J Hum Genet (2002) 47:262–265

SHORT COMMUNICATION

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Association of autism in two patients with hereditary multiple exostoses caused by novel deletion mutations of *EXT1*

Received: December 20, 2001 / Accepted: February 18, 2002

Abstract Two boys from separate families presented with hereditary multiple exostoses (EXT) and autism associated with mental retardation. Their fathers both expressed a clinical phenotype of hereditary multiple exostoses milder than those of the patients and without the associated mental disorder. The *EXT1* and *EXT2* genes from lymphocytes of the affected individuals were analyzed by using denaturing high-performance liquid chromatography and direct sequencing. A novel deletion mutation, 1742delTGT-G in exon 9 of *EXT1*, causing a frameshift was detected in one boy and his father. Another novel deletion mutation, 2093delTT in exon 11 of *EXT1*, causing transcription termination was detected in the other affected boy and his father. *EXT1* is expressed in the brain, and both EXT1 and EXT2 proteins are associated with glycosyltransferase activities required for the biosynthesis of heparan sulfate, which also has activity in the brain. The coincidental association of mental disorders in the boys was not completely excluded. However, these results suggest the involvement of EXT1 in the development of mental disorders, including mental retardation and autism.

Key words Hereditary multiple exostoses · Mental retardation · Autism · *EXT1* · *EXT2*

Introduction

Hereditary multiple exostoses (EXT; MIM 133700) is an autosomal dominant inherited bone disorder that is characterized by the formation of cartilage-capped exostoses, short stature, and skeletal deformities. Various mutations in *EXT1* on 8q24.1 and *EXT2* on 11p11–12 have been reported in EXT families (Phillippe et al. 1997; Wells et al. 1997; Wuyts et al. 1998; Dobson-Stone et al. 2000; Francannet et al. 2001; Seki et al. 2001). *EXT1* and *EXT2* are thought to be responsible for EXT in more than 70% of EXT patients.

Although the association of developmental disorders with EXT has not been reported to date, tricho-rhino-phalangeal syndrome (TRPS) type II (MIM 150230) is associated with exostoses and mental disorder. TRPS that is characterized by a dysmorphic appearance involving the face, scalp, hair, and skeletal system is assigned to the *TRPS1* gene (Lüdecke et al. 2001), which resides on 8q24.12. Unlike TRPSI and TRPSIII, TRPSII presents with mental retardation (MR) in most cases and a deletion mutation including 8q24.11–q24.13 involving *TRPS1* and *EXT1* has been detected in TRPSII. These findings suggest that some gene other than *TRPS1* in the deletion area is the likely cause of MR, and *EXT1* is one candidate. Ishikawa-Brush et al. (1997) reported a patient who presented with autism, MR, and EXT with a balanced translocation 46,X,t(X;8)(p22.13;q22.1). Two genes at the breaking point were identified, and neither were responsible for the EXT or autism. No information was presented concerning EXT1 in this patient, but the association of exostoses and developmental disorders in this patient suggested that EXT-related genes can play an important role in the developing brain. Here, we describe two Japanese families with a deletion mutation of *EXT1*, wherein the probands showed developmental disorders.

Subjects and methods

Patient 1. Patient 1 was a 5-year-old boy. He began to develop multiple bony tumors in early infancy and was diagnosed as having EXT. He was also diagnosed as having autism, according to DSM-IV criteria (American Psychiatric Association 1994), associated with moderate MR. No dysmorphic phenotype of TRPS was observed. His

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developmental quotient was approximately 40. His father had developed multiple exostoses in his teens, but the father's condition was much milder than that of his son. The father's intelligence was within the normal range. The patient's mother and his brother were unaffected. The paternal grandfather and two brothers had EXT, but detailed clinical information was not available. None of the relatives showed mental disorder.

Patient 2. Patient 2 was a 5-year-old boy. Within a month after birth, he was found to have bony tumors which led to a diagnosis of EXT. He was also diagnosed as having autism associated with mild MR according to the DSM-IV criteria. No dysmorphic phenotype of TRPS was observed. His developmental quotient was 65. His father developed multiple exostoses in his second decade and had normal intelligence. His mother and a sister were unaffected. His father's sister developed EXT in her teens. There was no information about his paternal grandparents. No relatives showed mental disorder.

