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Inherited erythromelalgia due to mutations in *SCN9A*: natural history, clinical phenotype and somatosensory profile

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Inherited erythromelalgia, the first human pain syndrome linked to voltage-gated sodium channels, is widely regarded as a genetic model of human pain. Because inherited erythromelalgia was linked to gain-of-function changes of sodium channel $Na_v1.7$ only a decade ago, the literature has mainly consisted of reports of genetic and/or clinical characterization of individual patients. This paper describes the pattern of pain, natural history, somatosensory profile, psychosocial status and olfactory testing of 13 subjects with primary inherited erythromelalgia with mutations of *SCN9A*, the gene encoding $Na_v1.7$. Subjects were clinically profiled using questionnaires, quantitative sensory testing and olfaction testing during the in-clinic phase of the study. In addition, a detailed pain phenotype for each subject was obtained over a 3-month period at home using diaries, enabling subjects to self-report pain attacks, potential triggers, duration and severity of pain. All subjects reported pain and heat in the extremities (usually feet and/or hands), with pain attacks triggered by heat or exercise and relieved mainly by non-pharmacological manoeuvres such as cooling. A large proportion of pain attacks (355/1099; 32%) did not involve a specific trigger. There was considerable variability in the number, duration and severity of pain attacks between subjects, even those carrying the same mutation within a family, and within individuals over the 12–13 week observation period. Most subjects (11/13) had pain between attacks. For these subjects, mean pain severity between pain attacks was usually lower than that during an attack. Olfaction testing using the Sniffin'T test did not demonstrate hyperosmia. One subject had evidence of orthostatic hypotension. Overall, there was a statistically significant correlation between total Hospital Anxiety and Depression Scale scores ($P = 0.005$) and pain between attacks and for Hospital Anxiety and Depression Scale Depression scores and pain between attacks ($P = 0.001$). Hospital Anxiety and Depression Scale scores for five subjects were below the threshold for mild anxiety or depression and none of the 13 subjects were severely anxious and/or depressed. Quantitative sensory testing revealed significantly increased detection thresholds for cold and warm stimuli at affected, compared to unaffected sites. By contrast, significantly decreased cold and heat pain thresholds were found at unaffected sites. Sensory profiles varied considerably between affected and unaffected sites, suggesting the existence of small fibre neuropathy in symptomatic sites. This in-depth clinical characterization of a well-defined inherited erythromelalgia population indicates the importance of characterizing the pain phenotype in individuals before undertaking clinical trials, given the inherent variability of pain both between and within inherited erythromelalgia subjects, even those within a family who carry the same mutation.

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Abbreviations: HADS = Hospital Anxiety and Depression Scale; IEM = inherited erythromelalgia; NRS = Numerical Rating Scale; PCS = Pain Catastrophizing Questionnaire; QST = quantitative sensory testing

Introduction

Inherited erythromelalgia (IEM), the first human pain syndrome linked to voltage-gated sodium channels, is regarded as a genetic model of human pain (Dib-Hajj *et al.*, 2013). IEM is genetically linked to dominant gain-of-function mutations of the *SCN9A* gene encoding voltage-gated sodium channel $Na_v1.7$ (Cummins *et al.*, 2004; Yang *et al.*, 2004; Dib-Hajj *et al.*, 2005). This channel is highly expressed in the peripheral nervous system including dorsal root ganglion neurons, sympathetic ganglion neurons (Toledo-Aral *et al.*, 1997; Rush *et al*, 2006) and olfactory sensory neurons (Ahn *et al.*, 2011; Weiss *et al.*, 2011). It is recognized as key to setting the gain in pain-signalling neurons (Waxman 2006; Dib-Hajj *et al.*, 2013) and in olfaction (Weiss *et al.*, 2011). IEM is associated with episodic pain and reddening of the skin, typically localized to the distal extremities (feet and hands) (Drenth and Waxman, 2007). Consistent with the vasomotor changes (erythema of distal extremities) seen in IEM patients during their attacks, available evidence indicates that IEM mutations render sympathetic ganglion neurons hypoexcitable (Rush *et al.*, 2006). Pain in IEM is reported to occur as early as 1 year old (early onset), in the second decade (delayed onset) of life, and in adults (Dib-Hajj *et al.*, 2007; Drenth and Waxman, 2007). A correlation has been reported between the magnitude of the hyperpolarizing shift in channel activation (Han *et al.*, 2009) or slow-inactivation (Cheng *et al.*, 2011) and age of onset of symptoms. There is a need to know more about the natural history and pain phenotype of the disease, the temporal pattern of painful episodes and the effect of IEM associated with $Na_v1.7$ mutations on psychosocial status and olfaction. A series of 168 cases of erythromelalgia including natural history, presentation and outcome was reported by Davis *et al.* (2000), based on retrospective medical record review with a follow-up survey questionnaire; however, this report preceded the availability of genetic testing, and thus included patients with and without identified genetic mutations.

IEM was first linked to gain-of-function mutations of *SCN9A*/$Na_v1.7$ a decade ago and, since then, fewer than two dozen mutations have been identified in individuals or families with IEM, functionally characterized, and published, usually with brief clinical descriptions of the patients, giving only a partial picture, if any, of the clinical features and longitudinal aspects of the disease. The present paper describes the results of a systematic clinical phenotyping study of 13 subjects, each studied extensively over a 3-month period, representing four families with well-characterized clinical features of IEM and confirmed pathogenic (gain-of-function) mutations in the *SCN9A* gene.

Subjects and methods

Subject recruitment

A total of 28 patients carrying gain-of-function mutations of $Na_v1.7$, that were included in a database of genotyped and phenotyped IEM subjects maintained in the Department of Neurology at Yale University, had documented gain-of-function mutations of $Na_v1.7$ and who resided in the USA or Canada, were invited to participate in this study. All subjects had previously been referred to Yale Medical School for Institutional Review Board (IRB)-approved studies. Subjects were consented for participation in the study, in accordance with the Declaration of Helsinki 2008, with assent obtained for minors. The study was approved by an Independent Ethics Review Board. Adolescent subjects (12–18 years old) were included in the questionnaires, olfaction testing and pain diary completion. Quantitative sensory testing (QST) was optional for subjects aged 16–18 years, two of whom declined to participate. All adult subjects consented to QST. All participants had been clinically diagnosed with erythromelalgia and experienced episodes of pain in the extremities, associated with reddening and or swelling and increased heat of the skin. Family history of IEM was documented. Subjects who had other pain disorders not due to IEM, or who had severe psychiatric disorders (determined by medical history) that would limit participation in the study, were excluded.

Prior to entry in this study, all subjects had mutations in the *SCN9A* gene previously confirmed by Sanger sequencing, as part of the Yale study protocols. The mutations in the $Na_v1.7$ sodium channel had been functionally characterized and produced gain-of-function changes including hyperpolarizing shifts in activation of the channel and induction of hyperexcitability in dorsal root ganglion neurons expressing the mutant channel (Dib-Hajj *et al.*, 2010). In several subjects, genetic confirmation of a *SCN9A* gene mutation in family members had been performed.

Study design

This non-interventional (no drug treatment) clinical study was conducted in two parts; an in-clinic phase performed over 2–3 days (Part A) and a 3 month at-home phase where subjects completed a daily pain diary (Part B). Subjects were not required to discontinue pain medications or concomitant treatments for this study.

A detailed medical history including prior and current clinical conditions, use of non-pharmacological and pharmacological treatments to manage the pain of IEM and other concomitant medications was obtained at the in-clinic screening visit. Physical examination, including vital signs, was performed. Supine and standing blood pressure and pulse rate were measured. Clinical chemistry and haematology tests were not performed.

