Graefe's Arch Clin Exp Ophthalmol
(2001) 239:722–728

CLINICAL INVESTIGATION

DOI 10.1007/s004170100342

Toshiaki Abe
Koji Abe
Takehide Tsuda
Yasuto Itoyama
Makoto Tamai

Ophthalmological findings in patients with spinocerebellar ataxia type 1 are not correlated with neurological anticipation

Received: 12 April 2001
Revised: 27 June 2001
Accepted: 27 June 2001
Published online: 11 September 2001

T. Abe (✉) · M. Tamai
Department of Ophthalmology,
Tohoku University School of Medicine,
1-1 Seiryoumachi Aobaku Sendai,
Miyagi 980-8574, Japan
e-mail: toshi@oph.med.tohoku.ac.jp
Tel.: +81-22-7177298
Fax: +81-22-7177294

K. Abe
Department of Neurology,
Okayama University, Okayama, Japan

T. Tsuda · Y. Itoyama
Department of Neurology, T
ohoku University School of Medicine,
Miyagi, Japan

Abstract *Background:* Optic atrophy, attenuation of the oscillatory potentials (OPs) of the electroretinogram (ERG), and enlargement of corneal endothelial cells, have been reported in patients with spinocerebellar ataxia type 1 (SCA1). These patients have a trinucleotide repeat expansion in the *SCA1* gene and show neurological anticipation. The purpose of this study was to determine whether the ophthalmological findings are correlated with the neurological disorders, and whether ophthalmological anticipation is present in patients with SCA1. *Methods:* The visual acuity, ERGs, and corneal endothelial cell density were examined in 14 patients whose DNA analysis revealed an expanded trinucleotide repeat in an allele of the *SCA1* gene. The results of the tests were compared with the trinucleotide repeat number and the duration of the neuronal disease. *Results:* The neurological disorders in the patients showed anticipation. The negative correlation between the trinucleotide repeat number and the neurological disorder was statistically significant ($P<0.0001$). However, the correlations between trinucleotide repeat number and visual acuity, amplitude of OPs, and corneal endothelial cell density were not significant. Statistically significant correlations were found between the duration of the neuronal disease and the visual acuity, OPs, and corneal endothelial cell density ($P<0.0001$, $P=0.0004$, and $P<0.0001$, respectively). The ophthalmological disorders were prominent in patients who had neuronal disease for more than 10 years. *Conclusion:* Unlike the neurological findings, the ophthalmological disorders in patients with SCA1 were not correlated with the trinucleotide repeat number of the *SCA1* gene. The ophthalmological findings were most highly correlated with the duration of the neuronal disease.

Introduction

Spinocerebellar ataxia type 1 (SCA1) is classified as one of the autosomal dominant cerebellar ataxias (ADCAs) that are hereditary and heterogeneous neurodegenerative disorders with cerebellar phenomena and ophthalmoparesis [17]. Several classifications of ADCAs have been made using clinical [12] or pathological [17] features. In the eye, optic atrophy [1, 11], electroretinographical abnormalities [2, 1], pigmentary macular dystrophy [12, 17], and corneal endothelial abnormalities [4, 2] are associated with the ADCAs. These ophthalmological characteristics are at times important for classifying the disease process.

The genes responsible for these diseases have been examined using advanced molecular biological techniques such as linkage analysis [10, 13, 14, 16, 22, 24]. One feature of these genes is that they have an unstable trinucleotide repeat of CAG, which is expanded in one of the alleles of the affected patients. This feature is also observed in patients with other types of neurodegenerative diseases [6, 18, 19, 23]. Patients who have longer

expanded CAG repeats have an earlier age of onset and have more severe neurological symptoms than patients who have shorter repeats. This phenomenon of earlier onset with longer trinucleotide repeat expansion of the responsible gene is referred to as anticipation.

Patients who have trinucleotide repeat expansion of the *SCA7* gene also show pigmentary retinal degeneration with neurological anticipation. We have reported that the macular degeneration in patients with SCA7 showed ophthalmological anticipation [5].

Patients with trinucleotide repeat expansion in the *SCA1* gene have been shown to have neurological anticipation. Ophthalmological disorders such as decreased visual acuity due to optic atrophy, attenuation of the OPs, and enlargement of the corneal endothelial cells also have been reported in patients with SCA1 [2]. However, to the best of our knowledge, ophthalmological anticipation in patients with a mutation of the *SCA1* gene has not been reported. Thus, the purpose of this study was to determine whether the ophthalmological findings are correlated with the neurological disorders, and whether ophthalmological anticipation is present in patients with SCA1.