Denaturing high-performance liquid chromatography (DHPLC) screening. Lymphocytes were obtained from the patients and their parents with the informed consent of the parents, and genomic DNA was extracted. Each exon of the *EXT1* and *EXT2* genes was amplified by polymerase chain reaction (PCR) by using primer sets described by Wells et al. (1997) and Philippe et al. (1997), respectively. Heteroduplex formation was induced by heat denaturation of PCR products at 94°C for 5 min, followed by gradual reannealing from 94° to 25°C over 45 min. DHPLC analysis was performed with the WAVE DNA-fragment analysis system (Transgenomic, Omaha, NB, USA). PCR products were eluted at a flow rate of 0.9 ml/min with a linear acetonitrile gradient. Heterozygous profiles were detected as distinct elution peaks from homozygous wild-type peaks. PCR products shown to be heteroduplexes were subjected to direct sequencing analysis.

Results

We screened all exons of *EXT1* and *EXT2* of two patients by DHPLC. In Patient 1, exon 3 and exon 9 of *EXT1* and the 3′-untranslated region (UTR) of *EXT2* showed heteroduplexes. Direct sequencing showed 1742delTGT-G in exon 9 of *EXT1* (Figure 1B), which caused a frameshift from 581 valine and resulted in a stop codon after five amino acids. A similar DHPLC pattern in exon 9 was detected in the boy's father but not in his mother (Figure 1A), and the 1742delTGT-G deletion was also detected in his father. Direct sequencing of the boy's sample also detected a base substitution, C1065T, in exon 3 of *EXT1*, which has already been reported as a polymorphism, and another, G2210A, in the 3′-UTR of *EXT2*. Both base substitutions were inherited from his mother. We screened for G2210A in the 3′-UTR of *EXT2* in 23 normal controls and detected it in six subjects (data not shown). Patient 2 showed a heteroduplex in exon 11 of *EXT1*, and direct sequencing detected a two-base deletion, 2093delTT (Figure 2B). This deletion caused a frameshift from 698 phenylalanine resulting in a stop codon after 31 amino acids. A similar DHPLC pattern in exon 11 was detected in his father but not in his mother or in a normal control (Figure 2A), and this same 2093delTT deletion was detected in his father, but not in his mother or sister.

Discussion

A novel two-base deletion, 1742delTGT-G in exon 9 of *EXT1*, detected in Patient 1, and another novel two-base deletion, 2093delTT in exon 11 of *EXT1*, detected in Patient 2, were associated with the expression of exostoses in each family. Both two-base deletions induced a frameshift predicted to result in premature termination of the

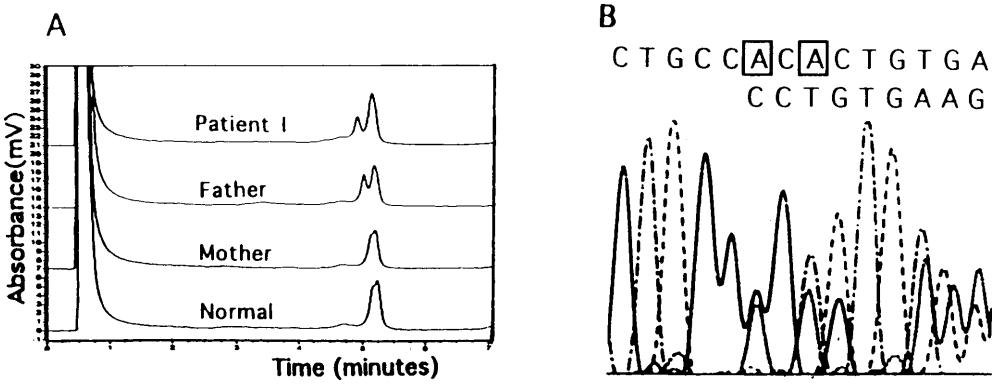

Fig. 1. A The denaturing high-performance liquid chromatography (DHPLC) elution profiles of exon 9 of *EXT1*. Profiles of *Patient 1*, his *parents*, and a *normal control* are shown. **B** The results of direct sequencing of exon 9 of *EXT1* at the deleted site detected in Patient 1. The deleted bases are enclosed in *boxes*. The reverse sequence is shown because the deleted site was too close to the primer-setting region to read in a forward direction. The reverse sequence shows 1742delACA-C. The *lower sequence* is that of the mutated allele

