Ross syndrome: a rare or a misknown disorder of thermoregulation? A skin innervation study on 12 subjects

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Ross syndrome is described as a rare disorder of sweating associated with areflexia and tonic pupil. Since Ross’s first description in 1958, ~40 cases have been described. We assessed the involvement of cutaneous innervation in 12 subjects with Ross syndrome using quantitative sensory testing, sweating assessment and immunohistochemical study of anhidrotic and hyperhidrotic skin. This evaluation was repeated over time in 4 out of 12 subjects. In addition, we enrolled four subjects with Holmes–Adie syndrome (areflexia and tonic pupil) to investigate similarities between the two conditions. We found in Ross patients a complex and progressive involvement of cutaneous sensory and autonomic innervation underlying the impairment of heat production and heat dissipation through both loss of sweating and loss of cutaneous blood flow regulation. In Holmes–Adie subjects we found a mild impairment of sweating without thermoregulatory problems. The persistence of a sudomotor vasoactive intestinal peptide-immunoreactive (VIP-ir) innervation, although deranged and poor, definitely differentiated Holmes–Adie from Ross patients. Ross syndrome is a progressive and complex disorder of thermoregulation difficult to differentiate from the probably pathogenetically related Holmes–Adie syndrome. Sweating assessment and skin biopsy are suitable tools to define a boundary between them. Owing to the large number of Ross patients observed in only 5 years, and to the long and complex medical history of most of them, doubts arise on the effective rarity of this condition, and we warn family doctors and other specialists, besides neurologists, to become aware of this complex disorder.

Keywords: skin biopsy; Ross syndrome; autonomic nervous system; Holmes–Adie syndrome; thermoregulation

Abbreviations: AVAs = arteriovenous anastomoses; DβH = dopamine-β-hydroxylase; ENF = epidermal nerve fibres; IME = intrapapillary myelinated endings; ir = immunoreactive; MC = Meissner corpuscles; PGP = protein gene product; QST = quantitative sensory testing; SIT = Silastic imprint test; SSR = sympathetic skin response; TST = thermoregulatory sweat test; VIP = vasoactive intestinal peptide

In the last years, cutaneous innervation has been studied in patients with Ross syndrome by means of punch biopsy (Bergmann et al., 1998; Sommer et al., 2002; Perretti et al., 2003). Using this method Sommer et al. (2002) found a selective loss of cholinergic sudomotor fibres in four Ross patients while we found, in a previous study of three subjects with the same disorder, besides a marked loss of cholinergic sudomotor fibres, an involvement (although less severe) of other populations of autonomic and sensory nerves (Perretti et al., 2003).

In the present study, we enlarged the assessment of function and morphology of cutaneous innervation to 12 subjects with Ross syndrome, who arrived at our laboratory in the last 5 years, and we studied 4 of them over time. In addition, we enrolled four subjects with Holmes–Adie syndrome and we used a broad panel of antibodies including a selective marker for noradrenergic fibres (Donadio et al., 2006).

The aim of our study was to better define the involvement of peripheral nervous system in a larger cohort of subjects with Ross syndrome, to evaluate this involvement over time and to investigate possible common abnormalities of skin innervation between Ross and Holmes–Adie subjects.

**Subjects and methods**

**Subjects**
Clinical features of our patients are described in Table 1. Twelve subjects (six female and six male, mean age = 44.3 ± 10.5, range = 31–63 years) with Ross syndrome (1R–12R) were enrolled. This
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population included three patients (1R, 6R and 7R) described previously (Perretti et al., 2003) in order that their whole histological material could be reexamined and some of their unprocessed skin tissue could be processed with new antibodies.

All Ross patients arrived at our observation because of heat intolerance, except a 46-year-old woman (11R) who complained of a severe segmental hyperhidrosis in the left groin causing social embarrassment and a 34-year-old woman (12R) who suffered from sudden episodes of tachycardia with consequent panic attacks. Both of them, questioned about heat intolerance, admitted adopting several strategies to face hot weather.

