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Evaluation of Pulpal Vitality in Patients with Hereditary Sensory and Autonomic Neuropathy Type IV or V

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Abstract

Introduction: The aim of this study is to reveal the characteristics of the pulpal sensation and innervation in teeth of Japanese patients aged 6 to 33 years with hereditary sensory and autonomic neuropathy (HSAN) type IV or V in comparison to outpatients with no neuropathy (controls).

Methods: Pulpal vitality was examined with electric pulp testing (EPT). The minimally carious primary permanent incisors, premolars, and molars were prepared for histopathological analysis.

Results: Pulpal sensation and innervation in the patients with HSAN-IV was notably different from those of HSAN-V or controls. In comparison with HSAN-V or controls, HSAN-IV teeth had an overall marked reduction in pulpal sensation and the absence of large nerve bundles and subodontoblastic plexus in pulpal innervation, these nerve fibers have a well-established role in nociceptive processing. The pre-pain sensation of almost patients with HSAN-IV was not induced as unpleasant sensation of dental pulp using EPT, whereas the dental pulp vitality was confirmed with the same vital test in healthy controls and patients with HSAN-V. These findings were reconfirmed by morphological analyses with silver-, Klüver•Barrera-staining, and immunohistochemistry.

Conclusions: The present research suggests marked reduction or absence of myelinated and unmyelinated fibers in the dental pulp of HSAN-IV patients, providing a morphological basis for analgesia, and that HSAN-IV patients suffer from more widespread disturbances of sensation than has been previously recognized.

Keywords: HSAN Type IV, HSAN Type V, Nociceptive sensation, Pulpal vitality, Pulpal innervation, Human

Introduction

Hereditary sensory and autonomic neuropathy (HSAN) refers to a group of rare congenital disorders manifesting in a loss of pain sensation that results from peripheral neuropathy and has been classified into types I-V [1,2]. Recently, HSAN type VI was newly added to the list [3]. Among them, a relatively large proportion of patients with HSAN-IV is reported from Japan [4]. Congenital insensitivity to pain with anhidrosis (CIPA), or HSAN type IV, is a rare autosomal recessive disorder associated with consanguinity [5]. Clinical findings of HSAN-IV are characterized by a generalized loss of pain and thermal sensation, a lack of sweating, and are associated with variable degrees of intellectual disability and/or learning deficits. Absence of reaction to painful stimuli and anhidrosis is due to the absence of afferent neurons activated by tissue-damaging stimuli and a loss of innervation of sweat glands, respectively. HSAN-IV is the consequence of loss-of-function mutations in the *NTRK1* gene encoding TrkA (tropomyosin-related kinase A), a receptor tyrosine kinase for nerve growth factor (NGF) located on chromosome 1 (q21-q22) [6]. The *NTRK1*/NGF regulates the activities of both nociceptive sensory and autonomic sympathetic neurons as well as cholinergic neurons of basal forebrain. The inability to sense pain does not spare particular areas and even affects cranial visceral nerves [7].

The prevalence of HSAN type V amongst the Japanese population is unknown, except for one English paper reporting a Japanese patient with HSAN-V [8]. HSAN-V is also an autosomal recessive disorder characterized by a loss of pain and thermal sensation. In contrast with HSAN-IV, sweating and mental development are usually normal in these patients. In northern Sweden, a large family with six members affected by HSAN-V has

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been reported, with mutations in the *NGFB* (nerve growth factor, β subunit) gene detected [9]. According to the epidemiological data reported by Haga et al. [4], the number of Japanese patients with HSAN types IV and V is estimated as 130-210 and 30-60 patients, respectively.

Teeth are vital for survival and therefore the presence of a protective sensory innervation is of great importance. During normal development, teeth acquire a sensory innervation from the trigeminal ganglion through a series of steps which occur in a strictly controlled pattern. However, the formation of terminal branches in teeth is delayed until post-natal stages, and this event is coordinated with the developmental maturation of the teeth. In mammals, the shift from the primary to the permanent dentition necessitates a second period of *de novo* innervation of a new and larger set of teeth from local nerves in the jaws at a very late stage in post-natal development [10,11]. The dental pulp is a richly innervated tissue supplied by Aβ-, Aδ-, and C-fibers. Afferent impulses from pulpal fibers predominantly result in pain perception irrespective of the nature of sensory stimulus.