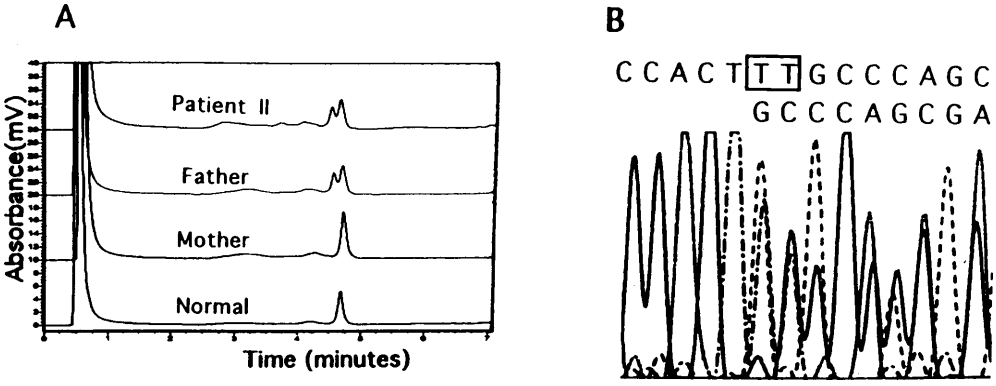

Fig. 2. A The DHPLC elution profiles of exon 11 of *Patient 2*, his *parents*, and a *normal control*. **B** The direct-sequencing data of Patient 2 showed a 2093delTT deletion causing a frameshift mutation (forward sequences are shown). The *box* indicates the deleted bases. The *lower sequence* is that of the mutated allele

translation of *EXT1*. Both exon 9 and exon 11 are in the C-terminal region of *EXT1*. The majority of mutations in *EXT1* have been reported in exon 1, which harbors a membrane-binding region and catalytic domain of the EXT protein, and fewer mutations have been reported in exons in the C-terminal region. In exon 9, five mutations have been reported to date. Despite the fact that the majority of missense mutations have been reported in the N-terminal side of the central region, the H627del mutation in exon 9 has been shown to abolish heparan sulfate synthesis (Cheung et al. 2001). Cheung et al. (2001) suggested that another important functional domain may reside within this region. 1742delTGT-G was at the 581st amino acid, which results in termination before this possibly active site is reached. In exon 11, only one nonsense mutation, C2101T(R701X), has been reported in a Japanese patient (Seki et al. 2001). The 2093delTT deletion was eight bases upstream from this nonsense mutation. The existence of patients with EXT having a mutation in this region suggests that this C-terminal region has important functions, such as in protein–protein interactions (Cheung et al. 2001).

The coincidental association of these mutations with a developmental disorder and EXT cannot be excluded in light of the general susceptibility of males to developmental disorders. The general prevalence of autism is approximately 5/10,000 people, while that of EXT is 1–2/100,000 (Ishikawa-Brush et al. 1997). Thus, the likelihood of a coincidental association of EXT and autism in these two patients is quite low.

EXT1 is expressed ubiquitously in adult tissues, including the brain (data not shown). Despite this ubiquitous nature, functional activity of EXT1 has not been detected except in the actively growing bone. *EXT1* is a putative tumor suppressor gene. The exostoses can be explained by the dysfunction of this tumor suppressor function, which is likely caused by the loss of function of one allele. Both the EXT1 and EXT2 proteins are associated with glycosyltransferase activities required for the biosynthesis of heparan sulfate (McCormick et al. 2000). Heparan sulfate is an important side chain of the proteoglycans, which receives signaling molecules, and modulates a wide spectrum of regulation processes not only in the bony tissues but also in the developing brain (Bandtlow and Zimmermann 2000). The gene homologous to *EXT1* in *Drosophila melanogaster* has been linked to hedgehog signaling (Bellaiche et al. 1998). Therefore, accumulating data suggest that EXT1 is functioning in the developing tissues during embryogenesis. Thus, the association of autism and MR in our two boys with deletion mutations in *EXT1* suggests that the *EXT1* gene is involved in the development of these conditions.

Anticipation has been observed in some families with EXT (Francannet et al. 2001), although the underlying mechanism is not known. Our two families also showed anticipation, with earlier onset in patients than in their fathers. The difference that the patients had autism and mental retardation but their fathers did not may result from some unknown mechanism of *EXT1* or from a combination with some other gene's effect.

To our knowledge, this is the first report of patients who developed developmental disorders in association with EXT. There are several possible explanations for this association. First, the association may be coincidental, although the likelihood is low, as discussed above. Second, in most cases of exostoses, a mild developmental abnormality might be missed due to the lack of follow up by a neurologist or pediatric neurologist. Third, a mutation in a gene other than *EXT1* and *EXT2* may have been involved in the development of the mental disorders. Autism is considered to be a polygene disease. Therefore, EXT1 may be one gene relating to autism pathogenesis. Further studies of the function of EXT proteins in the developing brain and additional pediatric patients with EXT and mental disorders may allow a novel function of EXT proteins to be identified.

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