There were several common features in the clinical history of all our patients: an unpleasant feeling in a warm environment since childhood; excessive sweating in some areas of the body that had been getting better over time; frequent episodes of fever and sometimes fainting during the summer with an increased heart rate just before the heat became unbearable; dryness of the skin especially of the legs and hands that worsened over time with progressive loss of the ability to leaf through a book; the need to wear sun glasses outside; early fatigue during physical exercise; gastrointestinal disorders such as irritable bowel syndrome or constipation.

Four of these subjects (1R–4R) underwent a follow-up evaluation 1–4 years after the first one. At the second visit they appeared less frustrated about their disease. In fact, after becoming aware of their thermoregulatory disorder, they had learned to manage it, and, for the limited period of observation, they appeared unaware of a possible worsening of their condition. Two of them (1R and 3R) had developed skin disorders and were therefore under dermatological treatment. We also recruited four subjects with Holmes–Adie syndrome (1A–4A) casually observed during the period of enrolment. We also recruited four subjects with Holmes–Adie syndrome (1A–4A) casually observed during the period of enrolment of Ross patients. None of these subjects complained of heat intolerance, but when thoroughly asked about their behaviour in warm environment, they described some strategies for avoiding heat.

A total number of 120 healthy volunteers (age range = 30–60 years) was used as a control group. Of this population 100 subjects represented the control group for quantitative sensory and sudomotor testing and 20 subjects formed the control group for morphological data.

Methods

All patients underwent neurological and electrophysiological examination and brain and spinal cord MRI. Electrophysiological evaluation included recording, via surface electrodes, of antidromic sensory conduction velocity along median, ulnar and sural nerves and motor conduction velocity along median, ulnar and peroneal nerves; H-reflex from soleus muscle by stimulation of sciatic nerve at popliteal fossa; F-wave from abductor pollicis brevis by supramaximal stimulation of median nerve at wrist; short latency somatosensory evoked potentials by bipolar electrical stimulation of median and tibial nerves at wrist and at medial malleolus, respectively. The assessment of autonomic and sensory function was performed by means of cardiovascular reflexes testing, sympathetic skin response (SSR), thermoregulatory sweat test (TST), Silastic imprint test (SIT) and quantitative sensory testing (QST). Sensory and autonomic nerve fibre morphology was evaluated by means of immunohistochemical techniques applied to punch skin biopsies. Since the bulk of the present paper is the study of function and morphology of cutaneous innervation, we described in detail methods and results related to these aspects. Informed consent was obtained from all the subjects included in the study.

Assessment of cutaneous autonomic and sensory function

Sweating function

SSR. SSRs were recorded from both hands and feet using surface electrodes after delivering random electrical stimuli at wrist along median nerve and at ankle along peroneal nerve.

TST. Subjects were unclothed and the entire body surface, except face and genitalia, was covered with a 2% alcoholic solution of iodine and with rice starch powder. Subjects stayed in a sweat cabinet (air temperature = 45–50°C, relative humidity of 35–45% until oral temperature increased by 1°C). The colour of rice starch powder changes from white to black in the presence of sweat. Digital pictures of subjects were taken in order to calculate the per cent of anhidrotic areas per entire body surface.

SIT. Sweat droplets were counted using a Silastic mould technique (Kennedy et al., 1984) after 5 min of 1% pilocarpine iontophoresis in two body sites (dorsum of hand and foot).

Quantitative sensory testing

Dorsum of foot and hand, thigh and leg were tested. If one of these sites was hyperhidrotic, a contralateral site was tested too in order to detect possible differences between hyperhidrotic and anhidrotic sites. Cold, warm, cold pain and heat pain thresholds were evaluated using a thermal sensory analyser (Medoc, TSA 2001, Israel) with a Peltier probe of 3 × 3 cm and the method of limits.

Tactile thresholds were assessed using a series of 18 calibrated nylon monofilaments (Semmes–Weinstein) moving stepwise from the thicker toward the thinner filament in order to detect the thinnest one perceivable 5 times out of 10.