Study visits

During the in-clinic phase, subjects were administered questionnaires on the day after screening. The Erythromelalgia Questionnaire (EMQ) had three sections related to diagnosis and family history (10 questions), symptoms (33 questions) and treatments (five questions) (Supplementary Table 1). Baseline subjective psychosocial aspects were assessed using the Hospital Anxiety and Depression Scale (HADS) and the Pain Catastrophizing Questionnaire (PCS).

The HADS is a self-reported screening tool for anxiety and depression in non-psychiatric clinical populations consisting of 14 items (seven each for anxiety and depression) (Zigmond and Snaith, 1983). Responses to the HADS were based on the relative frequency of symptoms over the preceding week.

The PCS has 13 questions with categorical responses, and taps three dimensions of catastrophizing: rumination, magnification and helplessness. A total score of $\geqslant 30$ indicates a clinically relevant level of catastrophizing (Sullivan *et al.*, 1995).

DNA Sanger sequencing of the 26 coding exons of *SCN9A* was performed during this study to independently confirm each subject's previously identified *SCN9A* mutation. Olfaction was assessed in all subjects using the Sniffin'T odour threshold test (Hummel *et al.*, 2007), performed on two consecutive days. The test was performed with *n*-butanol, with a single-staircase, triple forced choice procedure, using a 1:2 dilution series beginning with 4%. Threshold was defined as the mean of the last four of seven staircase reversal points. Data were compared with normative values (Hummel *et al.*, 2007), converted to Z-scores and represented graphically.

QST was performed on two consecutive days during the in-clinic phase of the study according to the protocol of the German Research Network on Neuropathic Pain (DFNS) (Rolke *et al.*, 2006). Thirteen parameters are measured through seven tests, comprising: (i) thermal detection (cold detection threshold, CDT, warm detection threshold, WDT) and thermal pain thresholds (cold pain threshold, CPT, heat pain threshold, HPT) and the number of paradoxical heat sensations (PHS) using a TSA 2001-II thermode (MEDOC; contact area: 9 cm^2, baseline temperature 32°C, cut-offs 0°C, 50°C); (ii) the mechanical detection threshold (MDT, modified von Frey hairs, 0.25–512 mN, Optihair$_2$-Set, Marstock Nervtest); (iii) the mechanical pain threshold (MPT, weighted pinprick stimulators, 8–512 mN, MRC Systems); (iv) a stimulus/response function including mechanical pain sensitivity (MPS) for pinprick stimuli and dynamic mechanical allodynia (DMA, cotton wisp ~3 mN, Q-tip fixed to an elastic strip ~100 mN, brush ~200–400 mN); and (v) the vibration detection threshold (VDT, Rydel-Seiffer tuning fork, 64 Hz, 8/8 scale). To minimize the subject's burden in this study, the wind-up ratio and pressure pain threshold tests were omitted from the original QST protocol.

In each subject, one affected and one unaffected body site were assessed during a non-attack period. QST was performed on the unaffected area first, followed by the affected area (Supplementary Fig. 1). The affected site was the foot ($n = 7$) or hand ($n = 4$) and the face in one subject. The cheek was used as the unaffected site except in four subjects who also reported face pain. In these subjects, the area over the trapezius muscle was used as an unaffected site. The same sites in each subject were used on two consecutive days to assess variability between study days. Data were collected from 12/13 subjects as QST was optional for subjects aged 16 to 18 years and not performed in subjects <16 years of age. If the subject experienced a pain attack with a pain intensity of $\leqslant 8/10$ (where 0 = no pain and 10 = worst pain possible) on the Numerical Rating Scale (NRS) on one or both days, there was an option, with the subject's consent, to perform QST during the attack (five subjects with eight QST assessments) (Supplementary Fig. 1).

Skin temperature was measured at each test site, using an infra-red thermometer (Dermatemp ASM Model DT1000) (Exergen Corp), immediately before performing the QST battery.

The 12 week at-home evaluations (Part B of the study) consisted of a daily paper diary completed by the subjects, who were trained on diary completion during the in-clinic phase of the study. The purpose of the diary was to facilitate assessment of pain characteristics over time. It was constructed to capture the number and duration of pain attacks, location of attacks (foot/hand or other part of the body), severity of pain during attacks (using the NRS), incidence and severity of pain between attacks, likely attack triggers, concomitant pain medications, non-pharmacologic therapies and exercise regimen. The NRS was used because of its ease of use by subjects over the relatively long period (12–13 weeks) of diary-keeping. If the subject did not experience pain attacks on any given day, this was also recorded.

Statistical methods

This study was open to all IEM subjects who met study inclusion and exclusion criteria. Medical history and demographic data were recorded. Vital signs, core body and skin temperatures were reviewed and summarized descriptively.

For each subject, the daily, weekly and monthly total number of pain attacks obtained from the pain diary were tabulated and plotted longitudinally. The severity of pain during an attack, duration of pain on each attack and severity of pain between attacks was plotted longitudinally on a day-to-day basis for each subject. The location (right hand/left hand/right foot/left foot/other body location) and number of pain attacks was tabulated by week. The subject's *SCN9A* mutation type was specified on the longitudinal plots and in summary tables.

Psychosocial questionnaire (HADS, PCS) raw data scores for each subject were presented in tabular form. The HADS Anxiety and Depression scores for each subject were listed separately in addition to individuals' summed scores. Scores were summarized descriptively [mean, median, standard deviation (SD), minimum and maximum] for all subjects.

The PCS raw data scores for each subject for each parameter were listed separately in addition to summed scores of the three parameters for each subject. Subscale and total scores were additionally summarized in terms of the mean, median, SD, minimum and maximum.

Olfaction data (odour threshold) were analysed by calculating Z-scores using the equation described for QST testing above and normative data published by Hummel *et al.* (2007). The Z-score transformed data were plotted for each subject.

QST results were calculated using EQUISTA (Casquar) based on published reference data for face, foot and hand (Magerl *et al.*, 2010), and for the trunk (Pfau *et al.*, 2014). To align QST

data for age, gender and test site, raw data or logarithmically transformed raw data, depending on the particular QST parameter, were transformed into Z-scores using the equation: Z-score = $(value_{patient} - mean_{controls})/SD_{controls}$ (Rolke *et al.*, 2006; Magerl *et al.*, 2010). A Z-score of 0 corresponds to the mean of the reference group. Z-scores between −1.96 and 1.96 represent the normal range of healthy subjects (95% confidence interval); values outside this range are regarded as abnormal. Z-scores >1.96 indicate hyperaesthesia, (when considering detection thresholds) and hyperalgesia (when considering pain thresholds); Z-scores <-1.96 indicate hypoaesthesia (when considering detection thresholds) and hypoalgesia (when considering pain thresholds) compared to the reference group. PHS and DMA were not z-transformed. Z-score profiles were plotted for the whole study group and groups of patients with the same mutation (V400M, S241T, F1449V) for the affected and unaffected site, as well as for the affected site during a pain attack. Z-score profile data from both days were averaged for each site for each patient, prior to averaging across the patients; data for the second affected site were included with the affected site in this calculation. As a normal distribution within z-transformed parameters is given (Rolke *et al.*, 2006), the Z-scores of the whole group's affected and unaffected site were compared using paired *t*-tests, with two-sided 5% significance level. PHS parameter Z-score data between affected and unaffected site were compared with a two-sided Fisher's Exact test at the 5% significance level.

Results

Clinical features

From the initial group of 28 IEM patients invited to participate in the current study, 13 subjects, six males and seven females, aged 15–77 years, consented to participate and were enrolled. Seven subjects (all from one family, previously described by Dib-Hajj *et al.*, 2005) carried the F1449V mutation, three subjects from another family (previously described by Fischer *et al.*, 2009) carried the V400M mutation, two subjects from a different family carried the S241T mutation, and one subject carried both the I848T mutation and S449N single nucleotide polymorphism (SNP). Although all subjects carried the clinical diagnosis of IEM, and some provided anecdotal evidence for triggers, mental state and general quality of their pain, they had not been studied systematically by an approach similar to what we employed in our current study.

