A mutation in the nerve growth factor beta gene (NGFB) causes loss of pain perception

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Identification of genes associated with pain insensitivity syndromes can increase the understanding of the pathways involved in pain and contribute to the understanding of how sensory pathways relate to other neurological functions. In this report we describe the mapping and identification of the gene responsible for loss of deep pain perception in a large family from northern Sweden. The loss of pain perception in this family is characterized by impairment in the sensing of deep pain and temperature but with normal mental abilities and with most other neurological responses intact. A severe reduction of unmyelinated nerve fibers and a moderate loss of thin myelinated nerve fibers are observed in the patients. Thus the cases in this study fall into the class of patients with loss of pain perception with underlying peripheral neuropathy. Clinically they best fit into HSAN V. Using a model of recessive inheritance we identified an 8.3 Mb region on chromosome 1p11.2–p13.2 shared by the affected individuals in the family. Analysis of functional candidate genes in the disease critical region revealed a mutation in the coding region of the nerve growth-factor beta (NGFB) gene specific for the disease haplotype. This NGF mutation seems to separate the effects of NGF involved in development of central nervous system functions such as mental abilities, from those involved in peripheral pain pathways. This mutation could therefore potentially provide an important tool to study different roles of NGF, and of pain control.

INTRODUCTION

Pain insensitivity can vary from pain syndromes associated with dysfunction in various neurological processes including serious peripheral neuropathy, self-mutilation and mental retardation to more pure forms of analgesia (1). In most cases of pain insensitivity, an underlying neuropathy leads to the inability to perceive pain and is denoted insensitivity to pain. In some rare cases of pain insensitivity peripheral neuropathy is absent. These patients have normal peripheral responses to pain but do not seem to perceive the sensation of pain. This syndrome is therefore denoted congenital indifference to pain (2). The absence of peripheral neuropathy has become a criterion for distinguishing congenital indifference to pain from congenital insensitivity to pain (3). So far no genes have been identified for the pure forms of indifference to pain. For peripheral neuropathies, with pain insensitivity combined with other neurological dysfunctions like the hereditary sensory and autonomic neuropathies (HSANs), a number of genes are known today. HSAN is a group of peripheral neuropathies characterized by loss of pain sensation in combination with other sensory and/or autonomic abnormalities (3,4). Five types of HSAN have been identified. HSAN I is associated with distal loss of pain sensitivity and thermal sensitivity, ulcers and in some kinships also deafness (5). HSAN I has been shown to be caused by a mutation in the Serine Palmitoyltransferase, long-chain base subunit 1, SPTLC1, gene (6). For HSAN II, with autonomic dysfunctions and severely impaired sensory functions already leading to trophic ulcers in infancy, there is still no causative gene identified (7). Individuals affected with HSAN III, also called familial dysautonomia or Riley–Day syndrome, display a variety of symptoms, including decreased sensitivity...
to pain and temperature, cardiovascular instability, recurrent pneumonias, vomiting crises and gastrointestinal dysfunction (8–10). HSAN III has been shown to be caused by a mutation in the IκB kinase complex associated protein, IKBKAP, gene (11,12). HSAN IV is a congenital insensitivity to pain with anhidrosis, also called CIPA. HSAN IV is characterized by profound loss of pain sensitivity, leading to injuries, self-mutilation and osteomyelitis, and also by anhidrosis and mental retardation (13). Point mutations in the nerve growth factor receptor gene TRKA have been identified in HSAN IV patients, implicating the NGF/TRKA pathway in the pathogenesis (14,15). HSAN V is a rare disorder, with only a few described cases. The disease is characterized by loss of pain perception and impaired temperature sensitivity, ulcers, and in some cases self-mutilation. The autonomic involvement is variable (7). The genetic background of HSAN V is still unclear.