Mechanical pain perception was evaluated by applying on the skin, 10 times for 1–2 s, a calibrated monofilament, with a bending force of 95 mN, connected to a sharp non-penetrating probe (50-µm tip). The per cent of stimuli perceived as painful was recorded and so was the pain magnitude using a visual analogue scale.

Skin biopsy

Punch skin biopsies of 2–3 mm in diameter were taken from the fingertip (III digit), thigh and leg after intradermal injection of 1% xylocaine. In Ross subjects, if one of these sites was hyperhidrotic (right leg in 2R and left thigh in 4R), the contralateral site was biopsied too. Otherwise, additional samples were taken from hyperhidrotic skin (foot in 1R and 3R and 12R, gluteus in 5R and 11R, sacral area in 6R, axilla in 7R, shoulder in 8R, neck in 9R, back in 10R and 2R at follow-up) and contralateral anhidrotic areas. Results from thigh, leg and fingertip were compared with findings from 20 normal age- and sex-matched subjects. Results from the additional sites were compared with findings from homologous contralateral samples. Biopsies were fixed overnight in Zamboni solution, cut into 80-µm-thick sections using a freezing slide microtome (Leica 2000R) and processed using indirect immunofluorescence techniques as described previously (Kennedy and Wendelschafer-Crabb, 1993). A panel of primary antibodies (Table 2) and an endothelium binding agglutinin (Ulex europaeus, Vector, Burlingame CA, USA) were used, and antigens were
Confocal digital images were acquired using a CARV confocal system (ATTO Biosciences, Rockville, MD, USA) connected to an Axioskop 2 Mot Zeiss microscope (Jena, Germany) using ·10, ·20 and ·100 Plan Apochromat and ·40 F-Fluar objectives. Quantification of epidermal nerve fibres (ENFs), intrapapillary myelinated endings (IMEs) and Meissner corpuscles (MCs) was performed using Neurolucida (Microbrightfield, Colchester VT, USA) and ScionImage (Scion Corporation, Frederick, MD, USA) software as described previously (Kennedy et al., 1996; Nolano et al., 2001, 2003).

Statistical analysis
We used Student’s t-test for paired data to compare findings from baseline and follow-up observations and ANOVA to compare functional and morphological data from patients and controls.

Results
Neurological examination of our patients was normal except for areflexia and tonic pupils. Electrophysiological evaluation showed normal sensory and motor conduction velocities and normal sensory evoked potentials. F-wave latency was normal while H-reflex was of low amplitude in Patient 10R and absent in all the remaining ones. Cardiovascular reflexes and brain and spinal cord MRI were normal in all of them.

Sweating assessment
Ross patients
SSR was absent in 11 out of 12 of our subjects. The only patient presenting a normal SSR (Patient 12R) had some areas of residual sweating on the hands and feet. TST provided evidence of a generalized anhidrosis accounting for >80% of total skin surface (Table 1). Residual sweating areas were usually localized between T5 and T12 sudomotor dermatomes. Using SIT, a marked loss of sweat drops per square centimetre was observed in both the examined sites in all subjects (Fig. 1). In the four patients studied over time we observed a further reduction in the residual sweating area (mean value decreased from 7.9 to 1.37% of total body surface) and in the number of activated sweat glands per square centimetre (from 103.3 to 60.1 and from 47.7 to 39.9 at hand and foot, respectively).

Holmes–Adie patients
All patients showed a normal SSR, but TST demonstrated the presence of segmental anhidrosis (Table 1) and SIT demonstrated the presence of a reduction in sweat drop density (statistically significant at foot) although not as severe as in Ross patients (Fig. 1).

Quantitative sensory testing
Ross patients
Although our patients did not complain of sensory disturbances, we detected in all of them an impairment of at least two sensory modalities with no differences between anhidrotic and hyperhidrotic areas. Statistical analysis showed a significant increase in tactile thresholds and mechanical pain perception in Ross subjects, compared with controls, in all the examined sites. A significant increase in warm threshold value was found at thigh and foot where heat pain threshold was also significantly impaired (Fig. 2).