The majority of subjects (11/13) had onset of clinical signs of IEM within the first decade of life, while the remaining two subjects (S241T mutation) experienced onset of IEM within the second decade. Subjects had previously consulted a variety of medical specialists with a clinical diagnosis of IEM confirmed anywhere from the same year as onset of clinical signs to an average of ∼20 years after the onset of first episodes of pain. Co-morbidities included diabetes mellitus (three subjects), hypothyroidism (three subjects), hypertension (six subjects) and hyperlipidaemia (two subjects).

Clinical features of IEM, the most commonly identified triggers of pain attacks, and concomitant pain medications are listed in Table 1. Feet and hands were the most commonly affected parts of the body, with reddening, swelling and heat in the skin presenting as consistent clinical features. Four of 13 subjects also reported involvement of the face (facial pain), and several subjects reported pain attacks affecting other parts of the body, for example arms, knees, elbows and thighs. Eleven subjects reported intermittent pain and two subjects reported constant pain, based on self-recall. Five subjects reported that their pain had become worse over time whereas eight subjects reported that pain had either stayed the same (four subjects) or improved over time (four subjects). The majority of subjects (11/13) used non-pharmacological manoeuvres such as ice, cold air fans or immersion of affected extremities in cold water to alleviate pain; however, two subjects reported that cold, for example, going out in snow or cold weather, aggravated their pain. Subjects reported taking a variety of medications to manage their pain (Table 1). Eleven of 13 subjects had taken acetylsalicylic acid previously for pain management; five subjects stated they had no response (38.5%) and six subjects self-reported that they had some response or a good clinical response (46.2%). Two subjects carrying the V400M mutation reported that carbamazepine reduced the intensity of pain and the number of weekly pain attacks.

Pain phenotype

The average pain NRS score during pain attacks, across all subjects was 5.7, ranging from a minimum score of 1 to a maximum score of 10. For those subjects who reported pain between attacks, the average pain NRS score was 2.69 (maximum score 7.0). To better understand if there was any relationship between mutation type and pain phenotype, pain data were further analysed and are presented by mutation type/family group.

Number of pain attacks

There was considerable variation between subjects with the same mutation (either F1449V, S241T, V400M or I848T) in the number of pain attacks per week (means of 0.9 to 15.3 attacks per week) over the 12–13 week pain diary period (Table 2). Some subjects reported no pain attacks or one pain attack per week over certain weeks with other subjects experienced up to 20 pain attacks per week. Some subjects reported substantial week-to-week variation in number of attacks. Two subjects, who reported no attacks during some weeks, reported six or seven attacks during other weeks. No subject in this study had more than three pain attacks per day (Supplementary Table 2).

There was considerable variability within family groups as to the number of pain attacks experienced over the recording period. For example, Subject 1004 (S241T) experienced at least one pain attack per day with two pain

Table 1 IEM subject clinical features

Subject	Gender	Mutation	IEM age of onset	Affected body sites	Pain attack trigger	Pain intermittent (I), constant (C) better/worse with time	Pain relief by cooling	Current pain medication
1001	F	F1449V	4–5 yr	F + H + face	Heat, exercise, standing	C, worse with time	Yes	Acetaminophen + hydrocodone, morphine, Naproxen, paracetamol
1002	F	F1449V	7 yr	F + H	Heat, exercise, standing	I, better over time	Yes	Acetylsalicylic acid
1003	F	F1449V	6 yr	F + H	Heat, exercise, standing	I, better over time	Yes	Ibuprofen
1004	M	S241T	17 yr	F + H, legs, arms and face, excluding torso	Heat, exercise, standing	I, worse over time	Yes	None
1005	F	S241T	17 yr	H + F	Heat, exercise, standing	I, better over time	Yes	Ibuprofen
1006	M	F1449V	6 yr	F + H	Heat, exercise	I, stayed the same over time	Yes	None
1007	F	F1449V	< 2 yr	F + H + face	Heat, exercise, standing, cold	I, stayed the same over time	No, cold makes it worse	Ibuprofen
1008	F	F1449V	< 6 yr	H + F	Heat, exercise, standing	I, stayed the same over time	Yes	Acetaminophen, acetylsalicylic acid, pregabalin, gabapentin
1009	M	V400M	4 yr	F + H, arms, thighs	Heat, exercise, standing Cold	I, worse over time	No, cold makes it worse	Ibuprofen, acetaminophen + codeine
1010	M	V400M	< 10 yr	H + F + thighs	Heat, exercise	I, stayed the same	Yes	Carbamazepine
1011	M	V400M	18 months	F + H	Heat, exercise, standing	I, better over time	Yes	Acetaminophen, carbamazepine, ibuprofen
1012	F	F1449V	5 yr	H + F, face, knees, elbows	Heat, exercise, standing	I, worse over time	Yes	Acetaminophen + hydrocodone, tramadol, acetaminophen, pregabalin (neuropathy + IEM)
1013	M	I848T/S449N	4 yr	F + H	Heat, exercise, standing	C, worse over time	Yes	Mexiletine

Clinical features of IEM, including concomitant treatments for pain management. Data derived from the Erythromelalgia Questionnaire administered at screening and the daily pain diaries recorded by subjects over a 12–13 week at-home period. F = feet; H = hands.

Table 2 Number of pain attacks per week and duration of pain attacks (min)

Subject	Mutation	Number of pain attacks per week					Mean duration of pain attacks (min)				
		n^a	Mean	SD	Min	Max	n^b	Mean	SD	Min	Max
1001	F1449V	13	7.6	1.61	5	10	90	150.4	72.82	30	330
1002	F1449V	13	0.9	1.26	0	4	8	76.9	29.39	30	120
1003	F1449V	13	2.5	1.45	1	5	31	47.7	30.98	15	150
1004	S241T	13	11.8	2.64	5	16	83	378.3	203	60	780
1005	S241T	12	2.8	1.75	0	7	33	56.1	53.02	5	240
1006	F1449V	12	15.3	1.76	13	18	176	76.9	37.82	30	210
1007	F1449V	13	6.8	3.22	3	14	87	76.6	69.37	15	300
1008	F1449V	11	13.4	4.86	5	20	144	184.9	143.86	15	670
1009	V400M	13	1.9	1.19	0	4	20	59.8	102.99	5	480
1010	V400M	13	4.6	1.80	1	7	60	22.2	23.72	5	180
1011	V400M	13	1.8	1.83	0	6	23	41.1	27.47	10	120
1012	F1449V	13	8.9	2.29	6	13	59	228.5	94.35	60	540
1013	I848T/S449N	13	6.7	1.11	7	3	87	171.9	64.20	160	995

[a]Number of weeks in which data were recorded.
[b]Number of pain attacks reported per subject over the 12–13 week data collection period.
Min = minimum; Max = maximum.