Patients and methods

Fourteen patients from 11 unrelated pedigrees whose DNA analysis revealed an allele of the *SCA1* gene with expanded CAG repeats were examined neurologically and ophthalmologically. The ophthalmic examinations included best corrected visual acuity, slit-lamp biomicroscopy, visual field analysis, color vision testing, specular microscopy, fundus examination, and electroretinography. Specular microscopy and data analyses were performed by Konan specular microscopy ROBO-CA (Konan, Tokyo, Japan).

The ERGs were recorded using the guidelines of the International Society for Clinical Electrophysiology of Vision [8]. A standard white flash (20 J) was used to elicit the maximum responses of the rods and cones after 30 min of dark-adaptation.

The ocular media were clear in all patients, and none of the patients had a history of eye surgery, eye disease, or trauma. As in our previous study [2], we analyzed the visual acuity, the amplitude of the OPs of the ERG, and the corneal endothelial cell density. For control, we analyzed the amplitude of the OPs in 77 eyes and the corneal endothelial cell density in 55 eyes of healthy people who showed normal ocular findings except for mild senile cataracts and who had no history of trauma or intraocular surgery.

DNA and sequence analysis with polymerase chain reaction (PCR)

DNA was extracted from the peripheral blood lymphocytes (about 20 ml) of each patient[1]. PCR and analysis of repeat numbers were performed, as previously reported [1]. PCR was carried out with the thermocycler (Perkin Elmer, Norwalk, Conn, USA) on 50 µl of reaction mixture containing 1 µg of the patient's genomic DNA and 20 µM of each primer (Rep-1: 5′-AACTGGAAATGTGGACGTAC-3′ and Rep-2: 5′-CAACATGGGCAGTCTGAG-3′) or fluorescein isothiocyte (FITC)-labeled primer of Rep-2; 200 µM each of dATP, dCTP, dGTP, and TTP; 50 mM of KCl; 10 mM of Tris-Cl (pH 8.3); 1.5 mM of $MgCl_2$; 0.001% gelatin; and 2.5 units of Taq polymerase. The reaction cycles were 35. In each case, the amplified DNA was separated on 3% agarose gel (SeaKem, FMC BioProducts, Rockland, Me, USA) containing 0.05 µg/ml ethidium bromide, or on 6% denaturing polyacrylamide gel with an automated DNA sequencer (Pharmacia LKB ALF DNA sequencer, Pharmacia, Uppsala, Sweden).

Statistical analysis

The ophthalmological findings, neurological findings, and the number of trinucleotide repeats were statistically compared by unpaired *t*-tests and Pearson's correlation coefficients. AP <0.05 was considered significant.

Human research

The tenets of the Declaration of Helsinki were followed, and informed consent was obtained from all subjects who participated in this study.

Results

All patients exhibited an autosomal dominant family history (Table 1). The ophthalmologic findings at successive follow-up examinations in 6 of the 14 patients (cases 1-2, 4-2, 8-2, 9-2, 13-2, and 14-2) are also presented in Table 1. Seven patients were men and seven were women. Their ages at the initial ophthalmological examination ranged from 24 to 77 years. One patient (case 11) was considered an asymptomatic carrier.

The CAG repeat number was between 22 and 37 from 122 chromosomes of 61 neurologically healthy controls. All patients had an expanded allele of the *SCA1* gene (Table 1). The repeat number of the expansion was different between patients, even in those from the same family. Cases 1 and 12 were from the same family and had 44 and 49 repeats respectively, and cases 3, 4, and 5, from another family, had 46, 43, and 47 repeats, respectively. The shortest repeat was 40 (case 6) and the longest was 58 (cases 10 and 14).

Ophthalmological data were analyzed from both eyes. A negative correlation between the trinucleotide repeat number and the age of neurological onset was found and was statistically significant (n=13, r=-0.895, P<0.0001). Case 11 (age=31 years) was an asymptomatic carrier and was excluded from this analysis (Fig. 1). The neurological onset was earlier and more severe in patients who had longer repeats than in those with shorter repeats.