At follow-up evaluation, in all four Ross subjects, a further impairment of thermal and tactile thresholds with significant increase in cold and heat pain thresholds at hand dorsum and in cold threshold at leg was observed (Fig. 3).

Holmes–Adie patients
We found abnormalities of tactile thresholds and mechanical pain perception in all subjects, whereas we did not observe abnormalities of thermal thresholds (Fig. 2).

Skin biopsies
Normal subjects
A rich network of vasoactive intestinal peptide-immunoreactive (VIP-ir) nerve fibres was present around sweat glands (Figs 4, A4 and 6, A2), hair follicles (Fig. 4,
A2), arteriovenous anastomoses (AVAs) (Fig 5, A2), along arrector pilorum muscles (Figs 4, A3 and 5, A1) and, particularly in glabrous skin, along dermal vessels (Fig. 4, A1); in addition, Merkel cells in the epidermis basal layer appeared intensely stained with VIP (Fig. 4, A1); DBH-ir fibres were abundant and very bright along arrector pilorum muscles (Figs 5, A1 and 6, A4) and around AVAs (Figs 5, A2 and 6, A3) while they accounted only for a small amount of the sudomotor network. Both DBH-ir and VIP-ir fibres showed around the canal of AVAs a typical encircling pattern and a discontinuous course (Fig. 5, A2). Protein gene product (PGP) 9.5 staining showed a regular subepidermal plexus in the upper dermis with tiny branches crossing the basement membrane to reach the epidermis. ENFs were present at close and regular intervals in hairy skin (Fig. 7A), in cluster on the apex of dermal papillae in glabrous skin where MCs and IMEs appeared regularly-distributed (Fig. 7D). A regular presence of calcitonin gene-related peptide (CGRP) and substance P-immunoreactive (SubP-ir) fibres, with few fibres reaching the epidermis, was found in the dermis of both glabrous and hairy skin.

**Ross patients**

We found a complete lack of VIP-ir fibres in anhidrotic skin where only Merkel cells appeared intensely VIP-ir (Fig. 4, C1). Blood vessels (Figs 4, C1 and 5, C2 and D2), hair follicles (Fig. 4, C2), arrector pilorum muscles (Figs 4, C3 and 5, C1 and D1) were devoid of VIP-ir fibres in both anhidrotic and hyperhidrotic skin. Very rare VIP-ir fibres were found around sweat glands in skin samples from areas of residual sweating (Fig. 4, C4) while they were completely absent around sweat glands in anhidrotic skin (Fig. 6, C2). In addition, we observed in hyperhidrotic skin a marked loss of DBH-ir fibres in arrector pilorum muscles (Fig. 5, D1) and around the canal of AVAs (Fig. 5, D2) while in anhidrotic skin these structures (Fig. 5, C1 and C2 and Fig. 6, C3 and C4) appeared completely devoid of fibres. A sparse innervation of dermal structures appeared with PGP staining (Fig. 6, C1–C4). Compared with normal, a generalized reduction of ENFs was observed in thigh, leg and fingertip, regardless of the sweating capability of the skin (Table 1 and Fig. 7C and F). In the additional sites, we did not find significant differences in ENF density between anhidrotic and hyperhidrotic skin. Although a significant loss of receptors was evident only in four subjects, MCs and their myelinated fibres presented several abnormalities of structure and position (Fig. 7G–K). Some MCs appeared simplified, misshapen and deranged (Fig. 7G and H) and sometimes clearly atrophic; others were located near the base of the dermal papillae instead of at the apex, indicating a structural remodelling of the corpusscles. Degenerative aspects of myelinated fibres, such as wrinkling, swelling, paranodal demyelination, calibre variability and dishomogeneous aspects of myelin were frequently observed in both glabrous (Fig. 7F–I) and hairy skin. SubP-ir fibres in dermis and epidermis appeared to be rare compared with those in normal subjects. CGRP-ir fibres appeared more preserved and sometimes showed aspects of hyperinnervation. Chaotic aspects of hyperinnervation (Fig. 7J) with a peculiar elongation of dermal papillae were observed with PGP staining in the papillary dermis of 4 out of 12 patients (1R, 2R, 4R and 12R). This process was particularly evident in glabrous skin where dermal papillae doubled the normal depth with a secondary elongation of papillary nerves (Fig. 7J) and then of the internodal length (mean value = 200 versus 78 µm) of myelinated MC afferences (Fig. 7, Ka and Kb compared with Kc and Kd). Following the shape of dermal papillae, in these subjects, capillary loops also appeared elongated and showed a complex course with dilations and frequent anastomoses (Fig. 7L).
These aspects of abnormalities of vascular structure with various degrees of dilation and complexity were present in all Ross patients in both glabrous and hairy skin.

