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# Acoustic impairment is a distinguishable clinical feature of Asidan/SCA36

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ABSTRACT

*Objective:* To investigate acoustic function of Asidan/spinocerebellar ataxia type 36 (SCA36) in which sensorineural hearing loss may be found as one of extracerebellar symptom that can be a distinguishable feature from other degenerative ataxias.
*Methods:* Acoustic function in the groups of normal control ($n=31$), Asidan/SCA36 ($n=13$), cortical cerebellar atrophy (CCA, $n=28$), multiple system atrophy of cerebellar predominance (MSA-C, $n=48$), SCA31 ($n=4$), and other forms of SCAs ($n=14$) was evaluated by pure tone average (PTA) calculated by the results of audiogram and brainstem auditory evoked potentials (BAEPs).
*Results:* PTA was significantly decreased in Asidan/SCA36 in comparison to normal control and other ataxic groups, but not significant within other ataxic groups and normal control. In comparison to other groups, Asidan/SCA36 showed a constant depression at 7 different frequencies in audiogram, especially at 4000 and 8000 Hz. BAEPs in 2 Asidan/SCA36 cases suggested possible involvement in the inner ear or the peripheral part of the auditory system. PTA in Asidan/SCA36 cases significantly correlated with their severity of ataxia.
*Conclusions:* In addition to signs for motor neuron involvement, acoustic impairment in Asidan/SCA36 is another characteristic clinical feature that is distinguishable from other forms of SCAs.


## 1. Introduction

Both sporadic and hereditary forms of ataxias are a heterogeneous group of neurodegenerative disorders characterized by gait and truncal instability, limb incoordination, and dysarthria [1]. In dominantly inherited spinocerebellar ataxias (SCAs), more than 30 different loci involving 23 genes have been reported for the SCA subtypes [2]. Although the increasing number of SCAs adds to the clinical heterogeneity, detailed characterization of the disease phenotypes including extracerebellar signs and symptoms is useful for making differential diagnosis of SCA and other ataxic disorders. Progressive deterioration of hearing acuity is one of characteristic symptoms in some hereditary ataxic disorders such as mitochondrial encephalomyopathy, however, acoustic involvement in other forms of ataxia has not been fully investigated to date. We have previously reported no significant difference in acoustic functions in SCA31, cortical cerebellar atrophy (CCA), multiple system atrophy of cerebellar predominance (MSA-C), and other forms of SCAs [3].

We recently identified a novel type of SCA, which is named SCA36 or Asidan and is caused by a hexanucleotide GGCCTG repeat expansion in intron 1 of the nucleolar protein 56 (*NOP56*) gene [4–6]. This Asidan/SCA36 frequently displays unique clinical features involving cerebellum and motor neurons characteristically found in the tongue and limbs [5]. Here, we present the results of audiogram and brainstem auditory evoked potentials (BAEPs) of Asidan/SCA36 patients in comparison to normal controls, CCA, MSA-C, SCA31, and other forms of SCAs to investigate sensorineural hearing loss that was frequently found in our Asidan/SCA36 patients. In addition to signs for motor neuron involvement, acoustic impairment in Asidan/SCA36 is an important clinical feature that is distinguishable from other forms of SCAs.

## 2. Materials and methods

We recruited both sporadic and dominant ataxia cases with healthy controls for this study. After obtaining informed consent, genomic DNAs of cases in dominantly inherited ataxia families were extracted from peripheral blood leukocytes, and screened for CAG triplet dynamic mutations in spinocerebellar ataxia (SCA) types 1–3, 6–8, and dentatorubral and pallidoluysian atrophy (DRPLA) using a method described in our previous reports [5,7,8]. A diagnosis of SCA31/16q-ADCA is investigated by confirming both an insertion of (TGGAA)n by long PCR analysis, and a C-to-T substitution at 5′ UTR of the *puratrophin-1* gene by conventional PCR-based restriction fragment length polymorphism (RFLP) analysis as reported in elsewhere [9]. A genetic diagnosis of Asidan/SCA36 was investigated by confirming a (GGCCTG)n repeat expansion in intron 1 of the *NOP56* gene either through repeat-primed PCR analysis or Southern blot analysis according to our previous report [4].

