

# Peripheral Sympathetic Nerve Dysfunction in Adolescent Japanese Girls Following Immunization with the Human Papillomavirus Vaccine

Tomomi Kinoshita<sup>1</sup>, Ryu-ta Abe<sup>1</sup>, Akiyo Hineno<sup>1</sup>, Kazuhiro Tsunekawa<sup>2</sup>,  
Shunya Nakane<sup>3</sup> and Shu-ichi Ikeda<sup>1</sup>

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## Abstract

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**Objective** To investigate the causes of neurological manifestations in girls immunized with the human papillomavirus (HPV) vaccine.

**Methods** During the past nine months, 44 girls visited us complaining of several symptoms after HPV vaccination. Four patients with other proven disorders were excluded, and the remaining forty subjects were enrolled in this study.

**Results** The age at initial vaccination ranged from 11 to 17 years, and the average incubation period after the first dose of the vaccine was  $5.47 \pm 5.00$  months. Frequent manifestations included headaches, general fatigue, coldness of the legs, limb pain and weakness. The skin temperature examined in 28 girls with limb symptoms exhibited a slight decrease in the fingers ( $30.4 \pm 2.6^\circ\text{C}$ ) and a moderate decrease in the toes ( $27.1 \pm 3.7^\circ\text{C}$ ). Digital plethysmograms revealed a reduced height of the waves, especially in the toes. The limb symptoms of four girls were compatible with the Japanese clinical diagnostic criteria for complex regional pain syndrome (CRPS), while those in the other 14 girls were consistent with foreign diagnostic criteria for CRPS. The Schellong test identified eight patients with orthostatic hypotension and four patients with postural orthostatic tachycardia syndrome. The girls with orthostatic intolerance and CRPS commonly experienced transient violent tremors and persistent asthenia. Electron-microscopic examinations of the intradermal nerves showed an abnormal pathology in the unmyelinated fibers in two of the three girls examined.

**Conclusion** The symptoms observed in this study can be explained by abnormal peripheral sympathetic responses. The most common previous diagnosis in the studied girls was psychosomatic disease. The social problems of the study participants remained unresolved in that the severely disabled girls stopped going to school.

**Key words:** human papillomavirus vaccine, complex regional pain syndrome, orthostatic hypotension, postural orthostatic tachycardia syndrome, sympathetic nerve dysfunction

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## Introduction

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The immunization of adolescent girls with the human papillomavirus (HPV) vaccine was initiated to prevent uterine and cervical cancer (1, 2). The first quadrivalent vaccine (Gardasil<sup>®</sup>, Merck & Co, Whitehouse Station, U.S.) was ap-

proved in 2006, after which a bivalent vaccine (Cervarix<sup>®</sup>, GSK, Brentford, UK) was introduced. By the end of 2011, approximately 130 million doses of Gardasil<sup>®</sup> and 44 million doses of Cervarix<sup>®</sup> had been distributed worldwide (3). In 2010, both vaccines were widely introduced for application in Japanese girls, and starting in April 2013, those 13 to 16 years of age were legally required to undergo vaccina-

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<sup>1</sup>Department of Medicine (Neurology and Rheumatology), Shinshu University School of Medicine, Japan. <sup>2</sup>Department of Plastic Surgery, Shinshu University School of Medicine, Japan and <sup>3</sup>Department of Clinical Research, Nagasaki Kawatana Medical Center, Japan

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Correspondence to Dr. Shu-ichi Ikeda, ikedasi@shinshu-u.ac.jp

tion. The introduction of a new vaccine invariably puts focus on its effectiveness as well as safety (4). Adverse events of HPV vaccination commonly include fever, headache and local pain at the injection site. Additionally, a relatively high incidence of chronic limb pain, frequently complicated by violent, tremulous involuntary movements, has been noted in Japanese girls following vaccination. In such cases, severe spontaneous pain affects one or more extremities and is consistently accompanied by coldness of the involved limbs, producing a marked disturbance in daily activities. This condition is thought to be a form of complex regional pain syndrome (CRPS), although this has not yet been confirmed. CRPS is usually classified into two forms (5): CRPS-I, which occurs without known nerve injury, and CRPS-II, which includes nerve injury.

CRPS-I, previously called reflex sympathetic dystrophy, is infrequently reported in individuals immunized against hepatitis B (6) and rubella (7), although a few cases of CRPS-I following HPV vaccination were recently reported from Australia (8). CRPS is characterized by persistent pain disproportionate to any inciting event and the presence of at least one sign of autonomic dysfunction (9). An abnormal sympathetic response after the initiating injury is generally considered to underlie the pathogenesis of this disorder (10). In order to clarify the cause of serious limb pain in such cases, we examined adolescent Japanese girls who visited us with a possible chief complaint of adverse events after HPV vaccination, paying special attention to the possibility of peripheral sympathetic nerve dysfunction.

## Materials and Methods

From June 21, 2013 to March 31, 2014, forty four girls visited us complaining of several symptoms after HPV vaccination, including 31 patients with a history of vaccination with Cervarix<sup>®</sup> and 13 with a history of receiving Gardasil<sup>®</sup>. All subjects underwent complete physical and neurological examinations and routine laboratory screening, including measurement of the serum levels of C-reactive protein (CRP) and creatine kinase (CK) as well as thyroid and adrenal gland hormones. If a patient exhibited one or both of the following findings, including hypotension and coldness of the limbs, they were evaluated with the Schellong test and a digital plethysmogram. The Schellong test was performed in conjunction with measurement of the plasma levels of catecholamines. A digital plethysmogram was recorded on the right second finger and right first toe, while checking the patient's skin temperature. In patients with limb weakness, routine motor and sensory nerve conduction studies were performed on the right ulnar and tibial nerves.

### **Histopathological and ultrastructural examinations of the intradermal nerves**

Punch biopsies of the skin were performed. The samples were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer at a pH of 7.4, postfixed in 1% osmium tetroxide in

the same buffer and embedded in epoxy resin. Sections measuring 1  $\mu$ m in thickness were cut, then stained with 1% toluidine blue and examined with light microscopy. Ultrathin sections were then prepared, stained with uranyl acetate and lead citrate and observed under a JEM 1400 electron microscope (JOEL, Tokyo, Japan).

### **Detection of autoantibodies to ganglionic acetylcholine receptor antibodies (gAChR)**

Serum was collected from each of the girls with informed consent. Serum antibodies to gAChR were detected using a luciferase immunoprecipitation assay, as described elsewhere (11, 12). This assay system can detect be used to autoantibodies against two main subunits,  $\alpha$ 3 and  $\beta$ 4, in human AChR (13).

The study protocol was approved by the local ethics committee.