When we compared the ophthalmological findings with the trinucleotide repeat number, no significant correlation was observed between the repeat number and most recent visual acuity (n=28, r=0.283, P=0.145; Fig. 2a), the amplitude of the OPs (n=24, r=0.250, P=0.242; Fig. 2b), and the corneal endothelial cell density (n=26, r=0.335, P=0.0945; Fig. 2c). Although these findings indicate that the ophthalmological severity is not correlated with the trinucleotide repeat number, significant correlations were found between the duration

Table 1 Ophthalmic findings in study patients with SCA1 [*Age/gend* age and gender of patients, *Repeat (expanded/normal)* ratio of the trinucleotide repeat number of expanded and normal allele of the SCA1 gene, *ERG* electroretinogram, *(a)* a-wave of ERG, *(b)* b-wave of ERG, *(OPs)* oscillatory potentials, *Endothel* corneal endothelial cell density, *nd* no examination was performed]

Case	Age/gend	Repeat (expanded/normal)	Duration (years)	Visual acuity		ERG (a) (μV)		ERG (b) (μV)		ERG (OPs) (μV)		Endothel (cells/mm²)	
				R	L	R	L	R	L	R	L	R	L
1	52/F	44/27	11.0	0.6	0.6	330	330	560	570	205	240	nd	nd
1–2	57/F	44/27	16.0	0.1	0.1	390	370	620	600	83	35	770	700
2	60/F	46/27	15.0	1.0	1.0	280	260	370	385	140	200	870	1090
3	45/F	46/27	11.0	0.7	0.7	282	282	400	400	165	141	874	890
4	58/F	43/27	14.0	0.4	0.15	370	280	470	380	125	95	nd	nd
4–2	63/F	43/27	19.0	0.08	0.07	320	300	390	390	47	53	668	620
5	53/F	47/27	11.0	0.6	0.6	nd	nd	nd	nd	nd	nd	1034	998
6	77/M	40/31	20.0	0.6	0.4	300	300	430	430	200	212	2415	1529
7	32/M	47/28	1.0	1.2	1.5	350	380	580	580	300	300	3115	3030
8	66/F	41/28	2.0	0.5	0.4	300	300	540	540	nd	nd	2049	2164
8–2	67/F	41/28	3.0	0.4	0.4	nd	nd	nd	nd	nd	nd	2040	2169
9	31/M	54/27	3.0	1.2	1.5	259	283	400	353	153	153	nd	nd
9–2	36/M	54/27	8.0	0.7	0.7	nd	nd	nd	nd	nd	nd	1597	852
10	24/M	58/29	3.0	1.0	1.0	271	253	429	406	200	183	2777	2991
11	31/M	43/29	0.0	1.2	1.2	420	460	560	620	171	200	2262	2610
12	44/F	49/27	8.0	0–2	0.3	280	270	400	390	176	206	nd	nd
13	32/M	51/28	5.0	1.5	1.2	400	430	610	650	223	241	2500	2824
13–2	34/M	5J/28	7.0	1.2	1.2	nd	nd	nd	nd	nd	nd	2008	2421
14	25/M	58/30	2.0	1.5	1.5	405	380	575	545	235	235	2577	2688
14–2	31/M	58/30	8.0	0–7	0.6	340	320	480	420	219	170	2409	2506

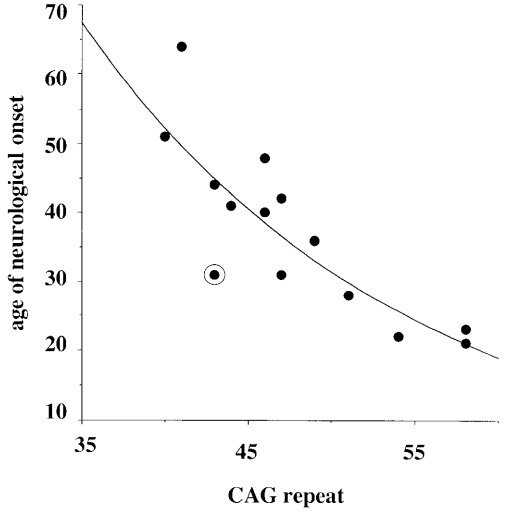

Fig. 1 The CAG trinucleotide repeat number (X-axis) and the age of neurological onset (Y-axis) are plotted. A *dot in a circle* shows the asymptomatic carrier. The coefficient was calculated for an exponential regression (r=-0.895).

