Human Mutation

MUTATION UPDATE

Molecular Basis of Congenital Insensitivity to Pain with Anhidrosis (CIPA): Mutations and Polymorphisms in *TRKA (NTRK1)* Gene Encoding the Receptor Tyrosine Kinase for Nerve Growth Factor

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ABSTRACT

Congenital insensitivity to pain with anhidrosis (CIPA), also referred to as hereditary sensory and autonomic neuropathy type IV (HSAN-IV), is an autosomal recessive hereditary disorder characterized by recurrent episodic fever, anhidrosis (inability to sweat), absence of reaction to noxious stimuli, self-mutilating behavior, and mental retardation. The *TRKA (NTRK1)* gene located on chromosome 1 (1q21-q22), consists of 17 exons and spans at least 23 kb. *TRKA* encodes the receptor tyrosine kinase (RTK) for nerve growth factor (NGF) and is the gene responsible for CIPA. Defects in NGF signal transduction at the TRKA receptor lead to failure to support survival of sympathetic ganglion neurons and nociceptive sensory neurons derived from the neural crest. Thirty-seven different *TRKA* mutations, identified in patients in various countries, including nine frameshift, seven nonsense, seven splice, and 14 missense mutations, are distributed in an extracellular domain involved in NGF binding, as well as in the intracellular signal-transduction domain. Extensive analysis of CIPA mutations and associated intragenic polymorphisms should facilitate detection of CIPA mutations and aid in the diagnosis and genetic counseling of this painless but severe genetic disorder with devastating complications. In addition, naturally occurring *TRKA* missense mutations with loss of function provide considerable insight into the structure-function relationship in the RTK family. Further, molecular pathology of CIPA would provide unique opportunities to explore critical roles of the autonomic sympathetic nervous system as well as peripheral sensory nervous system that transmit noxious stimuli in humans. Hum Mutat 18:462–471, 2001.

KEY WORDS:

congenital insensitivity to pain with anhidrosis; CIPA; hereditary sensory and autonomic neuropathy type IV; HSAN-IV; *NTRK1*; *TRKA*; nerve growth factor; NGF; receptor tyrosine kinase; RTK; uniparental disomy; UPD

DATABASES:

NTRK1 – OMIM, 191315, 256800 (CIPA); GDB:127897; GenBank: AB019480-AB019488, M23102, NM_002529; HGMD NTRK1

INTRODUCTION

Congenital insensitivity to pain with anhidrosis (CIPA; MIM# 256800), also classified as hereditary sensory and autonomic neuropathy type IV (HSAN-IV), is an autosomal recessive genetic disorder that is characterized by recurrent episodic fever, anhidrosis, absence of reaction to noxious stimuli, self-mutilating behavior, and mental retardation [Swanson, 1963; Dyck, 1984]. Pain serves an important protective function and warns of injury that should be avoided or treated. When children with CIPA injure themselves severely, the injury may go unnoticed, the result being permanent damage. Sweating is important to maintain body temperature in hot environments, especially for humans. Anhidrosis (inability to sweat) causes disturbance of the homeostasis responsible for body temperature and recurrent febrile episodes can occur. The anomalous pain and temperature sensation and anhidrosis in CIPA are due to the absence of afferent neurons activated by tissue-damaging stimuli and a loss of sympathetic innervation of eccrine sweat glands, respectively [Rafel et al., 1980; Langer et al., 1981].

Nerve growth factor (NGF; see MIM #162030) induces neurite outgrowth and promotes survival of embryonic sensory and sympathetic neurons [Levi-Montalcini, 1987]. The sensory neurons that respond to tissue damage (nociceptors) are NGF-dependent. Human *TRKA (NTRK1)* gene (MIM# 191315) encodes a receptor tyrosine kinase (RTK) that is phosphorylated in response to NGF [Kaplan et al., 1991; Klein et al., 1991]. Mice lacking the gene for NGF or TRKA (TrkA) share dramatic phenotypic features of CIPA, including loss of responses to painful stimuli, although anhidrosis is not apparent in these animals [Crowley et al., 1994; Smeyne et al., 1994]. Using a candidate gene strategy, the genetic basis for CIPA was identified when loss-of-function mutations in *TRKA* were noted in patients [Indo et al., 1996]. In view of the fact that defects in TRKA cause CIPA, the NGF–TRKA systems has a crucial role in the development and function of nociceptive reception as well as establishment of thermoregulation via sweating in humans.