Although patients with HSAN-IV have been reported for over half a century, the majority of related articles has examined differences in peripheral sensory nerve function between HSAN-IV patients and healthy controls by basic quantitative clinical neurological inspection of the sense of touch/pressure, vibration, joint position, and two point discrimination. On the contrary, no study to date has systematically investigated peripheral sensory nerve function of dental pulp. According to our knowledge, this study is the first report that compares physiological findings of dental pulp with histopathological data in patients with HSAN-IV or -V. In the present paper, we examined 16 and 5 Japanese patients with HSAN-IV or -V, respectively, focusing on the status of pulpal sensation/innervation and evaluated differences in peripheral sensory nerve function of dental pulp among HSAN-IV, -V patients, and healthy controls.

Materials and Methods

Investigation design

A total of 16 clinically diagnosed HSAN-IV (CIPA) patients (eleven males and five females) aged 6 to 33 years (mean age, 14.6 years) and 5 clinically diagnosed HSAN-V patients (one male and four females) aged 11 to 21 years (mean age, 15.4 years) participated in this study (Tables 1 and 2). The permanent teeth used in the present study were extracted under local or general anaesthesia. This study was approved by the Ethical Committee

No.	Gender	Age (Years)	Pre-pain in R1*1	Pre-pain in L1*2	Genetic analysis	MR	Histopathological examination
1	M	6	80	80		±	
2	M	7	80	80		+	
3	M	8	80	80		+	
4	M	8	80	80		±	
		9					○
5	M	11	80	80		++	
6	M	11	80	80		+	
		14					○
7	M	13	80	80		+++	
8	M	15	80	80	○	+	
9	M	26	pulpless	pulpless		++	○
10	F	7	80	80		+	
11	F	11	80	80	○	++	○
		14	80	80			
12	F	11	80	80			
13	F	14	80	80		+++	
14	F	15	80	80		+	
15	F	23	63	56		++	
		24	51	52			
		26					○
16	F	33	68	pulpless		++	

F female; M male; *1 right upper central incisor; *2 left upper central incisor; MR mental retardation
(±: IQ76~85; +: IQ50~75; ++: IQ35~49; +++: IQ20~34); ○ examination performed; nosigned not performed

Table 1: List of HSAN-IV (CIPA) patients.

No.	Gender	Age (Years)	Pre-pain in R1*1	Pre-pain in L1*2	MR	Histopathological examination
1	F	11	19	37	+	
2	F	13	26	25	Nr	
3	F	13	32	26	Nr	
4	F	19	27	24	Nr	○
5	M	21	49	48	++	

F female; *M* male; *1 right upper central incisor; *2 left upper central incisor; *MR* metal retardation
(Nr: normal; ±: IQ76~85; +: IQ50~75; ++: IQ35~49; +++: IQ20~34); ○ examination performed

Table 2: List of HSAN-V patients

of the Tokyo Medical and Dental University (approval number 914) and the Tsurumi University School of Dental Medicine (approval number 617), respectively. The general health of the patients was thoroughly examined by physicians, including comprehensive neurophysiological assessment. The diagnosis was further verified by laboratory assays and genetic tests for some patients. They were introduced into the Dental Hospital of the Tokyo Medical and Dental University Graduate School for oral examination and care. We diagnosed carefully time-lapsed progress of oral manifestations over a long period of time. Furthermore, a lateral incisor and four third molars from 5 unrelated healthy outpatients (two males and three females) aged 10-25 years (mean age, 19.2 years) were used as control subjects for histopathological examination at the Dental Hospital of the Tokyo Medical and Dental University Graduate School under sufficient informed consent in accordance with approved guidelines set by the Ethical Committee of Human Subjects Research at Tokyo Medical and Dental University or Tsurumi University. They had orthodontic indication for extraction. The handling of patients followed the guidelines of the Ethical Committee of Human Subjects Research, set according to the Helsinki declaration (2008) for human beings. We gave informed consent to all participants prior to participation.

Physiological analyses

The threshold of pre-pain sensation in dental pulp of anterior teeth was examined in fifteen patients with HSAN-IV and five ones with HSAN-V by electric pulp testing (EPT) with the Analytic Technology Pulp Tester® (Analytic Technology Redmond, WA, USA). The pre-pain is, generally, measured as somatic sensation of dental pulp using EPT under 50 scores, the maximum of its threshold sensitized in the healthy controls [12]. The pre-pain sensation of patients with HSAN-IV was measured until 80 scores, the maximum threshold able to be measured using EPT and the dental pulp measured up to 80 scores with EPT was diagnosed non-vital.