## Results

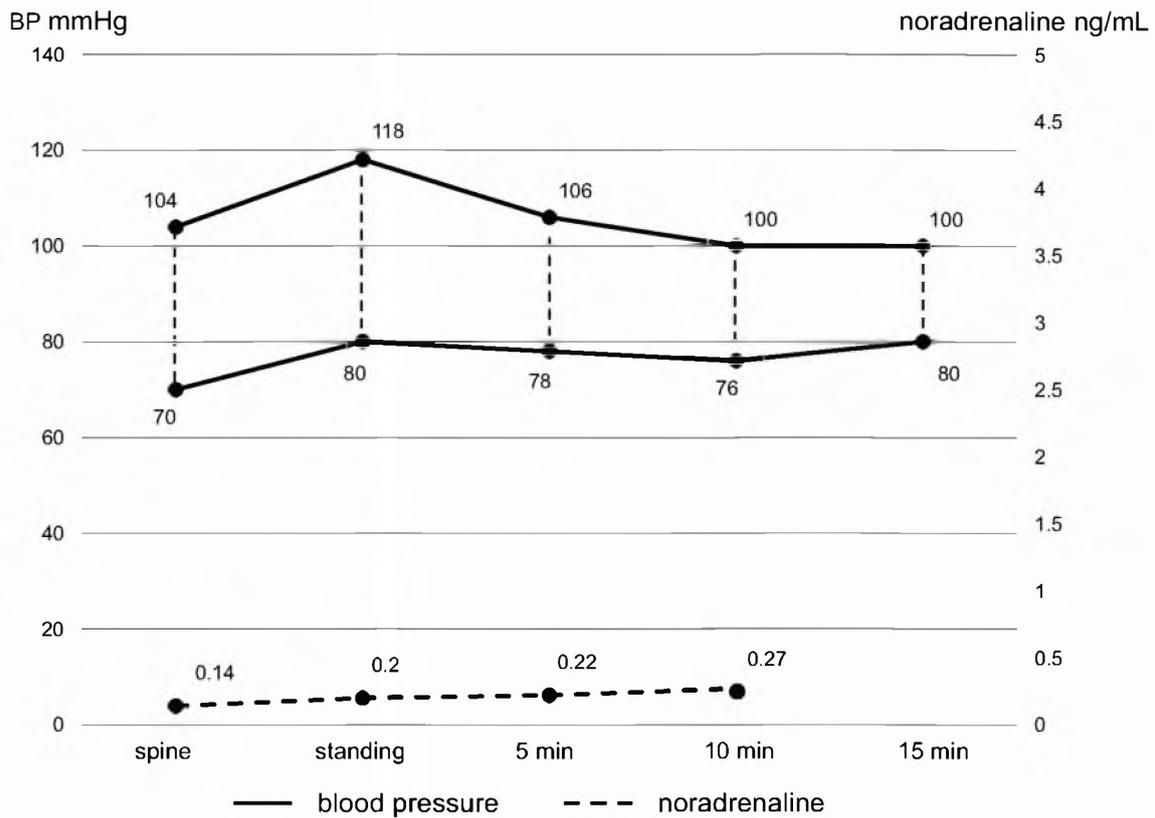
### **Clinical picture**

Among the 44 girls examined, four definitely diagnosed as having another disease at different hospitals were excluded, including two patients with systemic lupus erythematosus, one patient with schizophrenia and one patient with measles vaccine-related cerebellitis. The age at initial vaccination among the 40 girls examined ranged from 11 to 17 years (average age: 13.7 $\pm$ 1.6 years), and the average incubation period after the first dose of vaccine was 5.47 $\pm$ 5.00 months. Clinical manifestations of the 40 girls included headaches (n=28, 70%), general fatigue (n=21, 53%), coldness of the legs (n=21, 53%), limb pain (n=20, 50%), limb weakness (n=19, 48%), difficulty in getting up (n=19, 48%), orthostatic fainting (n=17, 43%), a decreased ability to learn (n=17, 43%), arthralgia (n=17, 43%), limb tremors (n=16, 40%), gait disturbances (n=16, 40%), disturbed menstruation (n=14, 35%) and dizziness (n=12, 30%). Headaches and general fatigue were more prominent in the morning, frequently leading to difficulty in getting up, while persistent fatigue required a long period of sleep. The most common combination of symptoms was limb coldness, pain, tremors and a gait disturbance. Less frequent but noteworthy complaints included the following: 1. polyarthralgia, primarily involving the wrist, knee and ankle joints, that lasted for several days and subsided spontaneously, although it occasionally recurred; 2. menstrual abnormalities, including amenorrhea for a few months after immunization; 3. a decreased ability to learn associated with reduced memory and concentration at school and/or while doing homework, thus resulting in a poor school record.

### **Representative case reports**

**Case 1** (serial patient number: 37 in tables)

In August 2013, an 18-year-old woman visited our hospital with complaints of continuous headaches and severe gen-



**Figure 1.** Changes in blood pressure and the plasma level of noradrenaline on the Schellong test in Case 1. OH was not detected; however, the basal plasma level of noradrenaline was below the normal range, and the increase in the plasma concentration of noradrenaline during positional changes (57% increase at five minutes after standing) was low.

eral fatigue. She had received her first dose of Cervarix<sup>®</sup> at the first week of August, 2011, and a few days later, began to suffer from severe epigastric pain and headaches. She underwent brain magnetic resonance image (MRI) at a local hospital, which showed no abnormalities; however, her headache persisted, accompanied by psychological instability and insomnia. Since she was unable to go to school, she was referred to a mental clinic and diagnosed as being inadaptable. During the first week in October, she received the second dose of the vaccine and her epigastric pain and headache worsened; her headache and general fatigue were more remarkable in the morning, and the administration of several types of drugs, including non-steroidal anti-inflammatory drugs (NSAIDs), failed to relieve these symptoms. The patient underwent an endoscopic examination and was given a diagnosis of superficial gastritis, possibly due to psychological stress. In mid-February, 2012, she received the third dose of the vaccine. At that time, her daily activity was severely disturbed by a difficulty in getting up, continuous heavy headaches and general fatigue. Finally, she stopped going to her regular high school and enrolled in a correspondence course at the beginning of May, 2012. She was also treated by a psychiatrist; however, her symptoms did not improve. She subsequently underwent detailed gynecological examinations due to the exacerbation of symptoms in the pre-menstrual period, the results of which showed no

abnormalities. A few months prior to admission, she began to experience frequent episodes of orthostatic fainting.

On a physical examination performed at our hospital, the patient was 146 cm tall and weighed 45 kg. Her pulse rate was 72 beats per minute (bpm), with a blood pressure (BP) of 98/50 mmHg in the sitting position. The findings of general physical and neurological examinations were normal, except for slight tenderness in the left occipital area. Her skin temperature was 23.0°C in the right first toe and 28.5°C in the right second finger at a room temperature of 23.5°C, and plethysmograms of both toes and fingers disclosed a reduced height of the waves in the toes. The changes in the patient's BP and plasma level of noradrenalin on the Schellong test are presented in Fig. 1. She did not exhibit orthostatic hypotension (OH), although the basal plasma level of noradrenaline was low and the increase in the plasma noradrenaline level was poor after changing positions from a supine to sitting position. These symptoms were consistent with the diagnostic criteria for orthostatic dysregulation (OD) proposed by the Japan Paediatric Society (14), including two major symptoms and four minor symptoms. The oral administration of amezinium metilsulfate (amezinium metilsulfate) (10 mg, two times per day) relieved all of the patient's symptoms, and her daily activities significantly improved.

**Case 2** (serial patient number: 14)

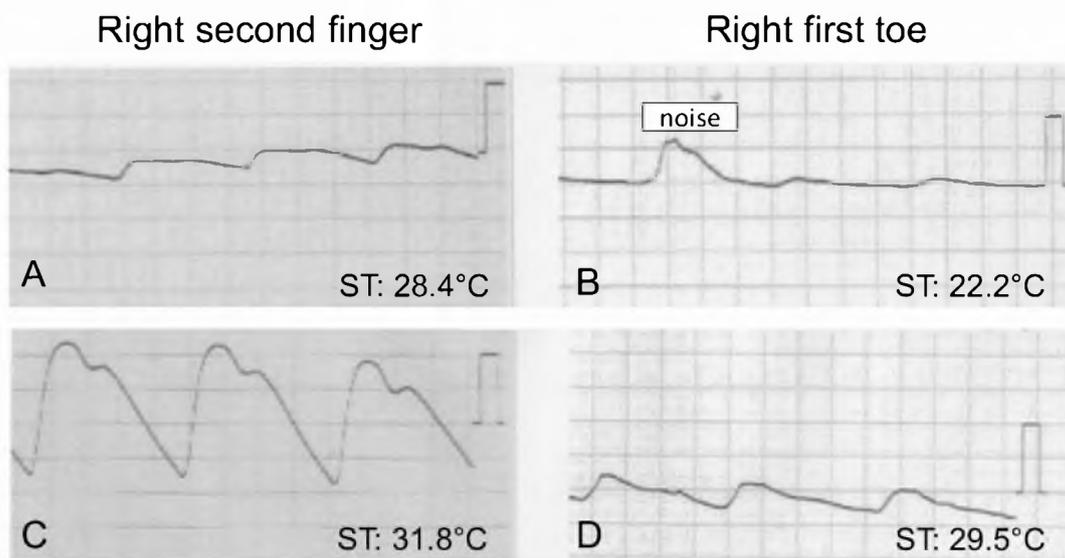
In November 2013, a 15-year-old girl was referred to our hospital due to continuous headaches, limb pain and a gait disturbance. She had received her first dose of Cervarix<sup>®</sup> at the end of February, 2011 and the third dose at the end of September, 2011. Her daily life had remained unchanged for a while; however, at the beginning of June, 2012 she began to complain of sharp pain in the eye balls and double vision, and, in the early morning in mid-July, she fell down after waking up due to left-sided limb weakness. She was transferred to an emergency hospital, where her consciousness was found to be clear, and an MRI scan of the brain and routine laboratory tests were all within the normal lim-

its. The left-sided limb weakness recovered, and she was discharged. After this event, she frequently began to skip school due to an uncertain inching pain and clumsy movement in hands, which prevented her from writing or using chopsticks (Fig. 2A). Additionally, she became very sensitive to sound and skin stimuli, such as touch and using the shower in the bathroom, both of which easily induced a panic state, with involuntary tremulousness of the limbs, and she thus visited several hospitals, where she was given a diagnosis of a psychiatric or anxiety disorder. In early July, 2013, she developed severe anorexia, possibly due to very uncomfortable sensations in the limbs, and was hospitalized for two weeks. Although she was treated for an eating disorder, her condition did not improve, and after discharge, she remained at home.