Subsequently, mutations causing CIPA have been detected in patients of various ethnic groups [Greco et al., 1999; Mardy et al., 1999; Yotsumoto et al., 1999; Miura et al., 2000a; Shatzky et al., 2000; Bodzioch et al., 2001; Mardy et al., 2001; Indo et al., 2001].

THE *TRKA (NTRK1)* GENE AND ITS PRODUCT

NGF, one of the first growth factors to be characterized, was discovered as a target-derived survival factor for the developing peripheral nervous system [Levi-Montalcini, 1987]. NGF supports the survival of sympathetic ganglion neurons and nociceptive sensory neurons in dorsal root ganglia derived from the neural crest and ascending cholinergic neurons of the basal forebrain [Thoenen and Barde, 1980; Levi-Montalcini, 1987]. *TRKA (NTRK1)* (GeneBank accession no. NM_002529; AB019480-AB019488) was isolated from a colon carcinoma as a potential new member of the tyrosine kinase gene family [Martin-Zanca et al., 1986] and was later found to be a high-affinity receptor for NGF [Kaplan et al., 1991; Klein et al., 1991]. Human *TRKA* maps to chromosome 1q21-q22 [Weier et al., 1995] and is divided into 17 exons and 16 introns [Martin-Zanca et al., 1989; Greco et al., 1996; Indo et al., 1997]. The entire sequence was estimated to span at least 23 kb, coding for a protein of 790 or 796 amino acid residues. Six amino acid residues encoded by exon 9 are in the extracellular domain of the neuronal-specific TRKA receptor [Barker et al., 1993]. A single transmembrane domain divides the TRKA protein into an extracellular and an intracellular domain [Schneider and Schweiger, 1991; Barbacid, 1995]. The extracellular domain is important for specific NGF binding and includes a signal peptide, three tandem leucine-rich motifs flanked by two cysteine clusters, and two immunoglobulin-like domains (or motifs). The intracellular domain, including a juxtamembrane region, a tyrosine kinase domain, and a very short carboxy-terminal tail, is phosphorylated in response to NGF and is critical for intracellular signaling.

The binding of NGF to TRKA stimulates homodimer formation and activation of tyrosine kinase activity. Phosphorylated tyrosine residues in the TRKA cytoplasmic domain serve as anchors for binding downstream signaling molecules [Barbacid, 1995; Bothwell, 1995]. *In vitro* assessment has identified TRKA tyrosine residues - 490, 670, 674, 675, and 785 as autophosphorylation sites [Stephens et al., 1994]. NGF binding to TRKA stimulates receptor transphosphorylation, resulting in the recruitment of a series of signaling proteins to docking sites on the receptor. These proteins include Shc, which activates Ras through Grb-2 and SOS, Suc1-associated neurotrophic factor target (SNT, also called FRS-2), rAPS, SH2-B, phospholipase Cγ-1 (PLC□γ-1), and Csk homologous kinase (CHK) [Kaplan and Miller, 2000; Patapoutian and Reichardt, 2001]. Shc and SNT (FRS-2) bind to phosphorylated

Tyr-490, while PLC□γ-1 and CHK bind to phosphorylated Tyr-785. TRKA activates phosphatidylinositol 3-kinase (PI3K) through the Ras and the Gab-1/IRS-1/IRS-2 family of adapter proteins.

Use of gene-targeting mutant mice demonstrated an important role for NGF and its specific receptor, TRKA, in the maturation and function of nociceptive sensory neurons as well as sympathetic neurons [Crowley et al., 1994; Smeyne et al., 1994]. The phenotype of *TRKA* knockout mutant mice is consistent with a specific role for TRKA protein in transducing cellular responses to NGF. In these animals, there is a dramatic loss of small diameter sensory neurons, combined with insensitivity to painful stimuli, and almost all the sympathetic neurons of the superior cervical ganglia neurons.

Recent evidence suggests that NGF, in addition to its neurotrophic functions, acts as an immunomodulator mediating “cross-talk” between neuronal and immune cells, including lymphocytes [Ehrhard et al., 1993a; Torcia et al., 1996] and monocytes [Ehrhard et al., 1993b]. These findings implicate NGF as an autocrine and/or paracrine factor in the development and regulation of immune response.

