Genetic and epidemiological aspect of Complex Regional Pain Syndrome

Rooij, A.M. de

7

No mutations found in the voltage-gated $Na_V1.7$ sodium channel α1 subunit gene *SCN9A* in familial Complex Regional Pain Syndrome

Annetje M. de Rooij [*a]

Florencia M. Gosso [*b]

Elisenda Alsina-Sanchis [b]

Johan Marinus [a]

Jacobus J. van Hilten [a]

Arn M.J.M. van den Maagdenberg [a,b]

* Both authors contributed equally

[a] Department of Neurology, Leiden University Medical Center, Leiden, The Netherlands

[b] Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands.

European Journal of Neurology 2010; Jan 12 Epub.

Abstract

Gain-of-function mutations in the voltage-gated $Na_V1.7$ Na^+ channel $\alpha 1$ gene *SCN9A* have been linked to two different familial painful disorders: inherited primary erythromelalgia (PE) and paroxysmal extreme pain disorder (PEPD). These two disorders show clinical overlap with Complex Regional Pain Syndrome (CRPS), a condition characterized by pain in association with different combinations of vasomotor, sudomotor, sensory, and motor disturbances. Here we investigate the involvement of the *SCN9A* gene in familial CRPS. We performed a mutation analysis of the gene in DNA from index cases from four CRPS families. All 26 coding exons and adjacent sequences of the *SCN9A* gene were analyzed for mutations using direct sequencing analysis. No causal gene mutations were identified in the *SCN9A* gene in any of the patients. We did not find any evidence that the *SCN9A* gene plays a major role in CRPS. It cannot be excluded, however, that the *SCN9A* gene plays a role in sporadic CRPS, or perhaps even a minor role in familial CRPS.

Introduction

Complex Regional Pain Syndrome (CRPS) is a painful disorder that usually is inflicted by a trauma to an extremity like a fracture or operation. A spontaneous onset has been reported in about 10% of cases.[1] CRPS is characterized by burning pain and sensory disturbances like allodynia and hyperalgesia. Apart from pain, the affected extremities may show: (i) discolorations, temperature changes and edema (vasomotor disturbances); (ii) alterations in perspiration (sudomotor disturbances); and/or (iii) loss of muscle strength and movement disorders like dystonia (motor disturbances).[2,3]

The etiology of CRPS is incompletely understood. Aberrant inflammation[4,5] as well as neuronal plasticity involving both peripheral and central pain pathways are believed to play a role.[6,7] There are several indications that CRPS has a genetic component. First, while CRPS is usually sporadic, families with CRPS have been reported.[8,9,10,11,12,13,14] Second, CRPS patients with a more severe phenotype, for instance those with multiple affected extremities or who exhibit CRPS in combination with fixed dystonia, have a younger age at onset compared to patients in whom the disease remits or stabilizes.[15,16] Third, a recent study calculating the sibling risk showed an increased risk to develop CRPS for siblings younger than 50 years.[17] Despite the fact that several genetic association studies – mainly examining the involvement of the HLA gene cluster on chromosome 6 – have suggested that genetic factors indeed play a role in CRPS,[18,19,20,21,22] the genetic component of CRPS has not been well investigated.

Here we want to investigate the involvement of the *SCN9A* gene in familial CRPS. Mutations in the *SCN9A* gene that encodes the α1 subunit of voltage-gated $Na_V1.7$ Na^+ channels have been linked to three disorders in which, similar to CRPS, altered pain sensitivity and vasomotor changes are prominent features.[23,24,25] Primary erythromelalgia (PE) (MIM 133020) is associated with several missense mutations, causing an enhanced activation of $Na_V1.7$ Na^+ channel functioning.[26,27] PE is characterized by attacks of symmetrical burning pain, as well as red discoloration, edema and increased temperature of the skin of certain extremities. Attacks are provoked by exercise, prolonged standing and exposure to warmth. In paroxysmal extreme pain disorder (PEPD) (MIM 167400),[28,29] missense mutations in *SCN9A* lead to impaired inactivation of $Na_V1.7$ Na^+ channels.[30] PEPD, formerly known as familial rectal pain syndrome, is primarily characterized by attacks of burning pain in the rectal, ocular and mandibular areas which can spread over the whole body. The pain is