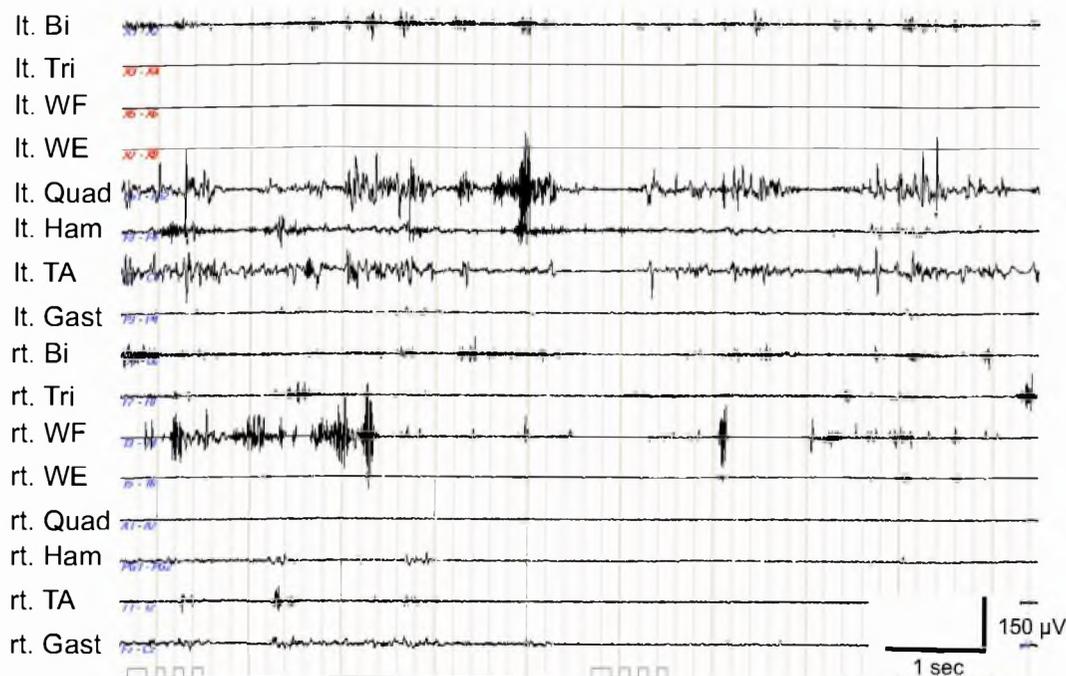
On a physical examination performed at our hospital, the patient was 150 cm tall and weighed 50 kg. Her pulse rate was 80 bpm, with a BP of 90/55 mmHg in the sitting position. Her body temperature was 37.1°C. In general, physical findings of coldness in both legs were noted, although the neurological gross power of the limb muscles was normal. However, violent limb tremors were easily elicited whenever an examiner touched the patient's thigh or legs. Due to this severe tremors, the patient was unable to walk alone. Her skin temperature was 22.2°C in the right first toe and 28.4°C in the right second finger at a room temperature of 23.5°C, and plethysmograms of the toe and finger showed markedly reduced heights of the waves (Fig. 3A, B). In contrast, the findings of peripheral nerve conduction studies were normal. Surface electromyograms recorded from the involved limbs indicated that the involuntary movements were not tremors, but rather myoclonus (Fig. 4, 5). The patient's reports of persistent inching pain, sensory changes with hyperesthesia



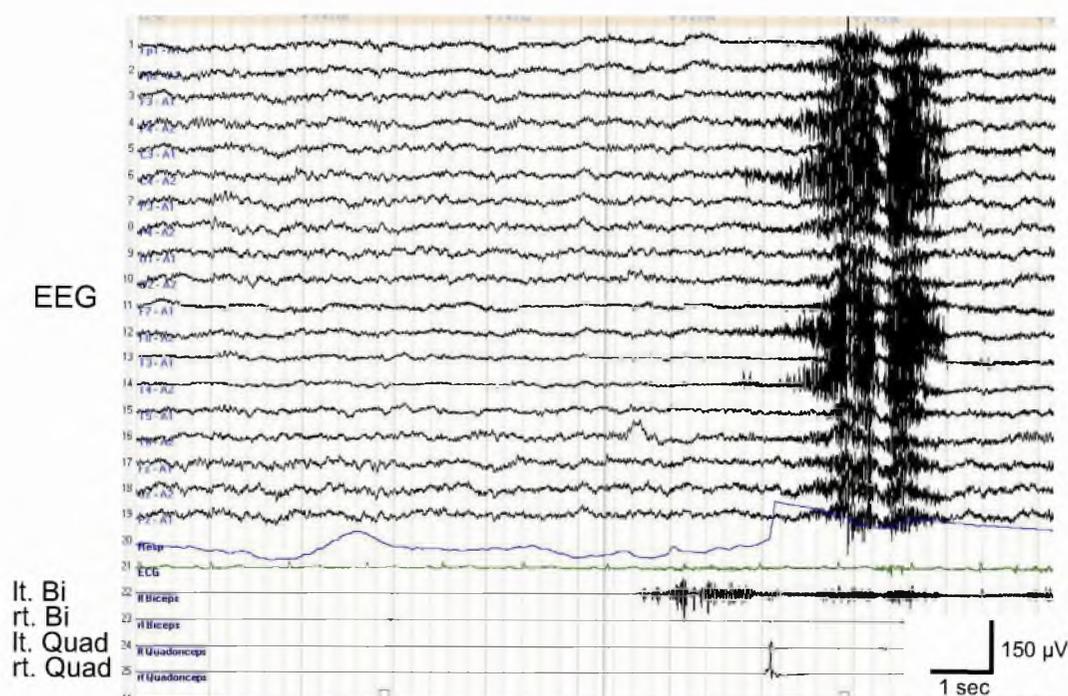
**Figure 2.** Impaired writing of letters in Case 2. A: On admission, the patient's writing was poor due to hand tremulousness. The irregular lines are noteworthy. B: After treatment, the patient's writing improved considerably. Permission for presentation of these images was obtained from the girl and her parents.



**Figure 3.** Digital plethysmograms in Case 2. A, B: On admission, C, D: After intravenous injection of alprostadil. On admission, digital plethysmograms showed a peripheral plateau pattern, with the height of wave for the toe being very low. After treatment, the pattern of waves normalized, and the skin temperature of the toe dramatically increased. ST is an abbreviation for skin temperature.



**Figure 4.** Surface electromyograms of the involved right upper and lower limbs in Case 2. Repetitive bursts appear at irregular intervals and the muscle contractions are not synchronous with each other, indicating that the involuntary movement is a myoclonic jerk.



**Figure 5.** Polygraph (EEG and EMG) in Case 2. The myoclonic EMG discharge of the left biceps muscle is not associated with any particular EEG activity. EEG is an abbreviation for electroencephalogram and EMG is an abbreviation for electromyogram.

and motor dysfunction with limb weakness and tremors, as well as our observation of these findings, were consistent with a diagnosis of CRPS-I, according to the guidelines presented in Table 1. On the Schellong test, her heart rate and BP changed from 78 bpm and 110/58 mmHg to 102 bpm and 67/48 mmHg, respectively, at eight minutes after stand-

ing. She was therefore diagnosed with OH. The intradermal nerve pathology observed in the first toe and second finger is described in a separate section. The intravenous administration of physiological saline (100 mL) and alprostadil (alprostadil; 5 μg) was employed to treat the leg coldness. The patient subsequently felt warmth in her limbs and the re-

**Table 1A. Japanese Diagnostic Hallmark for CRPS**


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A. Disease history must contain more than 2 in the following subjective symptoms
1. Trophic change in skin, nail or hair
2. Decreased range of joint motion
3. Persistent aching pain or shooting pain and/or hyperpathy
4. Sweating changes
5. Oedema
B. Must display at least two at time of evaluation in the following objective findings
1. Trophic change in skin, nail or hair
2. Decreased range of joint motion
3. Allodynia or hyperalgesia in pin-pricking
4. Sweating changes
5. Oedema

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**Table 1B. Proposed Clinical Diagnostic Criterion for CRPS**


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1. Continuing pain which is disproportionate to any inciting event
2. Must report at least one symptom in three of the four following categories
Sensory: reports of hyperaesthesia and/or allodynia
Vasomotor: reports of temperature asymmetry and/or skin colour changes and/or skin colour asymmetry
Sudomotor/oedema: reports of oedema and/or sweating changes and/or sweating asymmetry
Motor/trophic: reports of decreased range of motion and/or motor dysfunction (weakness, tremor, dystonia) and/or trophic changes (hair, nail, skin)
3. Must display at least one sign at time of evaluation in two or more of the following categories
Sensory: evidence of hyperaesthesia and/or allodynia
Vasomotor: evidence of temperature asymmetry and/or skin colour changes and/or skin colour asymmetry
Sudomotor/oedema: evidence of oedema and/or sweating changes and/or sweating asymmetry
Motor/trophic: evidence of decreased range of motion and/or motor dysfunction (weakness, tremor, dystonia) and/or trophic changes (hair, nail, skin)
4. There is no other diagnosis that better explains the signs and symptoms