In this report we describe a large multi-generational family from northern Sweden suffering from loss of pain perception but with most other neurological functions intact. The patients suffer from a severe loss of deep pain perception that prevents them from feeling pain from bone fractures and joints, heat perception is also impaired. Decreased deep pain perception leads to destroyed joints (Charcot joints) in childhood. Most other neurological functions including sweating and mental abilities are intact. A number of additional family members suffer from a less severe phenotype presenting as joint problems mostly in the feet and knees, and resulting in Charcot joints later in life. Neurophysiologic and neuropathologic findings show that the patients in this family suffer from a peripheral neuropathy with a severe reduction of unmyelinated nerve fibers and a moderate loss of thin myelinated nerve fibers. Thus the cases in this study fall into the class of patients with loss of pain perception with underlying peripheral neuropathy. Clinically they best fit into HSAN V. We describe the mapping and identification of the mutation responsible for pain insensitivity in this northern Swedish family. The mutation is particularly interesting, as it apparently does not interfere with development of functions of the central nervous system such as mental abilities, while peripheral pain pathways are severely affected.

RESULTS

Clinical description

In the studied family three severe, juvenile cases have been identified and investigated with respect to orthopedic, neurologic, neuropathologic and neurophysiologic status.

Case 1 (individual 8; Fig. 1). Case 1 is a 38-year-old man born to consanguineous parents. At the age of 7 he presented with painless effusion in his right knee. At 8, he was operated for right ankle osteochondritis and at that time a low sensitivity to pain was noticed. At 8, arthropathy was noticed in the ankles. At 15 he suffered from multiple painless metatarsal fractures. He has had low back pain since the age of 21 and at 34 he was diagnosed with progressive changes in his back joints. He feels a burning sensation in lukewarm water. He does not suffer from anhidrosis. Sural nerve biopsy revealed moderate loss of thin myelinated nerve fibers and a severe reduction of unmyelinated nerve fibers. Peripheral nerve conduction and R-R variation test was normal but vibratory and temperature thresholds were increased and SSR was absent.

Case 2 (individual 11; Fig. 1). Case 2 is a 20-year-old woman born to consanguineous parents. At 15, she presented with osteoarthritis of the right ankle and knee. She has suffered repeated painless fractures. She lacks protective reflexes against burns, but can feel burning pain after a latency of about 1 s. Cuts do not hurt, but pinpricks do. She does not suffer from anhidrosis. She faints easily when rising. She has difficulty emptying the bladder and a slight urinary incontinence and also has gastrointestinal problems with abdominal pains, diarrhea or constipation. She has developed a slight finger tremor. Sural nerve biopsy revealed moderate loss of thin myelinated nerve fibers and severe reduction of unmyelinated nerve fibers. Peripheral nerve conduction and vibration threshold were normal, but temperature threshold was increased and SSR was absent.

Case 3 (individual 4; Fig. 1). Case 3 is a 12-year-old boy born to consanguineous parents. He showed normal early motor milestones but was shown to exhibit multiple fractures at the age of 4. Recurrent septic arthritis and osteochondritis has resulted in the development of neuropathic malformations in several joints. He can feel a burning sensation even in a normal temperature shower, but only slight pain when he does burn himself. He does not suffer from anhidrosis. He does not faint nor has he had any gastrointestinal or urinary problems. Sural nerve biopsy revealed moderate loss of thin myelinated nerve fibers and severe reduction of unmyelinated nerve fibers. Peripheral nerve conduction, SSR, R-R variation test and vibration thresholds were normal but temperature threshold was increased.

