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A 1-Mb critical region in six patients with 9q34.3 terminal deletion syndrome

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Abstract Patients with 9q34.3 terminal deletion usually show a clinically recognizable phenotype characterized by specific facial features (microcephaly, flat face, arched eyebrows, hypertelorism, short nose, anteverted nostrils, carp mouth and protruding tongue) in combination with

severe mental retardation, hypotonia, and other anomalies. We analyzed six unrelated patients with a various 9q34.3 terminal deletion. While having different-sized 9q34.3 deletions, all of these patients shared several distinctive anomalies. These anomalies are likely to arise from a commonly deleted region at distal 9q34.3. Fluorescence in situ hybridization (FISH) analysis using a dozen BAC clones mapped at the 9q34.13-q34.3 region defined the shortest region of deletion overlap (SRO) as a 1-Mb segment proximal to 9qter containing eight known genes. Possible candidate genes delineating specific phenotypes of the 9q34.3 terminal deletion syndrome are discussed.

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Introduction

Subtelomeric chromosomal rearrangements comprise about half of segmental aneusomies and are one of the major causes of mental retardation (MR). A recent review showed that cryptic subtelomere rearrangements were detected in 131 (5.1%) of 2,585 children with MR (Flint and Knight 2003) and submicroscopic deletions of 4p, 5p, 9p, 16p, 17p, 1p, 2q, and 22q were frequently observed (De Vries et al. 2003). We previously reported five unrelated patients with a 9q34.3 deletion (Dawson et al. 2002; Iwakoshi et al. 2004). In addition, we found another, which was previously reported as a MCA/MR case of satellited chromosome 9 (Harada et al. 1989). All of these patients shared several distinctive anomalies, which are likely to have their origin in a deletion overlap region at distal 9q34.3. Definition of shortest region of deletion overlap (SRO) is therefore very important to delineate the critical region for 9q34.3 terminal deletion syndrome. In the present study, we identified the SRO as a 1-Mb segment proximal to 9qter and discuss candidate genes likely to be responsible for the clinical features manifested in the 9q terminal deletion syndrome.

Furthermore, haplotype analysis in order to determine parental origin of the deletion was performed in three patients.

Materials and methods

Subjects

We analyzed six patients with 9q34.3 terminal deletions, and their karyotypes were 46,XY,der(9)t(9;22)(q34.3;q11.21)de novo (Case A: originally described as a case report by Harada et al. 1989), 46,XX, ish del(9)(q34.3)de novo (Case B: described as Patient 1 in Iwakoshi et al. 2004), 46,XY, ish del(9)(q34.3)de novo (Case C: described as N03R003 and Patient 2 in Harada et al. 2004 and Iwakoshi et al. 2004, respectively), 46,XX, ish der(9)t(5;9)(q35.3;q34.3)pat (Case D: described as Patient 1 in Dawson et al. 2002), 46,XX, ish del(9)(q34.3)de novo (Case E: described as Patient 2 in Dawson et al. 2002), and 46,XY, ish der(9)t(6;9)(p25;q34.3)de novo (Case F: described as Patient 3 in Iwakoshi et al. 2004). Clinical features of Cases B, C, D, E, and F were previously described in detail (Iwakoshi et al. 2004). Case A was clinically reevaluated in the present study because 9q34.3 terminal deletion was newly confirmed (Table 1).