In addition, *TRKA*-derived oncogenes are also detected in human breast tumor cells [Kozma et al., 1988] or in papillary thyroid carcinoma [Butti et al., 1995; Greco et al., 1995]. These oncogenes are activated by somatic rearrangements juxtaposing their tyrosine kinase domain to the 5’-end sequences derived from unrelated loci and producing chimeric oncogenes whose products display a constitutive and ectopic tyrosine kinase activity. Breakpoints producing some oncogenes often involve a specific region of the *TRKA* gene and part of its sequence has been described [Greco et al., 1993]. The region frequently involved in the rearrangements is located in exons 8 through 12 of the *TRKA* [Indo et al., 1997].

MUTATIONS AND POLYMORPHISMS

Mutations

A total of 37 different mutations have been detected in CIPA families from various countries (Table 1). The mutations are located in the extracellular and intracellular domains, including nine frameshift, seven nonsense, seven splice, and 14 missense mutations (Fig. 1). Most of them are private mutations, but relatively common mutations have been reported for particular ethnic groups such as Japanese and

Israeli-Bedouins [Miura et al., 2000a; Shatzky et al., 2000], as described below. At least eight missense mutations have been confirmed to cause defects in NGF-stimulated autophosphorylation of the TRKA protein by *in vitro* expression studies [Greco et al., 1999; Greco et al., 2000; Mardy et al., 2001] (Table 1). The effect of other missense mutations remains to be determined.

Polymorphisms

A number of polymorphisms have been reported [George et al., 1998; Cargill et al., 1999; Gimm et al., 1999; Mardy et al., 1999; Miura et al., 2000a; Shatzky et al., 2000; Bodzioch et al., 2001; Mardy et al., 2001] (Table 2). Six non-synonymous amino acid substitutions have been described. Three, R85S, H598Y, and G607V, were detected as double and triple mutations [Mardy et al., 1999]. But these three are probably polymorphisms in a particular ethnic background according to an *in vitro* expression study [Mardy et al., 2001] and various mutation and polymorphism searches [Gimm et al., 1999; Shatzky et al., 2000; Bodzioch et al., 2001]. The effect of other non-synonymous amino acid substitutions (G18E, G608V, and R774Q) remains to be determined.

Application of Intragenic Polymorphic Sites for Detection of Unexpected Mutations

The IVS7-33T>A mutation is located upstream of the consensus splice acceptor site and was detected in five chromosomes from five Japanese patients [Miura et al., 2000a]. Initial screening for *TRKA* mutations failed to identify this mutation. Analyses of intragenic polymorphic sites indicated that this mutation is linked to a very rare haplotype. Re-examination of DNA from patients revealed that the intronic mutation is involved in a putative branch-site critical for intron excision. Subsequently, the exon trap analysis showed that this causes an aberrant splicing *in vitro*. Thus, analysis of intragenic polymorphic sites do aid in identifying an unexpected mutation that can often escape detection.

BIOLOGICAL SIGNIFICANCE

Functional Studies of *TRKA* Mutants

Functional studies of *TRKA* mutants using cell lines obtained from patients are usually difficult since abundant expression of *TRKA* is limited to sympathetic or

afferent neurons that transmit painful sensations. It is feasible to analyze the *TRKA* transcript, using EB virus transformed lymphoblastoid cells [Indo et al., 1996]. However expression level is low and a nested PCR technique is needed for analysis. Seven putative splice mutations have been reported on the basis of the gene's structure (Table 1) and four are confirmed to cause aberrant splicing *in vitro* [Indo et al., 1996; Mardy et al., 1999; Miura et al., 2000a].

Eight of 14 putative missense mutations were confirmed to show diminished NGF-stimulated autophosphorylation. Two mutants (L93P and L213P) in the extracellular domain were aberrantly processed and showed diminished autophosphorylation [Mardy et al., 2001]. Five mutants (G516R, G571R, R643W, R648C, and G708S) in the tyrosine kinase domain were processed as wild type TRKA but showed significantly diminished autophosphorylation [Mardy et al., 2001]. The G571R mutant was also studied by another group [Greco et al., 2000]. The other mutant (R774P) in the tyrosine kinase domain also showed significantly diminished autophosphorylation [Greco et al., 1999]. Mutated residues (except for residue Arg-774) in the tyrosine kinase domain are conserved in various RTKs and probably contribute to critical functions of these proteins. Thus, naturally occurring *TRKA* missense mutations with loss of function provide considerable insight into the structure-function relationship in the RTK family.