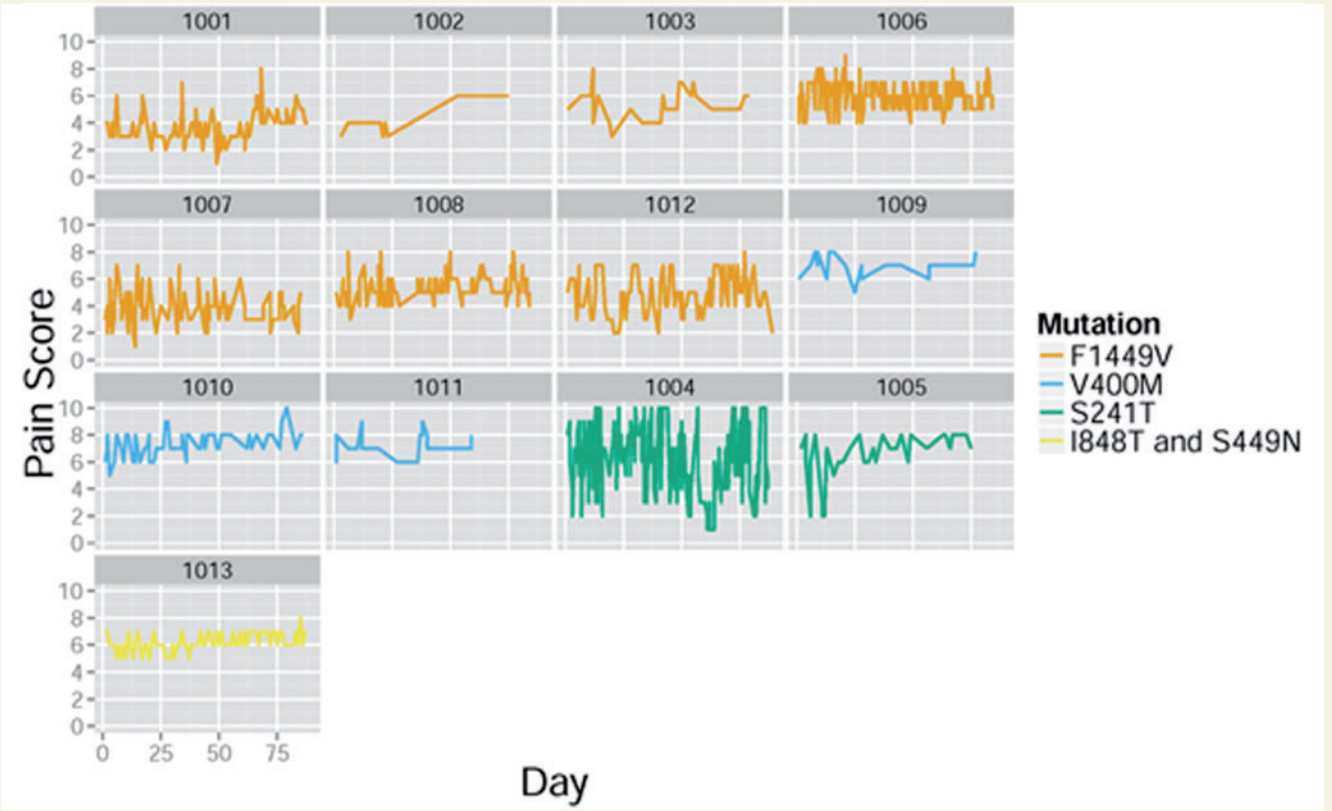

Figure 1 Pain severity during pain attacks. Pain attack severity data derived from subjects' daily diary recorded over a 12–13 week period at home. Subjects rated their pain on an 11-point NRS where 0 = no pain and 10 = worst pain possible.

attacks per day occurring on 47 (54%) of the 87 days over which they recorded pain attacks, whereas a first-degree relative (Subject 1005, S241T) experienced no pain attacks on the majority of days (47 days; 60%) during their 78 day recording period.

Duration of pain attacks

Pain attacks were variable in duration, both within subjects with the same mutation and across all subjects (Table 2). Over the 12–13 week diary period, subjects experienced pain attacks lasting from 5 min up to 17 h (995 min) per attack. There was considerable within-subject variation with regard to duration of pain attacks, for example, Subject 1009 (V400M) reported pain attacks that lasted a minimum of 5 min up to a maximum of 480 min with a mean of 60 min (SD 103 min). Within the same family, different subjects demonstrated variability in attack duration, for example, Subject 1004 (S241T) had pain attacks lasting a minimum of 60 min to a maximum of 780 min, whereas Subject 1005 (S241T) had much shorter duration pain attacks (minimum 5 min, maximum 240 min).

Pain severity during attacks

Nine of 13 subjects recorded an average pain severity score of 5 on the NRS (0–10). Pain severity during attacks ranged from a minimum score of 1 to a maximum of 10 for some subjects (Supplementary Table 3). Subjects described their pain using descriptors such as 'burning', 'like being on fire', 'constant pain, like frostbite that is warming up', 'blowtorch-like', 'hot', 'throbbing', 'aching', 'tingling', or 'pins and needles'. Pain severity reports over the 12–13 week data collection period at home demonstrated attack-to-attack variation in pain severity for each subject within each family group (Fig. 1).

Ongoing pain between attacks

Eleven of 13 subjects reported ongoing pain between attacks, with 8 of 11 subjects having a mean pain score of > 2 on the NRS (Supplementary Table 3 and Fig. 2). The mean pain score between attacks was further investigated by assessing whether the subject had zero, one or more pain attacks per day (maximum number of attacks experienced by any subject was three per day) to determine if those subjects who had more frequent pain attacks experienced worse pain between attacks. There was no consistent relationship between the number of pain attacks per day and severity of pain between attacks for each subject (Supplementary Fig. 2). With regard to severity of pain during and between attacks, the majority of subjects (11 of 13 who reported ongoing pain) had a 3–4 point

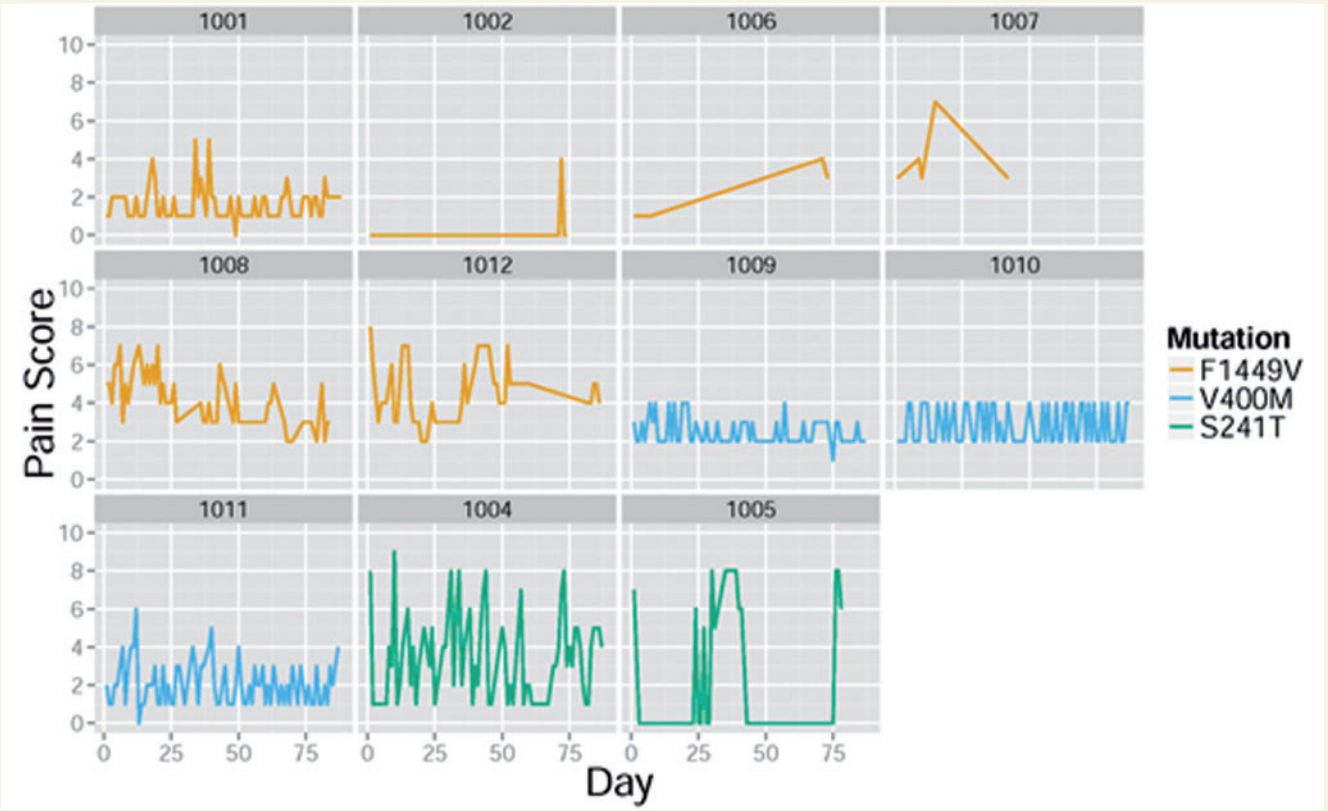

Figure 2 Pain severity between pain attacks (ongoing pain). Pain between attack data derived from subjects' daily diary recorded at home over a 12–13 week period. Eleven subjects self-reported ongoing pain between attacks. Two subjects (Subjects 1003 and 1013) did not report ongoing pain between attacks. Pain scores were recorded using an 11-point pain NRS where 0 = no pain and 10 = worst pain possible.

difference on the NRS, that is, pain during attacks was more severe by 3–4 points than pain between attacks. Two subjects (Subjects 1007 and 1012) did not differentiate pain severity during attacks from pain severity between attacks.