In the four Ross patients that we examined over time, we observed a further reduction of PGP-ir fibres around sweat glands and other dermal annexes. ENF density remained heavily reduced and no significant further reduction was detected (Fig. 8). MCs and, in particular, their myelinated fibres were markedly reduced (Fig. 8) although their density was in two subjects still within the normal range. In two of these four subjects (1R and 2R) we observed a further marked increase in the papillary length already elongated at first evaluation.

Holmes–Adie patients
VIP-ir sudomotor innervation appeared poor and showed aspects of disarrangement in anhidrotic areas (Figs 4, 5 and 6) while both VIP and DBH-ir fibres around vessels (Figs 5B and 6B) and in arrector pilorum muscles (Figs 5B1 and 6B4) appeared normally represented. ENF density was normal at thigh and leg, regardless of the sweating capability of the skin, and reduced at fingertip (Table 1 and Fig. 7B and E). MCs and IMEs were normal in density (Table 1) but showed frequent occurrence of slight abnormalities (Fig. 7E).
Discussion

The results of the present paper add new insight into the comprehension of characteristics of Ross syndrome besides confirming our previous findings (Perretti et al., 2003) in a larger population of patients. This disorder arrives at clinical observation for a thermoregulatory impairment but it involves cutaneous innervation in a complex fashion. In fact, besides the observed cholinergic sudomotor damage

Fig. 4 Digital confocal images of glabrous (A1, B1 and C1, ×20) and hairy skin innervation (A2–C3, ×40 and A4–C4, ×100). In controls (A) VIP immunoreactivity is present, besides in Merkel cells, in perivascular network, which is more evident in glabrous skin (A1) and is very intense around hair follicle (A2), arrector pilorum muscle (A3) and sweat glands (A4). In anhidrotic skin of Holmes–Adie subjects (B) VIP-ir fibres appear well represented in the perivascular network of glabrous skin (B1), in hair follicles (B2) and along arrector pilorum muscles (B3), while they appear poorly represented and deranged around sweat glands (B4). In Ross patients (C) the most typical finding is the loss of VIP-ir fibres in the anhidrotic (C1–C3) and hyperhidrotic skin (C4). Note the disappearance of VIP-ir perivascular network in the upper dermis (C1) while Merkel cells in the epidermis basal layer maintain VIP immunoreactivity. The loss of VIP-ir fibres involves hair follicle (C2), arrector pilorum muscle (C3) and sweat glands (C4). In C4, rare VIP-ir fibres were found around a sweat gland tubule in hyperhidrotic skin. Bar equals 50 μm in ×20 images, 25 μm in ×40 images and 10 μm in ×100 images. Site and patient correspondence: B1 = Patient 1A fingertip; C1 = Patient 5R fingertip; B2 = Patient 4A thigh; C2 = Patient 4R right thigh; B3 = Patient 4A thigh; C3 = Patient 10R thigh; B4 = Patient 4A thigh; C4 = Patient 11R left gluteus.
we found an involvement of pilomotor and vasomotor fibres and, although to a lesser extent, of somatic unmyelinated and myelinated nerve fibres.