Fluorescence in situ hybridization (FISH) analysis

Fluorescence in situ hybridization (FISH) analysis was performed using 13 RPCI-11 human BAC clones (RP11-89B23, 92B21, 265M2, 166H7, 98L5, 432I20, 83N9, 370H5, 769N4, 350O14, 974F22, 417A4, and 424E7) mapped at 9q34.13-q34.3 according to Human Genome Browser 2003 July version (<http://www.genome.ucsc.edu/cgi-bin/hgGateway?db=hg11>) and a subtelomeric BAC clone, GS-135I17 (Knight et al. 2000) (Fig. 1). BAC clone DNAs labeled with either SpectrumOrange-dUTP or SpectrumGreen-dUTP (Vysis, Downers Grove, IL, USA) by nick translation were denatured and hybridized to metaphase chromosomes of patients at 37°C for 16 h and washed. In order to confirm the common telomeric structure of deleted chromosomes, FISH using telomere-specific probes containing TTAGGG repeats generated by PCR (Ijdo et al. 1991) and labeled with a similar manner as BACs was performed in Cases E and F. Fluorescence images were captured using a monochrome CCD camera (Carl Zeiss, Jena, Germany) on Axioplan 2 fluorescence microscope (Carl Zeiss) with appropriate filters and were analyzed with ISIS version 3.4.5 software (MetaSystems, Altlußheim, Germany).

Haplotype analysis

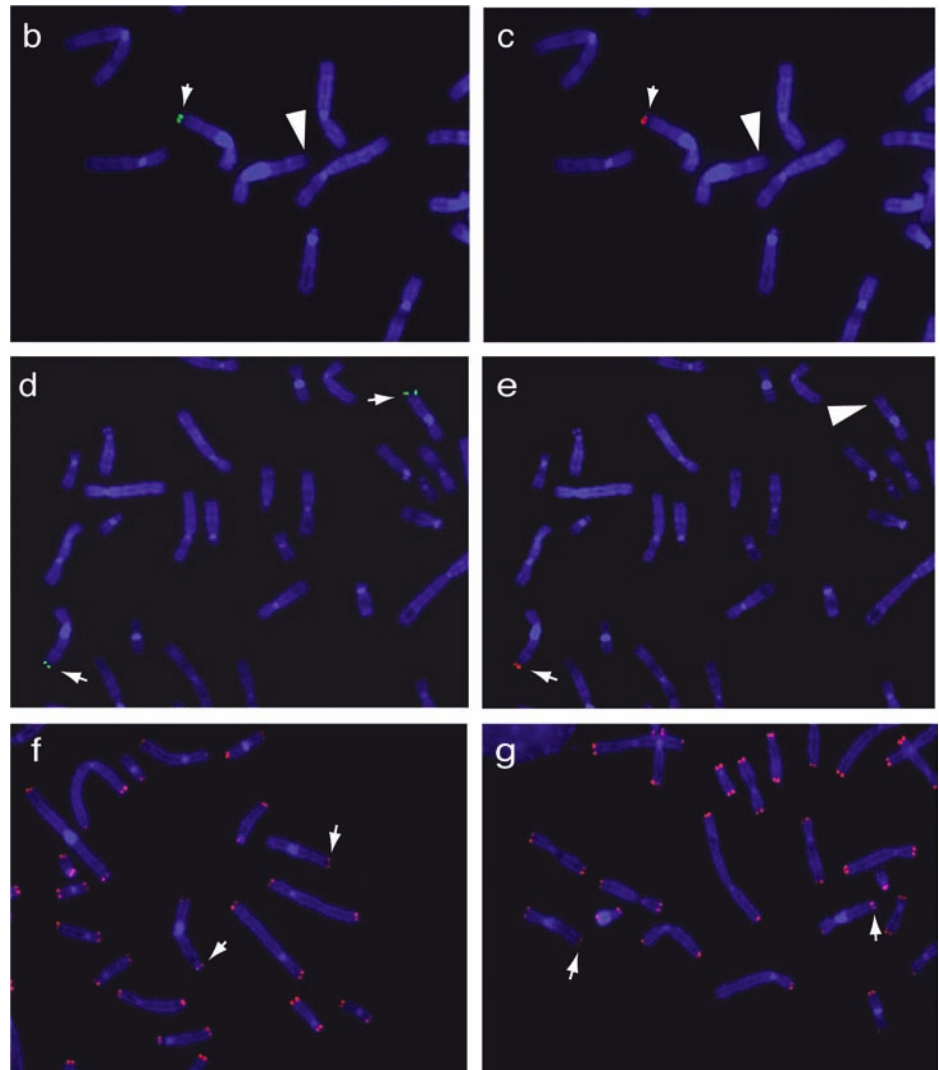
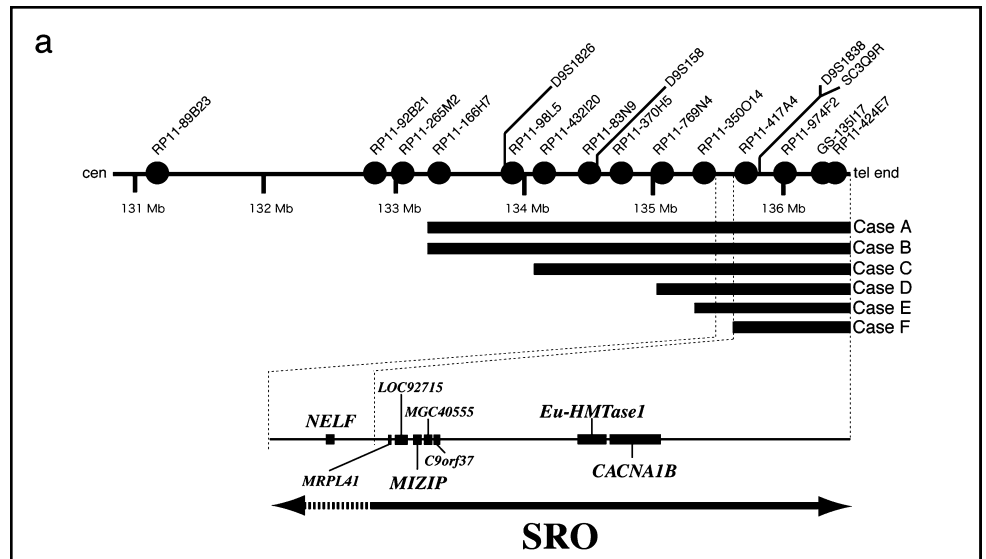
In order to confirm parental origin of 9q deletion, we performed haplotype analysis using available parental