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Individuals with MSA-C fulfilled the diagnostic criteria of probable MSA according to the second consensus statement [10]. Sporadic CCA was defined as an adult onset (>20 years old), progressive pure cerebellar ataxia with little or no overt involvement of other CNS parts. CCA cases did not fulfill the criteria for MSA, and their brain MRI or CT showed confined cerebellar atrophy [11]. Ataxia cases with established symptomatic factors that can cause cerebellar ataxia (alcoholism, cerebrovascular, metabolic, neoplastic, autoimmune or inflammatory diseases, thiamine or vitamin E deficiency, chronic intake of antiepileptic drugs) were excluded from this study. Severity of ataxia in each subject was evaluated by the scale for assessment and rating of ataxia (SARA) [12].

Acoustic function in cases with ataxia was evaluated using audiogram and BAEPs. An audiogram was performed in 13 cases with Asidan from 9 families, 4 cases with SCA31 from 3 families, 1 SCA2, 3 SCA3/Machado–Joseph disease (MJD), 6 SCA6 and 4 DRPLA cases from independent families, 28 CCA cases, and 48 MSA-C cases. Ages were matched between controls and Asidan, SCA31, CCA, and MSA-C except for other SCAs. Genders were all matched among controls, Asidan, SCA31, other SCAs, CCA, and MSA-C groups. BAEPs were recorded in 2 Asidan cases from independent families. Each subject was interviewed for occupational experience and history of otological diseases to rule out the presence of any pathological conditions that will affect the auditory function. Subjects with noise-induced hearing loss caused by occupational noise exposure or past medical history of otological disorders such as infection in auditory system or administration of drugs that can affect acoustic function were excluded from this study. The pure-tone air-conduction hearing threshold were collected for each ear at frequencies of 125, 250, 500, 1000, 2000, 4000, and 8000 Hz using a duly calibrated diagnostic audiometer measured by trained audiometric technicians. The pure-tone average (PTA) of air-conduction hearing thresholds was measured as the average of hearing thresholds at the four frequencies (500, 1000, 2000, and 4000 Hz) [13]. BAEPs were recorded from both earlobes (A1 and A2) against Cz, following condensant-click stimulation at 65 dB above the individual click hearing threshold and at 10 Hz. One thousand responses were averaged twice to confirm reproducibility, and the latencies of the first five positive peaks and interpeak latencies were measured.

Statistical analysis among respective groups was evaluated by one-way ANOVA followed by a Tukey–Kramer post-hoc multiple comparison test. $p$ values $<0.05$ were considered statistically significant.

## 3. Results

Table 1 summarizes the demographic data and the results of PTA evaluated by audiogram of participants in this study. Average age at onset of ataxia in the group of other SCAs (44.1 ± 13.4 years old) was significantly younger than CCA (54.7 ± 11.5 years old) and MSA-C (60.4 ± 8.2 years old). The duration of illness in MSA-C (2.6 ± 2.3 years) was significantly shorter than Asidan (13.5 ± 5.7 years), other SCAs (10.3 ± 5.1 years), and CCA (7.9 ± 6.5 years). Ages at examination were matched between controls and each of other groups except for other SCAs. Age at examination in other SCAs group (54.6 ± 13.6 years) was significantly younger than Asidan (66.5 ± 7.2 years, $p<0.01$), CCA (63.4 ± 9.9 years, $p<0.05$), MSA-C (63.3 ± 8.3 years, $p<0.05$) and controls (64.0 ± 4.1 years, $p<0.05$) (Table 1).