Pain attack triggers

Over the 12–13 week diary period, subjects recorded pain attack triggers. If there was no identifiable pain attack trigger, they were asked to record that as a specific category. Exercise and heat were the most common triggers for pain attacks, followed by hot humid weather, physical activities such as chores and wearing clothing (usually closed toe shoes or socks). Cold triggered 17 attacks (out of a total of 1099 attacks; Table 3) in two subjects. Of the 1099 pain attacks recorded, 32% ($n = 355$) had no identifiable trigger. The more common triggers (exercise, heat) tended to be associated with attacks of relatively brief duration (mean 1.2 h, 2.8 h, respectively) (Table 3).

Psychometric tests

Across the group of 13 subjects, based on results of HADS, mean scores for anxiety were 8.2 (range 3–13; SD 3.56) and for depression 4.5 (range 0–11; SD 3.99), respectively. Scores recorded from individual subjects indicated that two subjects had mild anxiety and five subjects had moderate anxiety (Table 4). Two subjects had mild depression and another two subjects had moderate depression based on scores. Five subjects recorded scores below the threshold for mild depression and anxiety. For those subjects who had ongoing pain between attacks ($n = 11/13$), there was a significant correlation between pain severity between attacks and total scores for HADS ($P = 0.005$) and between pain scores between attacks and HADS depression scores ($P = 0.001$) (Supplementary Fig. 3A and B).

Three subjects had summed PCS scores of > 30 (Table 4). Across the group of 13 subjects, the mean score for helplessness was 8.9 (SD 5.35); for magnification, mean score was 3.2 (SD 1.59), and for rumination, the mean score was 10.3 (SD 3.84).

Olfaction testing

Olfaction threshold testing scores were plotted as Z-score profiles. There was no evidence of hyperosmia in subjects in this study. The majority of subjects displayed olfactory scores within the reference ranges proposed by Hummel *et al.* (2007).

Orthostatic hypotension

In 12 of 13 patients, there was no evidence of orthostatic hypotension (defined as a decrease of $\geqslant 20$ mmHg for systolic blood pressure or $\geqslant 10$ mmHg for diastolic blood pressure 2 min after standing from a supine position). One subject, (Subject 1012, female), had a decrease in diastolic blood pressure of 10 mmHg (supine to standing) and a decrease in systolic blood pressure of 22 mmHg from supine to standing position.

Table 3 Pain attack triggers and duration of pain attacks (h) overall for all subjects, stratified by attack triggers

Trigger[a]	*n*	Mean	SD	Median	Min	Max
Exercise	174	1.2	1.20	0.8	0	7
Humidity	45	6.5	6.81	3.8	0	24
Heat	167	2.8	3.99	1.0	0	24
Cold	17	4.9	6.47	2.0	0	24
Weather	64	5.3	5.94	3.2	0	24
Routine/household tasks	96	2.5	2.94	1.7	0	24
Clothes	81	5.6	4.12	8.0	0	13
Virus	31	5.7	7.37	2.8	1	24
Stress	17	3.3	2.28	2.8	1	9
Lack of sleep	4	8.6	10.36	4.5	2	24
Hormonal (females only)	9	4.6	4.35	2.0	1	12
Missing	32	3.1	5.60	1.5	0	24
Other conditions	7	7.5	7.13	4.0	1	17
No trigger	355	5.9	6.03	3.0	0	24

[a]Data derived from subjects' daily diary. *n* = number of pain attacks recorded. There was a category in the daily diary for no identifiable pain attack trigger for the subject to complete. 'Clothes' refers to wearing of clothes that warm the body and closed toe shoes.

QST and skin temperature results

The QST profiles of the affected and unaffected site differed significantly for a number of parameters (Fig. 3). Regarding the thermal detection thresholds, at the affected site, there were significantly lower Z-scores for cold and warm detection thresholds (CDT, WDT, $P < 0.01$, respectively) compared to the unaffected site, suggesting reduced small fibre function in this area. On an individual level, of the 12 subjects with QST data, six (50%) subjects had averaged Z-scores < -1.96 for the CDT and five subjects (42%) had averaged Z-scores of < -1.96 for the WDT at the affected site. There was only one subject with Z-scores of < -1.96 for both CDT and WDT at the unaffected site. Regarding perception of touch, the Z-score for the mechanical detection threshold (MDT) was lower at the unaffected site (though not statistically significant), suggesting reduced A-beta fibre function. Six subjects had abnormal averaged Z-scores < -1.96 for the MDT at the unaffected site, but only one subject had an abnormal MDT at the affected site. With respect to thermal pain thresholds, the Z-scores for cold and heat pain threshold (CPT, HPT) were significantly higher at the unaffected site (CPT: $P < 0.05$, HPT: $P < 0.01$) compared to the affected site, that is, there was hyperalgesia to cold and heat stimuli on the unaffected skin area. On an individual level, one subject had a Z-score value >1.96 for the CPT at the unaffected site, whereas five subjects (42%) had a Z-value >1.96 for the HPT at the unaffected site. Two subjects also had Z-values >1.96 for HPT on the affected site, one of whom had Z-values >1.96 at both affected and unaffected sites. There were no differences between the affected and unaffected site regarding vibration detection threshold (VDT), mechanical pain threshold (MPT), mechanical pain sensitivity (MPS), the magnitude of dynamic mechanical allodynia (DMA) and the number of paradoxical heat sensations (PHS).

Table 4 HADS and PCS scores listed by subject

Subject	Mutation	HADS scores		PCS – all subjects			
		Anxiety Score HADS-A	Depression Score HADS-D	Helplessness	Magnification	Rumination	Total Score[a]
1001	F1449V	5	1	4	1	4	9
1002	F1449V	7	0	3	2	9	14
1003	F1449V	12	4	8	5	12	25
1004	S241T	12	8	8	4	13	25
1005	S241T	8	1	3	1	5	9
1006	F1449V	5	5	9	1	12	22
1007	F1449V	12	6	21	4	14	39
1008	F1449V	11	11	12	4	12	28
1009	V400M	3	0	8	4	9	21
1010	V400M	4	1	7	4	11	22
1011	V400M	9	3	15	6	15	36
1012	F1449V	13	11	14	3	14	31
1013	I848T/S449N	5	8	4	3	4	11

[a]Total (summed) PCS scores for helplessness, rumination and magnification for each subject.
For each subject, HADS scores for Anxiety and Depression are listed separately.