Table 1 Clinical manifestations of a newly evaluated case (Case A) and other reported cases (Cases B–F) with 9q34.3 terminal deletion in this study. *HOCM* hypertrophic obstructive cardiomyopathy. Common features in six patients were shown in bold face

	Case A	Cases B–E
Gender	Male	Male (2)/female (3)
Flat face	+	5/5
High forehead	–	2/4
Arched eyebrows	+	5/5
Synophrys	–	3/5
Hypertelorism	+	5/5
Down-slanted palpebral fissures	–	2/5
Flat nasal root	+	3/5
Short nose	+	5/5
Anteverted nostrils	+	5/5
Short philtrum	+	2/5
Open carp mouth	+	5/5
Thin upper lip	+	4/5
Thick lower lip	+	3/3
Protruding tongue	+	3/3
Micrognathia	–	4/5
Pointed chin	–	3/3
Prognathism	–	1/3
Low-set ears	–	1/3
Malformed ears	–	3/5
Exudative otitis media	–	1/3
Partial alopecia	–	3/3
Brown thin soft hair	–	3/3
Abnormal toes	–	2/3
Cardiac anomaly	HOCM	VSD (3)/heart murmur (1)
Asthma	–	2/3
Anal atresia	–	1/3
Obesity	+	1/5
Hypothyroidism	–	1/3
Hypotonia	+	5/5
Epilepsy	–	3/5
MR	Severe	Severe (5)
Autistic behavior	–	3/4