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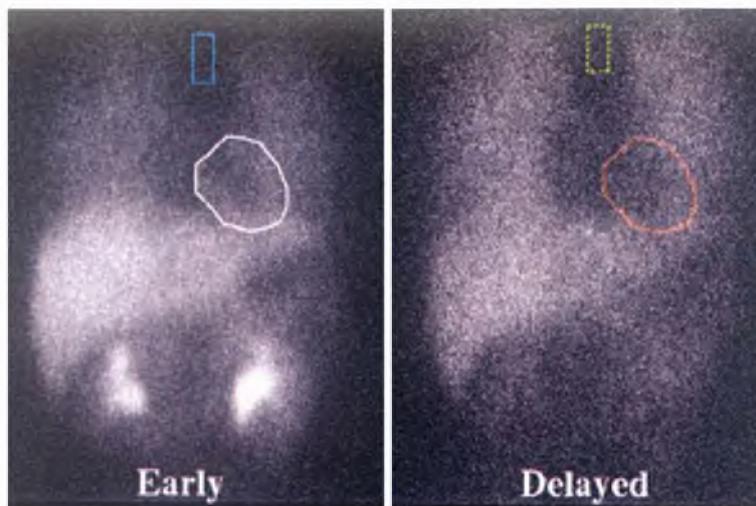
sults of digital plethysmograms exhibited a normal pattern (Fig. 3C, D). After discharge, she continued to receive this treatment at her neighboring hospital, and the limb tremors gradually subsided. In addition, the patient again became able to walk and write (Fig. 2B) and returned to school three months later.

#### Case 3 (serial patient number: 2)

In October 2013, a 13-year-old girl was referred to our hospital due to paroxysmal limb pain with headaches and a gait disturbance. She had a history of surgical removal of a left ovarian tumor at 10 years of age. She received her first dose of Gardasil® at the end of June, 2012, and two weeks later, began to suffer from a continuous high fever (39.0-40.0°C) and headaches. She was evaluated at a local hospital, where no abnormal findings were detected on a routine laboratory examination, endoscopy or CT. Various NSAIDs were prescribed; however, all were ineffective in relieving the patient's symptoms. She was tentatively diagnosed as having a psychosomatic fever and stopped participating in all sport activities on campus. At the end of January, 2013, she received the third dose of the vaccine. Her high body

temperature and general malaise gradually resolved; however, paroxysmal limb tremors subsequently appeared, especially while lying down, which caused the patient serious anxiety at night, resulting in insomnia. In early March, 2013, she developed severe limb pain and palpitations; the limb pain restricted her shoulder and thigh movement, sometimes accompanied by temporal paresis of the hands and legs, and the palpitations and chest discomfort were remarkably exacerbated when the patient changed from a sitting to standing position. Both conditions resulted in difficulties in writing and walking. The patient's condition was considered to be due to psychosomatic behavior at the hospital and at school. Therefore, she stopped going to school and had stayed home since late April, 2013.

On a physical examination conducted at our hospital, the patient was 155 cm tall and weighed 51 kg. Her pulse rate was 98 bpm, with a BP of 112/78 mmHg in the sitting position. Her body temperature was 37.1°C, and her general physical findings were normal. Neurologically, she complained of uncomfortable pain in the legs; however, manual muscle tests, objective sensory examinations and deep ten-



**Figure 6.** MIBG cardiac scintigram showing a decreased uptake of the isotope in both the early and delayed phases. A: The early phase was photographed 15 minutes after isotope injection: the heart to mediastinum (H/M) ratio is 1.98. B: The delayed phase was photographed at two hours: the H/M ratio was 1.56. Framed or circled areas are targets for counting the radioactivity of the isotope.

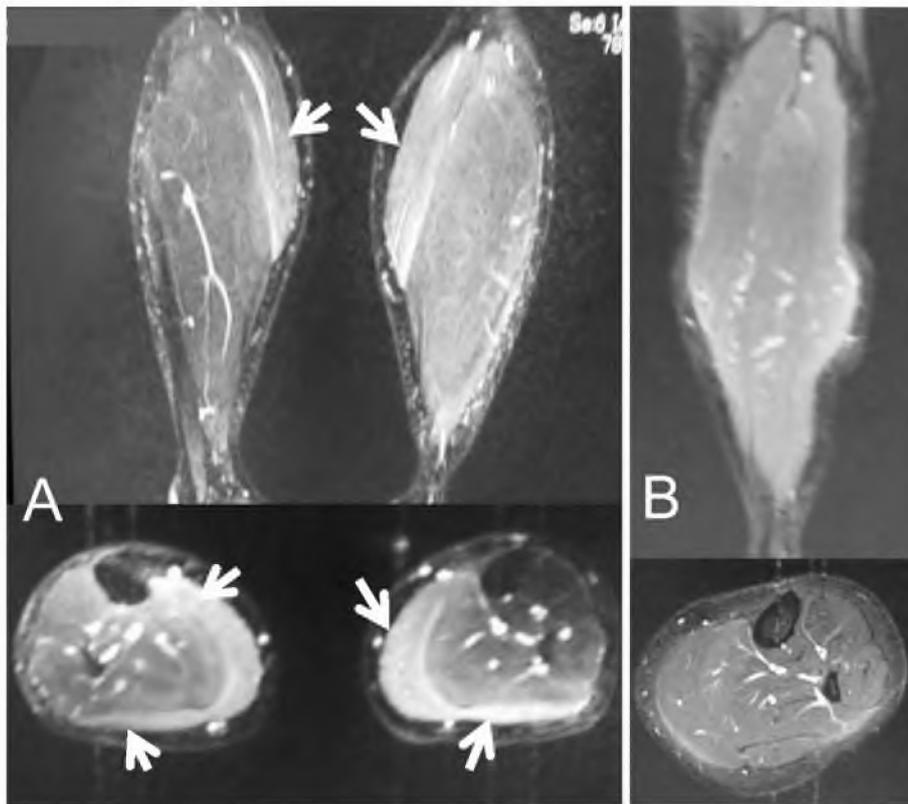
don reflex studies were all normal. No limb tremors were noted at that time. The patient was able to walk using a handrail for short distances, exhibiting a very unsteady posture that easily led to squatting. The awkward gait appeared to us to be of hysteric origin. Her skin temperature was 28.8°C in the right first toe and 30.8°C in the right second finger at a room temperature of 23.5°C, and plethysmograms of both the toes and fingers showed reduced heights of the waves in the toes. On the Schellong test, the patient's heart rate and BP changed from 91 bpm and 105/91 mmHg to 126 bpm and 98/59 mmHg, respectively, at nine minutes after standing. A cardiac scintigram obtained using <sup>123</sup>I-meta-iodobenzylguanidine (MIBG) revealed a reduced uptake of the isotope (Fig. 6), indicating the loss of post-ganglionic nerve terminals containing noradrenaline (15, 16). She was therefore diagnosed as having CRPS-I and postural orthostatic tachycardia syndrome (POTS) (17) and treated with the oral administration of bisoprolol fumarate (bisoprolol fumarate) at a dose of 2.5 mg daily. Four months later, her gait improved, and she was able to walk with the use of stick, although she did not return to her previous school life.

#### Case 4 (serial patient number: 24)

In December 2013, a 16-year-old girl was referred to our hospital due to polyarthralgia, leg pain and weakness. She had a history of bronchial asthma and atopic dermatitis in childhood. She received the first dose of Cervarix® in early October, 2011 and the third dose in early March, 2012. One month later, she noticed knee joint pain and general fatigue, with a slightly elevated body temperature. She visited a few orthopedic clinics, failing to obtain a definitive diagnosis. The arthralgia moved to other joints, and the patient began to suffer from paroxysmal limb tremors in autumn of that year. In early January 2013, she developed severe aching in both calves that resulted in the need for a wheelchair. She was examined at a local hospital, where positive anti-

cardiolipin antibodies were detected in the serum (17 U/mL, normal ≤10 U/mL). Treatment with prednisolone (10 mg/day) and clopidogrel sulfate (clopidogrel; 75 mg/day) was started; however, all of the patient's symptoms persisted and she stopped going to school.