accompanied by autonomic symptoms such as color changes and edema of the skin.[31,32] Finally, in channelopathy-associated insensitivity to pain (CIP) (MIM 243000) that is due to truncating, inactivating *SCN9A* mutations[33] patients are insensitive to pain stimuli but this is not accompanied by prominent vasomotor symptoms. Whereas in PE and PEPD, increased activation of $Na_V1.7$ Na^+ channels in primary sensory neurons of dorsal root and trigeminal ganglia as well as in certain sympathetic ganglion neurons increases the sensitivity of nociceptors to painful stimuli,[34] in CIP, on the contrary, a decreased activation of $Na_V1.7$ Na^+ channels results in insensitivity to painful stimuli.

We hypothesized that the clinical manifestations spontaneous pain, allodynia and hyperalgesia that are commonly observed in CRPS patients may be the result of an *SCN9A* mutation. Allodynia – where a normally innocuous stimulus is perceived as painfull – can result from either an enhanced activity of spinal nociceptive pathways (central sensitization) or lowering of pain receptor activation thresholds of nociceptors on $Na_V1.7$-expressing sensory neurons when exposed to products of tissue damage and inflammation (peripheral sensitization) [35]. Hyperalgesia – an increased sensitivity to pain –, on the other hand, is thought to be due to sensitization of nociceptors.[36]

Because of an overlap in clinical symptoms in CRPS, PEPD and PE, and the potential role $Na_V1.7$ Na^+ channels may play in determining increased sensitivity to pain in CRPS, we investigated the role of *SCN9A* in familial CRPS. To increase the chance of identifying a causal mutation, we scanned four CRPS patients in whom CRPS also occurred in multiple family members.

Patients and Methods

Patients

The index cases of four CRPS families were included in the mutation analysis. Clinical characteristics of the probands and affected family members are given in Table 1. The clinical information of families 1-3 was published before,[37] but is here included to allow for optimal comparison with clinical information of family 4. All patients met the IASP criteria for CRPS.[2]

The study was approved by the Medical Ethical Committee of the Leiden University Medical Center. All patients gave written informed consent before participation.

Details of the index case and family members of newly collected family 4

The proband (III-10) of newly collected family 4 developed CRPS at the age of 47 years after a sprain of her right ankle. Soon after the injury, she developed symptoms in her left arm after an intravenous administration of mannitol. Her sister (III-15) developed CRPS in her left arm after a complete dislocation of her elbow when she was 54 years old. The cousin (III-8) developed CRPS after a wound of his right hand when he was 57 years old. The niece of the proband (IV-19) developed CRPS in her left arm after an accident at the age of 19. Nine years later she developed CRPS in her left leg after a contusion (Figure 1).

Figure 1: Pedigree of CRPS family 4

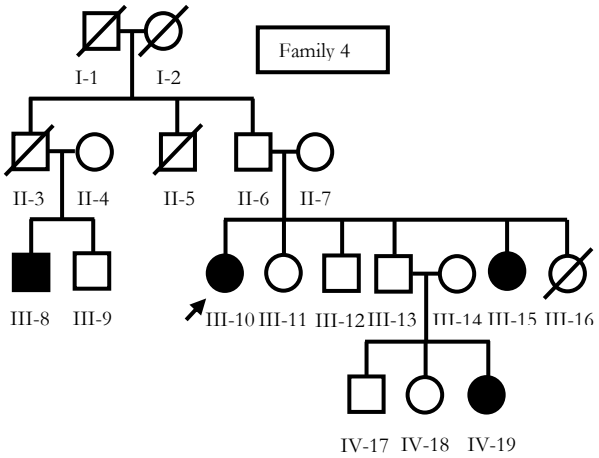

Figure represents the pedigree of CRPS family 4. Circles indicate females; squares indicate males; crossed-out symbols: deceased individuals. Black symbols indicate individuals with CRPS. The arrow indicates the proband of the family.