Histopathological and immunohistochemical analyses

The pulpal innervation was examined in incisors, premolars, or molars extracted from each type of patients for pathological tooth mobility. One lower lateral incisor and four lower third molar teeth obtained from a total of 5 healthy persons were used as a control as above-mentioned. The teeth were immersed in the fixative of 0.1 M phosphate-buffered (pH 7.4) 4% paraformaldehyde containing 15% saturated picric acid for at least 1 week at 4˚C. After fixation, the tissue blocks were decalcified with buffered 10% ethylenediaminetetraacetic acid (pH 7.5; EDTA; Wako, Osaka, Japan), and then embedded in paraffin. Sections (5 μm-thick) were cut and collected on MSA-coated slides (Matsunami, Osaka, Japan). After deparaffinization, they were stained with hematoxylin and eosin (HE), silver impregnation technique, Klüver•Barrera-staining, or immunohistochemistry for protein gene product 9.5 (PGP9.5) or βIII tubulin.

Silver impregnation technique – Bodian method

The Bodian method has been used as the routine staining for studying nervous elements. Sections were taken through xylene and graded ethanols to water. They were then rinsed in distilled water and placed in Coplin jars, each containing 50 ml of 1% silver proteinate in one of solutions. 2.5 g of fine copper gauze was added to the solution in each jar. Impregnation was varied out in the dark (usually for 24 h at 37°C). After impregnation the sections were rinsed briefly in distilled water and developed until no further darkening could be observed, in a solution consisting of 1 g of hydroquinone and 5 g of sodium sulfite (anhydrous) in 100 ml tap-water. The sections were washed in distilled water for 4 min. The water changed twice to prevent precipitation at the next stage. Next, they were covered with 1% gold chloride, acidified with 3 drops of glacial acetic acid in 50 ml for 5 min. After being rinsed in distilled water, the sections were transferred to a solution consisting of 2 g of oxalic acid and 1 ml of formalin in 100 ml of tap-water. They were left in this solution for 7 min. Sections were washed for 4 min in tap-water, which was changed twice. After rinsing briefly in distilled water and fixing in 5% sodium thiosulfate for 5 min, they were washed in running tap-water, dehydrated through graded ethanols to xylene, and mounted with Permount® (Fisher Scientific, Fair Lawn, NJ, USA).

Klüver•Barrera-staining

We hydrated the sections to 95% alcohol and then stained them with 0.1% luxol fast blue solution at 56-60°C overnight. Sections were rinsed in 95% alcohol to remove excess stain. After rinsing briefly in distilled water they were differentiated by quick immersion in 0.05% lithium carbonate solution and by putting through several changes of 70% alcohol solution. The sections were thoroughly washed in distilled water. Next, they were stained with 0.1% cresyl echt violet solution at 37°C for 6 min, which was filtered and preheated to 37°C just before use. They were differentiated in several changes of 95% alcohol, dehydrated in absolute alcohol and in xylene, and mounted with Permount® (Fisher Scientific).

Immunohistochemistry for PGP9.5 or βIII tubulin

The immunoreactive products were visualized with the streptavidin-biotin-peroxidase complex (SAB) method [13]. Rabbit polyclonal antibodies against PGP9.5 or βIII tubulin (Abcam, Cambridge, MA, USA) were used. We performed heat mediated antigen retrieval via the pressure cooker before commencing with immunohistochemical staining protocol. Sections were treated with 0.3% hydrogen peroxide (H_2O_2) in 100% methanol for 20 min to block endogenous peroxide activity and then incubated with 20% normal goat serum in PBS for 30 min to block non-specific binding. Primary antibodies were applied at appropriate concentrations (PGP9.5 1:400; βIII tubulin 1:100 or 1:400) in PBS with 1% bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO, USA) and 0.03% Triton X-100 (Serva, Heidelberg, Germany) for 2 hrs at 37˚C. The sections were incubated in biotinylated goat anti-rabbit Ig (1:800, Dako, Copenhagen, Denmark). Sections were rinsed with PBS with Triton X-100 (Serva) between each step. Immunoreactions were visualized using 0.025% 3, 3'-diaminobenzidine tetrahydrochloride (DAB; Sigma-Aldrich) and 0.01% H_2O_2 in 0.05M Tris-HCl buffer (pH 7.3) for 7-10 min. After being counterstained with hematoxylin, they were dehydrated and mounted with Permount® (Fisher Scientific).

As immunohistochemical controls, sections were processed by replacing the primary antibodies with normal non-immune serum. The immunoreactions were completely absent in control sections.