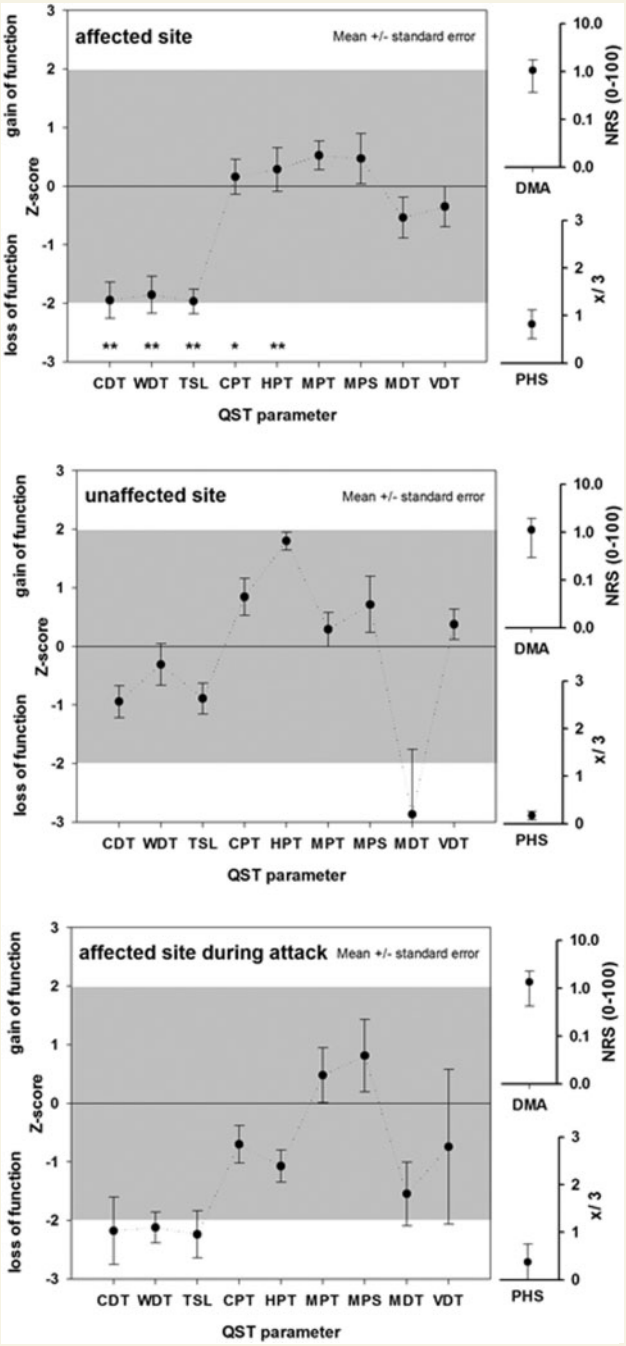

Figure 3 QST, Z-score profiles. Sensory profiles of the affected and unaffected site (n = 12 subjects who were eligible and consented to QST, tested on two separate occasions and individually averaged before group comparison) and for the affected site during a pain attack (n = 5 subjects, two with single QST assessment, three tested on two separate occasions; these data were individually averaged before group comparison). All values are normalized for age, gender and testing site on a Z-scale. Z-scores between −1.96 and 1.96 represent the normal range of healthy subjects (grey area). Z-scores > 0 indicate a gain of sensory function; i.e. the subject detects the stimulus at a lesser stimulus intensity than healthy controls in case of detection thresholds (hyperaesthesia) or the subject feels a painful sensation at a lesser stimulus intensity than healthy controls in case of pain thresholds (hyperalgesia). By contrast, Z-scores < 0 indicate a loss of sensory function; i.e. the subject detects the stimulus at a greater stimulus intensity than healthy controls in case of detection thresholds (hypoaesthesia) or the subjects feels a painful sensation at a greater stimulus intensity than healthy controls in case of pain thresholds (hypoalgesia). $^{*}P < 0.05$, $^{**}P < 0.01$ paired t-test, affected versus unaffected site. CDT = cold detection threshold; CPT = cold pain threshold; DMA = dynamical mechanical allodynia; HPT = heat pain threshold; MDT = mechanical detection threshold; MPT = mechanical pain threshold; MPS = mechanical pain sensitivity; PHS = paradoxical heat sensations; TSL = thermal sensory limen; VDT = vibration detection threshold; WDT = warm detection threshold.

During a pain attack, Z-scores for the CPT and HPT as well as for the MDT were lower compared to the QST profile of the affected site in-between attacks. No subject experienced a pain attack as a consequence of QST testing for heat pain threshold (HPT).

Comparing the QST profiles of patients with specific mutations among each other (Fig. 4), MDT displayed the greatest difference. Subjects with F1449V mutations showed numerically lower Z-scores with larger variability at the unaffected site than subjects with V400M and S241T mutations. Subjects with S241T mutations showed higher Z-scores for the HPT, that is, heat hyperalgesia at the affected and unaffected sites, in contrast to subjects with F1449V and V400M mutations who only showed heat hyperalgesia at the unaffected site. All other parameters did not exhibit any notable differences between the different mutation types.

Skin temperatures were recorded in six subjects just prior to performing QST on both days and ranged from 28.6°C to 36.6°C, with a mean temperature of 32°C (reference range 32°C– 35°C; Freitas 1999).

Discussion

Although 19 IEM and functionally characterized mutations of $Na_v1.7$ have been described in the literature (Dib-Hajj *et al.*, 2013), there has been no systematic study of the pattern of pain, natural history or the effect of IEM associated with $Na_v1.7$ mutations on psychosocial status or olfaction. In this study, we undertook detailed clinical phenotyping of 13 subjects with IEM, each studied over a 12–13 week period. All *SCN9A* mutations in these subjects had been previously demonstrated on functional testing *in vitro* to be pathogenic (Cummins *et al.*, 2004; Dib-Hajj *et al.*, 2005; Lampert *et al.*, 2006; Fischer *et al.*, 2009; Yang *et al.*, 2012).

The majority (11/13) of subjects in this study, all carrying gain-of-function mutations of $Na_v1.7$, had early onset of pain symptoms due to IEM, starting in the first decade of life, apart from two subjects who first experienced pain attacks in the second decade. This contrasts with later ages of onset ranging from 30 to 50 years in patients with clinical diagnosis of erythromelalgia and no mutation