**Sweat glands innervation and sudomotor function**

In rodents, during development, sympathetic innervation of sweat glands is noradrenergic and switches to cholinergic phenotype in response to signals derived from the target organ (Tian et al., 2000; Stanke et al., 2006). Both catecholaminergic and cholinergic neurotransmission are required for the acquisition of secretory responsiveness (Tian et al., 2000) but sudomotor function of adult animals relies only on cholinergic fibres. In our controls, as described previously (Uno, 1977; Donadio et al., 2006), sweat glands showed, besides VIP-ir cholinergic innervation accounting for most of PGP-ir fibres, a DBH noradrenergic component, that, when present, accounted only for a small amount of it. In Ross patients sweat glands in anhidrotic areas showed only a slender network of PGP-ir fibres that did not express the markers of cholinergic (VIP) or noradrenergic (DBH) axons. Despite the severe loss of sudomotor fibres, sweat glands maintained a normal morphology as already reported in Ross syndrome (Bergmann et al., 1998; Sommer et al., 2002). Moreover, these glands, unable to sweat by increasing body temperature, maintained the capability to secrete sparse tiny sweat drops in response to pharmacological stimulation with pilocarpine by iontophoresis. Experimental models of denervation of sweat glands in rodents have demonstrated that cholinergic innervation is required for the maintenance of sudomotor function (Grant et al., 1995), and in humans denervation has been shown to cause loss of sweating capability (Kennedy et al., 1984). We can speculate that the surviving PGP-ir fibres around sweat glands in Ross patients might play a role in the maintenance of gland trophism and in the survival of some muscarinic receptors that might still be responsive to direct pharmacological stimulation. It remains unclear how few VIP-ir sudomotor fibres in the hyperhidrotic areas can induce profuse sweating. This could be a compensatory phenomenon but we cannot exclude an hyperactivity of the sudomotor function, due to the post-ganglionic partial denervation, that could last until cholinergic sudomotor degeneration is complete. This hyperactivity might be due, as Sommer et al. (2002) hypothesized, to an early loss of cholinergic M2 inhibitor presynaptic autoreceptors, whose presence has been demonstrated in rats (Haberberger and Bodenbenner, 2000), that precedes the loss of cholinergic fibres leading to anhidrosis. During the follow-up period, we observed in our patients a further impairment of sudomotor function (loss of sweating body surface and a further loss of density of sweat drops after pilocarpine stimulation), indicating a progressive process of which they were only partially aware.

![Fig. 5 Noradrenergic and cholinergic innervation in arrector pilorum muscles (A1–D1, ×40) and in AVAs (A2–D2, ×40) of a control (A), an Holmes–Adie subject from normohidrotic skin (B) and a Ross patient from anhidrotic (C) and hyperhidrotic (D) skin. In controls and in Holmes–Adie subjects both DBH and VIP-ir fibres are abundant and encircle AVAs and run longitudinally along arrector pilorum muscles. Both nerve populations have a dotted aspect probably owing to a dishomogeneous distribution of the antigens within axons. In anhidrotic skin of Ross patients there is a complete lack of both DBH and VIP-ir fibres, while in hyperhidrotic skin some DBH-ir fibres persist along muscle and around AVAs. Bar equals 25 μm. Site and patient correspondence: B1 = Patient 2A thigh; B2 = Patient 2A fingertip; C1 = Patient 2R thigh; C2 = Patient 1R right foot; D1 = Patient 2R back; D2 = Patient 1R left foot.](http://brain.oxfordjournals.org/
We observed a severe loss of VIP cholinergic nerves as well as DBH noradrenergic nerves in arrector pilorum muscles in all of our patients. PGP staining showed denervation of variable degree in samples from different subjects, in samples from different sites of the same subject and in the same sample from muscle to muscle. This implies that piloerection...
also could be impaired in a patchy fashion and sometimes preserved as it was in the patient that Ross (1958) described. We could not evoke piloerection in our patients, and when we asked them about goose bumps, they had not been able to recall this phenomenon since many years. Patient 3R described the occurrence of very painful goose bumps limited to his thigh evoked by cold water bathing. Together with the loss of piloerection, most of our patients described an
inability to shiver. Goose bumps and shivering are both thermogenetic responses to cooling, but the first in humans is rudimentary. Arrector pilorum denervation explains the lack of piloerection but not of shivering; therefore, an impairment along thermal sensory afferent pathways and/or along the efferent motor pathways at spinal level, through an involvement of inhibitory interneuronal circuitry, can be hypothesized. This hypothesis is supported by the sensory abnormalities and the absence of H-reflex observed in our patients.