Results

Clinical findings

On the clinical examination, the patients described here (Table 1) were associated with variable degrees of mental retardation or learning deficits and exhibited oral features characteristic of HSAN-IV. Clinical examination revealed that the proband presented a developmental delay. Psychomotor development tests performed on IQ test showed variable levels of mental retardation (Table 1). There was no reported history of pain associated with the carious dentition or acute dentofacial abscess. Scrach marks, erosions and cicatrization healing (scars) due to self-inflicted soft tissue trauma were often found on their face. An absence of sweating with dry warm skin was consistently noted during the febrile periods. Oral examination often revealed a full-thickness loss of the tongue tip, which was ulcerated, and there was also considerable tissue loss from the lower lip (Figure 1). The patients had decreased ability to open their mouths that were attributed to fibrous bands of scar tissue in the cheeks and lips. Early tooth loss was noted, including almost all primary teeth except for some primary and permanent teeth. In the case of mandibular osteomyelitis of 11-year- and six-month-old proband with HSAN-IV, the panoramic radiograph showed that numerous permanent teeth were missing. In this case, all of the teeth examined were non-responsive to EPT. Genetic analysis of the *NTRK1* gene was performed in two patients, which is known to be responsible for HSAN-IV (No. 8, 11 in Table 1) [6,7]. A loss of base C at nucleotide 1726 (c.1726delC; R548fs) or two mutations at intron 7 (IVS7-33T>A) were confirmed, respectively.

In the patients with HSAN-V examined in this study, sweating and mental development in some patients were normal but others were associated with variable degrees of mental retardation (Table 2).

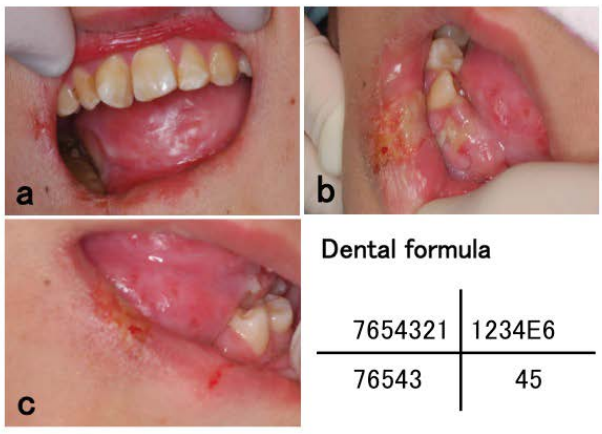

Figure 1: Oral manifestations of the proband (female; 11-year- and 5-month-old) with HSAN type IV. Oral examination showing a full-thickness loss of the tongue tip (**a**), tissue loss from the lower lip (**b**), and the ulceration of oral tissues (**c**). Thus, the patient seems to be decreased ability to open her mouth that are attributed to fibrous bands of scar tissue in the cheeks and lips. She loses eight teeth including lower central incisors, a left lower canine tooth, and left molars due to mandibular osteomyelitis caused by self-exfoliation of a left mandibular lateral incisor.

	Age (years)	Number of tooth	Minimum	Maximum	Average	SD
1) Healthy control*						
	6-7	53	30	80	59.2	13.34
	8-11	51	20	70	50.8	11.21
	12-18	60	18	52	38.5	7.91
	Over 18	60	12	45	24.9	9.60
2) HSAN-IV (CIPA) patients						
	6-7	6	80<	80<	-	-
	8-11	12	80<	80<	-	-
	12-18	10	80<	80<	-	-
	Over 18	5	51	68	58	7.31
3) HSAN-V patients						
	8-11	2	19	37	28	12.73
	12-18	4	25	32	27.25	3.20
	Over 18	4	24	49	37	13.34

* This table was drawn up from the data of Otawa et al. (1986) [12].

Table 3: List of EPT-value

Pre-pain sensation to dental pulp

The threshold of pre-pain sensation to dental pulp of anterior teeth was examined by EPT with analytic technology pulp tester. The pre-pain sensation was induced as unpleasant sensation and measured as somatic sensation of dental pulp using EPT under about 50 scores in healthy controls (Table 3) and patients with HSAN-V (Table 3). Contrastingly, the pre-pain sensation of patients with HSAN-IV was measured until 80 scores, the maximum threshold measured using EPT. Two of 16 HSAN-IV patients exhibited pre-pain sensation of the dental pulp, although its threshold values were higher in the HSAN-IV patients of the same age than in the control and HSAN-V groups (Table 3).

Histopathological examination

The general histological features of the tissues including dentine, predentin, odontoblasts, blood vessels, fibroblasts and some myelinated nerve fibers were seen in the HE-stained sections of controls (Figure 2a) and HSAN-V tooth (Figure 4a) but in some sections the predentine or odontoblasts were not obvious. In the HE-stained section of coronal pulp, furthermore, four distinct zones were distinguished: (1) the odontoblastic zone at the pulp periphery; (2) a cell-free zone of Weil beneath the odontoblasts; (3) a cell-rich zone, where cell density is high; and (4) the pulp core, which is characterized by the major vessels and nerves of the pulp (Figure 2a). In the same way, four distinct zones (odontoblastic, cell-free, cell-rich, and pulp core) were observed on the surface of the dentin matrix of HSAN-IV teeth (data not shown). However, the pulp core did not exhibitted any nerve bundles in the HE-stained sections of the HSAN-IV teeth (Figure 3a).

