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Address for correspondence and reprints: Dr. Tetsuo Ashizawa, Department of Neurology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030. E-mail: tetsuoa@bcm.tmc.edu

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Homogeneity of Kerato-Epithelin Codon 124 Mutations in Japanese Patients with Either of Two Types of Corneal Stromal Dystrophy

To the Editor:

Granular corneal dystrophy Groenouw type 1 (CDGG1; OMIM 121900 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?121900]) is an autosomal dominant form of corneal dystrophy that is characterized by discrete deposits of gray-white material in the subepithelial and stromal layers of the cornea (Mannis et al. 1997). The precise nature and source of these deposits are unclear. Amyloid deposits have been detected in the corneas of older individuals with typical CDGG1 (Garner 1969; Akiya and Brown 1970). Lattice corneal dystrophy type 1 (CDL1; OMIM 122200 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?122200]), which is clinically distinct from CDGG1, is characterized by linear amyloid deposits in the subepithelial and stromal layers of the cornea (Mannis et al. 1997). However, granular-lattice corneal dystrophy recently has been described as having the clinical and histological features of both granular and lattice corneal dystrophies (Folberg et al. 1988, 1994; Holland et al. 1992; Rosenwasser et al. 1993). This condition has been named "Avellino corneal dystrophy" ("ACD"; OMIM 121900 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?121900]), because most of the affected individuals could trace their origin to the Italian province of Avellino (Holland et al. 1992).

Recently, four corneal disorders with autosomal dominant traits, CDGG1, CDL1, ACD, and Reis-Bücklers corneal dystrophy (CDRB; OMIM 121900 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?121900]), have been mapped to chromosome 5q31 (Stone et al. 1994; Gregory et al. 1995; Korvatska et al. 1996; Small et al. 1996). More recently, these four corneal dystrophies have all been shown to result from mutations in the kerato-epithelin gene (Munier et al. 1997). The mutations detected in Caucasian patients in Europe were R124C, in two families with CDL1; R124H, in two families with ACD; R555W, in one family with CDGG1; and R555Q, in one family with CDRB. Each of these four mutations affects the CpG dinucleotide of an arginine codon. Kerato-epithelin genes with R124 mutations may form amyloidogenic intermediates that precipitate in the cornea.

We have demonstrated the deposition of amyloid, a feature of ACD, in corneal specimens from six of eight Japanese patients with a clinical diagnosis of CDGG1 (Konishi et al. 1997). To investigate whether the high frequency of amyloid deposition in Japanese patients with CDGG1 is attributable to a specific mutation in the kerato-epithelin gene or to other conditions, such as aging, we screened kerato-epithelin genes from Japanese patients with CDGG1, ACD, or CDL1 (primary corneal amyloidosis).

The study subjects comprised 6 Japanese patients with ACD (of whom 1, with recurrent disease, was the offspring from a consanguineous marriage), diagnosed by histological examination; 10 patients with a clinical diagnosis of CDGG1 (of whom 2, with recurrent disease, were the offspring from consanguineous marriages); and 6 patients with CDL1. None of the patients were related. The mean age ($\pm$SD) of the 6 patients with ACD was 70.5 ($\pm$14.2) years, at the time of surgery, and that of the 10 patients with CDGG1 was 50.1 ($\pm$18.0) years, at the time of their first visit to us. Individual exons (exons 4–16) of the kerato-epithelin gene were amplified from genomic DNA from each subject, by PCR with primers described elsewhere (Munier et al. 1997), and the amplification products were sequenced on both strands. We detected a homozygous R124H (np418G→A) mutation in the one ACD and two CDGG1 patients with consanguineous parents and a heterozygous R124H (np418G→A) mutation in the remaining five ACD and eight CDGG1 patients (fig. 1, *top*). Patients with the homozygous mutation experienced rapid progression of clinical manifestation and visual deterioration at an early age and required keratoplasty in the 1st decade of life. These severe corneal conditions resembled a variant of superficial granular corneal dystrophy (Haddad et al. 1977; Owend et al. 1992; Sajjadi and Javadi 1992). A heterozygous R124C (np417C→T) mutation was identified in the six patients with CDL1 (fig. 1, *bottom*). To exclude the possibility of contamination with mutated DNA in PCR or sequence-reaction mixtures, we also analyzed DNA from control subjects, at the same time as our analysis of patient DNA, and obtained normal nucleotide sequences from the controls. We also screened 40 Japanese subjects without corneal dystrophy and failed to detect any of the mutations identified in the patients.

