Corneal Endothelial Degeneration in Dentatorubral-Pallidoluysian Atrophy

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Background: Dentatorubral-pallidoluysian atrophy (DRPLA) is an autosomal dominant spinocerebellar degeneration that exhibits a variety of neurologic manifestations. However, only a few reports have studied disturbances outside the central nervous system. We described 2 unrelated patients with DRPLA accompanied by corneal endothelial degeneration.

Patients and Methods: A 52-year-old man presented with cerebellar ataxia and dementia. Magnetic resonance imaging of the brain showed cerebellar atrophy. Dentatorubral-pallidoluysian atrophy was diagnosed because of the detection of expansion of CAG repeats at the DRPLA locus. On admission, his visual acuity was severely impaired. Specular microscopy showed decreased endothelial cell density (500 cells/mm²) compared with that of healthy subjects. The second patient was a 69-year-old man with cerebellar ataxia. Magnetic resonance imaging of the brain showed cerebellar and brainstem ataxia. The diagnosis of DRPLA was based on expanded CAG repeats of the DRPLA gene. Specular microscopy showed significant decrease of endothelial cell density (1506 cells/mm²). Reverse transcriptase-polymerase chain reaction analysis showed DRPLA gene expression in corneal endothelial cells.

Conclusions: Mutant DRPLA protein may be directly associated with corneal endothelial degeneration. Corneal endothelial cell loss is an important sign of DRPLA, and the corneas of patients with DRPLA should be examined.

Arch Neurol. 2002;59:289-291

Dentatorubral-pallidoluysian atrophy (DRPLA) is a member of the family of autosomal dominant cerebellar ataxias. Patients may have a variety of symptoms, including myoclonus, seizures, choreiform movements, cerebellar ataxia, dementia, and psychiatric disturbance.1 Only a few reports have studied disturbance of the extracerebral system in autosomal dominant cerebellar ataxia. Recently, Abe et al2,3 reported corneal endothelial changes in patients with spinocerebellar ataxia type 1 (SCA1) and DRPLA, and suggested a significant correlation between decreased corneal endothelial cell density and autosomal dominant cerebellar ataxias. In the present study, we report 2 cases of DRPLA accompanied by corneal endothelial cell loss and the expression of the DRPLA gene in corneal endothelial cells in unrelated patients and discuss the molecular pathogenesis of corneal endothelial degeneration in DRPLA.

REPORT OF CASES

CASE 1

A 52-year-old Japanese man had been well until 49 years of age. His grandfather had died of cerebellar ataxia with severe visual disturbance (a genetic analysis was not performed). The patient first noticed gait disturbance and dysarthria at 50 years of age, and these symptoms gradually progressed for 2 years. At 52 years of age, he was admitted to Keio University Hospital, Tokyo, Japan, for the first time. The results of a neurologic examination showed dysarthria, ataxic gait, disturbance of coordination in the upper and lower limbs, pyramidal tract signs, and dementia. Magnetic resonance imaging of the brain showed cerebellar atrophy and dilation of the cerebral ventricles. Electromyographic studies demonstrated slightly low conduction velocity of the motor nerve. Genetic analysis showed that the expanded allele had 62 CAG repeats at the DRPLA locus, whereas the normal allele contained 18 repeats.

At admission to our hospital, he complained of visual disturbance. His best corrected visual acuity was 20/25 in his right eye and 20/70 in his left eye. An examination of the pupils, ocular media, and fundus showed normal findings, but severe nystagmus was observed. The results of a slitlamp examination showed corneal edema and guttae, which are excrescences of the Descemet membrane produced by abnormal endothelial cells. On specular microscopy, the corneal endothelial cells were enlarged, and the endothelial cell density (300 cells/mm² OD and 500 cells/mm² OS) was severely de-
creased compared with that of age-matched healthy sub-
jects (mean [± SD] for age-matched healthy subjects,
2685±94 cells/mm²).

**CASE 2**

A 69-year-old Japanese man first noticed gait disturbance
at 61 years of age. His father and elder sister had similar cer-
ebellar ataxias (genetic analysis was not performed). He vis-
ited our hospital at 66 years of age because of dysarthria and
worsening of his gait disturbance. The results of a neuro-
logic examination showed dysarthria, ataxic gait, and dis-
turbance of coordination in the upper and lower limbs. Mag-
netic resonance imaging of the brain showed cerebellar
and brainstem atrophy. An analysis of DNA revealed that
he had an expanded allele of the **DRPLA** gene with a nor-
mal allele (expanded/normal CAG repeats, 56/11).

At 63 years of age, he had keratitis due to herpes simplex
and corneal transplantation in his right eye, but his left eye
was not affected then. At 69 years of age, his corrected
visual acuity was 20/20 OS. On specular microscopy, the
endothelial cell density of the cornea in his left eye (1506
cells/mm² OS) was significantly decreased compared with
that of healthy subjects (mean [± SD] for age-matched
healthy subjects, 2711±121 cells/mm²) (**Figure 1**). An
examination of the pupils, ocular media, and fundus and
electroretinography showed normal findings.