of the neuronal disease and the visual acuity (n=40, r=–0.619, P<0.0001), the amplitude of OPs (n=30, r=–0.596, P=0.0004), and the corneal endothelial cell density (n=32, r=-0.707, P<0.0001) (Fig. 3). These data were obtained from both eyes, including the asymptomatic carrier, and from successive follow-up periods. In addition, patients who had a longer duration of neurological disease were more likely to show severe ophthalmological findings. Thus, the ophthalmological findings in patients with a duration neuronal disease of less than 10 years were significantly less severe for the visual acuity, amplitude of the OPs, and decrease of the corneal endothelial cell density (P=0.0007, P=0.0018, and P<0.0001 respectively; Fig. 4) than in patients with a duration of more than 10 years.

The amplitude of the OPs and corneal endothelial cell density were also analyzed in both the normal healthy controls and the patients (Fig. 5). Statistically significant correlations of the amplitude of OPs and age were observed in both normal controls and patients (n=77, r=–0.445, P=0.0002; and n=30, r=–0.405, P=0.0285 respectively; Fig. 5a). However, the patients with SCA1 showed lower mean amplitude of the OPs than those of the normal controls at all ages. Statistically significant correlation of the corneal endothelial cell density and age also was observed in both normal controls and patients (n=55, r=–0.470, P=0.0002; and n=32, r=–0.540, P=0.0037 respectively; Fig. 5b). The mean corneal endothelial cell density in patients with SCA1 was lower than that of normal controls at any age.

Discussion

Recently, several genes associated with ADCAs have been cloned [10, 13, 14, 16, 22, 24], and the *SCA1* gene is located on the short arm of chromosome 6. This gene

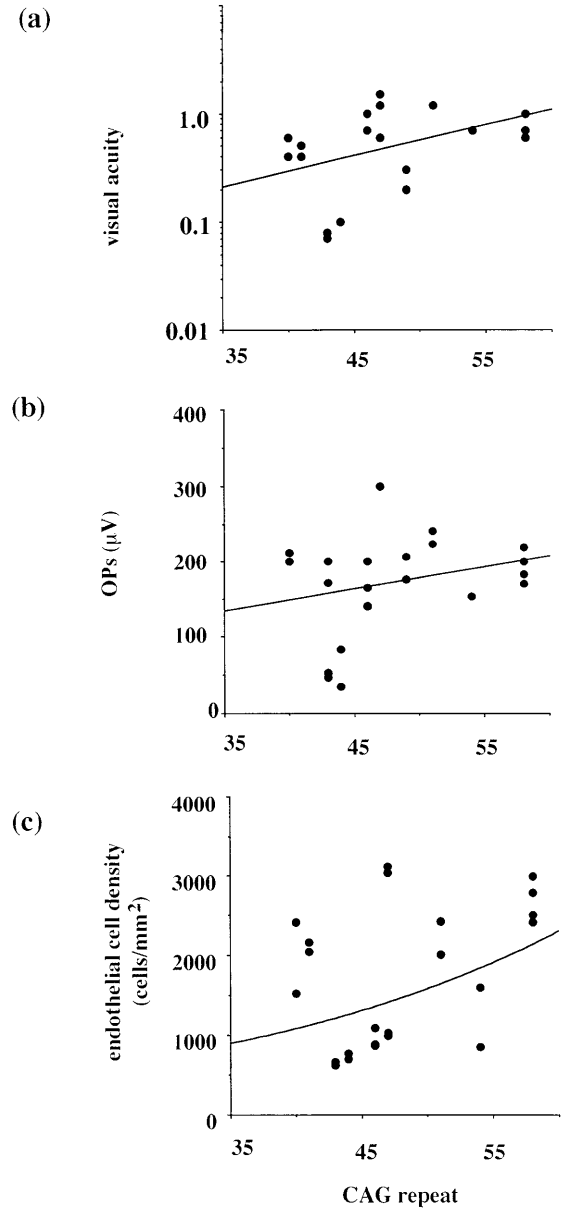

Fig. 2 **a** The CAG trinucleotide repeat number (X-axis) and visual acuity (Y-axis) are plotted. The visual acuity was from most recent examination. **b** The CAG trinucleotide repeat number (X-axis) and the amplitude of oscillatory potentials (OPs) (μV) are plotted. **c** The CAG trinucleotide repeat number (X-axis) and corneal endothelial cell density (cells per square millimeter) are plotted. The fitted regression lines are either linear (**a** and **b**) or exponential (**c**)

