deletion 22q11.2 syndrome, except for synostosis of the metopic suture. This is the first report of a relationship between deletion 22q11.2 syndrome and trigonocephaly. Craniosynostosis of the metopic suture might be a minor complication of deletion 22q11.2, although coincidental occurrence cannot be ruled out.

Key words: familial; deletion 22q11.2; trigonocephaly; metopic suture; polymicrogyria

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INTRODUCTION

Deletion 22q11.2 syndrome is well documented and its etiology has been well established. It occurs at a frequency of 1 in 4,000 live births [Emanuel et al., 2001], and has been identified in DiGeorge syndrome, velocardiofacial syndrome, and conotruncal anomaly face syndrome. The list of symptoms is extensive and varies from patient to patient. Homologous recombination events between low copy repeats (LCR) mediate the same 3-Mb deletion in 80%–90% of the patients [Spiteri et al., 2003]. McDonald-McGinn et al. [2005] reported four patients with a deletion 22q11.2 who presented craniosynostosis of the coronal suture. Recently, we encountered a unique patient with a familial deletion 22q11.2 associated with trigonocephaly derived from craniosynostosis of the metopic suture rather than the coronal suture. This is the first report of a relationship between deletion 22q11.2 and trigonocephaly.

CLINICAL REPORT

The patient, a boy, was born to non-consanguineous parents at 36 weeks of gestation, weighing 2,300 g (−0.7 SD), measuring 44.5 cm (−1.1 SD), and with OFC of 31 cm (−0.8 SD). Heart murmurs were noted, and cardiac examination revealed a ventricular septal defect, double-chambered right ventricle, and mitral regurgitation. At age 5 months, he was referred to us because of trigonocephaly. His OFC was 40.5 cm (−1.4 SD). He had a face with almond-shaped palpebral fissures, hypertelorism, edematous

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