Mutation analysis of the SCN9A gene

Genomic DNA was isolated from peripheral blood cells according to a salting-out method.[38] All 26 coding exons and adjacent sequences of the *SCN9A* gene were analyzed for mutations using direct sequencing analysis. Exons were amplified by PCR using exon-specific primers sets (Table 2). Reactions were performed in a 25 µL reaction volume, containing 10 pmol of each primer, 1xPCR buffer (3 mM Tris-HCl,

75 mM NH_4SO_4, 7.5 mM $MgCl_2$ with a pH of 8.5) (Invitrogen, Breda, The Netherlands), 3 mM dNTPs, 0.25 U AmpliTaq DNA polymerase (Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands), and 50 ng of genomic DNA. PCR conditions were performed as follows: 3 min at 94◦C, followed by 33 cycles of 30 sec at 94◦C, 30 sec at 60◦C, and 1 min at 72◦C, and an additional extension step of 10 min at 72◦. Unincorporated dNTPs and primers were removed by incubation at 37°C for 2 hours with shrimp alkaline phosphatase (SAP) (USB Corporation, Cleveland, Ohio, USA) and exonuclease (ExoI) (USB Corporation, Cleveland, Ohio, USA), followed by a deactivation step of 95°C for 15 min. For dideoxy sequencing, purified PCR product (15 to 25 ng) was used with 6 pmol forward or reverse primer in a final volume of 12 µl. Sequencing reactions were run on an automated sequencer (ABI3730, Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands). Sequence analysis was performed using ContigExpress software (a component of vector NTI Suite V9.0.0; Invitrogen, Leek, The Netherlands).

Table 2. Primer sequences for the SCN9A gene

Exons	**Strand**	**Primer sequence**
SCN9A_ex1	F	5'-GAACATGTTACCTTTTGTAGTTGAAA-3'
	R	5'-GCCAACAGAAACTGACCACTG-3'
SCN9A_ex2	F	5'-GGAGTCTGATGGCAGTGTTTC-3'
	R	5'-TTCTCTGAGACCCATGACAAA-3'
SCN9A_ex3	F	5'-TTAATATAATTTGTCATGGGTCTCAG-3'
	R	5'-GGAAGGTAAAGATTCCCCATCT-3'
SCN9A_ex4	F	5'-GGGTGTGTCCATAACCCAAC-3'
	R	5'-TTTGAAAAATTTAGTATTTGCCTATCA-3'
SCN9A_ex5	F	5'-CCCAAACGTAGAAAATACCTTG-3'
	R	5'-CATCCTAAAGACCCATGCAA-3'
SCN9A_ex6	F	5'-TGACTTTCTAGGAAAGCTTGTGTTT-3'
	R	5'-TTTGAAATGATTAAAATGAAAGCA-3'
SCN9A_ex7	F	5'-CACCTAAACATAAAGGAGGTCTCA-3'
	R	5'-TTTGCTGTGGGACAGAATGA-3'
SCN9A_ex8	F	5'-GCCAAATCATGTATTCTTAATCCTT-3'
	R	5'-GAGTTTCCTCCATTCTCAAATAAA-3'
SCN9A_ex9	F	5'-TTGATAACATGAGATTATACCAAACTG-3'
	R	5'-AGCCCAGAATTGGTTCCTTT-3'
SCN9A_ex10	F	5'-TGCACCATGTTGTTATGCTAAT-3'
	R	5'-TTTCCATAAATGCCTAGCGATAC-3'
SCN9A_ex11	F	5'-GCCAGTGGGTTCAGTGGTAT-3'
	R	5'-TGCAGAGCCATTCACAAGAC-3'
SCN9A_ex12	F	5'-GCCATAATTTGAACCCAGCA-3'
	R	5'-TTCCTAAGAATTTCATGTGCCTA-3'