On a physical examination conducted at our hospital, the patient was 157 cm tall and weighed 69 kg. Her pulse rate was 78 bpm, with a BP of 118/78 mmHg in the sitting position. She exhibited reddish skin with dryness on the cheek and limbs, possibly due to atopic dermatitis. Additionally, she complained of mild tenderness without swelling in the shoulder, elbow, wrist, knee and ankle joints, and a similar degree of pain was evoked in both calves by grasping. On a neurological examination, the patient's limb power and sensations were all normal; however, she was unable to walk by herself and was thus confined to a wheelchair. Her skin temperature was 31.2°C in the right first toe and 31.4°C in the right second finger at a room temperature of 24.5°C, and plethysmograms of both the toes and fingers showed reduced heights of the waves. On the Schellong test, severe OH was induced; 10 minutes after standing, she fainted with pallor (her BP decreased from 107/73 mmHg to 77/35 mmHg). On laboratory tests, the serum CRP level was 0.20 mg/dL and the serum CK level was 47 IU/L. In addition, the serum titer of anti-cardiolipin antibodies was 10 U/mL (normal ≤10 U/mL), whereas other auto-antibodies, including rheumatoid factor and anti-double strand DNA antibodies, were undetectable. Although a needle electromyogram showed normal patterns in both the upper and lower limbs, MRI disclosed an abnormal high signal intensity in both calves (Fig. 7A), indicating the presence of myofasciitis. Two weeks after discontinuing the dose of clopidogrel sulfate, the patient again underwent MRI of the legs and a muscle biopsy of the left gastrocnemius, which showed that the abnormal signals had disappeared (Fig. 7B) and the his-



**Figure 7.** MRI findings of the calf muscles in Case 4. A: On admission, both gastrocnemius muscles showed high-signal lesions on flair-weighted images with gadolinium enhancement. B: Two weeks later, the lesions were undetectable on the same type of images.

tology of the muscle was normal. The intradermal nerve pathology observed in the same leg area is described in a separate section. The patient was therefore diagnosed as having CRPS-I and OH and was transferred to another hospital for gait rehabilitation.

#### Case 5 (serial patient number: 19)

In February 2014, a 15-year-old Japanese girl visited our hospital complaining of transient limb weakness and orthostatic fainting. At the beginning of May, 2010, she had received her first dose of Gardasil<sup>®</sup> in a clinic in the U.S. since she was living there at the time. In early December, 2010, she received the third dose of the vaccine. A few days later, she felt pain and weakness in the lower limbs, especially in the left leg, leading to difficulty in walking. This symptom subsided within the following three days; however, after one month, she developed numbness and weakness in both hands that lasted for two days. Transient weakness repeatedly appeared in both the hands and legs, and the patient subsequently experienced orthostatic fainting and abdominal discomfort. She returned to Japan in April, 2012 and was examined at a local hospital, where no specific findings were noted. In addition to recurrent limb weakness, the patient newly exhibited a decreased ability to learn at school; she was unable to memorize different themes simultaneously and her understanding of textbooks was incomplete, both of which were noticed by her mother. The patient and her family were seriously worried about her symp-

toms.

On a physical examination conducted at our hospital, the patient was 162 cm tall and weighed 47 kg. Her pulse rate was 74 bpm, with a BP of 94/62 mmHg in the sitting position. Her general physical findings were normal, although a neurological examination showed slight weakness in both hands and the left leg (grip power: 18 kg in the right hand; 10 kg in the left hand). Her skin temperature was 21.8°C in the right first toe and 31.1°C in the right second finger at a room temperature of 27.0°C, and plethysmograms of both the toes and fingers showed reduced heights of the waves in the toes. The findings of peripheral nerve conduction studies of the left median and tibial nerves were normal. On the Schellong test, the patient's heart rate and BP changed from 70 bpm and 105/62 mmHg to 109 bpm and 102/52 mmHg, respectively, at seven minutes after standing. The WAIS-III disclosed the following scores: FIQ=82, VIQ=88, PIQ=79, VC=92, PO=70, WM=85, AS=105. Furthermore, the patient had remarkable difficulty in quickly understanding long sentences. She was therefore diagnosed with CRPS-I and POTS, and her slight cognitive decline was thought to be potentially related to POTS. She was treated with the oral administration of limaprost alfadex at a dose of 5 mg (limaprost alfadex) three times daily, and her limb symptoms disappeared.

**Table 2. Results of Schellong Test Including the Changes of Plasma Noradrenaline Level**

Patient No.	Age	NA at supine position (ng/mL)	Increasing rate of NA (%)	Maximum change		Diagnosis	
				decreased SBP mmHg	increased HR /min	OH	POTS
1	13	0.13	15	42	-	+	-
2	13	0.14	129	8	36	-	+
3	13	0.16	106	48	-	+	-
5	13	0.16	44	13	22	-	-
8	14	0.17	65	7	21	-	-
13	15	0.11	45	16	24	-	-
14	15	0.26	42	43	-	+	-
17	15	0.16	100	40	-	+	-
18	15	0.22	100	1	48	-	+
19	15	0.36	67	16	39	-	+
22	16	0.09	122	5	44	-	+
24	16	0.17	59	30	-	+	-
25	16	0.14	86	6	28	-	-
26	16	0.18	50	-	11	-	-
27	16	0.33	64	45	-	+	-
28	17	0.16	119	7	27	-	-
29	17	0.41	49	15	15	-	-
32	18	0.09	166	52	-	+	-
36	18	0.12	58	1	18	-	-
37	18	0.14	57	4	6	-	-
38	18	0.14	43	24	-	+	-

NA: noradrenaline, SBP: systolic blood pressure, HR: heart rate, OH: orthostatic hypotension, POTS: postural orthostatic tachycardia. Normal plasma level of NA at supine position is 0.15-0.57 ng/mL. Increasing rate of plasma NA after standing is more than 60% of basal level after 5 minutes.

**Table 3. Evaluation Results of Skin Temperature and Digital Plethysmogram**

Patient No.	Skin temperature (°C)		Digital plethysmogram	
	2nd finger	1st toe	2nd finger	1st toe
2	30.8	28.8	normal	↓
3	27.5	28.5	↓	↓
5	30.6	28.4	↓	↓
8	27.2	24.7	↓	↓
10	32.8	35.8	normal	normal
11	30.8	27.6	normal	normal
13	27.3	23.9	↓	↓
14	28.4	22.2	↓	↓
16	31.0	28.8	normal	normal
17	33.6	23.8	normal	normal
18	33.2	24.1	↓	↓
19	31.1	21.8	normal	↓
21	30.9	28.0	n.e.	n.e.
22	33.1	33.5	normal	normal
23	32.4	29.3	normal	↓
24	31.4	31.2	↓	↓
25	28.7	26.8	↓	↓
26	26.0	31.6	normal	↓
27	23.9	21.5	↓	↓
28	31.5	27.8	normal	↓
29	30.0	24.4	↓	↓
31	33.9	25.7	normal	normal
32	34.2	31.7	normal	normal
33	28.6	24.5	normal	normal
34	33.1	24.7	↓	↓
36	31.2	32.0	↓	↓
37	28.5	23.0	normal	↓
40	29.5	24.5	↓	↓

n.e.: not examined. ↓: decreased height of waves. The room at examination of skin temperature was kept at 23-25°C.

### Schellong test and the plasma levels of noradrenaline

Twenty-one girls underwent these examinations. OH was defined as a decrease of more than 20 mmHg in systolic blood pressure and/or 5 mmHg in diastolic blood pressure within a few minutes after standing (14, 18). POTS was defined as an increase in heart rate of more than 30 bpm within the first 10 minutes of standing or tilting the head upwards (17). Since the plasma levels of noradrenaline have been reported to increase approximately 60-120% when changing from a reclining to standing position in normal subjects (19), patients who exhibited an elevation of less than 60% within five minutes were classified as having an abnormal response in this study.

OH was detected in eight patients, two of whom demonstrated orthostatic syncope during testing. POTS was noted in the four other girls. A low response in the plasma level of noradrenaline to postural change was seen in 10 girls, including four with OH (Table 2).

### Skin temperature and plethysmogram of the right first toe and right second finger

The skin temperature was examined in 28 girls; the average temperature of the right second finger was  $30.4 \pm 2.6^\circ\text{C}$ , while that of the first right toe was  $27.1 \pm 3.7^\circ\text{C}$ . There is no standard diagnostic criterion for diagnosing a diminished skin temperature; therefore, a value below room temperature was judged to be abnormal. The digital plethysmogram findings were evaluated according to the classification of the wave pattern, as follows (20): peripheral plateau pattern in-

volving a reduced height of the waves in the right second finger, as detected in 13 girls, and a peripheral plateau pattern in the first right toe, as observed in 19 girls (Table 3).

### **Evaluation of CRPS**

The diagnosis of CRPS-I was made based on the patient's history and clinical examination findings in reference to two different sets of diagnostic criteria: the Japanese clinical diagnostic criteria proposed by the research community in 2008 (21) (Table 1A), and the criteria used widely in foreign countries (9) (Table 1B), the recent revision of the International Association for the Study of Pain (IASP) criteria for CRPS originally described in 1999 (22). The limb symptoms were compatible with the Japanese criteria for CRPS in four girls and the foreign diagnostic criteria for CRPS in the remaining 14 girls (Table 4). Limb pain, paresis, tremulousness and gait disturbances were common manifestations in the girls diagnosed based on the revision of the IASP criteria. Meanwhile, the girls who displayed these symptoms in addition to abnormal sweating fulfilled the Japanese criteria.