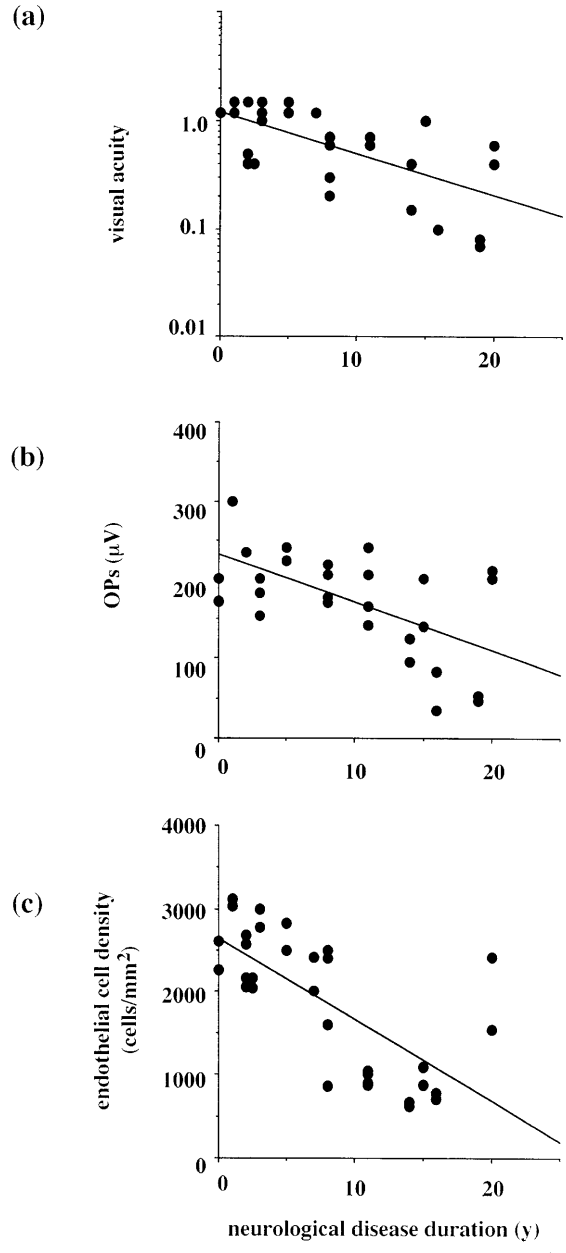

Fig. 3 **a** The duration of the neurological disease (X-axis) and the visual acuity (Y-axis) are plotted. The fitted linear regression line has a coefficient of r=–0.619. **b** The amplitude of the oscillatory potentials (OPs)(r=–0.596), **c** corneal endothelial cell density (cells/mm^2)(r=–0.707) are plotted.

has CAG trinucleotide repeat in the coding region similar to the other ADCA genes. The repeat is expanded in some patients, and the younger members show neurological anticipation [7, 15, 20, 22]. The expanded trinucleotide of CAG code polyglutamine and the mutant protein (ataxin-1) has been reported to localize in the nucleus and create a novel function that would lead to cell-specific neuronal degeneration [9].

The decrease of corneal endothelial cell density has been observed not only in patients with SCA1 but also in patients with dentatorubral-pallidoluysian atrophy (DRPLA) [4]. Decreased lens epithelial cell density, with typical lens opacity in patients with myotonic dystrophy (MD) whose DNA analysis showed trinucleotide repeat expansion of the MD gene, has also been reported [3]. These observations suggest a cellular dysfunction in