A Lesson from Human *TRKA* Mutation

A characteristic feature in CIPA is anhidrosis. Sweating is important to maintain body temperature under hot environments, especially for humans. Mice lacking the gene for TRKA share dramatic phenotypic features of CIPA, including loss of responses to painful stimuli, although anhidrosis is not apparent in these animals [Smeyne et al., 1994]. Rodents probably maintain body temperature by other mechanisms such as panting or saliva-spreading rather than sweating. Mutations of the *TRKA* gene in CIPA patients suggest that TRKA is essential for innervation of eccrine sweat glands by sympathetic neurons [Indo et al., 1996]. Thus, the NGF–TRKA system probably plays a crucial role in the development and function of the nociceptive reception as well as the establishment of thermoregulation via sweating systems in humans. Elucidation of the molecular basis for the human genetic disorder provides considerable insight into formation of neural circuits and physiology in humans.

CLINICAL RELEVANCE

Genotype/Phenotype Relationship

Fundamental features of CIPA consist of a combination of anhidrosis (inability to sweat), absence of reaction to noxious stimuli, and mental retardation. Levels of mental retardation are variable among CIPA patients. Anhidrosis is explained by a loss of innervation of eccrine sweat glands by sympathetic neurons [Langer et al., 1981]. The absence of reaction to noxious stimuli is attributed to absence of small-diameter afferent neurons responsible for tissue-damaging stimuli in the dorsal ganglia [Swanson et al., 1965; Rafel et al., 1980]. The neurological basis of mental retardation remains to be elucidated. Hyperthermia, self-mutilating behavior, and repetitive trauma due to these basic features result in devastating complications, often leading to crippling or fatal consequences. Other clinical features include recurrent episodic fever, poor wound healing, limb amputation, recurrent infections, osteomyelitis, Charcot joints, corneal ulceration, and abnormal sympathetic skin response [Swanson, 1963; Swanson et al., 1965; Rosemberg et al., 1994; Shatzky et al., 2000; Indo et al., 2001]. Some patients may show a multisystem involvement besides the nervous system, including bone fracture with slow healing, immunologic abnormalities, such as low response to specific stimuli, and chronic inflammatory state ending in systemic amyloidosis [Toscano et al., 2000].

Genotype/phenotype correlation in the patients was not observed in a study of limited number of subjects [Mardy et al., 1999; Miura et al., 2000a; Indo et al., 2001]. The accumulated data favor the notion that there seems to be no genetic heterogeneity in CIPA. However, one cannot completely rule out the possibility that mutation(s) in other gene(s) are responsible for clinical phenotypes similar to CIPA, as suggested [Shatzky et al., 2000]. CIPA may present with genetic heterogeneity that can cause difficulties in locating the mutations. But a rare cause or mechanism of this autosomal recessive disorder should be considered, as described below, when a linkage analysis is performed to detect transmission of a mutant gene.

Relatively Common Mutations in Particular Ethnic Backgrounds

TRKA mutations are detected in CIPA patients from various countries and most of them are private mutations (Table 1). But a relatively common founder mutation (R548fs) was noted in the Japanese [Miura et al., 2000a]. Mutation analysis of *TRKA*

indicated that more than 50% of 46 CIPA chromosomes share the frameshift mutation. Haplotype analysis of the *TRKA* gene, based on intragenic polymorphic sites, showed that this mutation apparently shows linkage disequilibrium with a rare haplotype in normal chromosomes. These findings strongly suggest that it is a common founder mutation in the Japanese population. Another relatively common mutation (P615fs) was found in 16 of 19 unrelated CIPA families from Israeli-Bedouin people [Shatzky et al., 2000].

Application of Molecular Tools for Clinical Diagnosis of CIPA

Routine clinical and laboratory examinations usually do not show abnormalities in CIPA. Previously, the diagnosis of CIPA was established on the basis of clinical findings, pharmacological tests, and peripheral-nerve biopsy. These examinations, especially nerve biopsy, were done by a specialist familiar with these procedures, thus limiting the number of facilities where an accurate diagnosis could be made. A comprehensive strategy to screen for *TRKA* mutations has been established on the basis of the gene's structure and organization and substantial numbers of mutations have been reported from various countries, as described above. Molecular diagnosis is clearly a plausible choice for making a diagnosis.