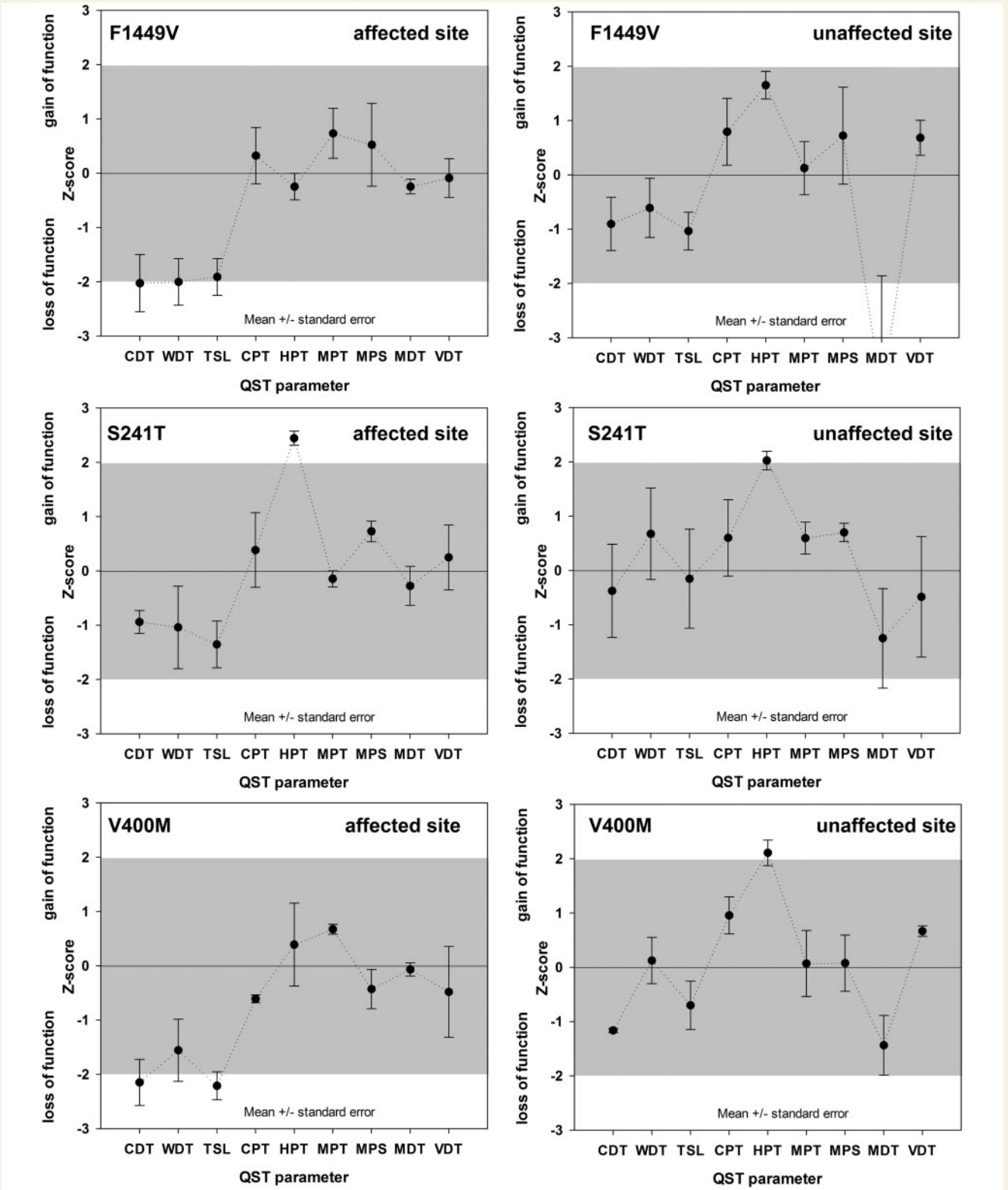

Figure 4 QST, Z-score profiles by mutation type. Sensory profiles of the affected and unaffected site for different mutations (F1449, $n = 7$ subjects; S241T and V400M, $n = 2$ subjects, respectively). All subjects were tested on two separate occasions and individually averaged. Z-scores between −1.96 and 1.96 represent the normal range of healthy subjects (grey area). All values are normalized for age, gender and testing site on a Z-scale. Z-scores > 0 indicate a gain of sensory function; i.e. the subject detects the stimulus at a lesser stimulus intensity than healthy controls in case of detection thresholds (hyperaesthesia) or the subject feels a painful sensation at a lesser stimulus intensity than healthy controls in case of pain thresholds (hyperalgesia). By contrast, Z-scores < 0 indicate a loss of sensory function; i.e. the subject detects the stimulus at a greater stimulus intensity than healthy controls in case of detection thresholds (hypoaesthesia) or the subjects feels a painful sensation at a greater

(continued)

in the open reading frame of *SCN9A*, reported by Namer *et al.* (2015) and the later age of onset (56 years) in the patient with the I228M $Na_v1.7$ mutation carrying a diagnosis of IEM and small fibre neuropathy (Namer *et al.*, 2015). It has been reported that pain in IEM may become more severe with increasing age (Finley *et al.*, 1992). In the current study, eight out of 13 subjects self-reported that pain had either stayed the same or diminished with increasing age. The majority of subjects experienced at least one pain attack per day, with seven subjects recording up to three pain attacks per day. Pain during attacks tended to be moderate to severe (pain score of at least five on the NRS) for the majority of subjects. It has been reported that pain symptoms may vary between family members (Finley *et al.*, 1992; Michiels *et al.*, 2005). In the current study, over a period of 12–13 weeks, the number, frequency, duration and severity of pain attacks was markedly variable, both between and within subjects in a family, supporting this observation on more limited numbers of subjects by previous authors.

The majority of subjects in our study reported minimal benefit from existing pain medications. Two subjects carrying the V400M mutation had obtained a reasonable degree of pain control and a reduction in the number of weekly pain attacks with carbamazepine (Fischer *et al.*, 2009) although, based on the data from this study, they still experienced an average of two to five pain attacks per week, with moderate to severe pain (scores ranging from 5 to 10 on the NRS). The majority of subjects gained relief from non-pharmacological manoeuvres such as cooling the affected parts of the body in cold water, using ice, or with cool air fans. These situational remedies, however, imposed limited mobility on these subjects and restrictions on normal daily activities.

Pain attacks in erythromelalgia have been reported to be triggered by heat, clothing, exercise and humidity (Finley *et al.*, 1992; Michiels *et al.*, 2005; Drenth and Waxman, 2007). Our subjects reported similar triggers for pain attacks in this study; however, ~32% of pain attacks in the present group of subjects occurred in the absence of a specific trigger. It is noteworthy that no subject experienced a pain attack as a consequence of QST testing for HPT. It is possible that pain attacks were not evoked during this thermal testing because the stimulus is applied to a relatively small area of the skin, a general limitation for QST. Pain attacks in IEM have also been reported to be episodic. It is noteworthy that in the current study, 11 of 13 subjects experienced ongoing pain between attacks, with this ongoing pain being generally less severe than that experienced during pain attacks (Supplementary Table 3 and Figs 1 and 2). There appeared to be no relationship between the number of pain attacks experienced per day by a subject and the severity of ongoing pain between attacks (Supplementary Fig. 2).

In contrast to patients with small-fibre neuropathy associated with $Na_v1.7$ mutations who display a spectrum of autonomic abnormalities (Faber *et al.*, 2012), patients with IEM tend to display redness of the skin during attacks in the absence of other autonomic symptoms (Drenth and Waxman, 2007). It has been demonstrated by Rush *et al.* (2006) that a single IEM mutation in $Na_v1.7$ can produce hyperexcitability in sensory neurons and hypoexcitability in sympathetic ganglion neurons, providing a molecular basis for the sympathetic dysfunction underlying the abnormal reddening of the skin during attacks that has been observed in erythromelalgia. In the current study, we assessed all subjects for evidence of orthostatic hypotension. One subject out of 13 studied, had blood pressure measurements consistent with orthostatic hypotension. While this may be a rare phenomenon in IEM subjects, our findings suggest the need to screen for orthostatic hypotension as patients with IEM are studied.

$Na_v1.7$ plays a crucial role in odour perception and is present in axons of human olfactory sensory neurons (Weiss *et al.*, 2011). Anosmia or hyposmia has been frequently reported in patients with congenital insensitivity to pain due to mutations in *SCN9A* (Goldberg *et al.*, 2007). In the present study it was hypothesized that patients with IEM associated with gain-of-function mutations in the *SCN9A* gene might have hyperosmia. Heimann *et al.* (2013) recently assessed linkage between two SNPs in *SCN9A* [one of which had been previously associated with enhanced excitability of dorsal root ganglion neurons and higher pain scores (Estacion *et al.*, 2009; Reimann *et al.*, 2010) and olfaction]. They reported a wild-type *SCN9A* haplotype composed of rs41268673C/rs6746030C alleles (found in a sample of random Caucasian subjects at an allelic frequency of 82.5% in their study) linked with comparatively reduced olfactory acuity as well as pain perception. Although none of their subjects reported any subjective perception of smell sensitivity differing from normal status, an increasing number of non-mutated haplotype alleles was associated with a higher olfactory threshold, i.e. with the detection limit of volatile phenylethylethanol at lower dilutions, a result they interpret as suggesting an association of the minor alleles with enhanced olfactory function. Following administration of the Sniffin'T test to subjects with IEM and gain-of-function

Figure 4 Continued

stimulus intensity than healthy controls in case of pain thresholds (hypoalgesia). Due to the small sample size within the subgroups, no statistical comparisons were carried out between affected and unaffected sites. CDT = cold detection threshold; CPT = cold pain threshold; DMA = dynamical mechanical allodynia; HPT = heat pain threshold; MDT = mechanical detection threshold; MPT = mechanical pain threshold; MPS = mechanical pain sensitivity; PHS = paradoxical heat sensations; TSL = thermal sensory limen; VDT = vibration detection threshold; WDT = warm detection threshold.

mutations of $Na_v1.7$ and comparison of subjects' results with normative values (Hummel *et al.*, 2007), we did not find evidence of hyperosmia in this study. Whether the different results are the result of different study designs, different populations of subjects, the presence of hyposmia in three of the 25 subjects with wild-type alleles at both loci in the Heimann *et al.* (2013) study, or differences in the effects of different $Na_v1.7$ variants on olfactory function, is not known.