Exons	**Strand**	**Primer sequence**
SCN9A_ex13	F	5'-TGCTTCATCTAGGCAACGAA-3'
	R	5'-CCAGAAACTTGACTCTACACAT-3'CC
SCN9A_ex14	F	5'-GGGAAGAGCAATACCTAATTTACA-3'
	R	5'-AAGTGCATTTGTCAATGAATGG-3'
SCN9A_ex15	F	5'-TGCTTTACCCTTTGAACAAAAA-3'
	R	5'-CATCACAAAATAATTTCCACAGAGA-3'
SCN9A_ex16	F	5'-CGTTGCTGGTTTGTATTGTG-3'
	R	5'-GTGAGGCTGGGATTGTGAAT-3'
SCN9A_ex16	F	5'-CCTTACGTGAATTTATTCTAAAAGCAT-3'
	R	5'-TCACGCACAAAAACTATCCATC-3'
SCN9A_ex17	F	5'-AAGGTTACTTGACCTCAATATGTGT-3'
	R	5'-TGCCATAGATGTGGAAACTCA-3'
SCN9A_ex18	F	5'-CAACTATTTAGAAAACATCATGCAAA-3'
	R	5'-TCATTGGCACTAATCATAGGG-3'
SCN9A_ex19	F	5'-CAGCACTGGTGAGAAGGCTA-3'
	R	5'-TTTACATTTGTTGAACGATTTCTG-3'
SCN9A_ex20	F	5'-ATTTCCTTACAGAAACATCCATCT-3'
	R	5'-GAAACAACTCATAATTTCTACATACCC-3'
SCN9A_ex21	F	5'-AAGGGCGGCTTTTCTAATTT-3'
	R	5'-GCCAAGACTGGCACTGTTTTA-3'
SCN9A_ex22	F	5'-TGGACATGTTGAATACAGCAAA-3'
	R	5'-TCAGGCTCTATCTCCAACTGC-3'
SCN9A_ex23	F	5'-CCTCAACAATGCTATGGCTTCv
	R	5'-TTGTTTTCTGTGCAAAAATGAAT-3'
SCN9A_ex24	F	5'-CTGTGTTTGGAGACCCATGT-3'
	R	5'-CAAAGGCTTGATTTGTCACTAGG-3'
SCN9A_ex25	F	5'-TCATTTTTAATGCACATCTTTAATTTC-3'
	R	5'-GCTTTGGGGCCTGTAGTGTA-3'
SCN9A_ex26-I	F	5'-TTCTATTATTTTCCTCCACATACAGG-3'
	R	5'-TTCCAACAGATGGGTTACCA-3'
SCN9A_ex26-II	F	5'-AACAGTAAGCCACCCGACTG-3'
	R	5'-ATGGATCCGGTCACCACTAA-3'
SCN9A_ex26-III	F	5'-AACCCAACAAAGTCCAGCTC-3'
	R	5'-TTCCCTTTGTCTTCCTTTTCTG-3'
SCN9A_ex26-IV	F	5'-AACAGATGCCACTTCATCCAC-3'
	R	5'-TGAAAAGATGACAAGGCAGATG-3'
SCN9A_ex26-V	F	5'-CGAAGGCAGTGCAGTCACTA-3'
	R	5'-AAAAGTCAAGCTCCCTAATAATG-3'C

Results

CRPS probandi

All four probands developed symptoms after a noxious event and had more than one affected extremity. All patients reported allodynia, hyperalgesia, discoloration, edema and changed temperature of the skin. Every one of the symptoms was also observed at the time of the examination except for the allodynia in the proband of family two and the discoloration in the proband of family three.

CRPS families

We collected four families with CRPS, three of which had been published before.[39] In family one there are two affected individuals among the 21 first- and second-degree relatives of the probands (10%). In family two there are four affected individuals among the 29 first- and second-degree relatives of the probands (14%). In family three there is one affected individual among the fourteen first- and second-degree relatives of the probands (7%). The fourth family was recently collected and has two affected individuals among the 21 first- and second-degree relatives of the probands (10%). The other affected individuals in all these families are more distantly related from the probands.

No clear mendelian segregation pattern was observed in any of the CRPS families. A conventional linkage analysis in CRPS is therefore less appealing. CRPS in these families can still be caused by high-penetrant mutations, because there may be non-penetrant cases. This is not unlikely, since exposure to an initiating event such as a fracture or soft tissue injury is often required to express the syndrome. In these four families 89% (15 of 18) of all affected individuals reported a trauma before the onset of the condition in the first affected extremity.

Mutation analysis of the SCN9A gene

We hypothesized that a candidate gene sequencing strategy to identify possible high-penetrant causal mutations in the CRPS families is the most realistic approach. Therefore, we performed a mutation analysis in the *SCN9A* gene by direct sequencing of all 26exons. No causal gene mutations were identified in *SCN9A* gene in any of the four index cases.