Prenatal Diagnosis

CIPA is a painless but severe genetic disorder associated with devastating complications, often leading to crippling or fatal consequences. No specific therapy is available for CIPA because survival and maintenance of specific neurons take place during embryogenesis. Repeated profound traumatic complications, as well as hyperthermia, necessitate frequent medical treatments. Thus, care of children with CIPA causes a burden to family members. If a *TRKA* mutation responsible for CIPA is identified in a family, it is now technically possible to do prenatal genetic testing. Genetic counseling should be provided and consideration given to ethical issues when performing the procedure. Prenatal diagnoses have been requested by some families and such was done after the identification of the *TRKA* mutations and results were described [Shatzky et al., 2000].

Uniparental Disomy of Chromosome 1 as a Mechanism for Non-Mendelian Inheritance of Mutant *TRKA*

Uniparental disomy (UPD) is defined as the presence of a chromosome pair that derives from only one parent in a diploid individual [Engel, 1980]. UPD has been recognized as a genetic mechanism for non-Mendelian inheritance of autosomal recessive disease from a single carrier parent. A complete paternal isodisomy for chromosome 1 was found to be the cause of reduction to homozygosity of the *TRKA* mutation, leading to CIPA [Miura et al., 2000b]. This case was the paternal UPD for chromosome 1 in a male patient with CIPA who developed normally at term and showed no overt dysmorphisms or malformations. This finding further supports the idea that there are no paternally imprinted genes on chromosome 1 with a major effect on phenotype. Another putative paternal UPD was also reported to be the cause of reduction to homozygosity of the *TRKA* mutation, leading to CIPA [Indo et al., 2001]. Thus, when conducting genetic testing, UPD must be considered a rare but possible cause of autosomal recessive disorders.

FUTURE PROSPECTS

Mental retardation of variable severity is one of basic symptoms observed in CIPA, but the neurological basis remains to be elucidated. In *TRKA (Trk)*-deficient mice, cholinergic projections of the basal forebrain to the hippocampus and cerebral cortex are severely decreased [Smeyne et al., 1994]. The autonomic ganglia and brain appeared normal in a patient with CIPA [Swanson et al., 1965], but additional pathological studies on CIPA patients are required, including analysis of these ganglia as well as the central nervous system sites of *TRKA* expression in humans. Rapid progress in imaging techniques such as magnetic resonance imaging (MRI) or positron emission tomography (PET) used for the brain region will facilitate and support analyses of the central nervous system in CIPA.

In addition, studies on CIPA may have clinical implications in treating acquired ‘complex regional pain syndrome’ including ‘causalgia’ and ‘reflex sympathetic dystrophy.’ It is unknown why these anomalous painful conditions are induced and persist irrespective of extensive medical interventions, including analgesic procedures. In these conditions pain is often associated with abnormal skin color, temperature change, abnormal sudomotor activity, or edema [Stanton-Hicks et al., 1995], thereby suggesting an implication that the sympathetic nervous system is maintained by the NGF–TRKA system. Many factors or mechanisms may underlie these devastating clinical conditions. Accumulated studies on *TRKA* mutations in

CIPA will provide clues in the development of a specific drug that targets the clinically important pain-related conditions, as based on a structure-based design.

Further, molecular pathology of CIPA will provide unique opportunities to explore critical roles of autonomic sympathetic nervous systems as well as sensory nervous systems that transmit noxious stimuli. Both *NGF* and *TRKA (trkA)* knockout mice resulted in dramatic phenotypes [Crowley et al., 1994; Smeyne et al., 1994]. Most animals of both phenotypes die within the first month of life, and behavioral studies are hampered. Human patients with CIPA often survive into the adulthood if they receive careful medical attention. Behavioral studies of CIPA patients will reveal unexpected roles of sympathetic and afferent neurons in the human physiology.

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FIGURE LEGEND

FIGURE 1. Location of human *TRKA* mutations associated with congenital insensitivity to pain with anhidrosis (CIPA). Abbreviations listed below show the domain structures encoded by the corresponding exon(s) [Schneider and Schweiger, 1991; Indo et al., 1997]. SP = signal peptide; CC-1 and CC-2 = the first and second cysteine clusters, respectively; LRMs = leucine-rich motifs; Ig-1 and Ig-2 = the first and second immunoglobulin-like motifs, respectively; TM = transmembrane; JX = juxtamembrane; TK = tyrosine kinase. Amino acid numbering of the TRKA protein and structure of the *TRKA* gene are described, according to Martin-Zanca et al. [1989] and Indo et al. [1997], respectively. A nomenclature system for human gene mutations is used, according to the recommendations of Antonarakis and Nomenclature Working Group [1998] and den Dunnen and Antonarakis [2000]. Mutations in more than one family are indicated by a number (the number of families) in brackets.