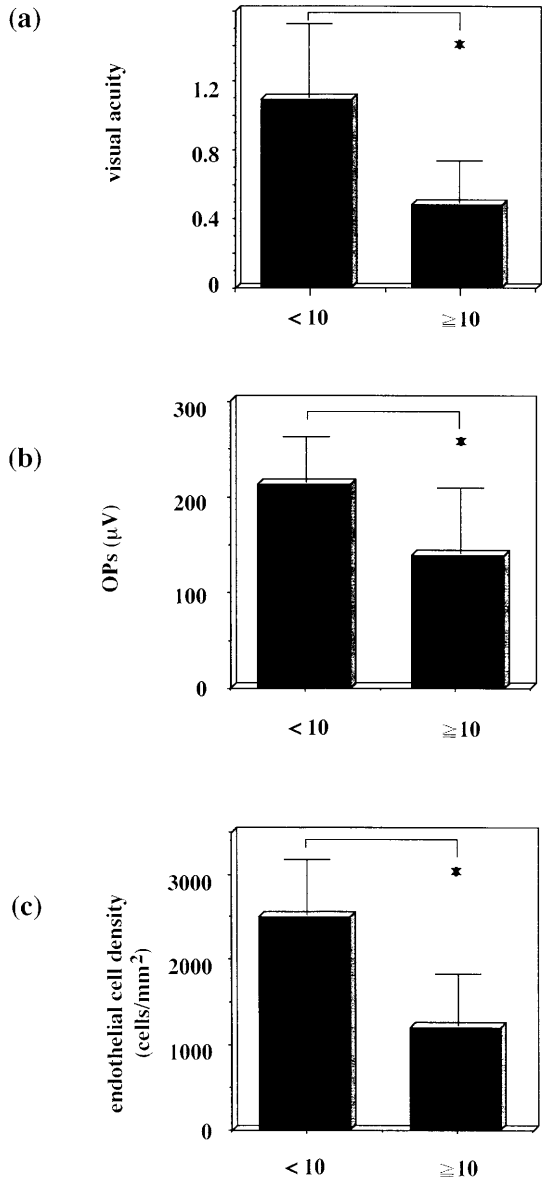

Fig. 4 **a** Visual acuity, **b** oscillatory potentials (OPs), and **c** corneal endothelial cell density are compared in patients who suffered from the disease for less than 10 years (<10) and patients with the disease for more than 10 years (≥10). Statistically significant correlations were observed (*asterisks*)

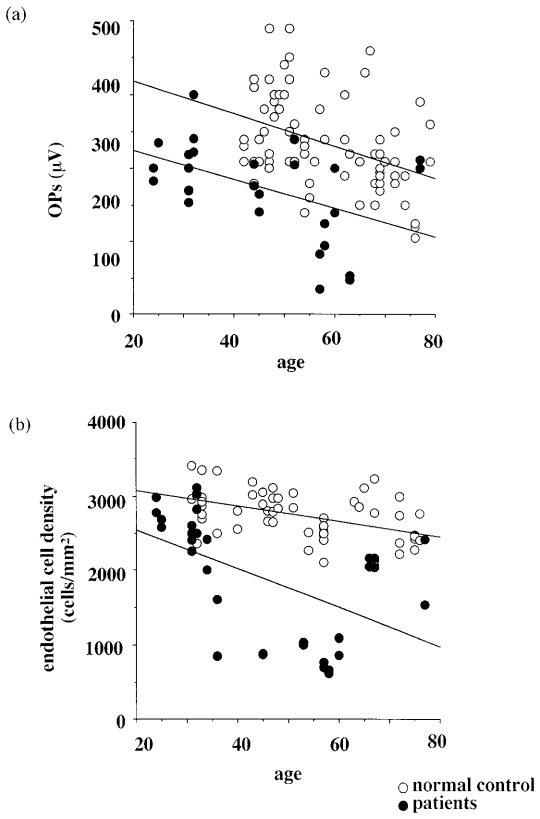

Fig. 5 **a** Age of the patients or normal control (X-axis) and amplitude of oscillatory potentials (OPs) are plotted. The coefficients are calculated for linear regression (r=-0.405 and –0.445, respectively). **b** Age of the patients or normal control (X-axis) and corneal endothelial cell density (cells/mm^2) are plotted (r=–0.504 and –0.470 respectively)

these diseases where the trinucleotide repeat expansion of each responsible gene may be expressed. Although the distribution of the *SCA1* gene in ocular tissues is still unknown, the presence of optic atrophy, attenuated OPs, and decreased corneal endothelial cell density [2] may provide a clue on the localization of the gene expression.

The neurological symptoms in this disorder usually begin during the third or fourth decade of life, and the disease gradually worsens, often resulting in complete disability and death 10–20 years after the onset. One man (case 11, 31 years old), who had the short repeats (43 CAG repeats) and normal ophthalmological and neurological findings, was an asymptomatic carrier. As seen in Fig. 1, this patient may develop neurological disorder at around age 45 and may show the typical ophthalmological disorders if we continue to follow him for another 10 years.