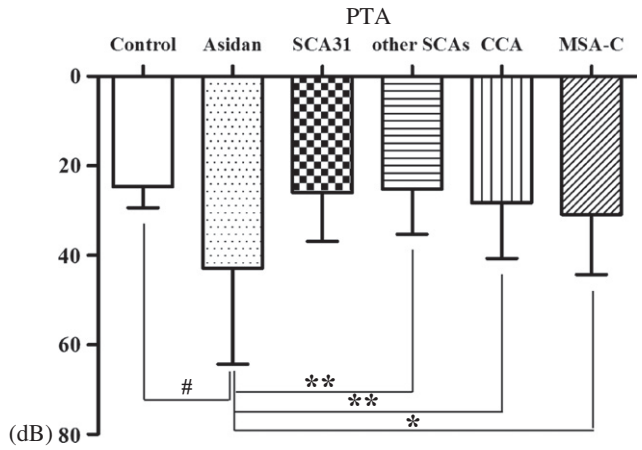


**Fig. 1.** Comparison of the pure tone average (PTA) in respective groups. Note that the PTA in Asidan is significantly decreased in comparison to normal controls, other SCAs, CCA, and MSA-C. Data are expressed as mean ± SD. #$p<0.01$ vs. control, *$p<0.05$ and **$p<0.01$ vs. Asidan.

The average hearing levels expressed by PTA are significantly decreased in Asidan (42.9 ± 21.5 dB) in comparison to controls (24.7 ± 4.7 dB, #$p<0.01$), other SCAs (25.2 ± 10.1 dB, **$p<0.01$), CCA (28.3 ± 12.5 dB, **$p<0.01$), and MSA-C (30.8 ± 13.5 dB, *$p<0.05$). The average PTA in SCA31 cases (26.0 ± 10.9 dB) is lower than Asidan, but is not significant due to the limited number of SCA31 cases (Table 1 and Fig. 1). There was no statistical difference of PTA between controls and SCA31, other SCAs, CCA or MSA-C. The average hearing thresholds measured at 7 different frequencies in each group are shown in Fig. 2a. The 7 hearing thresholds in Asidan were constantly higher at respective frequencies than other groups. In particular, the depression at higher frequencies (4000 Hz and 8000 Hz) was much larger in Asidan patients than the control groups ($p<0.05$). Correlation between the PTA and the severity of ataxia evaluated by SARA in 12 Asidan patients are shown in Fig. 2b. There was significant positive correlation between them ($r=0.76$, $p<0.01$). Correlation between the PTA and the duration of illness in 13 Asidan patients are shown in Fig. 2c. There was significant positive correlation between them ($r=0.80$, $p<0.01$).

BAEPs in a typical control and 2 Asidan cases are shown in Fig. 3. Ages at onset with truncal instability of the Asidan cases 1 and 2 were both 55 years old, and duration of illness were 6 and 21 years, respectively. Ages at examination of the Asidan cases 1 and 2 were 61 and 76 years old, respectively. The Asidan case 1 showed mild acoustic impairment with a PTA of 34 dB, and verbal communication in daily life was sometimes affected. The Asidan case 2 showed severe

**Table 1**
Demographic data and the results of PTA in this study.

| | Control | Asidan | SCA31 | Other SCAs | CCA | MSA-C |
|---|---|---|---|---|---|---|
| No. of cases | 31 | 13 | 4 | 14 | 28 | 48 |
| Gender (M/F) | 15/16 | 7/6 | 3/1 | 7/7 | 15/13 | 24/24 |
| Age at onset (years) | – | 53.0 ± 3.6 | 52.7 ± 4.5 | 44.1 ± 13.4* | 54.7 ± 11.5 | 60.4 ± 8.2 |
| Duration (years) | – | 13.5 ± 5.7 | 8.7 ± 2.9 | 10.3 ± 5.1 | 7.9 ± 6.5 | 2.6 ± 2.3** |
| Age at exam. (years) | 64.0 ± 4.1 | 66.5 ± 7.2 | 64.3 ± 7.4 | 54.6 ± 13.6*** | 63.4 ± 9.9 | 63.3 ± 8.3 |
| PTA (dB) | 24.7 ± 4.7 | 42.9 ± 21.5**** | 26.0 ± 10.9 | 25.2 ± 10.1 | 28.3 ± 12.5 | 30.8 ± 13.5 |