samples of three cases (B, C, and F) by use of two STR markers located within the deleted region. Sequence information of PCR primers of *D9S1838* were described elsewhere (Cormier-Daire et al. 2003). Primer sequence of SC3Q9R, TGCTGTCAAAGGACCTACCC for forward and ACCCCATTCTGAACAAAGCA for reverse were originally designed based on the sequence information from the NCBI Homo sapiens genome view build 33 April 2003 database. PCR was performed in a 20 µl mixture containing 50–75 ng genomic DNA, 1 µM of fluorescent labeled primer and reverse primer, 0.5 U Takara *Ex Taq* polymerase (Takara Bio, Ohtsu, Japan), 0.2 mM each dNTP and 1X *Ex Taq* Buffer (Takara Bio). PCR conditions included initial denaturation at 98°C for 2 min, 35 cycles of 98°C for 10 s, 55°C for 30 s, 72°C for 30 s, and a final extension of 72°C for 7 min. PCR products were loaded for electrophoresis in an ABI Prism 377 DNA sequencer (Applied Biosystems, Tokyo, Japan) and analyzed by the manufacturer-supplied GeneScan Analysis and Genotyper Software (Applied Biosystems).

Fig. 1 Fluorescence in situ hybridization (FISH) studies confirming the shortest region of deletion overlap (SRO) in six cases of 9q34.3 terminal deletion. **a** Schematic presentation of the SRO. Top row depicts BAC clones and STR markers used in this study and their genomic location. *Solid thick bars* from top to bottom indicate deleted segments of Cases A–F, respectively. *Bi-directional arrow* indicates the SRO. Eight known genes exist within the SRO according to the Human Genome Browser, 2003 July version (<http://www.genome.ucsc.edu/cgi-bin/hgGateway?db=hg11>). *cen* centromere, *tel end* telomeric end. Results of FISH studies revealing the 1 Mb of minimal deletion: **b** and **c** show FISH studies using RP11-350O14 (*Green*) and RP11-417A4 (*Orange*) in Case E, respectively. **d** and **e** show FISH results using RP11-350O14 (*Green*) and RP11-417A4 (*Orange*) in Case F, respectively. *Arrow* and *arrowhead* indicate existence of a signal and absence of a signal, respectively. **f** and **g** show the results of FISH using the telomeric-repeat probe in Cases E and F, respectively, indicating that the common telomeric structure was preserved in both cases



Results and discussion

The FISH analysis revealed that the SRO was defined as a region within 1-Mb from the 9q telomere end (Fig. 1a,b,c,d,e). In addition, the intact common telomere structure with TTAGGG repeats was confirmed in Case E (Fig. 1f) and Case F (Fig. 1g). The SRO is likely to be responsible for typical facial features (flat face, arched eyebrows, hypertelorism, short nose, anteverted nostrils, and open carp mouth), hypotonia, and MR, as all six cases shared them (Table 1). Cormier-Daire et al. (2003) reported a de novo deletion of chromosome 9q34 in two unrelated MR children with early onset of obesity, distinctive facial features, sleep disturbance, and behavioral problems. Their FISH and STR analysis revealed that both children carried a similar-sized deletion of 3 Mb. However, these 3-Mb sized regions were not precisely defined due to lack of informative STR markers in the region between D9S1826 and D9S1838, and FISH analysis of this region was not performed. The SRO identified in this study contains several known genes (Fig. 1a). Among these, *NELF*, *MIZIP*, *Eu-HMTase1* and *CACNA1B* could be functionally important, but no mutations associated with human diseases have been reported. *NELF* encodes nasal embryonic LHRH factor (OMIM *608137). Murine *Nelf* is expressed in peripheral and central nervous systems during embryonic development, including olfactory sensory cells and LHRH cells, and is suggested to play roles in guidance for olfactory axon projections and neurophilic migration of LHRH cells (Kramer et al. 2000). Although the patients we are describing were not reported to have abnormal olfaction, careful olfactory evaluations may be necessary. *MIZIP* encodes a melanin-concentrating hormone receptor 1 interacting zinc-finger protein and is expressed ubiquitously, although a higher expression in the brain is reported (Bachner et al. 2002). *MIZIP* deletion may contribute to MR of 9q34.3 deletion syndrome. Euchromatic histone methyltransferase 1 (*Eu-HMTase1*, OMIM *607001) is a component of the E2F transcription factor 6 (*E2F6*, OMIM *602944) (Trimarchi et al. 1998) complex that represses transcription. *Eu-HMTase1* methylates lys-9 of histone H3, resulting in epigenetic transcriptional repression by recruiting heterochromatin protein 1 to methylated histones (Ogawa et al. 2002) and may maintain normal