**DETECTION OF DRPLA GENE EXPRESSION IN CORNEAL ENDOTHELIAL CELLS**

To study **DRPLA** gene expression in human corneal endo-
theelial cells, reverse transcriptase–polymerase chain reac-
tion (RT-PCR) analysis was performed. We produced
complementary DNA (cDNA) of the human corneal en-
dothelial cell from human corneal endothelial cells, which
were isolated from healthy donor cornea (obtained from
Rocky Mountain Lions Eye Bank, Denver, Colo) as de-
scribed previously. Total RNA was first isolated from hu-
man corneal endothelial cells using a monophasic solu-
tion of phenol and guanidine isothiocyanate (Isogen; Nipon
Gene, Toyama, Japan), then treated with RNase-free DN-
ase 1 (Stratagene, La Jolla, Calif) for 30 minutes. Human
brain cDNA was purchased (Maxim Biotech, Inc, San Fran-
cisco, Calif), and amplification of the 557-base pair frag-
ment of the **DRPLA** gene, which is designed to amplify
exons 9 to 10, was performed using the 5′ primer 5′-
AGGAGGACTACTACAGTCAC-3′ and 3′ primer 5′-
TGGTTTTGGTTGGGATGTTT-3′. For the negative
control of corneal endothelial cells, PCR was performed
in the absence of RT. The PCR buffer contained 1.5mM
magnesium chloride with 0.2mM of each deoxynucleo-
tide triphosphate, 2µM of each primer, and 2.5 U of Taq
polymerase (Takara Shuzo Co, Ltd, Shiga, Japan). The PCR
analysis consisted of 1 cycle of 5 minutes at 94°C; 35 cycles
of 1 minute at 94°C, 1 minute at 60°C, and 2 minutes at
72°C; and 1 cycle of 7 minutes at 72°C in a PCR system
(GeneAmp System 2400; Perkin Elmer, Foster City, Calif).
The PCR products were separated by means of electropho-
resis through an 8% polyacrylamide gel and visualized using
ethidium bromide under UV light.

As shown in **Figure 2**, RT-PCR of human brain and
corneal endothelial cells yielded the amplified band at the
expected size for the **DRPLA** gene. This PCR prod-
uct was also confirmed by restriction of the fragment
length using **MvaI** and **HhaI** (New England Biolabs, Bev-

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**Figure 1.** Corneal endothelial cells of patients with dentatorubral-pallidoluysian atrophy and spinocerebellar ataxia type 6 (SCA6) visualized under specular microscopy. **A**, Case 2 (1506 cells/mm²). **B**, A patient with SCA6 (3300 cells/mm²). The corneal endothelial cell density of case 2 was severely decreased compared with that of the patient with SCA6.
Abe et al.2,3 also described 2 related patients with DRPLA (patients with unknown genetic loci) (Figure 1). Recently, cerebellar ataxias (1 patient with SCA2, 2 patients with SCA6, and 2 patients with unknown genetic loci) (Figure 1). Recently, Abe et al.2,3 also described 2 related patients with DRPLA who exhibited a reduction in corneal endothelial cell density, had no such history. These decreases in cell density were significant compared with those of age-matched healthy subjects. The possibility of Fuchs endothelial dystrophy, which is characterized by a decrease in endothelial cell density and corneal guttae, must also be considered in these cases. Although the precise incidence of Fuchs endothelial dystrophy in the general Japanese population is unclear, Fuchs dystrophy has been reported to be extremely rare in Japan.4 Therefore, it seems unlikely that Fuchs dystrophy would be present in the 2 unrelated patients described herein. In addition, no corneal changes were detected in patients with other autosomal dominant cerebellar ataxias (1 patient with SCA2, 2 patients with Machado-Joseph disease, 1 patient with SCA6, and 2 patients with unknown genetic loci) (Figure 1). Recently, Abe et al.2,3 also described 2 related patients with DRPLA who exhibited a reduction in corneal endothelial cell density; corneal changes were not observed in the unaffected family members. Our findings provide an independent confirmation of these studies and strongly suggest that corneal endothelial cell loss is an important sign of DRPLA.

Another important finding of our report was that corneal endothelial cells clearly express the DRPLA gene (Figure 2). Although the DRPLA gene is reported to be widely expressed in various tissues, including unaffected organs, high levels of expression are observed predominantly in neuronal tissue.5 Because corneal endothelial cells are derived from the neuroectoderm,6 the presence of DRPLA gene expression in corneal endothelial cell is not surprising. Recent investigations suggest that the aggregation of mutant proteins containing expanded polyglutamine stretches plays an important role in neuronal degeneration.7 Our findings imply that the mutant DRPLA protein may be directly associated with corneal endothelial degeneration and neuronal degeneration.

Finally, further study is needed to elucidate whether decreased corneal endothelial cell density is associated with the duration of the disease and the number of expanded CAG repeats in the DRPLA gene. Whether the nuclear aggregation of mutant DRPLA protein occurs in corneal endothelial cells should also be investigated. In addition, we have evaluated the corneas of only 2 Japanese patients with DRPLA. The occurrence of corneal endothelial degeneration in patients with DRPLA should also be investigated in other populations. Nevertheless, the present findings suggest that the corneas of patients with DRPLA should be thoroughly examined.

Accepted for publication September 18, 2001.

Author contributions: Study concept and design (Drs Ito, Hamada, and Fukuuchi); acquisition of data (Drs Ito, Yamada, Kawai, and Hamada); analysis and interpretation of data (Drs Ito, Yamada, Kawai, and Usui); drafting of the manuscript (Dr Ito); critical revision of the manuscript for important intellectual content (Drs Yamada, Kawai, Usui, Hamada, and Fukuuchi); administrative, technical, and material support (Drs Ito, Yamada, Kawai, and Usui); study supervision (Drs Hamada and Fukuuchi).

We thank Kinya Ishikawa, MD, and Hidehiro Mizusawa, MD, Tokyo Medical and Dental University, for the genetic analysis in case 1, and Mamoru Shibata, MD, Department of Neurology, Keio University, for the genetic analysis in case 2.

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