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TABLE 1. List of Human *TRKA* Mutations

Region	Nucleotide change	Mutation Class	Mutation effect (predicted consequence)	Country (# of families)	Reference
Exon 1	c.109C>T	Nonsense	Q9X	Italy (1)	Mardy et al. [1999]
Exon 1	c.284delA	Frameshift	N67fs	Kuwait (1)	Mardy et al. [1999]
Exon 2	c.362T>C	Missense	L93P[a]	Japan (1)	Miura et al. [2000a]; Mardy et al. [2001]
Exon 4	c.475_476delTC	Frameshift	S131fs	Japan (1); Italy (1)	Miura et al. [2000a]; Indo et al. [2001]
Exon 4	c.610C>T	Nonsense	Q176X	Kuwait (2)	Indo et al. [2001]
Intron 4	IVS4-1G>C	Splice	[r.513_658del +512_513ins41][b]	UAE (1)	Mardy et al. [1999]
Exon 5	c.574G>T	Nonsense	E164X	Japan (1)	Miura et al. [2000a]
Intron 5	IVS5+1G>A	Splice	(Splice donor site)	Poland (1)	Bodzioch et al. [2001]
Exon 6	c.722T>C	Missense	L213P[a]	Canada (1)	Mardy et al. [1999]; Mardy et al. [2001]
Intron 6	IVS6+1G>C	Splice	(Splice donor site)	Japan (1)	Indo et al. [2001]
Intron 7	IVS7+1G>A	Splice	r.802_934del[b]	Kuwait (1)	Mardy et al. [1999]
Intron 7	IVS7-33T>A	Splice	r.934_935ins137[b]	Japan (5)	Miura et al. [2000a]
Exon 8	c.1008_1014del GCCGGCA	Frameshift	Q308fs	Canada (1)	Mardy et al. [1999]
Exon 8	c.1161C>A	Nonsense	Y359X	Japan (1)	Miura et al. [2000a]
Intron 9	IVS9-1G>A	Splice	(Splice acceptor site)	USA (1)	Indo et al. [2001]
Exon 13	c.1588_1589delCG	Frameshift	R502fs	Italy	Indo et al. [2001]
Exon 13	c.1596_1597ins GGGACATC	Frameshift	V505fs	Kuwait	Indo et al. [2001]
Exon 13	c.1630G>A	Missense	G516R[a]	Japan (1)	Miura et al. [2000a]; Mardy et al. [2001]
Exon 14	c.1726delC	Frameshift	R548fs	Japan (17)	Indo et al. [1996]; Miura et al. [2000a]; Miura et al. [2000b]; Yotsumoto et al. [1999]
Exon 14	c.1795G>C	Missense	G571R[a]	Japan (1)	Indo et al. [1996]; Greco et al. [1999]; Mardy et al. [2001]
Exon 14	c.1820delT	Frameshift	L579fs	Japan (1)	Miura et al. [2000a]
Exon 14	c.1825A>G	Missense	M581V	Japan (1)	Yotsumoto et al. [1999]
Exon 14	c.1870C>T	Nonsense	R596X	Japan (1)	Miura et al. [2000a]
Exon 15	c.1909G>T	Nonsense	E609X	Germany (1)	Bodzioch et al. [2001]
Exon 15	c.1915G>T	Missense	V611L	Germany (1)	Bodzioch et al. [2001]
Exon 15	c.1926_1927insT	Frameshift	P615fs	Israel (16)	Shatzky et al. [2000]
Exon 15	c.2011C>T	Missense	R643W[a]	Spain (1)	Mardy et al. [1999]; Mardy et al. [2001]
Exon 15	c.2026C>T	Missense	R648C[a]	Japan (1)	Miura et al. [2000a]; Mardy et al. [2001]
Exon 15	c.2086G>T	Missense	D668Y	Japan (4)	Miura et al. [2000a]; Mardy et al. [2001]
Intron 15	IVS15+3A>C	Splice	[r.1872_2112del +r.1966_2112del][b]	Ecuador (1)	Indo et al. [1996]
Exon 16	c.2150C>T	Missense	P689L	Israel (1)	Shatzky et al. [2000]
Exon 16	c.2206G>A	Missense	G708S[a]	Italy (1)	Mardy et al. [1999]; Mardy et al. [2001]
Exon 16	c.2210T>C	Missense	V709A	Japan (1)	Indo et al. [2001]
Exon 17	c.2337C>G	Nonsense	Y751X	Italy (1)	Indo et al. [2001]
Exon 17	c.2347C>T	Missense	R755W	Italy (1); Netherlands (1)	Indo et al. [2001]; Indo et al. [2001]
Exon 17	c.2393_2394insT	Frameshift	D770fs	Japan (1)	Miura et al. [2000a]
Exon 17	c.2405G>C	Missense	R774P[a]	Italy (1)	Greco et al. [1999]