### **Skin biopsy findings**

Biopsies were performed in three girls, including the above Cases 2 and 4 and serial patient No. 18. The skin sample of the first toe obtained from the third girl with CRPS-I lacked any signs of pathological findings. For example, on the light microscopic examination, three small nerve fascicles were identified in one skin sample (Fig. 8A). Some nerve fascicles were located near apocrine glands (Fig. 8B), where sudomotor autonomic unmyelinated nerves were surmised to be present. On the electron microscopic examinations, one fascicle was found to consist of a small number of myelinated nerve fibers (most of them being less than 5  $\mu\text{m}$  in diameter) and a large number of unmyelinated nerve fibers. In Case 2, skin samples were harvested from the first toe and second finger. On light microscopic observation, these samples commonly displayed endoneurial edema (Fig. 8C, D), which was more remarkable in the toe samples (Fig. 8D). On electron microscopic observation, the finger skin contained well-preserved numbers of both myelinated and unmyelinated nerve fibers, although electron-dense coarse granular materials were seen in some axons of the unmyelinated nerve fibers (Fig. 9), reflecting degenerative changes. In the toe skin, spaces without any structures occupied a significant portion of the endoneurium, indicating endoneurial edema (Fig. 10A), and the number of unmyelinated nerve fibers was markedly decreased, accompanied by denervated parallel Schwann cell processes and increased collagen pockets (Fig. 10B). Similar changes in intradermal nerves were noted in the calf skin obtained during the muscle biopsy in Case 4 (Fig. 11).

### **Detection of autoantibodies to gAChR**

All 14 girls who exhibited sympathetic dysfunction showed negative findings in these examinations.

### **Final diagnosis**

Our final diagnoses in the 40 girls examined are shown in Table 5. Twenty-nine girls were classified into our categorized diagnoses, while the remaining 11 were given a tentative diagnosis based on their symptoms and/or manifestations. The latter group consisted of three patients with a simple headache, three patients with polyarthritis, two patients with OD-like symptoms, one patient with lumbosacral pain, one patient with easy fatigability, chest discomfort and limb tremulousness and one patient with a fever, headache and upper limb paresthesia.

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## **Discussion**

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In the U.S., the routine vaccination of girls 11 to 12 years of age using three doses of Gardasil<sup>®</sup> began on June 1, 2006, and by December 31, 2008, more than 23 million Gardasil<sup>®</sup> doses had been administered. During these 2.5 years, the Vaccine Adverse Event Reporting System (VAERS) received 12,424 reports of adverse events following immunization (AEFI), for a rate of 53.9 reports per 100,000 doses. A total of 772 reports (6.2% of all reports) were judged to involve serious AEFI cases, including 32 reports of death (4). The rate of reporting per 100,000 vaccine doses was 8.2 for syncope, 7.5 for local site reactions, 6.8 for dizziness, 5.0 for nausea, 4.1 for headache, 3.1 for hypersensitivity reactions, 2.6 for urticaria, 0.2 for venous thromboembolic events, autoimmune disorders and Guillain-Barré syndrome, 0.1 for anaphylaxis and death, 0.04 for transverse myelitis and pancreatitis and 0.009 for motor neuron disease. A high frequency of syncope was observed immediately after injection (4). In Japan, a total of 8.75 million doses, in which Cervarix<sup>®</sup> predominated, were used during the past three years (3). Although similar public reports are not available in Japan, mass media largely reported that a significant number of Japanese girls suffered from severe spontaneous limb pain frequently accompanied by involuntary tremulous movements of the involved limbs and/or gait disturbances following vaccination. Recently, a social community has been established for these girls in Tokyo, where information concerning more than 230 affected girls has been collected (antihpvaccine@yahoo.co.jp). Such neurological manifestations have not been previously experienced at our hospital and we therefore investigated the pathogenesis of this unique disorder in this study.

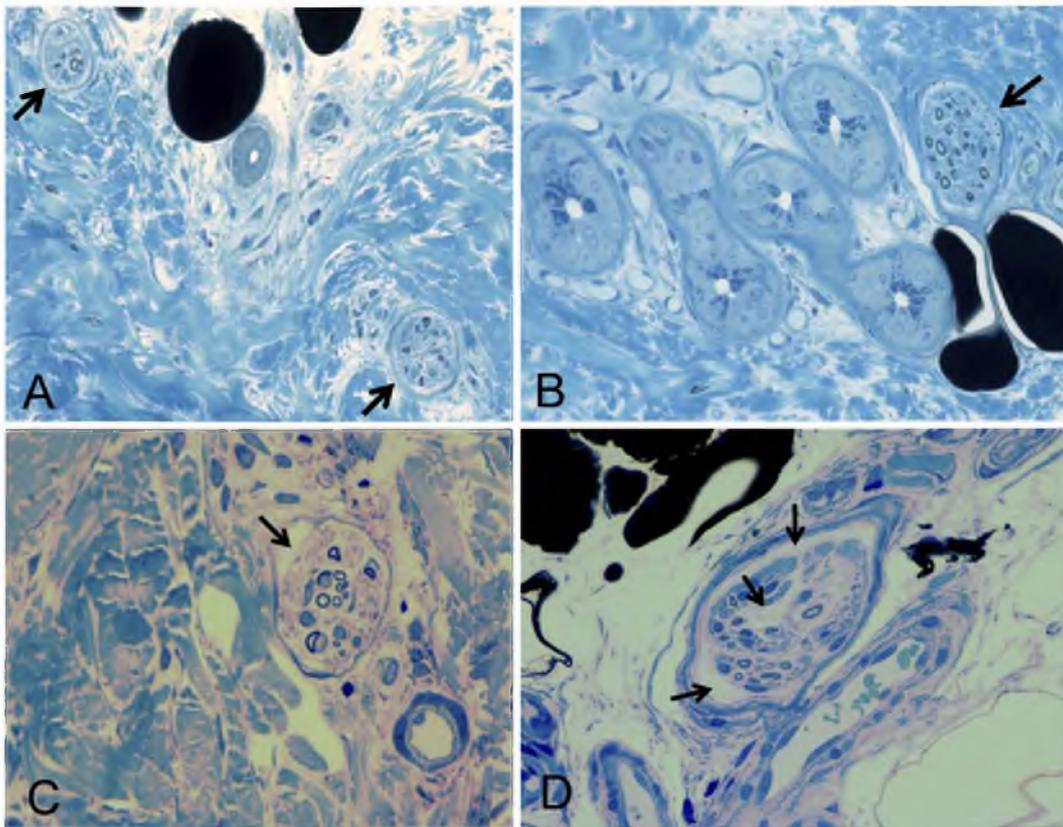
### **CRPS**

CRPS is characterized primarily by pain (with allodynia and hyperpathia), vasomotor changes, edema and a decreased range of motion (5). CRPS-I is being increasingly recognized in children and adolescents (23), with clinical features that are generally compatible with those observed in adults affected by this syndrome, including a female preponderance, with the highest incidence of disease around puberty and the lower extremities more often being involved.