PTA: pure tone average.
* $p<0.05$ compared with CCA, $p<0.01$ compared with MSA-C.
** $p<0.01$ compared with Asidan, other SCAs, or CCA.
*** $p<0.01$ compared with Asidan, $p<0.05$ compared with control, CCA, or MSA-C.
**** $p<0.01$ compared with control, other SCAs or CCA, $p<0.05$ compared with MSA-C.

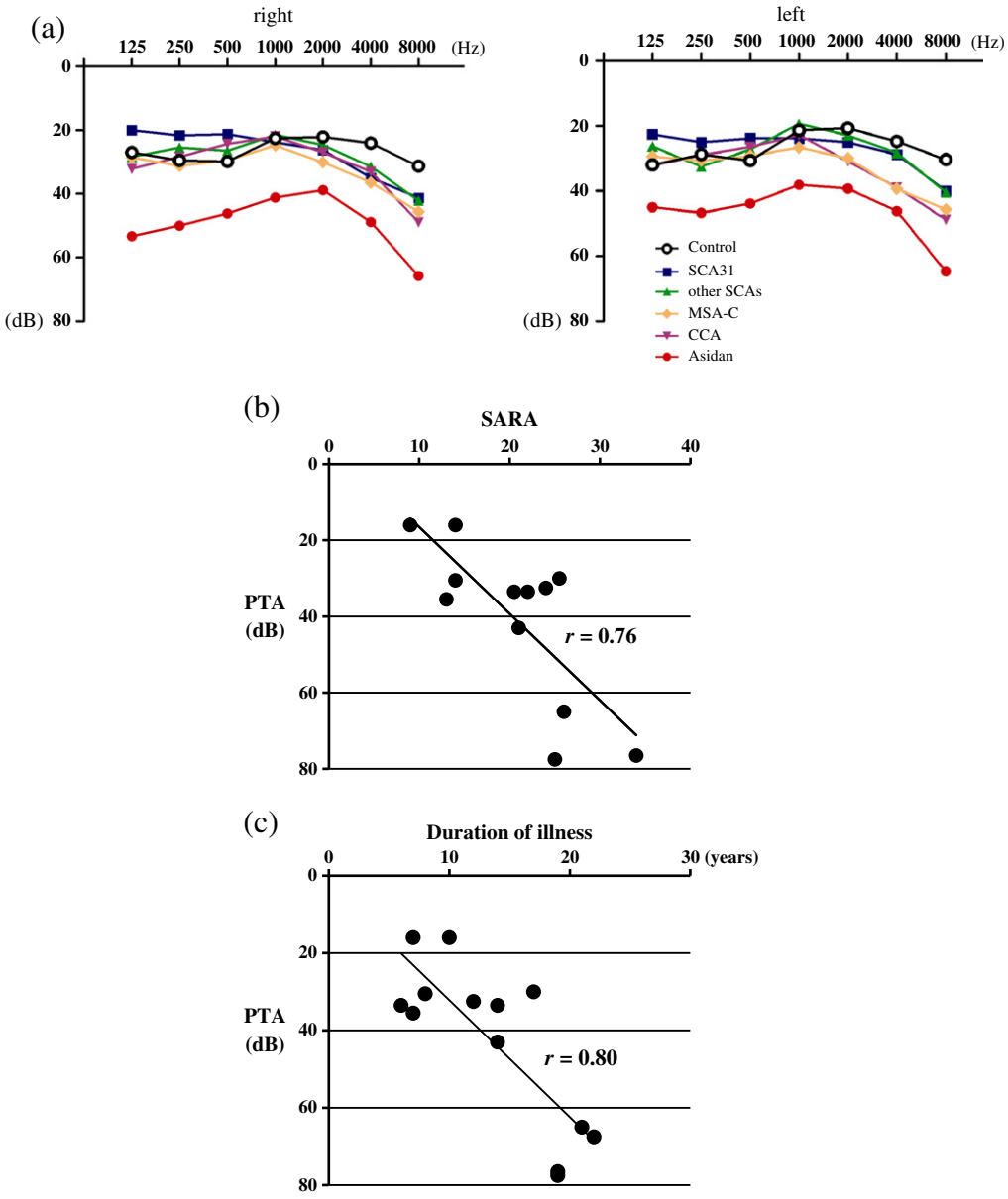


**Fig. 2.** Comparisons of mean hearing thresholds at 7 frequencies (125–8000 Hz) in respective groups evaluated by audiogram (a). Note that the 7 hearing thresholds were constantly depressed in Asidan at respective frequencies than other groups. Correlation between the pure tone average (PTA) and the scale for assessment and rating of ataxia (SARA, $r=0.76$, $p<0.01$) in Asidan cases ($n=12$) (b). Correlation between the PTA and the duration of illness ($r=0.80$, $p<0.01$) in Asidan cases ($n=13$) (c).

acoustic impairment with a PTA of 65 dB, and verbal communication in daily life was severely affected. The results of BAEPs in a control subject without known neurological disorders (80 years old) clearly showed I to V waves, and the latencies to each wave-peak and the I–III, III–V, and I–V interpeak latencies were all within normal range (Fig. 3, top panel). The results of BAEPs in the Asidan case 1 exhibited poor responses, but the I, III, and V waves were barely confirmed. The latencies to each wave-peak and the I–III, III–V, and I–V interpeak latencies were not obviously delayed (Fig. 3, middle panel). The results of BAEPs in Asidan case 2 exhibited that poor responses were recorded from both sides, and only the V wave was recognizable. The latency to the V wave-peak was prolonged at 6.40 ms (Fig. 3, bottom panel).

## 4. Discussion

This is the first detailed report showing that sensorineural hearing loss is characteristic for Asidan/SCA36 patients. Asidan/SCA36 is a dominant form of combined hereditary ataxia and motor neuron disease initially identified in families from an area along the river Asida, located in the western part of the mainland Japan. The responsible mutation was discovered as the hexanucleotide (GGCCTG)n repeat expansion in the *NOP56* gene located at chromosome 20p13 [4]. We had found 18 Asidan patients from 9 families with a size range of the repeat expansion from 1700 to 2300 repeats. The 9 Asidan families showed a significant founder effect. Clinical and neuropathological features in these families were reported in elsewhere [5].

The PTA in Asidan patients was significantly decreased at 42.9 dB with a remarkable depression at higher frequencies (4000 Hz and 8000 Hz) in comparison to control, other SCAs, CCA, and MSA-C (Table 1, Figs. 1 and 2a). Because of no significant difference of examination ages between Asidan and control, CCA or MSA-C, sensorineural hearing loss in Asidan is probably a specific symptom due to the disease process caused by the (GGCCTG)n expansion mutation. Asidan patients also showed that the PTA significantly correlated with the SARA scores and the duration of illness (Fig. 2b and c), indicating their sensorineural hearing loss might