In the study of Davis *et al.* (2000) a diagnosis of erythromelalgia was associated with significant physical and mental health issues with a decrease in survival versus age and sex-matched controls, and three patients had committed suicide with erythromelalgia listed as a secondary cause of death. In this study, we evaluated the psychosocial status of subjects living with IEM using the HADS and PCS scales. The HADS is a widely used and validated measure of a subject's anxiety and/or depression and has been used in both psychiatric and non-psychiatric populations (Bjelland *et al.*, 2002) and hospital and community settings. Despite their history of chronic pain, five of the 13 subjects in this study had scores below the threshold for mild anxiety or depression. Two subjects had both moderate anxiety and depression and the remaining subjects had either mild or moderate anxiety or depression. No subject was severely anxious and/or depressed. There was a significant correlation between total HADS scores and pain severity between attacks ($P = 0.005$) and between HADS depression scores and pain between attacks ($P = 0.001$). There was no notable correlation between pain during attacks for either total HADS scores or Anxiety and Depression scores. Subjects in the current population who experienced ongoing pain between attacks were more likely to be depressed, based on HADS scores. This conclusion is consistent with the previous findings that, in general, frequent pain episodes and pain that is refractory to treatment are associated with more depressive symptoms or severe depression (Bair *et al.*, 2003).

Individuals who score high on measures of pain catastrophizing (PCS) tend to report more intense pain and disability due to their pain (Sullivan *et al.*, 1995, 2005) and more severe depression and anxiety (Keefe *et al.*, 1989; Martin *et al.*, 1996). Only 3 of 13 subjects with IEM in this study had clinically significant levels of pain catastrophizing. There was no correlation between scores for each parameter and the severity of pain experienced by IEM subjects in the current study. Catastrophic thinking may be a significant determinant of pain experience associated with the affective component of neuropathic pain (Jensen *et al.*, 2002; Sullivan *et al.*, 2005). Evidence from previous studies (Vienneau *et al.*, 1999) suggested that the helplessness scale of the PCS was the strongest predictor in patients experiencing chronic pain for more than 4 years. The conclusion was that pain catastrophizing contributed to the prediction of functional disability in patients with neuropathic pain (Sullivan *et al.*, 2005). In the current study, subjects had, on average, highest scores for rumination, followed by helplessness and magnification. It is interesting that, in a previous study on musculoskeletal pain, the rumination scale of the PCS showed the strongest association with pain experience (Sullivan *et al.*, 1998).

A number of studies have been interpreted as suggesting the existence of small fibre neuropathy (SFN) in at least some patients with erythromelalgia (Davis *et al.*, 2003; Oaklander and Klein, 2013; Bennett and Woods, 2014; Themistocleous *et al.*, 2014). The present study used QST to evaluate both small and large nerve fibre function. QST can additionally reflect signs of sensory gain such as allodynia or thermal and mechanical hyperalgesia (Rolke *et al.*, 2006; Backonja *et al.*, 2013). The positive predictive value of QST for reduced intra-epidermal nerve fibre density in skin biopsies (a gold standard for diagnosis of SFN) has been reported to be high (Scherens *et al.*, 2009). Previous studies investigating sensory changes of affected skin areas in patients with erythromelalgia reported loss of cold and warm detection as well as heat hyper- or hypoalgesia (Orstavik *et al.*, 2004; Genebriera *et al.*, 2012). Additionally, a reduction of intra-epidermal nerve fibre density was reported in skin biopsies of patients with erythromelalgia (Davis *et al.*, 2006) but that study did not differentiate between patients with and without $Na_v1.7$ mutations. Although we did not assess the density of intra-epidermal nerve fibres as a criterion for SFN, in the group analysis of the current study sample, the detection thresholds for cold and warmth (CDT, WDT) were significantly elevated in the affected skin, suggesting a reduced number, or abnormal function of both Aδ- and C-fibres. Single subject evaluations revealed increased thermal detection thresholds, suggesting SFN in more than half of all subjects. Signs of SFN were not detected in unaffected skin areas. However, a more conclusive diagnosis awaits a study on skin biopsy in individuals with IEM.

Only 17% of subjects in this study showed heat hyperalgesia on the affected site, but 42% of subjects had heat hyperalgesia on unaffected skin areas. Whilst it has been clearly shown that the $Na_v1.7$ mutations in the subjects assessed in this study produce shifts in voltage-dependence of activation of $Na_v1.7$ and subsequent hyperexcitability of dorsal root ganglion neurons (Cummins *et al.*, 2004; Dib-Hajj *et al.*, 2005; Lampert *et al.*, 2006; Fischer *et al.*, 2009; Han *et al.*, 2009), it remains unclear why heat hyperalgesia is pronounced in otherwise asymptomatic areas. No less unexpected was the finding that 50% of the patients displayed tactile hypoaesthesia in unaffected skin areas. The extent of loss of the perception of touch is pronounced for the F1449V mutation (Fig. 4). Somatosensory function has previously been linked to TRPV-polymorphisms (Binder *et al.*, 2011); we suggest that there may be a similar association for $Na_v1.7$. It is also known that pain itself can be associated with tactile hypoaesthesia (Geber *et al.*, 2008), which might explain the increased MDT in subjects in the current study during a pain attack.

Additionally, the tactile hypoaesthesia in unaffected skin areas might partly be explained by the choice of the unaffected area for QST assessment; small sensory changes,

especially in the face, can cause large variations in Z-scores (Magerl *et al.*, 2010). However, a pathological or molecular explanation for the large differences between asymptomatic and symptomatic areas remains a possibility that merits further study. QST, according to the DFNS protocol, includes an extensive battery of both thermal and mechanical pain and detection threshold tests but is restricted to a relatively small area of skin and limited time for execution, thus effects of temporal and spatial summation cannot be tested. This may lead to an underestimation of the effects (Krumova *et al.*, 2012).

This study presents a detailed genetic and clinical evaluation of the phenotype of subjects with IEM. Our results demonstrate that pain is reliably evoked in patients with IEM with heat and/or exercise, and show substantial variability of pain both between and within subjects with IEM, even those who carry the same mutation within a family. Despite concerns that hyperactivity of the $Na_v1.7$ channel could lead to hyperosmia, there was no evidence of this in the study population. The QST results suggest that, for the specific mutations studied, SFN may occur together with IEM. Our findings provide a picture of the clinical syndrome associated with IEM and $Na_v1.7$ gain-of-function mutations, and underscore the importance of characterizing the pain phenotype in any given subject before undertaking clinical trials for new pain medications.

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Conflict of interest

A.M., Z.A. and S.T. are employees of Pfizer Inc. F.B. and S.C. are employees of Quanticate International, contracted by Pfizer Inc to provide statistical input into the study and manuscript. S.W., S.D-H. and B.S. are employed by Yale University and were engaged by Pfizer Inc to serve as sub-investigators on the study. T.M. has received speaker fees from Astellas Pharma GmbH, Grünenthal and Pfizer, a travel grant from Astellas Pharma GmbH, an award for scientific achievements sponsored by Grünenthal and a prize for rhetorical performance sponsored by Mundipharma.

Supplementary material

Supplementary material is available at *Brain* online.

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