**Table 4.** Summary of Clinical Picture in 29 Girls Showing Obvious Sympathetic Nerve Dysfunction

Patient No.	Age	Type of vaccine	Initial symptom	Headache	Limb pain	Limb paresis	Limb tremors	Gait disturbanc	Decreased skin temperature in toe	Hyperpathy	Over sweating	CRPS (Japan)	CRPS (IASP)	OD	OH	POTS
1	13	G	Fatigue	+	+	-	-	-	n.e.	-	-	-	-	+	+	-
2	13	G	Fever	+	+	+	+	+	-	-	-	-	+	+	-	+
3	13	G	Headache	+	+	+	+	+	-	-	-	-	+	+	+	-
5	13	C	Hyperventilation	+	+	+	+	+	-	+	-	-	+	+	-	-
8	14	G	Headache	+	-	-	-	-	+	-	-	-	-	+	-	-
12	15	C	Headache	+	-	+	-	+	n.e.	-	-	-	-	+	-	-
13	15	G	Headache, nausea, fever	+	+	+	-	+	+	+	-	-	+	+	-	-
14	15	C	Pain in eye ball, double vision	+	+	+	+	+	+	-	-	-	+	+	+	-
16	15	C	Fever	-	-	+	+	-	-	-	-	-	+	-	-	-
17	15	C	Headache	+	+	-	+	-	+	-	+	+	+	+	+	-
18	15	C	Limb pain	-	+	-	+	-	+	-	-	-	+	+	-	+
19	15	G	Limb pain and weakness	+	+	+	+	-	+	+	+	+	+	+	-	+
20	16	C	Limb pain	-	+	-	-	+	n.e.	-	-	-	+	-	-	-
21	16	C	Limb pain	+	+	+	-	+	-	+	-	-	+	-	-	-
22	16	C	Fatigue	+	-	+	-	+	-	-	-	-	-	+	-	+
23	16	C	Limb weakness	-	+	+	+	+	-	-	-	-	+	-	-	-
24	16	C	Arthralgia	-	+	-	+	-	-	+	-	-	+	+	+	-
25	16	C	Arthralgia	-	+	+	-	+	-	+	-	-	+	-	-	-
27	16	C	Fatigue, difficulty in getting up	-	-	-	-	-	+	-	+	-	-	+	+	-
28	17	C	Arthralgia	+	+	-	-	-	-	-	-	-	-	+	-	-
29	17	C	Limb pain and weakness, paresthesia	+	+	+	+	-	+	+	-	-	+	+	-	-
31	17	C	Nausea	+	-	-	+	-	-	-	+	-	-	+	-	-
32	18	G	Fatigue, limb paresthesia	+	-	+	-	+	-	-	-	-	-	+	+	-
34	18	C	Headache	+	-	-	-	-	+	-	-	-	-	+	-	-
36	18	C	Fever, gait disturbance	+	-	+	-	+	-	+	-	-	+	+	-	-
37	18	C	Abdominal pain	+	-	-	-	-	+	-	-	-	-	+	-	-
38	18	C	Difficulty in getting up	+	+	-	+	-	n.e.	+	+	+	+	+	+	-
39	19	C	Syncope	+	-	+	-	+	n.e.	-	-	-	-	+	-	-
40	19	C	Abdominal pain	+	+	+	-	+	+	+	+	+	+	+	-	-
Total number	29			19	17	16	12	14	11	10	6	4	18	24	8	4

G: Gardasil<sup>®</sup>, C: Cervix<sup>®</sup>, CRPS: complex regional pain syndrome, IASP: international association for the study of pain, OD: orthostatic dysregulation, OH: orthostatic hypotension, POTS: postural orthostatic tachycardia, n.e.: not examined, \*: limb tremors that could be observed at our examinations. Decreased skin temperature in 1st toe is defined as lower level than examination room temperature kept at 23-25°C.

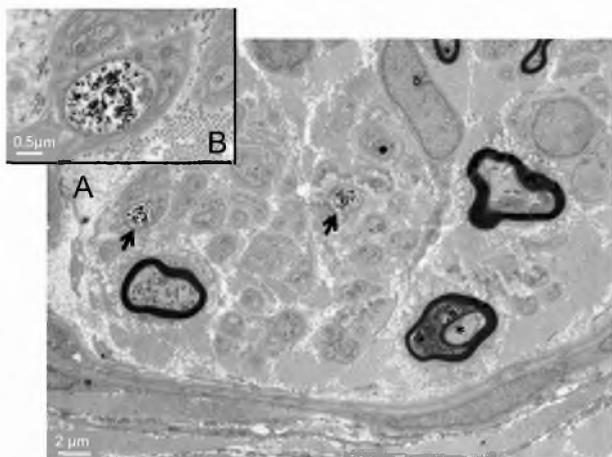


**Figure 8.** Light-microscopic findings of epon-embedded skin tissues stained with 1% toluidine blue. **A, B:** Findings for the first toe skin in patient No. 18, who was diagnosed with CRPS-I. The patient's symptoms were not severe and she was able to walk without difficulty. **A:** Two small nerve fascicles (indicated by arrows) are identified in the dermis. Original magnification  $\times 120$ . **B:** One nerve fascicle (indicated by an arrow) located the near apocrine glands and this fascicle was surmised to contain a sudomotor unmyelinated autonomic nerve. All nerve fascicles in **A** and **B** appear normal. Original magnification  $\times 280$ . **C, D:** Findings in Case 2 showing serious limb symptoms of CRPS-I. **C:** One nerve fascicle in the skin of the second finger showing slight subperineurial edema (indicated by arrow). Original magnification  $\times 500$ . **D:** One nerve fascicle in the first toe's skin showing more prominent subperineurial and endoneurial edema (indicated by arrows). Original magnification  $\times 500$ .

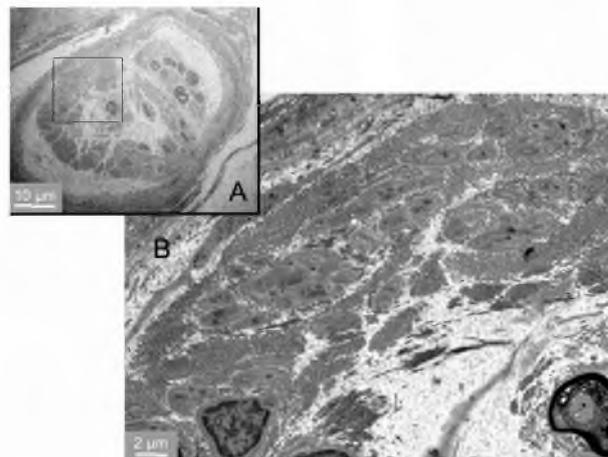
In this study, the diagnosis of CRPS-I was made based on the patient's history and clinical examination findings in reference to two different sets of diagnostic criteria: the Japanese clinical diagnostic criteria proposed by the research community (21) and the recent revision of the IASP criteria (9). The former set of criteria requires the detection of more diagnostic hallmarks. Our series of girls with chronic limb pain commonly showed a decreased skin temperature and abnormal digital plethysmogram findings, both of which indicate an impaired vasomotor tone in the affected limbs. Among these patients, only four fulfilled the Japanese diagnostic clinical criteria for CRPS-I, while the remaining 14, who lacked signs of sweating abnormalities, skin color changes and/or trophic changes, were diagnosed with CRPS-I based on the foreign diagnostic criteria. Concerning the pathogenesis of CRPS, cold skin is thought to be the result of vasospasms caused by sympathetic denervation supersensitivity (10), and the presence of electron microscopic abnormalities in peripheral nerves supports this hypothesis. For example, the loss of small-diameter unmyelinated fibers (C-

fibers) is the only finding observed in the sural nerves (24, 25), and one quantitative analysis of epidermal neurite density revealed reduced (on average by 29%) axonal densities at CRPS-affected sites in the limbs (26). Additionally, an intact population of large myelinated fibers was observed in the examined nerves in that study, explaining why the conduction velocities of neurophysiological peripheral nerves are normally preserved in the involved limbs. Among our 18 girls with a confirmed diagnosis of CRPS-I who underwent neurophysiological studies, none showed abnormal electromyography or conduction velocity findings. Our morphologic study results for the intradermal nerves obtained from affected limbs that showed endoneurial edema and selective degeneration of unmyelinated fibers coincided well with the findings mentioned above.

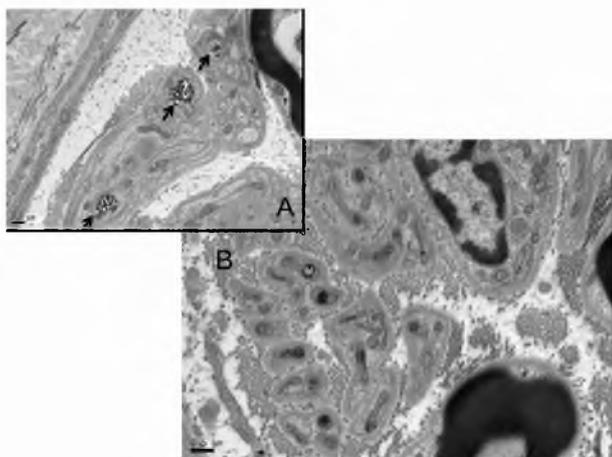
One important disease that must be differentiated from CRPS-I in vaccinated patients is macrophagic myofasciitis (27, 28). This disorder develops as an unusual reaction to the intramuscular injection of aluminium-containing vaccines and is characterized by common manifestations of diffuse



**Figure 9.** Electron-microscopic findings of the intradermal nerves in the second finger in Case 2. A: The number of myelinated and unmyelinated nerves was preserved, although some unmyelinated nerve axons (indicated by arrows) contained electron-dense coarse granular materials, possibly indicating degeneration. B shows an enlarged image of the unmyelinated axon indicated by the left arrow. Coarse granular structures with a diameter ranging from 200 to 500 Å. Their morphology coincided with that of glycogen granules (49). Original magnification A  $\times 7,500$ , B  $\times 20,000$ .



**Figure 10.** Electron-microscopic findings of the intradermal nerves in the first toe of in Case 2. A is a low-magnification image of one fascicle with extensive structure-less space in the endoneurium, indicating edema. B is a high-magnification image of the framed area in A: Denervated Schwann cell's cytoplasmic processes are surrounded by proliferating bundles of collagen fibers (indicated by arrows). Both findings indicate severe degeneration of the nerve fascicle. Original magnification A  $\times 7,500$ , B  $\times 1,800$ .