Pulpal innervation evaluated using silver- or Klüver•Barrera-staining

Freshly extracted healthy, HSAN-IV, or -V teeth were subjected to silver- or Klüver•Barrera-staining. Bundles of nerve fibers were labeled in the center of the dental root of control (Figure 2b) and HSAN-V teeth (Figure 4c) using silver-staining. Furthermore, the Klüver•Barrera-staining revealed numerous nerve bundles in the pulp core of healthy and HSAN-V teeth (Figure 4b). The both methods showed a lot of thick myelinated nerve fibers distributed in the dental pulp tissues of control and HSAN-V teeth. On the

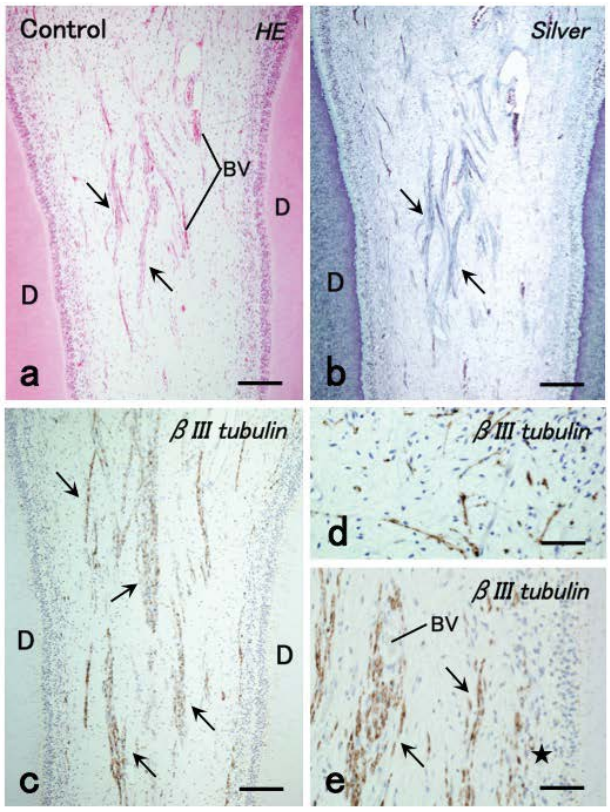

Figure 2: Histopathological examination of normal teeth. **a**. Dentine (*D*), predentin, odontoblasts, blood vessels (*BV*), fibroblasts and some myelinated nerve fibers (*arrows*) are clearly identified in the dental pulp of the right lower lateral incisor in a 10-year-old healthy control girl on a HE-stained section. **b**. Silver-stained tooth tissue of a control sample is showing a large number of myelinated nerve fibers (*arrows*) in the dental pulp. **c**. Immunohistochemistry for βIII tubulin labels numerous posivie myelinated nerve fibers (*arrows*) in the pulpal tissue of the same person as **a** and **b**. **d** βIII tubulin-stained nerve fibers represent varicose appearance in the dental pulp of left lower molar of 19-year-old person. **e** Pulpal large nerve bundles (*arrows*) passing from the coronal to the root area, often in association with blood vessels (*BV*), and continueing towards the pulp horns giving branches peripherally to form a subodontoblast plexus (*star*) in the same control samples as **d**. *D* dentin; *Bars* = 200 μm for Figures. **a**-**c**; *bars* = 50 μm for Figures **d**, **e**.