Table 1: Signs and symptoms of the patients from the four described families

					Sensory				Vasomotor		Sudomotor		Motor/Trophic		
family	person	extr	age onset	trauma	pain	allodynia	hyperest	hyperal	color	temp	edema	sweat	trophic	dystonia	tremor
1	**II-5**	**RL**	**39**	**+**	**+**	**ob.**	**ob.**	**ob.**	**ob.**	**ob.**	**ob.**	**ob.**	**ob.**	**ob.**	**-**
		LL	**44**	**+**	**+**	**ob.**	**ob.**	**ob.**	**ob.**	**ob.**	**re.**	**ob.**	**ob.**	**ob.**	**re.**
		RA	**54**	**-**	**+**	**ob.**	**ob.**	**ob.**	**re.**	**ob.**	**-**	**ob.**	**ob.**	**ob.**	**ob.**
		LA	**56**	**-**	**+**	**ob.**	**ob.**	**ob.**	**re.**	**ob.**	**re.**	**ob.**	**ob.**	**ob.**	**re.**
	II-7	LA	58	+	+	ob.	ob.	ob.	ob.	ob.	ob.	ob.	ob.	ob.	re.
	II-8	RA	51	-	+	-	ob.	ob.	-	re.	-	-	-	-	-
		LA	51	-	+	-	ob.	ob.	-	re.	-	-	-	-	-
		RL	51	-	+	-	ob.	ob.	-	ob.	-	-	-	-	-
		LL	51	-	+	-	ob.	ob.	-	re.	-	-	-	-	-
	III-12	LA	21	+	+	re.	-	re.	ob.	ob.	ob.	ob.	ob.	-	-
	III-13	RL	14	-	+	-	ob.	ob.	-	-	-	ob.	-	-	-
		LL	14	-	+	-	ob.	ob.	-	-	-	ob.	-	-	-
		RA	19	-	+	ob.	ob.	ob.	-	-	-	ob.	ob.	-	re.
		LA	19	-	+	ob.	ob.	ob.	-	-	-	ob.	ob.	-	re.
2	II-3	RA	70	+	+	ob.	-	ob.	re.	ob.	ob.	ob.	ob.	re.	-
	II-5	LB	65	+	+	-	-	-	ob.	ob.	ob.	ob.	ob.	-	-
	II-7	LB	45	+	+	ob.	re.	ob.	ob.	ob.	re.	ob.	ob.	re.	re.
		RB	50	-	+	re.	ob.	ob.	ob.	ob.	ob.	ob.	ob.	ob.	re.
		LA	54	-	+	ob.	ob.	ob.	ob.	ob.	ob.	ob.	ob.	-	-
	III-10	**RB**	**24**	**-**	**+**	**re.**	**ob.**	**-**	**ob.**	**ob.**	**-**	**ob.**	**ob.**	**-**	**re.**
		LB	**26**	**+**	**+**	**re.**	**ob.**	**-**	**ob.**	**ob.**	**ob.**	**ob.**	**ob.**	**ob.**	**re.**
3	III-12	LA	29	+	+	ob.	-	re.	ob.	ob.	ob.	-	ob.	-	-
	III-13	LL	40	+	+	-	-	re.	re.	re.	re.	re.	re.	-	re.
	IV-21	LA	17	+	+	re.	re.	re.	re.	re.	re.	-	re.	re.	-
	IV-25	**RA**	**17**	**+**	**+**	**ob.**	**ob.**	**ob.**	**re.**	**re.**	**re.**	**re.**	**re.**	**ob.**	**ob.**
		LL	**20**	**+**	**+**	**ob.**	**ob.**	**ob.**	**re.**	**ob.**	**ob.**	**re.**	**ob.**	**ob.**	**ob.**
		RL	**23**	**-**	**+**	**ob.**	**ob.**	**ob.**	**re.**	**re.**	**re.**	**-**	**re.**	**ob.**	**ob.**
	IV-26	LA	21	+	+	re.	ob.	ob.	re.	re.	ob.	ob.	ob.	-	-
4	III-8	RA	57	+	+	-	-	-	re.	ob.	re.	re.	ob.	re.	re.
	III-10	**RL**	**47**	**+**	**+**	**-**	**ob.**	**ob.**	**ob.**	**ob.**	**ob.**	**-**	**-**	**-**	**-**
		LA	**47**	**+**	**+**	**re.**	**-**	**-**	**-**	**-**	**-**	**re.**	**-**	**-**	**-**
	III-15	LA	54	+	+	re.	ob.	ob.	re.	ob.	re.	ob.	ob.	-	-
	IV-19	LA	19	+	+	re.	-	-	-	re.	-	-	re.	-	-
		LL	28	+	+	re.	-	re.	re.	-	re.	-	-	-	-

RA=Right arm; LA=Left arm; RL=Left leg; LL=Left leg; + = present; - = absent; ob. =observed; re. = reported; **Bold** = family probands; Extr = extremity; hyperest = hyperesthesia; hyperal = hyperalgesia; temp = temperature.