We reported that macular degeneration in patients with SCA7 started earlier in patients with longer trinucleotide repeat expansion in the *SCA7* gene than in those with shorter trinucleotide repeat expansion [5]. Patients who had earlier onset of the disease also typically have a more rapid progression of the phenotype, and death at a younger age [7]. This means that the follow-up periods in patients with longer trinucleotide repeat may be limited. If we could follow patients with SCA1 for more than 10 years, we may find a higher incidence and more severe ophthalmological disorders. This is one reason why we could not examine ophthalmological disorders among successive generations in a family.

From the strong negative correlation between the trinucleotide repeat numbers and the age of neurological onset in our patients, we had expected that ophthalmological anticipation would be present in our patients with

SCA1. However, when we compared the ophthalmological disorders with the trinucleotide repeat numbers of the *SCA1* gene, no significant correlation was observed. However, ophthalmological findings did progress during the successive follow-up examinations (cases 1, 4, 8, 9, 13, and 14; Table 1), and were more prominent 10 years after the onset of neurological disorder (Fig. 4). From these results we conclude that, unlike the ophthalmological findings in patients with SCA7, the ophthalmological findings in patients with SCA1 do not show anticipation as did the neurological findings. However, it should be remembered that, unlike the macular degeneration in the patients with SCA7, the ophthalmological changes in SCA1 are less obvious to the patient and do not require a visit to an ophthalmologist. Thus, an earlier onset may be missed in patients with SCA1. In addition, SCA1 patients with higher repeat numbers tend to die earlier of neurological disorders, and the severity of the ophthalmological findings would not be detected.

Systemic neurodegeneration may demonstrate a predisposition for ophthalmological abnormalities. In *Pcd/pcd* (Purkinje cell degeneration) mice an autosomal recessive mutation leads to an almost complete loss of Purkinje cells between the ages of 3 and 5 weeks and photoreceptor cell degeneration of only 25% to 30% at 2 months of age. [21] This slower degeneration of cells in the retina than those in the cerebellum in a systemic neurodegeneration mouse model is similar to our patients with SCA1.

The transcriptional and translational regulations of ataxin-1 are complex and the expression in skeletal muscle is variable [7]. It is also possible that the expression of this protein may be different between neuronal tissues and ocular tissues. Moreover, reports have described a variation of the CAG repeat number in different tissue [15], and thus the repeat number may be different between ocular tissues and neural tissues. Further examination of the transcription or translation of the gene may reveal differences between neural and ocular tissues.

Acknowledgements This work was supported in part by grants 12671694 from grant-in-aid for Scientific Research from the Ministry of Education and Culture of the Japanese Government, Tokyo, Japan (Dr. Abe); and the Research Committee on Chorioretinal Degenerations, Ministry of Health and Welfare of the Japanese Government, Tokyo, Japan (Dr. Tamai). We are indebted to the families studied for their cooperation and support.