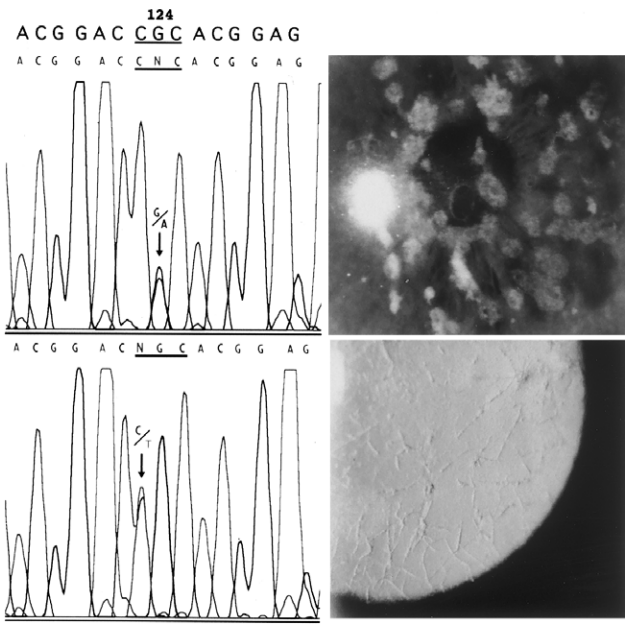

Figure 1 Direct-sequencing analysis of kerato-epithelin gene of patients with ACD (*top;* heterozygous G→A mutation at codon 124) and CDL1 (*bottom;* heterozygous C→T mutation at codon 124). The top line shows the normal DNA sequence.

Our results suggested two interesting genetic phenomena in corneal stromal dystrophy in Japanese patients. First, the R124H (np418G→A) mutation was identified in 16 unrelated Japanese individuals with a clinical diagnosis of CDGG1 or a histological diagnosis of ACD. The term "ACD" was proposed relatively recently (Holland et al. 1992); ACD is a variant of CDGG1. Histological examination is required for diagnosis. In the present study, individuals were diagnosed as having ACD on the basis of histological examination. The earliest clinical feature of ACD is the appearance of discrete granular deposits, which is typical of CDGG1, and is followed by the appearance of the lattice lesions. The youngest patient identified by Holland et al. (1992) as showing evidence of three typical lesions of ACD was 50 years old. Thus, individuals <50 years old with ACD may be misdiagnosed with CDGG1. The mean age of the 10 patients with a clinical diagnosis of CDGG1 at the time of their first visit to us was 50.1 (±18.0) years. The responsible mutation in most Japanese patients with a clinical diagnosis of CDGG1 may be R124H, which is the same mutation as that associated only with the ACD reported by Munier et al. (1997). This observation may explain the high frequency of amyloid deposition, a feature of ACD, in corneal specimens of Japanese patients with a clinical diagnosis of CDGG1 (Konishi et al. 1997). As mentioned above, patients with clinically typical CDGG1 are often found to have amyloid deposits, on the basis of histological examination (Garner 1969; Akiya and Brown 1970; Folberg et al. 1994). Indeed, in addition to discrete gray-white opacities typical of CDGG1, in the subepithelial stroma, several of the older patients with a clinical diagnosis of CDGG1, in the present study, showed a few whitish fusiform, stellate, or spicular opacities, suggestive of amyloid deposits, in the midstroma (Holland et al. 1992; Rosenwasser et al. 1993). Thus, clinical diagnosis of the different types of corneal stromal dystrophy is difficult, especially for CDGG1 and ACD. Direct corneal examination is not sufficient to establish a diagnosis; histological examination or molecular characterization of the mutation is required. The presence of the R124H mutation indicates that, in the present study, the individuals diagnosed with CDGG1 actually have ACD. The corneal appearance of the CDGG1 patient carrying a R555W mutation, described by Munier et al. (1997), differs from that of our 16 patients with the R124H mutation.