There are also individuals in the family with symptoms that could represent a milder expression of the phenotype. Out of these, three individuals have been examined in more detail. These cases belong to another branch of the family that was not included in the genetic analysis and is therefore not present in Figure 1. They developed joint problems in knees, feet and ankle in their twenties to thirties and bone deformities and destructive arthropathies first in mid life. In contrast to the severe cases, no severe loss of deep pain perception or painless fractures is observed in these individuals. Nerve biopsy of one of the adult-onset cases reveals reduction of both A-delta and C-fibers, but not to the extent observed in the juvenile cases. In a number of additional family members various joint problems that could reflect mild expression of the phenotype are observed. The parents of the juvenile cases do not display any symptoms except for individual 1, the mother of individual 4 (Fig. 1), who suffers from Chondromalacia patella. A brother of individual 2 has Charcot atrophy. The father of individual 10 has hip osteoarthritis. Owing to the difficulty of assigning a correct status for the milder cases, only the juvenile cases were considered in the genetic analysis.

Genetic analysis

The inheritance pattern for the three severe cases is consistent with an autosomal recessive transmission of the disease. Extensive inbreeding and the fact that the parents of the severe cases are related support this inheritance pattern (Fig. 1).
The number of possible cases present in the family, however, suggests a gene dosage effect resulting in less pronounced symptoms in heterozygote individuals. Based on the hypothesizing of a recessive inheritance of the severe form of the disease, we screened for shared homozygosis regions in the three severely affected individuals. Only individuals with the more severe form, presenting in childhood, were considered as affected in the genetic analysis (Fig. 1). A genome-wide screen was performed on the three severely affected individuals, their parents and siblings when available, using an ABI panel set with an average distance of 10 cM. The genotypes for each individual were then ordered into whole-chromosome haplotypes and regions of homozygosity were assessed visually. The analysis revealed a common haplotype, on chromosome 1p11.2–p13.2, for which all three severely affected individuals were homozygous (Fig. 1). The disease haplotype was also observed in unaffected or mildly affected family members, but in heterozygote form only (Fig. 1 and data not shown). No other marker loci tested fulfilled the criteria of a recessive gene identical by descent, including markers covering chromosomal regions harboring the genes SPTLC1, IKBKAP, TRKA, implicated in HSAN I, HSAN III and HSAN IV, respectively (data not shown).

The disease gene could be restricted to an 8.3 Mb region, close to the centromere, flanked by the SNP markers rs2490334 and rs2275607 (Fig. 1).

**Mutation analysis**

The disease critical region contained 66 genes and a number of hypothetical transcripts according to the Celera database.
Among these, nerve growth factor beta (NGFB) constituted a plausible candidate gene. The NGFB gene was analysed by direct sequencing of all exon and exon–intron boundaries. The mutation analysis of NGFB indeed revealed a point mutation in exon 3 of the gene specific for the disease haplotype (Fig. 2A). The mutation (661C→T) changed a basic arginine (CGG) to a non-polar tryptophan (TGG) at position 211 in the NGFB polypeptide corresponding to amino acid 100 in the mature protein (16,17) (Fig. 2B). The substituted amino acid was located in a region of the protein that is highly conserved between different neurotrophins as well as NGFs of different species (Fig. 2C). We screened for the mutation in 100 control individuals, as well as spouses and unaffected members of the family. The mutation could only be detected on the disease-associated haplotype, strongly suggesting that the identified mutation in NGFB is the cause of disease in this family. This is further supported by the fact that one of the two NGFB receptors, TRKA, has been shown to be involved in HSAN IV a pain insensitivity syndrome characterized by profound loss of pain sensitivity leading to injuries, self-mutilation and osteomyelitis, anhidrosis and mental retardation (13).

DISCUSSION

In this paper we describe the mapping and identification of the gene responsible for loss of pain perception in a family from northern Sweden. The causative mutation is located in a conserved region of the NGFB gene and gives rise to a phenotype that is associated with severe loss of pain perception and impaired temperature sensation, but with most other neurological functions, including sweating, intact, thus best fitting HSAN V.