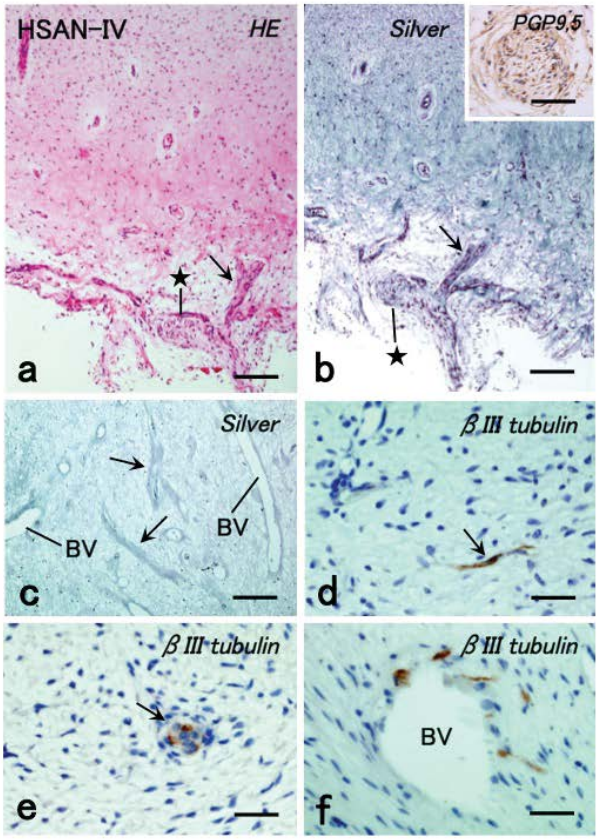

Figure 3. Histopathological examination of the probands with HSAN-IV. There is almost disappearance of immunoreactive products within the radicular and coronal pulp. **a**, **b**An innervation with PGP9.5-immunoreactive fibers (***Inset*** in **b**) is found in the neighboring cross section (*star* in **a**, **b**) of HE-stained (*arrow* in **a**) or silver-stained (*arrow* in **b**) nerve bundles in the left upper first premolar of 14-year old HSAN-IV patient. Only a few positive fibers (*arrows*) are labeled with anti-βIII tubulin in the same proband (**d**-**f**). **c** Nerve bundles (*arrows*) are detected in the right lower third molar of 26-year-old patient with HSAN-IV using silver-staining. *BV* blood vessel; *Bars* = 100 μm for Figures**a**-**c**; *Bar* = 50 μm for an inset of Figure**b**; Bars = 20 μm for Figures**d**-**f**.

contrary, no neuroelements were detected in the pulpal tissue of almost patients with HSAN-IV, whereas the pulp tissues of HSAN-IV teeth exhibited a few nerve bundles in two 14- and 26-year-old patients (No. 6 and 15 in Table 1) using silver-staining (Figure 3b and c).

Pulpodentinal innervation revealed with immunohistochemistry for PGP9.5 or βIII tubulin

The paraffin-sections of freshly extracted healthy, HSAN-IV, or -V teeth were treated with immunohistochemistry for PGP9.5 or βIII tubulin. The fixation and processing methods revealed the principal features of the tissues in all of the teeth examined but the intensity of the staining showed some variability. Immunostaining for βIII tubulin was intense and revealed prolific pulpal innervation in the control teeth (Figure 2c-e). Pulpal large nerve bundles were seen passing from the coronal to the root area, often in association with blood vessels, and continued towards the pulp horns giving branches peripherally to form a subodontoblast plexus (star in Figure 2e). Nerve fibers in the subodontoblast plexus terminated either there, in the odontoblast layer, or in the dentine; the majority terminated before reaching the dentine. These nerve fibers had often varicose appearances (Figure 2d and e). In the midcoronal zones, only a few nerve fibers branched from the subodontoblast plexus to reach the odontoblast layer. In the cervical part of the crown nerve fibers branched from the subodontoblast plexus and the greater part of them terminated in the odontoblast layer. In some areas a marginal plexus of nervous tissue was observed between the odontoblasts and the predentine; intratubular nerve fibers appeared to arise either from this plexus or directly from the pulp.

The pulpal innervation visualized in the HSAN-IV teeth was markedly different from controls or patients with HSAN-V. There was almost disappearance of immunoreactive products within the radicular and coronal pulp and a complete absence of the subodontoblastic plexus in patients with HSAN-IV (Figure 3), whereas numerous nerve fibers were immunohistochemically labeled in controls (Figure 2c-e) or patients with HSAN-V (Figure 4d-g). An innervation with PGP9.5-immunoreactive fibers was found in silver-stained nerve bundles of the HSAN-IV