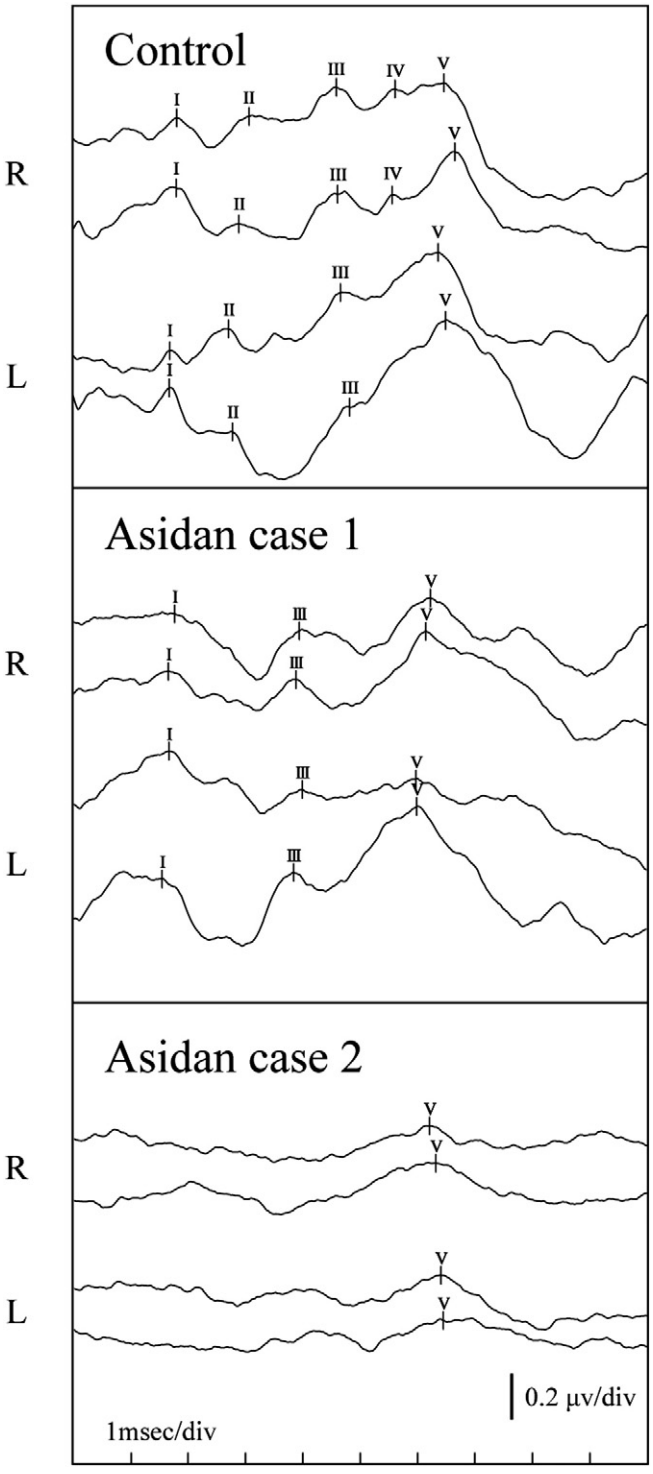


**Fig. 3.** Brainstem auditory evoked potentials (BAEPs) of a typical control (top) and 2 Asidan cases (middle and bottom). Note that the BAEPs in 2 Asidan cases exhibited poor responses.

deteriorate with the progression of cerebellar ataxia. Although a mild cognitive decline especially of frontal lobe function is present in Asidan patients [6], this decline cannot explain for the auditory disturbance (Fig. 2a). In fact, the results of BAEPs in 2 Asidan patients showing poor responses with normal interpeak latencies suggested possible involvement in the inner ear or the peripheral part of the auditory system (Fig. 3).

After we identified Japanese Asidan/SCA36 families, Garcia-Murias et al. reported Spanish SCA36 families with the (GGCCTG)n repeat expansion accumulated in the same village [14]. They performed audiometry on 6 Spanish SCA36 patients and found moderate to severe sensorineural hearing loss with a threshold over 40 dB beyond 2500 Hz, and severe decrease of waves I and II in BAEPs. Although detailed data of audiometric analysis was not exhibited, they suggested that sensorineural hearing loss could be a feature of SCA36 phenotype.