Vasomotor innervation

Ross (1958) found in his patient a normal vasomotor function evaluating changes of blood pressure to posture variations and to cold. The same findings have been later described by other authors (Weller et al., 1992; Pereon et al., 1993; Bergmann et al., 1998; Perretti et al., 2003). Although in our patients cardiovascular reflexes were normal, we found in most of them, around AVAs, a severe loss of PGP-ir fibres (Fig. 6, C3) that appeared markedly affected in their morphology (thinning, derangement) with a loss of DBH as well as VIP-ir fibres (Fig. 5, C2 and D2). At the same time, all the upper dermis vascular structure appeared dilated with complex and large vessels (Fig. 7L). These findings imply that the impairment of heat dissipation in Ross syndrome is due both to loss of sweating and loss of cutaneous blood flow regulation. In accordance with this, in most of our patients, skin, especially of the hands, was warm and red, and they reported no need to get repaired from cold even in very cold weather.

Sensory innervation

Sensory impairment was not mentioned by Ross (1958) in his first description, and this aspect has been disregarded or not evaluated by successive authors (Weller et al., 1992; Pereon et al., 1993; Reinauer et al., 1993; Diaz-Barreiros et al., 1995; Wolfe et al., 1995; Bergmann et al., 1998; Druschky et al., 1999; Shin et al., 2000; Sommer et al., 2002; Chakravarty et al., 2003; Beier et al., 2004; Serra Mitjans et al., 2004). Although only two of our patients complained of thermal and pain perception disturbances, QST allowed detection of a subclinical sensory impairment in all of them. This functional finding is in keeping with the loss of unmyelinated sensory fibres and with the abnormalities of sensory myelinated fibres that we found in all our patients. Aspects of wrinkling, swelling and paranodal demyelination of myelinated fibres suggest an axonal degenerative process that must proceed slowly since density of MCs and IMEs did not differ significantly from controls and it was still normal in two out of the four Ross patients studied over time. In this group the most evident findings were an increase in sensory abnormalities at QST and a loss of myelinated fibres following the predegenerative aspects observed at the first evaluation. These results indicate that, starting from a severe sudomotor disorder, there is a progression in the involvement of other subpopulations of autonomic and somatic cutaneous nerve fibres. The somatic involvement, through the damage of cutaneous thermoreceptors, might account for a further impairment of thermoregulation. The progressive involvement of additional subpopulations of nerves could explain the discrepancy of our findings with other authors who described a selective cholinergic damage (Bergmann et al., 1998; Sommer et al., 2002).

Skin changes

Skin abnormalities have been described in Ross patients (Heath et al., 1982). We observed skin disorders such as ichthyosis in Patient 1R and eczema in Patients 3R and 12R. In addition, in four of our patients (including 1R and 12R) we found an abnormal increase in dermal papillary length with subsequent lengthening of capillary loops and intrapapillary nerve fibres. We found interesting the effect of papilla changes on internodal lengthening of myelinated fibres directed to MCs. These internodes in fact doubled their length (Fig. 7, Ka and Kb compared with Kc and Kd). Proliferation of terminal cutaneous nerves is described in psoriasis (Raychaudhury and Raychaudhury, 2004). We do not know if there is a link between this disease and Ross syndrome or if the skin disorders described could be a secondary effect of anhidrosis.
Holmes–Adie subjects: similarities and differences with Ross