**Figure 11.** Electron-microscopic findings of the intradermal nerves in the calf in Case 4. A shows a few unmyelinated nerve axons (indicated by arrows) containing electron-dense coarse granular materials. B reveals denervated Schwann cell cytoplasmic processes. Both findings closely resemble those seen in Figs. 8 and 9. Original magnification A  $\times 12,000$ , B  $\times 20,000$ .

myalgia and arthralgia, as well as elevated levels of serum CK and CRP. In our series of girls, two demonstrated myalgia and grasping pain in the calves, and MRI disclosed abnormal signals in the involved muscles. However, the serum levels of CRP and CK were normal in both girls, and it was confirmed in the present Case 4 that the calf lesions on MRI spontaneously disappeared within a short period and that the histology of the involved muscle showed no inflammation. Such muscular lesions appear to reflect transient vasogenic edema caused by neurogenic dysregulation, and the histo-

**Table 5.** The Final Diagnosis of 40 Girls Examined

Diagnosis	No.
CRPS-1 alone	5
CRPS-1+OH	5
CRPS-1+OD	5
CRPS-1+POTS	3
OH alone	3
OD alone	7
POTS alone	1
Others	11

logical findings of intramuscular edema with degeneration have been previously reported in one patient with reflex sympathetic dystrophy (29).

**Orthostatic intolerance**

Orthostatic intolerance can be classified into four different conditions (30), including idiopathic orthostatic hypotension (IOH) and similar conditions associated with neurological disorders. The latter group is further divided into two diverse forms: peripheral sympathetic neuropathy and central nervous system defects in the sympathetic nerve activity. The fourth type is POTS. IOH and its related disorder, OD, are recognized to cause a variety of symptoms, such as recurrent dizziness, chronic fatigue, headaches and syncope, in school-aged children and adolescents (14). Approximately 80% of our affected girls had chronic headaches, which may have been causally related to IOH or OD in 67% of cases. Among these cases, Case 1 was the first in which all of the

patient's symptoms could be attributed to OD. In addition, the patient's peripheral sympathetic nerve response based on variations in the plasma noradrenalin level during position changes was inadequate (31).

POTS is a disorder of the autonomic nervous system that develops in adolescents and young people, with females being predominant. The orthostatic symptoms of POTS include lightheadedness, visual blurring or tunneling, palpitations, tremulousness and weakness, especially in the legs. Symptoms such as hyperventilation, anxiety, chest wall pain, acral coldness and pain occur less frequently (17, 32). Orthostatic stress can easily evoke intense fear or panic in some predisposed individuals (32). It is also noted that there is a considerable overlap between POTS and chronic fatigue syndrome (33), and patients with both disorders have been reported to complain of cognitive impairments, low energy and/or pronounced sleep disturbances, with the inability to complete normal educational activities or occupational duties (34). These symptoms are so severe that the lives of the patients and their families are seriously disrupted. However, these complicated conditions are not widely recognized among physicians or pediatric specialists, and many patients are misdiagnosed as having psychiatric diseases (34).

The present Cases 2 and 3, which involved orthostatic intolerance, are representative in that the patients were not adequately diagnosed and/or treated at previous institutes. For example, the visual symptoms and occurrence of transient left hemiparesis just after waking up seen in Case 2 may be caused by OH, while the chest discomfort and psychological symptoms noted in Case 3 may be produced by POTS. Additionally, the patient in Case 5, with a confirmed diagnosis of CRPS-I and POTS, exhibited slight cognitive decline, as shown on a conventional WAIS examination. Similar information regarding a decreased ability to learn was collected from 40% of the patients in our series.

Concerning the causative relationship between the above sympathetic nerve-mediated manifestations and the use of HPV vaccines, our patients had three main disorders, including CRPS-I, OH and POTS. OH is well known to develop spontaneously in many adolescent girls; however, OH was accompanied by CRPS-I in the present study. The combination of both disorders is rarely seen in the normal population of adolescent Japanese girls. Another orthostatic intolerance disorder, POTS, is classified as an attenuated form of acute autonomic neuropathy or acute pandysautonomia (35), and an autoimmune origin has been implicated in the pathogenesis of this disease. For example, viral infections very occasionally precede the onset of symptoms (17, 35), and the Mayo clinic's experience with 152 patients with POTS found that 90.5% of the patient's had a history of an antecedent viral infection (32). In addition, an interesting case of a patient who developed POTS after vaccination with Gardasil<sup>®</sup> was recently reported from the U.S. (36). In contrast, four of the present girls, including those in Cases 3 and 5, who were shown to have POTS, lacked any antecedent viral infections; therefore, a possible predisposing factor was

HPV vaccination.

### **Movement disorders**

One initial adverse event seen in post-vaccinated Japanese girls that was also noticed by mass media was violent limb tremulousness and bizarre gait disturbances. CRPS is known to be accompanied by various neurologic manifestations, including limb paresis, incoordination, tremors, myoclonus and dystonia (37). Similarly, tremulousness and limb weakness are frequently observed in patients with POTS (38). These involuntary movements can be diminished or abolished by successful sympathetic blockade; thus, increased peripheral afferent input associated with overactivity of the peripheral sympathetic nervous system appears to play an important role in the pathogenesis of this condition (34, 35). Denervated supersensitivity of peripheral adrenoreceptors is another candidate inducing the above abnormal movements (17, 25). The violent limb tremulousness observed in the affected girls temporally appeared in the early stage of the disease. Electromyograms recorded in our Case 2 indicated that the involuntary movements closely resembled myoclonus (39) and may be induced peripherally, since the myoclonic electromyographic discharge was not associated with any preceding electroencephalographic activity (40).

Concerning gait disturbances, symptoms such as limb pain, tremulousness and weakness are thought to be responsible. The limb weakness noted in 15 of our girls was disproportionate to the patient's muscle strength on manual muscle testing and was not attributable to disturbed neural control of the voluntary movement system, including the pyramidal tract and peripheral somatic motor nerves. It is therefore supposed that the limb weakness was initially related to CRPS or POTS and later enhanced by psychosomatic conditions, such as severe anxiety and/or prolonged asthenia. Consequently, 13 of our 15 girls with gait disturbances discontinued their normal school lives. A decreased ability to learn and/or not attending school among post-vaccinated girls is an important social problem.

### **Etiology**

It has been reported that some autoimmune neurological diseases have developed in post-vaccinated girls, including Guillain-Barré syndrome (4), acute disseminated encephalomyelitis (41) and multiple sclerosis (42, 43). Among these patients, 69 treated with Gardasil<sup>®</sup> vaccination who subsequently developed Guillain-Barré syndrome were collected in the U.S. between 2006 and 2009 (44). The onset of symptoms was within six weeks after vaccination, and the estimated weekly rate of reporting of post-Gardasil<sup>®</sup> Guillain-Barré syndrome within the first six weeks (6.6 per 10,000,000) was higher than that observed in the general population. Although CRPS is not generally accepted to be an autoimmune disorder, our patients with proven CRPS-I concomitant with OH or POTS had evidence of post-ganglionic sympathetic neuropathy, such as a decreased plasma level of noradrenalin (31), abnormal MIBG cardiac

scintigram findings, as observed in the present Case 3 (15, 16), and an ultrastructural pathology of intradermal unmyelinated nerve fiber degeneration, all of which support that this condition is a form of autonomic neuropathy. Furthermore, POTS is now regarded to be an immune-mediated disorder (32). Based on the temporal relationship between immunization and the development of symptoms, we cannot deny the possibility that immunization with HPV vaccines may secondarily induce sympathetically mediated disorders, including CRPS-I, OH and POTS.

Recently, the presence of gAChR antibodies in the serum has been noted to be involved in the pathogenesis of various autonomic disorders (45). Such antibodies act against neuronal acetylcholine receptors in the autonomic ganglia and are frequently found in patients with autoimmune autonomic ganglionopathy. Additionally, two rare cases of Rasmussen encephalitis with  $\alpha 7$  AChR antibodies have been reported (46). It has also been reported that a low titer of gAChR antibodies is detected in the serum in a small number of patients with POTS (32). In contrast, this autoantibody was undetectable in 14 of our girls, including four with POTS. The negative findings observed in our series of girls with autonomic symptoms indicates that the post-ganglionic peripheral sympathetic nerve lesions produced autonomic dysfunction in these cases. Finally, the above peripheral sympathetic nerve disorders have not been reported in foreign cohort studies of human HPV vaccines (47, 48) and it thus remains unclear why Japanese girls are more frequently involved. It is unlikely that the Japanese environment plays a role in the pathogenesis of this unique autonomic disorder, as the patient in our Case 5, who received vaccination in the U.S., developed similar adverse reactions. Studies with large-scale investigations and experimental approaches are needed to further answer these questions.

**The authors state that they have no Conflict of Interest (COI).**

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