The NGF/TRKA pathway has earlier been implicated in HSAN IV, a pain insensitivity syndrome characterized by profound loss of pain sensitivity leading to injuries, self-mutilation and osteomyelitis, anhidrosis and mental retardation (14). In all cases of HSAN IV analysed so far, mutations in the TRKA gene have been shown to be responsible for the syndrome. At least 37 different mutations in the TRKA gene have been implemented in HSAN IV to date, distributed in the extracellular domain involved in NGF binding as well as in the intracellular signal-transduction domain (15). As for many other forms of pain insensitivity, the loss of pain perception in HSAN IV is associated with various other neurological defects involving autonomic function as well as mental abilities. The NGF mutation described in this report gives rise to a less severe phenotype than the TRKA mutations associated with HSAN IV. The fact that a less severe effect is observed for the NGF mutation than mutations characterized in the TRKA receptor suggests that the mutation observed in this family does not completely abolish the ability of NGF to bind and activate TRKA. Alternatively, it affects the interaction with the low-affinity receptor p75, thereby inhibiting p75 promoted NGF activities. The latter hypothesis is supported by the fact that the mutation is located in a position highly conserved between NGF and the related neurotrophins, BDNF, NT 3 and NT 4 (Fig. 2C). The fact that they all bind to the p75 receptor might suggest that this region is important for their common ability to bind and/or activate p75.

Knock-out mice models have been generated for NGF as well as for the NGF receptors, TRKA and p75. Comparison shows that homozygous mice with disruptions in any of the three genes exhibit deficit in the nociceptive function (18–20). In accordance with this NGF, TRKA and p75 knock-out mice suffer from severe sensory neuropathies. The overall phenotype of the p75 null mice is, however, less severe than both that of TRKA and NGF null mice. Both the NGF and TRKA null mice die within a few weeks of birth, while the p75 null mice are viable and fertile. A loss of sympathetic neurons is observed in NGF and TRKA null mice, while the effect in p75 null mice seems to be restricted to the sensory nervous system. Furthermore, TRKA null mice exhibit a decrease in the cholinergic basal forebrain projections to the hippocampus and cortex, suggesting that the TRKA signaling pathway plays a crucial role in the development of both the peripheral and the central nervous systems (20). These phenotype differences could be consistent with the hypothesis that the mutation of NGF in our family affects the binding to p75 generating a less severe phenotype than would be expected if the NGF binding to the TRKA receptor was affected. The alternative hypothesis that the mutation affects the binding to and activation of TRKA can, however, not be ruled out. At least one mutation in TRKA has been shown to cause a less severe form of HSAN than is observed for other TRKA mutations (21). There are, however, differences between the phenotype described here and the phenotype resulting from the mutation in TRKA described by Houlden et al. (21) Their case had a phenotype less severe than HSAN IV but with anhidrosis, which was not observed in our cases. Furthermore, in opposite to our findings, they observed a less severe deficiency of unmyelinated axons but greater effect on small myelinated fibers.

The fact that the NGF mutation described here apparently separates the effects of NGF mediating a normal development of functions of the central nervous system such as mental abilities from effects on the nervous system involved in peripheral pain pathways is particularly interesting. This could potentially provide an important tool for studies aiming to separate the different roles of NGF. Furthermore, constituting one of the most potent growth factors for cholinergic neurons, NGF has been identified as a promising candidate for treating Alzheimer’s disease. In clinical trials for the treatment of Alzheimer’s disease, induction of pain has been one the major adverse events (22). In this perspective, elucidating the molecular basis for this dichotomy in the NGF function and its relationship to the identified mutation may provide new tools for Alzheimer therapy, circumventing some of the more severe side effects of such therapy.