In Holmes–Adie subjects TST provided evidence of the presence of a segmental anhidrosis, and this observation blurred the boundary between them and the patients with Ross syndrome. Moreover, we found that they shared with Ross patients other subclinical findings besides sweating disorders, such as increase in tactile thresholds, impairment of mechanical pain perception and reduction of fingertip ENFs. Owing to these similarities, and since segmental anhidrosis is the third feature of the triad characterizing Ross syndrome, should we consider this alternative diagnosis for our ‘Holmes–Adie’ patients? From our findings it appears that two main features sharply separate Ross from Holmes–Adie subjects: heat intolerance and absence of cutaneous VIP-ir fibres. In fact, despite the loss of ~50% of their sweating body surface and a significant reduction of functional sweat gland density, there was no heat intolerance in Holmes–Adie subjects. Conversely, this was the most clinically relevant characteristic of Ross patients who had <20% of their body surface still able to sweat. The absence of heat intolerance in Holmes–Adie syndrome could be partly explained by a high security factor protecting thermoregulation but also by a preservation of dry heat loss through cutaneous blood flow regulation as suggested by the presence of normally innervated vessels and AVAs. In addition, sweat gland innervation, although poor and disorganized, maintained in Holmes–Adie subjects a component of VIP-ir sudomotor fibres. SSR was a further feature that differentiated Ross from Holmes–Adie syndrome in our population: it was absent in 11 out of 12 Ross patients and present in all 4 Holmes–Adie subjects. Therefore, we suppose that Holmes–Adie and Ross syndrome are two clinically distinguishable conditions belonging to the same spectrum of autonomic and sensory ganglia involvement, in agreement with Shin et al. (2000). This gangliar damage can be the effect of an unknown noxa patogena but we cannot exclude the hypothesis that Ross and Adie syndromes are different expressions of a condition leading to an apoptotic process progressively involving populations of neurons derived from neural crest for a congenital lack of factors promoting their survival. Is Holmes–Adie an early stage or a mild expression of Ross syndrome? In the clinical history of three of our Ross patients (4R, 10R, 12R) the diagnosis of Holmes–Adie preceded the diagnosis of Ross of 10–15 years. Does this progression occur constantly? Further follow-up studies on a large population of Holmes–Adie are warranted to answer these questions.

Is Ross syndrome a rare condition?

The observation of 12 patients with Ross syndrome in 5 years led us to hypothesize that this disorder must be less rare than that described previously (Wilhelm et al., 1991; Weller et al., 1992; Wolfe et al., 1995). A possible explanation for this discrepancy can be that in a number of cases this condition remains undiagnosed, as suggested by the long and complex medical history of our patients. Most of them in fact received a diagnosis of Ross syndrome after many years and after having undergone several physical and instrumental examinations by different specialists. In particular, three of them (1R, 5R and 12R) had been treated with anxiolytic drugs and one (3R) with neuroleptics, as the fainting feeling and the sense of oppression with increased heart rate, had been considered as symptoms of panic attacks or anxiety crises. In addition, three of our patients had skin disorders and were under dermatological treatment; therefore, we advise other specialists besides neurologists and, above all, family doctors to become aware of this disorder.

Concluding remarks

Our findings allowed to better define the thermoregulatory problem in Ross patients, showing a complex and progressive impairment of heat dissipation as well as heat production through the involvement of thermal afferent pathways to hypothalamus.

Although the thermoregulatory impairment severely limits social life of patients with Ross syndrome, this condition does not seem to be a life-threatening disorder, since subjects, once aware of it, learn strategies to avoid excessive body heating.

Heat intolerance, absence of SSR and lack of VIP-ir fibres in skin could be additional criteria to better characterize Ross syndrome.

References

Skin innervation in Ross syndrome