Discussion

We analyzed the SCN9A gene in the index cases of four CRPS families. No causal gene mutation was identified in any of them. Consequently, our data suggest that the SCN9A gene does not play a major role in familial CRPS. It cannot be ruled out, however, that the SCN9A gene may play a role in sporadic CRPS, or perhaps even a minor role in familial CRPS. The possibility remains that certain mutations, such as exonic deletions, intronic mutations and/or promoter mutations may have been missed as a result of the applied PCR strategy. If the changed sensitivity to pain in CRPS would be due to a similar gain-of-function molecular mechanism as in PE or PEPD, which are due to missense mutations, our strategy most likely would have been successful in identifying these mutations. A mutation in the SCN9A gene promoter or in a regulatory element resulting in increased SCN9A gene expression, is theoretically possible and would remain unnoticed. Unfortunately, lack of $Na_V1.7$-expressing tissue of CRPS patients, precludes testing whether SCN9A expression levels are increased. The possibility of a lower or absent SCN9A expression level due to specific promoter mutations and/or (partial) gene deletions is rather unlikely, since loss-of-function mutations were shown to cause insensitivity and not increased sensitivity, to pain.

When large CRPS cohorts will become available, one may consider performing a well-designed case-control association study, testing for low-penetrant gene variants in SCN9A. At this moment no such variant has been identified in a pain-related disorder. Although the present study did not reveal a role for $Na_V1.7$ in CRPS , the rational for testing SCN9A was a relevant one. After all, a role of Na_V channels in pain mechanisms is well established (for a review see Cummins 2007).[40] For several Na_V isoforms, such as $Na_V1.3$, $Na_V1.7$ and $Na_V1.8$, a role in modulating the sensitivity of pain has been shown, as also revealed by experimental mouse models.

Although we did not find support for our hypothesis that the SCN9A is involved in CRPS, other genes involved in pain perception, such as non-$Na_V1.7$ voltage-gated Na_V channels (as discussed above) or GTP cyclohydrolase 1 (GCH1),[41] catechol-O-methyltransferase (COMT)[42] or transient receptor potential vanilloid 1 (TRPV1)[43] might be considered as candidates for future studies on genetic factors in CRPS.

Reference List

1. Veldman PH, Reynen HM, Arntz IE, and Goris RJ. Signs and symptoms of reflex sympathetic dystrophy: prospective study of 829 patients. Lancet 1993;342: 1012-1016
2. Merskey H and Bogduk N Complex Regional Pain Syndrome, Type I. In: Merskey, H. and Bogduk, N., editors. Classification of chronic pain: descriptions of chronic pain syndromes and definitions of pain terms. Seattle: IASP Press; 1994. pp. 41-42.
3. Veldman PH, Reynen HM, Arntz IE, and Goris RJ. Signs and symptoms of reflex sympathetic dystrophy: prospective study of 829 patients. Lancet 1993;342: 1012-1016
4. Huygen FJ, de Bruijn AG, De Bruin MT, Groeneweg JG, Klein J, and Zijistra FJ. Evidence for local inflammation in complex regional pain syndrome type 1. Mediators Inflamm 2002;11: 47-51
5. Birklein F and Schmelz M. Neuropeptides, neurogenic inflammation and complex regional pain syndrome (CRPS). Neurosci Lett 2008;437: 199-202
6. Maihofner C, Handwerker HO, Neundorfer B, and Birklein F. Patterns of cortical reorganization in complex regional pain syndrome. Neurology 2003;61: 1707-1715
7. Drummond PD and Finch PM. Sensory changes in the forehead of patients with complex regional pain syndrome. Pain 2006;123: 83-89
8. de Rooij AM, de Mos M, Sturkenboom MC, Marinus J, van den Maagdenberg AM, and van Hilten JJ. Familial occurrence of complex regional pain syndrome. Eur J Pain 2009;13: 171-177
9. Albert J and Ott H. Three brothers with algodystrophy of the hip. Ann Rheum Dis 1983;42: 421-424
10. Erdmann MW and Wynn-Jones CH. 'Familial' reflex sympathetic dystrophy syndrome and amputation. Injury 1992;23: 136-138
11. Veldman PH, Reynen HM, Arntz IE, and Goris RJ. Signs and symptoms of reflex sympathetic dystrophy: prospective study of 829 patients. Lancet 1993;342: 1012-1016
12. Galer BS, Henderson J, Perander J, and Jensen MP. Course of symptoms and quality of life measurement in Complex Regional Pain Syndrome: a pilot survey. J Pain Symptom Manage 2000;20: 286-292
13. Huhne K, Leis S, Schmelz M, Rautenstrauss B, and Birklein F. A polymorphic locus in the intron 16 of the human angiotensin-converting enzyme (ACE) gene is not correlated with complex regional pain syndrome I (CRPS I). Eur J Pain 2004;8: 221-225
14. Bruscas IC, Beltran Audera CH, and Jimenez ZF. [Polytopic and recurrent reflex sympathetic dystrophy in lower limbs in two siblings.]. An Med Interna 2004;21: 183-184