Positions of nucleotide change are from the transcription start site, as described in Martin-Zanca et al. [1989]. The ATG initiation codon is located at nucleotide position c.85. Structure and organization of human *TRKA* were described [Indo et al., 1997]. A nomenclature system for human gene mutation is used, according to the recommendations of Antonarakis and Nomenclature Working Group [1998] and den Dunnen and Antonarakis [2000].

[a]Missense mutations that have been confirmed to cause defects in NGF-stimulated autophosphorylation of the TRKA protein by in vitro expression studies.

[b]Splice mutations that have been confirmed to cause aberrant splicing.

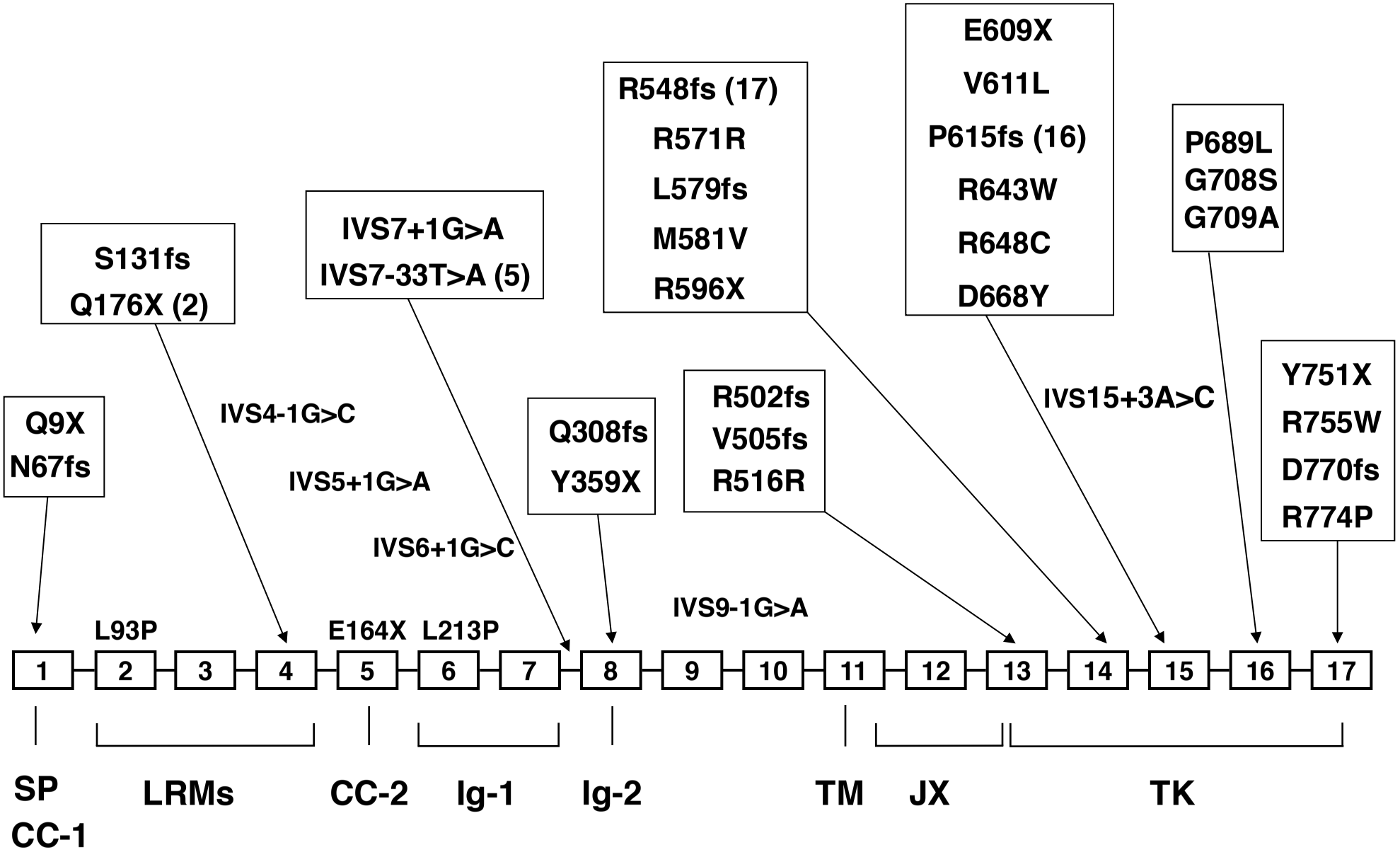
TABLE 2. List of Human *TRKA* Polymorphisms

