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BRIEF REPORT — POLYMORPHISM REPORT

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Novel polymorphisms in the βig-h3 gene

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Abstract We found three novel polymorphisms in the βig-h3 gene in patients with gelatinous drop-like corneal dystrophy: (1) a substitution from CTC to CTT at codon 472 that did not alter an amino acid; (2) a substitution from GCG (Ala) to GTG (Val) at codon 480; and (3) a substitution from C to T in intron 10, three nucleotides upstream from the acceptor site of exon 11. The allelic frequencies of the C:T polymorphism at codon 472 and in intron 10 in the Japanese population were estimated to be 0.778:0.222 and 0.954:0.046, respectively. Although the codon 480 substitution was not observed in 54 unrelated healthy Japanese people, the substation did not co-segregate with the disease phenotype, suggesting that this was a rare, non-deleterious alteration.

Key words βig-h3 gene · Polymorphism · Gelatinous droplike corneal dystrophy · Corneal dystrophy · Linkage analysis · Intragenetic marker

Introduction

βig-h3 was isolated as one of the genes induced by transforming growth factor beta in a human adenocarcinoma cell line (Skonier et al. 1992). Missense mutations of this gene are responsible for four kinds of corneal dystrophies; granular dystrophy Groenouw type 1 (CDGG1), Reis-Bucklers dystrophy (CDRB), lattice dystrophy type I (LCD1, a type of corneal amyloidosis), and Avellino dystrophy (ACD) (Munier et al. 1997). Gelatinous droplike dystrophy (GDLD) is a recessively inherited form of corneal amyloidosis (Buchi et al. 1994). To investigate whether abnormality of βig-h3 may be responsible for GDLD, we screened DNA from patients with GDLD for mutations of this gene. Although no deleterious mutation was detected, we found three novel polymorphisms and determined their allelic frequencies.

We amplified each exon of the βig-h3 gene by polymerase chain reaction (PCR), using primers reported by Munier et al. (1997), from the genomic DNA of probands of each of seven pedigrees carrying GDLD. For mutational screening by single stranded conformation polymorphism (SSCP), electrophoresis was carried out in polyacrylamide gels containing 0.5 × Tris-borate/EDTA (TBE), at 500 W for 12 h at 9°C. When PCR products corresponding to exon 11 were electrophoresed, we detected two aberrant conformers and determined their DNA sequences (Fig. 1).

One of the aberrant conformers was revealed to contain a silent substitution from CTC to CTT at codon 472. As this genetic alteration occurred at a *MnlI* restriction site (CCTC), we performed allele-specific restriction enzyme analysis of the PCR products to estimate the allelic frequency in the Japanese population. By examining the genotypes of 54 unrelated healthy Japanese controls, we estimated the allelic frequency of C:T to be 0.778:0.222 (Table 1). The other aberrant conformer was associated with an alteration at codon 480 that resulted in an alanine (GCG)-to-valine (GTG) substitution (Fig. 1). This base alteration occurred in a nucleotide sequence (CGCG) recognized by *Acc II*. In the 54 unrelated healthy Japanese

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Table 1 Allele frequencies of three novel polymorphisms of the βig-h3 gene in 54 unrelated healthy controls

Position	Allele	Frequency	Heterozygosity
Codon 472			
	Allele I (C)	0.778	0.296
	Allele II (T)	0.222	
In intron 10			
	Allele I (C)	0.954	0.092
	Allele II (T)	0.046	