References

1. Abe T, Ishiguro S, Saito H, Kiyosawa M, Tamai M (1992) Partially deficient glutamate dehydrogenase activity and attenuated oscillatory potentials in patients with spinocerebellar degeneration. Invest Ophthalmol Vis Sci 33:447–452
2. Abe T, Abe K, Aoki M, Itoyama Y, Tamai M (1997) Ocular changes in patients with spinocerebellar degeneration and repeated trinucleotide expansion of spinocerebellar ataxia type 1 gene. Arch Ophthalmol 115:231–236
3. Abe T, Sato M, Kuboki J, Kano T, Tamai M (1999) Lens epithelial changes and mutated gene expression in patients with myotonic dystrophy. Brt J Ophthalmol 83:452–457
4. Abe T, Yamada N, Abe K, Tamai M (1999) Corneal endothelial changes and trinucleotide repeat expansion of DRPLA gene. Br J Ophthalmol 83:124–125
5. Abe T, Tsuda T, Yoshida M, Wada Y, Kano T, Itoyama Y, Tamai M (2000) Macular degeneration associated with aberrant expansion of trinucleotide repeat of the SCA7 gene in 2 Japanese families. Arch Ophthalmol 118:1415–1421
6. Anonymous (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. Cell 72:971–983
7. Banfi S, Servadio A, Chung MY, Kwiatkowski TJ Jr, McCall AE, Duvick LA, Shen Y, Roth EJ, Orr HT, Zoghbi, HY (1994) Identification and characterization of the gene causing type 1 spinocerebellar ataxia. Nat Genet 7:513–520
8. Brigell M, Bach M, Barber C, Kawasaki K, Kooijman A (1998) Guidelines for calibration of stimulus and recording parameters used in clinical electrophysiology of vision. Calibration Standard Committee of the International Society for Clinical Electrophysiology of Vision (ISCEV). Doc Ophthalmol 95:1–14
9. Cummings CJ, Orr HT, Zoghbi HY (1999) Progress in pathogenesis studies of spinocerebellar ataxia type 1. Philosophical Transactions of the Royal Society of London – Series B: Biological Sciences 354:1079–1081
10. David G, Abbas N, Stevanin G, Durr A, Yvert G, Cancel G, Weber C, Imbert G, Saudou F, Antoniou E, Drabkin H, Gemmill R, Giunti P, Benomar A, Wood N, Ruberg M, Agid Y, Mandel JL, Brice A (1997) Cloning of the SCA7 gene reveals a highly unstable CAG repeat expansion. Nat Genet 17:65–70
11. de Jong PT, de Jong JG, de Jong-Ten Doeschate JM, Delleman JW (1980) Olivopontocerebellar atrophy with visual disturbances. An ophthalmologic investigation into four generations. Ophthalmology 87:793–804
12. Harding AE (1982) The clinical features and classification of the late onset autosomal dominant cerebellar ataxias. A study of 11 families, including descendants of the 'the Drew family of Walworth'. Brain 105:1–28
13. Imbert G, Saudou F, Yvert G, Devys D, Trottier Y, Garnier J M, Weber C, Mandel JL, Cancel G, Abbas N, Durr A, Didierjean O, Stevanin G, Agid Y, Brice A (1996) Cloning of the gene for spinocerebellar ataxia 2 reveals a locus with high sensitivity to expanded CAG/glutamine repeats. Nat Genet 14:285–291
14. Kawaguchi Y, Okamoto T, Taniwaki M, Aizawa M, Inoue M, Katayama S, Kawakami H, Nakamura S, Nishimura M, Akiguchi I (1994) CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. Nat Genet 8:221–228

15. Koefoed P, Hasholt L, Fenger K, Nielsen J E, Eiberg H, Buschard K, Sorensen SA (1998) Mitotic and meiotic instability of the CAG trinucleotide repeat in spinocerebellar ataxia type 1. Human Genetics 103:564–569
16. Koide R, Ikeuchi T, Onodera O, Tanaka H, Igarashi S, Endo K, Takahashi H, Kondo R, Ishikawa A, Hayashi T (1994) Unstable expansion of CAG repeat in hereditary dentatorubral-pallidoluysian atrophy (DRPLA). Nat Genet 6:9–13
17. Konigsmark BW, Weiner L P (1970) The olivopontocerebellar atrophies: a review. Medicine 49:227–241
18. La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH (1991) Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature 352:77–79
19. Mahadevan M, Tsilfidis C, Sabourin L, Shutler G, Amemiya C, Jansen G, Neville C, Narang M, Barcelo J, O'Hoy K (1992) Myotonic dystrophy mutation: an unstable CTG repeat in the 3' untranslated region of the gene. Science 255:1253–1255
20. Matilla T, Volpini V, Genis D, Rosell J, Corral J, Davalos A, Molins A, Estivill X (1993) Presymptomatic analysis of spinocerebellar ataxia type 1 (SCA1) via the expansion of the SCA1 CAG-repeat in a large pedigree displaying anticipation and parental male bias. Human Molecular Genetics 2:2123–2128
21. Mullen RJ, LaVail M (1975) Two types of retinal degeneration in cerebellar mutant mice. Nature 258:528–530
22. Orr HT, Chung MY, Banfi S, Kwiatkowski TJ Jr, Servadio A, Beaudet AL, McCall AE, Duvick LA, Ranum LP, Zoghbi HY (1993) Expansion of an unstable trinucleotide CAG repeat in spinocerebellar ataxia type 1. Nat Genet 4:221–226
23. Verkerk AJ, Pieretti M, Sutcliffe JS, Fu Y H, Kuhl D P, Pizzuti A, Reiner O, Richards S, Victoria MF, Zhang FP (1991) Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. Cell 65:905–914
24. Zhuchenko O, Bailey J, Bonnen P, Ashizawa T, Stockton D W, Amos C, Dobyns WB, Subramony SH, Zoghbi HY and Lee CC (1997) Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the alpha 1A-voltage-dependent calcium channel. Nat Genet 15:62–69