15. Veldman PH and Goris RJ. Multiple reflex sympathetic dystrophy. Which patients are at risk for developing a recurrence of reflex sympathetic dystrophy in the same or another limb. Pain 1996;64: 463-466
16. van Rijn MA, Marinus J, Putter H, and van Hilten JJ. Onset and progression of dystonia in complex regional pain syndrome. Pain 2007;130: 287-293
17. de Rooij AM, de Mos M, van Hilten JJ, Sturkenboom MC, Gosso MF, van den Maagdenberg AM, and Marinus J. Increased risk of Complex Regional Pain Syndrome in siblings of patients? J Pain 2009
18. Mailis A and Wade J. Profile of Caucasian women with possible genetic predisposition to reflex sympathetic dystrophy: a pilot study. Clin J Pain 1994;10: 210-217
19. Kemler MA, van de Vusse AC, van den Berg-Loonen EM, Barendse GA, van Kleef M, and Weber WE. HLA-DQ1 associated with reflex sympathetic dystrophy. Neurology 1999;53: 1350-1351
20. van Hilten JJ, van de Beek WJ, and Roep BO. Multifocal or generalized tonic dystonia of complex regional pain syndrome: a distinct clinical entity associated with HLA-DR13. Ann Neurol 2000;48: 113-116
21. Vaneker M, van der Laan L, Allebes WA, and Goris RJ. Genetic factors associated with complex regional pain syndrome 1: HLA DRB and TNF alpha promotor gene polymorphism. Disability medicine 2002;2: 69-74
22. de Rooij AM, Gosso MF, Haasnoot GW, Marinus J, Verduijn W, Claas FHJ, van den Maagdenberg AM, and van Hilten JJ. HLA-B62 and HLA-DQ8 are associated with Complex Regional Pain Syndrome with fixed Dystonia. Pain 2009;145: 82-85
23. Nassar MA, Stirling LC, Forlani G, Baker MD, Matthews EA, Dickenson AH, and Wood JN. Nociceptor-specific gene deletion reveals a major role for Nav1.7 (PN1) in acute and inflammatory pain. Proc Natl Acad Sci U S A 2004;101: 12706-12711
24. Mork C, Kalgaard OM, and Kvernebo K. Impaired neurogenic control of skin perfusion in erythromelalgia. J Invest Dermatol 2002;118: 699-703
25. Davis MD, Sandroni P, Rooke TW, and Low PA. Erythromelalgia: vasculopathy, neuropathy, or both? A prospective study of vascular and neurophysiologic studies in erythromelalgia. Arch Dermatol 2003;139: 1337-1343
26. Michiels JJ, Te Morsche RH, Jansen JB, and Drenth JP. Autosomal dominant erythermalgia associated with a novel mutation in the voltage-gated sodium channel alpha subunit Nav1.7. Arch Neurol 2005;62: 1587-1590
27. Yang Y, Wang Y, Li S, Xu Z, Li H, Ma L, Fan J, Bu D, Liu B, Fan Z, Wu G, Jin J, Ding B, Zhu X, and Shen Y. Mutations in SCN9A, encoding a sodium channel alpha subunit, in patients with primary erythermalgia. J Med Genet 2004;41: 171-174
28. Brown GE. Erythromelalgia and other disturbances of the extremities accompanied by vasodilation and burning. Am J Med Sci 1932;183: 468-484
29. Michiels JJ, van JT, and Vuzevski VD. Idiopathic erythermalgia: a congenital disorder. J Am Acad Dermatol 1989;21: 1128-1130