Region	Polymorphisms	Effect (predicted consequence)	NCBI assay ID	Reference
Promoter	-108 G/T	Unknown	ss1469255	
Exon 1	c.137 G/A	G18E	ss1469256	
Exon 2	c.337 C/A	R85S[a]		Mardy et al. [1999]; Mardy et al. [2001]
Intron 2	IVS2+49 G/T	Unknown	ss2421028	Miura et al. [2000a]
Intron 2	IVS2+84 G/A	Unknown	ss2421027	Miura et al. [2000a]
Exon 3	c.399 C/T	F105F	ss7968	Cargill et al. [1999]
Intron 4	IVS4+12 G/A	Unknown		Gimm et al. [1999]
Intron 5	IVS5+100 C/T	Unknown	ss2421029	Miura et al. [2000a]
Intron 10	IVS10-36 G/C	Unknown	ss7961	Cargill et al. [1999]
Intron 13	IVS13+118 T/C	Unknown	ss2421030	Miura et al. [2000a]
Exon 14	c.1740 G/A	Q552Q	ss7962	George et al. [1998]; Cargill et al. [1999]; Gimm et al. [1999]; Miura et al. [2000a]
Exon 14	c.1794 C/T	F570F	ss7963	Cargill et al. [1999]
Intron 14	IVS14-4A/delA	Unknown	ss2419780	Gimm et al. [1999]; Miura et al. [2000a]
Exon 15	c.1876 C/T	H598Y[a]	ss7964	Gimm et al. [1999]; Cargill et al. [1999]; Mardy et al. [1999]; Mardy et al. [2001]
Exon 15	c.1904 G/T	G607V[a]	ss7967	Gimm et al. [1999]; Cargill et al. [1999]; Mardy et al.[1999]; Shatzky et al. [2000]; Mardy et al. [2001]
Exon 15	c.1907 G/T	G608V		Bodzioch et al. [2001]
Exon 15	c.1926 C/T	G614G	ss7966	Cargill et al. [1999]
Intron 15	c.1953 C/T	A623A	ss7965	George et al. [1998]; Cargill et al. [1999]; Gimm et al. [1999]; Miura et al. [2000a]; Shatzky et al. [2000]
Intron 15	IVS15-16 T/C	Unknown		George et al. [1998]; Gimm et al. [1999]
Exon 16	c.2118 A/G	G678G		Shatzky et al. [2000]
Exon 17	c.2405 G/A	R774Q		Gimm et al. [1999]
Exon 17	c.2488 C/T	Unknown	ss1301099	

Positions of nucleotide change are from the transcription start site, as described in [Martin-Zanca et al., 1989]. The ATG initiation codon is located at nucleotide position c.85. Structure and organization of human *TRKA* have been documented [Indo et al., 1997].

[a]Non-synonymous amino acid substitutions that have been confirmed to cause NGF-stimulated autophosphorylation of the TRKA protein by *in vitro* expression studies [Mardy et al., 2001].

NCBI Assay ID, (http://www.ncbi.nlm.nih.gov/SNP/).


E609X
V611L
P615fs (16)
R643W
R648C
D668Y
R548fs (17)
R571R
L579fs
M581V
R596X
P689L
G708S
G709A
S131fs
Q176X (2)
IVS7+1G>A
IVS7-33T>A (5)
Y751X
R755W
D770fs
R774P
Q9X
N67fs
IVS4-1G>C
IVS5+1G>A
IVS6+1G>C
Q308fs
Y359X
R502fs
V505fs
R516R
IVS15+3A>C
IVS9-1G>A
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LRMs
CC-2
Ig-1
Ig-2
TM
JX
TK