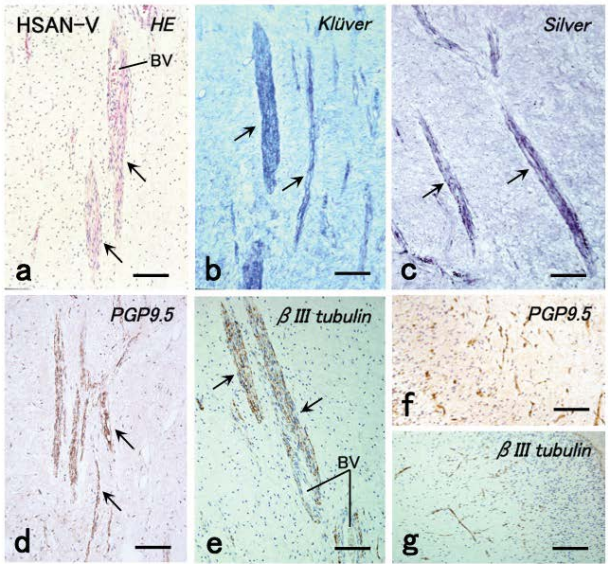

Figure 4. Histopathological examination of the proband with HSAN-V. The HE- (**a**) , Klüver•Barrera- (**b**), Silver- (**c**), PGP9.5- (**d**, **f**), or βIII tubulin-stained (**e**, **g**) lower third molar tissues of the 19-year-old patient with HSAN-V show a large number of myelinated nerve fibers (*arrows*) in the dental pulp. Pulpal large nerve bundles pass from the coronal to the root area, often in association with blood vessels (*BV*). PGP9.5- (**f**) orβIII tubulin-labeled (**g**) nerve fibers represent varicose appearance in the dental pulp. *Bars* = 200 m for Figures **a**, **b**, **d**; *Bars* = 100 μm for Figures **c**, **e**-**g**.

teeth examined in this study (Figure 3b; No. 6 in Table 1) and, furthermore, only a few positive fibers were labeled with anti-III tubulin (Figure 3d-f). There was no evidence of any PGP9.5- or βIII tubulin-immunolabeled fibers in the subodontoblastic layer or within the dentin of HSAN-IV probands.

Pulpodentinal innervation evaluated with both the EPT and histopathological methods

In the patients of HSAN-IV (No. 4, 6, 11, and 15 in Table 1) and of HSAN-V (No. 4 in Table 2), the pre-pain sensation and pulpal innervation in dental pulp of the same person was examined with EPT and histopathological methods, respectively. The vitality of the dental pulp in the HSAN-IV patient (No. 11) was diagnosed as non-vitality by the EPT. In the same patient, pulpal innervation was not confirmed with histopathological analysis. In the patient with HSAN-IV (No. 15 in Table 1), threshold values of the pre-pain sensation were higher compared to control but vital (Tabels 1 and 3), and histopathological technique labeled some nerve bundles (Figure 3c). In others with HSAN-IV (No. 4, 6, and 11 in Table 1), the pulpal pre-pain sensation was not detected using EPT. Histopathological analyses did not detect any neuroelements in No. 4 and 11 of HSAN-IV probands except for No. 6 (Figure 3b and d-f). To the contrary, in the patient with HSAN-V (No. 4 in Table 2), threshold values of the pre-pain sensation were almost same as control (Table 3) and histopathological examination, furthermore, confirmed developed pulpal innervation using HE-, Klüver•Barrera-, silver-staining, or immunohistochemistry for PGP9.5 or βIII tubulin (Figure 4).

Discussion

We have described the oral and dental manifestations of 16 patients with HSAN-IV and 5 patients with HSAN-V in the present study. Although some degree of phenotypic variability was observed in the HSAN-IV patients examined, overall they had typical features, including insensitivity to pain, anhidrosis, and mental retardation, confirming the diagnosis of HSAN type IV (CIPA). The healthy dental pulp is a richly innervated tissue supplied by Aβ-, Aδ-, and C-fibers. Afferent impulses from pulpal fibers predominantly result in pain perception irrespective of the nature of sensory stimulus. In HSAN-IV, where there is insensitivity to pain, one would expect pulpal innervation to exhibit markedly different features from normal. The most important characteristic of HSAN-IV is the self-mutilating behavior that shows bite wounds to the tongue, lips, and fingers, and conduct self tooth extraction that occasionally causes mandibular osteomyelitis; therefore, it is a disease for which dental treatment and care are important.

The innervation of pulp and dentin was examined in human permanent teeth obtained from normal persons and patients with HSAN-IV or -V using physiological and histopathological analyses. In HSAN-IV teeth, an immunohistochemical analysis showed lack of large nerve trunks entering or passing through the radicular pulp and a subodontoblast plexus (plexus of Raschkow), a predominant morphological feature observed in normal tooth pulps. In contrast, silver-staining labeled only a few large nerve trunks entering or passing through the radicular pulp. Part of the nerve trunks was positive to immunohistochemistry for PGP9.5 or βIII tubulin. In normal teeth, there is a prolific branching of nerve fibers within this plexus and emergent A-fibers lose their myelin sheaths before passing between the odontoblasts as free nerve endings [14]. In the present study, branches from the coronal subodontoblast plexus also reached the odontoblast layer and the dentine in the control teeth. Most of the nerve fibers terminated in the odontoblast layer. The histopathological findings in HSAN-IV teeth represented a markedly reduced pulpal innervation in comparison with normal [15] or HSAN-V teeth. The paucity of nervous tissue in the HSAN-IV teeth would indicate that dental sensitivity is likely to be greatly diminished or abolished in these patients.