30. Fertleman CR, Baker MD, Parker KA, Moffatt S, Elmslie FV, Abrahamsen B, Ostman J, Klugbauer N, Wood JN, Gardiner RM, and Rees M. SCN9A mutations in paroxysmal extreme pain disorder: allelic variants underlie distinct channel defects and phenotypes. Neuron 2006;52: 767-774
31. Hayden R and Grossman M. Rectal, ocular, and submaxillary pain; a familial autonomic disorder related to proctalgia fugaz: report of a family. AMA J Dis Child 1959;97: 479-482
32. Fertleman CR, Ferrie CD, Aicardi J, Bednarek NA, Eeg-Olofsson O, Elmslie FV, Griesemer DA, Goutieres F, Kirkpatrick M, Malmros IN, Pollitzer M, Rossiter M, Roulet-Perez E, Schubert R, Smith VV, Testard H, Wong V, and Stephenson JB. Paroxysmal extreme pain disorder (previously familial rectal pain syndrome). Neurology 2007;69: 586-595
33. Cox JJ, Reimann F, Nicholas AK, Thornton G, Roberts E, Springell K, Karbani G, Jafri H, Mannan J, Raashid Y, Al-Gazali L, Hamamy H, Valente EM, Gorman S, Williams R, McHale DP, Wood JN, Gribble FM, and Woods CG. An SCN9A channelopathy causes congenital inability to experience pain. Nature 2006;444: 894-898
34. Cummins TR, Howe JR, and Waxman SG. Slow closed-state inactivation: a novel mechanism underlying ramp currents in cells expressing the hNE/PN1 sodium channel. J Neurosci 1998;18: 9607-9619
35. Julius D and Basbaum AI. Molecular mechanisms of nociception. Nature 2001;413: 203-210
36. Woolf CJ and Costigan M. Transcriptional and posttranslational plasticity and the generation of inflammatory pain. Proc Natl Acad Sci U S A 1999;96: 7723-7730
37. de Rooij AM, de Mos M, Sturkenboom MC, Marinus J, van den Maagdenberg AM, and van Hilten JJ. Familial occurrence of complex regional pain syndrome. Eur J Pain 2009;13: 171-177
38. Miller SA, Dykes DD, and Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16: 1215
39. de Rooij AM, de Mos M, Sturkenboom MC, Marinus J, van den Maagdenberg AM, and van Hilten JJ. Familial occurrence of complex regional pain syndrome. Eur J Pain 2009;13: 171-177
40. Cummins TR, Sheets PL, and Waxman SG. The roles of sodium channels in nociception: Implications for mechanisms of pain. Pain 2007;131: 243-257
41. Tegeder I, Costigan M, Griffin RS, Abele A, Belfer I, Schmidt H, Ehnert C, Nejim J, Marian C, Scholz J, Wu T, Allchorne A, Diatchenko L, Binshtok AM, Goldman D, Adolph J, Sama S, Atlas SJ, Carlezon WA, Parsegian A, Lotsch J, Fillingim RB, Maixner W, Geisslinger G, Max MB, and Woolf CJ. GTP cyclohydrolase and tetrahydrobiopterin regulate pain sensitivity and persistence. Nat Med 2006;12: 1269-1277

42. Zubieta JK, Heitzeg MM, Smith YR, Bueller JA, Xu K, Xu Y, Koeppe RA, Stohler CS, and Goldman D. COMT val158met genotype affects mu-opioid neurotransmitter responses to a pain stressor. Science 2003;299: 1240-1243

43. Kim H, Neubert JK, San Miguel A, Xu K, Krishnaraju RK, Iadarola MJ, Goldman D, and Dionne RA. Genetic influence on variability in human acute experimental pain sensitivity associated with gender, ethnicity and psychological temperament. Pain 2004;109: 488-496