In our previous study, no significant difference of *NOP56* RNA or protein expression was found between Asidan/SCA36 and control lymphoblasts. Furthermore, RNA foci-containing aggregates of expanded GGCCUG transcripts were found in the nuclei of Asidan/SCA36 lymphoblasts, suggesting an RNA gain-of-function mechanism in the pathogenesis of this unique disease. To understand the reason why sensorineural hearing loss can occur in the present Asidan/SCA36 patients, it is necessary to investigate the molecular pathomechanism of degeneration of cerebellum, motor neurons, and auditory system leading to the characteristic symptoms of Asidan/SCA36 that are distinguishable from other types of dominant ataxias.

## Conflict of interest

Nothing to report.

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## References

[1] Schols L, Bauer P, Schmidt T, Schulte T, Riess O. Autosomal dominant cerebellar ataxias: clinical features, genetics, and pathogenesis. Lancet Neurol 2004;3:291-304.
[2] Durr A. Autosomal dominant cerebellar ataxias: polyglutamine expansions and beyond. Lancet Neurol 2010;9:885-94.
[3] Ikeda Y, Nagai M, Kurata T, Yamashita T, Ohta Y, Nagotani S, et al. Comparisons of acoustic function in SCA31 and other forms of ataxias. Neurol Res 2011;33:427-32.
[4] Kobayashi H, Abe K, Matsuura T, Ikeda Y, Hitomi T, Akechi Y, et al. Expansion of intronic GGCCTG hexanucleotide repeat in NOP56 causes SCA36, a type of spinocerebellar ataxia accompanied by motor neuron involvement. Am J Hum Genet 2011;89:121-30.
[5] Ikeda Y, Ohta Y, Kobayashi H, Okamoto M, Takamatsu K, Ota T, et al. Clinical features of SCA36: A novel spinocerebellar ataxia with motor neuron involvement (Asidan). Neurology 2012;79:333-41.
[6] Abe K, Ikeda Y, Kurata T, Ohta Y, Manabe Y, Okamoto M, et al. Cognitive and affective impairments of a novel SCA/MND crossroad mutation Asidan. Eur J Neurol 2012;19:1070-8.
[7] Ikeda Y, Shizuka M, Watanabe M, Okamoto K, Shoji M. Molecular and clinical analyses of spinocerebellar ataxia type 8 in Japan. Neurology 2000;54:950-5.
[8] Ikeda Y, Dalton JC, Moseley ML, Gardner KL, Bird TD, Ashizawa T, et al. Spinocerebellar ataxia type 8: molecular genetic comparisons and haplotype analysis of 37 families with ataxia. Am J Hum Genet 2004;75:3–16.
[9] Sato N, Amino T, Kobayashi K, Asakawa S, Ishiguro T, Tsunemi T, et al. Spinocerebellar ataxia type 31 is associated with "inserted" penta-nucleotide repeats containing (TGGAA)(n). Am J Hum Genet 2009;85:544-57.
[10] Gilman S, Wenning GK, Low PA, Brooks DJ, Mathias CJ, Trojanowski JQ, et al. Second consensus statement on the diagnosis of multiple system atrophy. Neurology 2008;71:670-6.
[11] Abele M, Minnerop M, Urbach H, Specht K, Klockgether T. Sporadic adult onset ataxia of unknown etiology: a clinical, electrophysiological and imaging study. J Neurol 2007;254:1384-9.
[12] Schmitz-Hubsch T, du Montcel ST, Baliko L, Berciano J, Boesch S, Depondt C, et al. Scale for the assessment and rating of ataxia: development of a new clinical scale. Neurology 2006;66:1717-20.
[13] Chia EM, Wang JJ, Rochtchina E, Cumming RR, Newall P, Mitchell P. Hearing impairment and health-related quality of life: the Blue Mountains Hearing Study. Ear Hear 2007;28:187-95.
[14] Garcia-Murias M, Quintans B, Arias M, Seixas AI, Cacheiro P, Tarrio R, et al. 'Costa da Morte' ataxia is spinocerebellar ataxia 36: clinical and genetic characterization. Brain 2012;135:1423-35.