MATERIALS AND METHODS

Subjects

Informed consent was obtained from all participating individuals and the study was approved by the local ethical committee at Umeå University. Three severe cases and at least 10 cases with various degrees of the ailment have been identified in a large multigenerational family from northern
Figure 2. Mutation analysis and sequence comparison of the NGFB gene. (A) Electropherogram showing part of exon 3 of the NGFB gene sequence containing the NGFB mutation associated with the disease phenotype. The arrow indicates the mutation at position 661 changing a basic arginine to a non-polar tryptophan. The affected individual (8, Fig. 1) is homozygous for the mutation, his unaffected parent (6, Fig. 1) heterozygous and an unaffected relative (3, Fig. 1) homozygous for the wild-type. (B) Structure of the NGFB protein, adapted from Rydén and Ibenez (23), with the alternative amino acid in position 100 of the mature protein, highlighted. (C) Sequence alignment of part of the NGFB protein sequence from different species as well as of human neurotrophins showing the conservation of the mutated amino acid in position 100 of the mature NGFB protein (boxed).
Sweden. In this study the three severe cases were set as affected in the genetic analysis.

Clinical, neurophysiologic and neuropathologic examination

History and clinical neurological examination with special emphasis on symptoms of polyneuropathy and autonomic dysfunction was done by the same neurologist (GS). Motor and sensory nerve conduction were measured in the median, superficial, radial, peroneal, tibial and sural nerves using conventional technique. Vibratory (100 Hz) and temperature thresholds were measured over the dorsum of the foot and in the hand. Autonomic function was assessed by the R-R variation test (reflecting the vagal parasympathetic arc) and the sympathetic skin response (SSR) that corresponds to the activation of sweat glands. Biopsies from the sural nerve were sampled under local anesthesia from behind the lateral malleolus. The biopsies were examined both with light and electron microscopy and also with morphometric analysis (Image J software) of fiber density, fiber area and circular fiber diameter distribution.

Genome-wide scan

Genomic DNA was prepared from whole blood using standard salt methods. The three severely affected individuals, together with their parents and healthy siblings when available, were analysed using an ABI panel set with an average spacing of 10 cM (ABI PRISM Linkage Mapping Set 10 cM version 2.5; Applied Biosystems, Foster City, CA, USA). The primers were amplified according to manufacturer’s instructions, utilizing multiplex PCR reactions. PCR products were resolved through 36 cm capillary arrays using POP-4 polymer on an ABI 3100 DNA sequencer and analysed using the Sequencing Analysis 3.7 software (Applied Biosystems, Foster City, CA, USA). SNP markers were typed using the Sequencing Analysis 3.7 software (Applied Biosystems, Foster City, CA, USA). Fine mapping was performed using additional STR markers D1S2809, D1S2789, D1S2837, D1S2453, D1S2669, D1S185 (DNA technology A/S, Aarhus, Denmark) and SNP markers rs2490334 and rs2275607 (assay-on-demand markers C_3138600 and C_1434549_1, Applied Biosystems, Foster City, CA, USA). SNP markers rs2490334 and rs2275607 (assay-on-demand markers C_3138600 and C_1434549_1, Applied Biosystems, Foster City, CA, USA). SNP markers were typed using the TaqMan assay system on the ABI PRISM 7900HT Sequence Detection System according to the manufacturer’s conditions (Applied Biosystems, Foster City, CA, USA).

Haplotype analysis

The genotypes for each individual were ordered into whole-chromosome haplotypes and regions of homozygosity were assessed visually using Cyrillic 2.1 (Exeter Software, NY, USA).

Sequencing

Sequences for exons and exon–intron junctions of candidate genes were retrieved from NCBI’s Entrez database and primers were designed using Whitehead’s Primer3 program. Primers used to amplify the region in exon 3 of the NGFB gene were forward: 5’-gatgccggttagcagtgcgctg-3’ and reverse 5’-cctccacctctacacgtcttaatttt-3’. Primers used to amplify other gene regions are available on request. PCR products were separated on agarose gels and purified using spin columns (Bio-Rad, Hercules, CA, USA). Sequencing was performed using the BigDye 3.0 kit according to manufacturer’s instructions (Applied Biosystems). Labeled products were resolved through 36 cm capillary arrays using POP-4 polymer on an ABI 3100 DNA sequencer and analysed using the Sequencing Analysis 3.7 software (Applied Biosystems, Foster City, CA, USA).

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