The absence of normal eccrine gland innervation and the lack of unmyelinated fibers in peripheral nerves, together with marked reduction in small-caliber myelinated fibers, is characteristic of HSAN-IV and can partly explain the observed anhidrosis and neurological disturbances of the peripheral autonomic and sensory nervous systems [16]. Nolano et al. [16] immunohistochemically revealed loss of nerve fibers (C and A fibers) in the epidermis and only a few hypotrophic and non-innervated glands in the dermis from the sural nerve of a HSAN-IV patient. In addition, ultrastructural studies have also revealed the loss of small myelinated and unmyelinated nerve fibers in HSAN-IV [17]. On the contrary, microneurography shows neural activity from Aβ sensory fibers while nociceptive and skin sympathetic C fiber activity is absent in HSAN-IV patients [16]. Neurological examination of 13 HSAN-IV patients by Shorer et al. [18] revealed that all patients exhibited normal motor (median and peroneal nerves) and sensory (digital branches of median and sural nerves) conduction velocities. Although nonnociceptive sensation, which is mediated by large-caliber myelinated Aβ fibers, has been reported to be normal in HSAN-IV patients [19], the recent findings reported by Iijima and Haga [20] suggest that HSAN-IV patients suffer from more widespread disturbances of sensation

than has been previously recognized. The current results on the pulpal sensation clarified by the present investigation strongly support their working hypothesis. Thus, impairment may not be restricted to the types of sensation conducted by peripheral sensory Aδ and C fibers.

The vitality of the dental pulp is determined clinically by the electric pulp testing (EPT), where the sensitivity of the pulp sensory nerves to an electrical stimulus is given by a score according to the patient's responses. This method, however, may not reveal real wellness of the pulp tissue because of the indirectness and subjectivity of the modality. In addition, because the EPT relies on the patient's responses and might induce as unpleasant sensation, it could result in a false-positive outcome, particularly in young patients. In some cases, such as traumatized or young permanent teeth, false-negative outcomes have also been reported due to elevated threshold levels in the pulp nerves [21,22]. In this study, two patients with HSAN-IV represented weak pre-pain sensation using EPT with analytic technology pulp tester, whereas the pulpal pre-pain responses were absent in other patients examined. The actions of neurotrophins are mediated by high-affinity receptors and one low-affinity receptor [23]. The high-affinity receptors include neurotrophic tyrosine kinase, receptor, type 1 (NTRK1), NTRK2, and NTRK3, which have an intracellular domain with enzymatic activity. Nerve growth factor (NGF) binds to NTRK1, brain-derived neurotrophic factor (BDNF) and neurotrophin-4/5 (NTF4/5) can bind to NTRK2, and the biological effects of NTF3 are mediated by NTRK3, although NTF3 also has affinity for NTRK1 and NTRK2. NGF is known to be one of several growth factors involved in the survival, growth, and differentiation of the nervous system [24]. NGF supports the survival of the sympathetic ganglion and nociceptive sensory neurons in dorsal root ganglia [25]. Also, mutation in the *TrkA* gene affecting the extracellular domain may prevent NGF binding, whereas mutations in the intracellular domain can interfere with signal transduction [26]. The present findings conform to the observation of previous reports that, in the patient with HSAN-IV, small myelinated and unmyelianated fibers are markedly diminished but not absent [17,18], which may account for the presence of diminished pain sensitivity in the dental pulp. In the present study, the dental pulp of some teeth in the HSAN-IV patient (No. 11 in Table 1) was diagnosed as non-vitality with the EPT, but the presence of pulp pulse of the same patient has been reported by the first author of this paper [27] using transmitted light plethysmography (TLP) in which the presence of pulpal blood flow can be objectively measured [28-30]. The TLP has been investigated to noninvasively assess "pulp vitality" [28]. The TLP provides information related to circulatory changes occurring inside the pulp chamber by measuring the transmitted light through a whole tooth [29,30]. Furthermore, in a patient with HSAN-IV (No. 6 in Table 1) the EPT inspection didn't show pulpal vitality at 11 years of age but histopathological analysis revealed myelinated nerves at 14 years of ages. The presnt findings may be supported by the facts that the EPT-value for pulpal vitality is gradually reduced with age, suggesting to become pulpal higher sensitivity.

In conclusion, this is the first study that has revealed lack of myelinated and unmyelinated fibers in the dental pulp of almost HSAN-IV patients, but only a few of them in some patients. Thus, HSAN-IV patients suffer from more widespread disturbances of sensation than has been previously recognized. Consequently, dental vital test with EPT and TLP should be considered as a primary diagnostic approach in patients with suspected HSAN-IV, instead of the invasive biopsy.

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