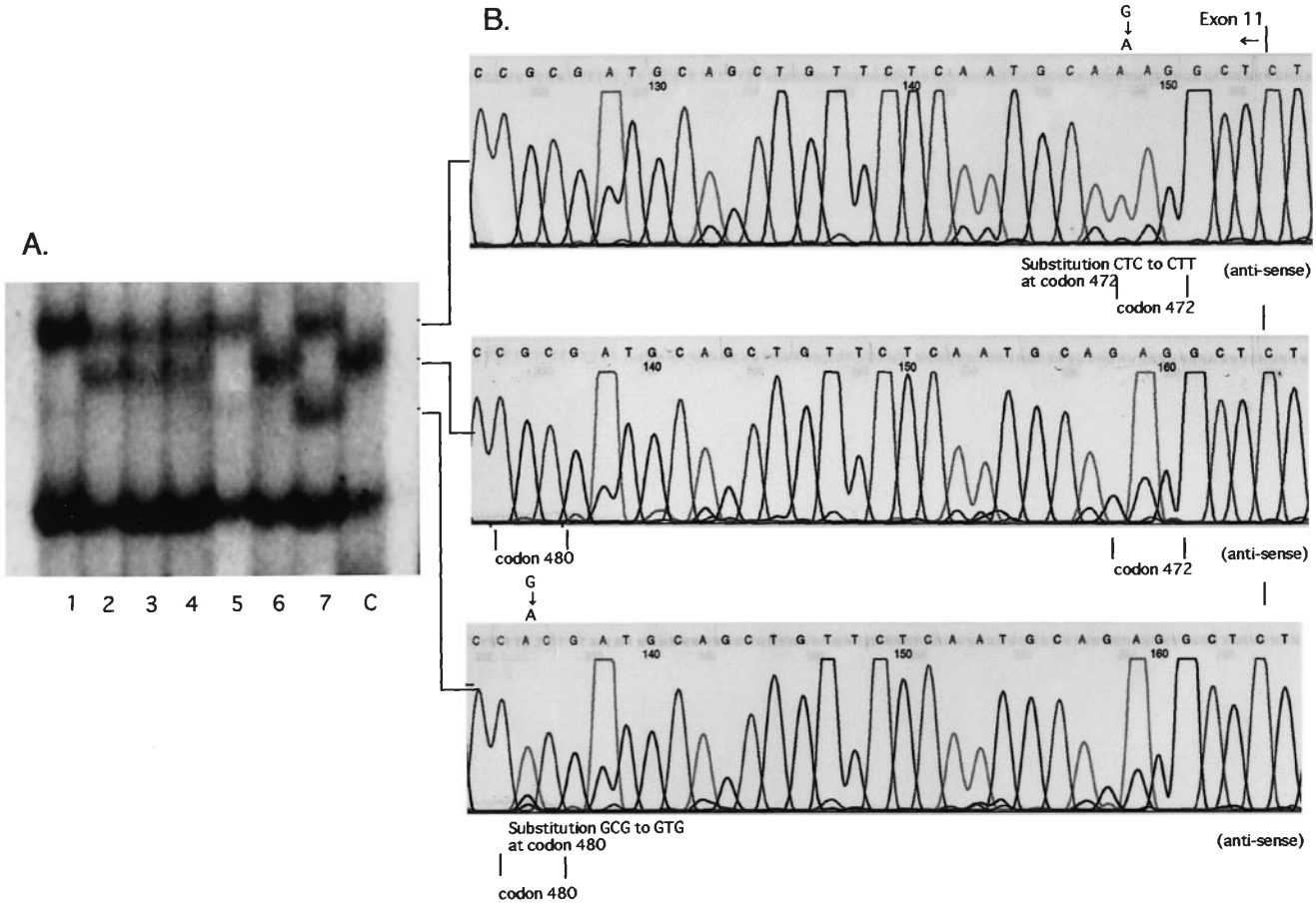

Fig. 1 **A** Results of single stranded conformation polymorphism (SSCP) analysis of exon-11 polymerase chain reaction (PCR) products from seven unrelated patients with gelatinous drop-like corneal dystrophy. Two aberrant bands were observed. *C*, Normal healthy control. **B** Sequence analysis of aberrant PCR products (anti-sense strands)

individuals, no GTG (valine) allele was observed. However, the alteration did not co-segregate with the disease phenotype in the family carrying GDLD (data not shown). Thus, we concluded that the substitution is not a disease-causative mutation but a rare polymorphism.

In addition, we found a nucleotide substitution from C to T in intron 10, three nucleotides upstream from the acceptor site of exon 11 (data not shown). Using a mismatch primer (TTCTCTGTCCCTCTTCTCT) which introduced a *Pst I* site into the wild-type amplicon, we examined the genotypes of the 54 normal Japanese controls. The allelic frequency of C:T in the normal Japanese population was estimated to be 0.954:0.046 (Table 1). Although the substitutions at codon 480 and in the intron both seemed to be rare polymorphisms, the relatively common *Mnl I* polymorphism will be useful as an intragenic marker for linkage analyses.

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