Second, our results demonstrate the homogeneity of the R124H and R124C mutations in the kerato-epithelin gene, in Japanese patients with ACD and CDL1, respectively. Another example of genetic homogeneity in Japanese patients is provided by Oguchi disease (Fuchs et al. 1995), a rare autosomal recessive form of congenital stationary night blindness. Most patients described as having Oguchi disease are from Japan. In Japan, five of six patients with Oguchi disease have showed homozygous deletion of nucleotide position 1147 (1147delA) in codon 309 of the arrestin gene, and this homogeneity has been suggested to be due to a single founder mutation (Fuchs et al. 1995). In contrast, homogeneity of the R124 mutations of the kerato-epithelin gene in Japanese patients with autosomal dominant types of corneal dystrophy might not be due to single founder mutations. The two mutations, R124H and R124C, detected in the present study also are present in European Caucasian patients with corneal dystrophies, despite different ethnic groups (Munier et al. 1997), and the R124-mutated kerato-epithelin gene certainly could be associated with amyloidogenic intermediates that precipitate in the cornea.

These results suggest the possibility that the unique mutations at codon 124 (R124H and R124C) in the kerato-epithelin gene may be responsible for ACD and CDL1, respectively. In this case, these R124 mutations probably occur independently in the Japanese population, similar to the nucleotide 11778 mutation of mtDNA in Leber's hereditary optic neuropathy (Brown et al. 1995; Howell et al. 1995). Haplotype analysis of the kerato-epithelin gene in individuals with ACD or CDL1 should clarify this issue. Further nationwide studies will be necessary to confirm the genetic uniqueness

of the kerato-epithelin mutation in individuals with these two corneal dystrophies.

Yukihiko Mashima,[1] Yutaka Imamura,[1,2]
Minako Konishi,[1] Akemi Nagasawa,[1,2]
Masakazu Yamada,[1] Yoshihisa Oguchi,[1]
Jun Kudoh,[2] and Nobuyoshi Shimizu[2]
Departments of [1]Ophthalmology and [2]Molecular Biology, Keio University School of Medicine, Tokyo

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Address for correspondence and reprints: Dr. Yukihiko Mashima, Department of Ophthalmology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan. E-mail: mashima@mc.med.keio.ac.jp

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Identification of an Interstitial Deletion in an Adult Female with Schizophrenia, Mental Retardation, and Dysmorphic Features: Further Support for a Putative Schizophrenia-Susceptibility Locus at 5q21-23.1

To the Editor:

Schizophrenia is a common and complex mental disorder. Family, twin, and adoption studies provide overwhelming but indirect evidence for a significant genetic contribution to the etiology of schizophrenia (Gottesman 1967, 1982, 1991; Murray et al. 1986; Kendler and Diehl 1993; Kendler et al. 1993). Cumulative evidence from genetic linkage studies is now available to suggest that schizophrenia-susceptibility genes may be found in relatively broad regions on chromosomes 22q, 8p, and 6p (Schizophrenia Collaborative Linkage Group 1996*a,* 1996*b*).

Evidence suggesting linkage at the 5q21-31 locus has also been provided independently by two groups (Schwab et al. 1997; Straub et al. 1997), and the implicated region consists of two partially overlapping regions, which extend to a combined distance of 30–40 cM, with the strongest evidence for linkage, under the