brain function/development. The calcium channel, voltage-dependent, N-type, alpha-1B subunit gene (*CACNA1B*, OMIM *601012) encodes an N-type calcium channel involving neurotransmitter release from neurons (Williams et al. 1992). Recently, Maeno-Hikichi et al. showed that enigma-like LIM domain protein interacts with protein kinase C-epsilon and *CACNA1B* to form a PKC ϵ -ENH-Ca²⁺ channel complex (Maeno-Hikichi et al. 2003). Heterozygous deletion of *CACNA1B* may result in the reduction of N-type channel activity and can be a contributing factor for MR and/or epilepsy.

An autosomal recessive neurological disorder, Joubert syndrome type 1 (JBTS1, OMIM #213300), was mapped close to *D9S158* at 9q34.3 (Saar et al. 1999). *D9S158* was identified within RP11-83N9, which was deleted in three of our six cases. JBTS1 presents with several clinical features similar to the 9q34.3 deletion syndrome. MR, hypotonia, and protruding tongue are observed in both syndromes, but cerebellar anomalies or ataxia were not evident in the 9q34.3 deletion syndrome. As the JBTS1 locus overlaps with the SRO shown in this study, it is possible that the 9q34.3 deletion syndrome phenotype might be caused by unmasking of a recessive allele for JBTS1. Although the linkage to 9q34.3 in JBTS1 is described in a single family so far, further study is necessary.

The results of the haplotype analysis in addition to previous studies regarding paternity of deletion are shown in Table 2. In Case A, paternal inheritance of his derivative chromosome 9 was traced by use of chromosomal polymorphism as a marker. In Case D, his derivative chromosome 9 originated from a paternal translocation. Cormier-Daire et al. (2003) reported a maternal origin of de novo 9q terminal deletion in one of two cases analyzed, while the other was paternal. We detected a parental origin of deletion in four of six cases in this study. The origin in the other two remains to be elucidated. Both paternal and maternal deletions have been found (Table 2), being supported by the fact that imprinting effects have not been established for chromosome 9 (Ledbetter and Engel 1995).

In conclusion, we delimited the SRO of the 9q34.3 terminal deletion syndrome to a region within 1-Mb from the 9q telomeric end that contains several genes important for human development. It remains to be

Table 2 Parental origin of 9q34.3 terminal deletion. *Pat* paternal inheritance, *Mat* maternal inheritance, *ND* not done, *NI* not informative

Patient	Karyotype	Conclusion	Detection method (informative marker)
Case A	der(9)t(9;22)(q34.3;q11.21)de novo	Pat	Chromosomal polymorphism
Case B	ish del(9)(q34.3)de novo	Pat	DNA polymorphism (D9S1838, SC3Q9R)
Case C	ish del(9)(q34.3)de novo	Pat	DNA polymorphism (D9S1838, SC3Q9R)
Case D	ish der(9)t(5;9)(q35.3;q34.3)pat	Pat	Chromosomal origin
Case E	ish del(9)(q34.3)de novo	-	ND
Case F	ish der(9)t(6;9)(p25;q34.3)de novo	NI	DNA polymorphism (D9S1838, SC3Q9R)
Case 1 ^a	ish del(9)(q34.3)de novo	Mat	DNA polymorphism (D9S1838)
Case 2 ^a	ish del(9)(q34.3)de novo	Pat	DNA polymorphism (D9S1838)

^aCases described by Cormier-Daire et al.2003

investigated whether the SRO as defined by this study is representative for other patients with the 9q34.3 deletion syndrome or can be confined to an even smaller segment.

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