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HotSpots

A life without pain? Hedonists take note

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An *SCN9A* channelopathy causes congenital inability to experience pain
Cox et al. (2006)
Nature 444: 894–898

Loss-of-function mutations in the $Na_v1.7$ gene underlie congenital indifference to pain in multiple human populations
Goldberg et al. (2007)
Clin Genet 71: 311–319

A stop codon mutation in *SCN9A* causes lack of pain sensation
Ahmad et al. (2007)
Hum Mol Genet 16: 2114–2121

'We cannot learn without pain.' – Aristotle

When most of us think of pain, we think of suffering. Most of us would not complain if offered a chance to eliminate it altogether. However, the physiological sensation of pain, otherwise known as nociception, serves as an adaptive way of avoiding harmful situations, as unpleasant as the experience may be. Organisms are conditioned to avoid adverse stimuli that cause them pain and potentially threaten their survival.

A series of recent papers describe patients who are unable to experience physical pain, and each group independently identifies the genetic locus responsible. Cox et al. described the index case of a 10-year-old Pakistani boy who regularly performed 'street theatre' that involved walking on burning coals or placing knives through his limbs. Unfortunately, this boy passed away before being assessed on his 14th birthday after jumping off a house rooftop. Other patients experience various types of injury, including self-mutilating oral and facial injury, burn injuries, and orthopedic complications resulting from undetected fractures.

Cox et al. were the first group to describe $Na_v1.7$ loss-of-function mutations in humans, and termed this condition as 'channelopathy-associated insensitivity to pain' (OMIM 243000). This term was chosen over the alternatives such as 'congenital indifference to pain' ((CIP); described in Goldberg et al.) for reasons described in detail by Cox et al. However, for simplicity, this condition will be referred to here as 'CIP'. CIP is an autosomal recessive disorder in which those affected lack the physical sensation of pain. They are indifferent to a variety of adverse stimuli that would normally result in inflammatory, burning, or visceral pain. Tactile stimuli are perceived but not described as painful. Other sensory modalities, such as proprioception, reflexes, and autonomic responses are normal. Patients are of normal intelligence, and the neurological examination is largely unremarkable, save for the absence of nociception. The features of CIP are distinct from other similar conditions. For example in congenital insensitivity to pain (OMIM 608654), also known as hereditary sensory and autonomic neuropathy type 5, impaired pain perception is accompanied by disturbances in other neurological modalities.

Studies in mice have found that a global knockout of $Na_v1.7$ results in early lethality, apparently owing to feeding difficulties. A 'nociceptor-specific' $Na_v1.7$ knockout mouse was created using a Cre-lox system and was viable and appeared normal. Pain thresholds to mechanical and thermal pain were increased, and responses to inflammatory pain stimuli were reduced or absent. Subsequent study demonstrated that mice lacking both $Na_v1.7$ and $Na_v1.8$ (another sodium channel implicated in nociception) in nociceptors demonstrated that while $Na_v1.7$ appears to be critical in inflammatory pain response, the development of neuropathic pain does not appear to require either $Na_v1.7$ or $Na_v1.8$. The early lethality of the global $Na_v1.7$ knockout mouse stands in contrast to the human CIP patients described by these three author groups, who appear normal apart from their inability to perceive pain. The tissue expression studies performed by Ahmad et al. described below may begin to explain the difference between species.

Three papers published within several months of each other have independently identified

mutations responsible for CIP. All three groups located the genetic locus to chromosome 2q and subsequently identified autosomal recessive loss-of-function mutations in *SCN9A*, which encodes the alpha subunit of a voltage-gated sodium channel, $Na_v1.7$. This channel is most highly expressed in the peripheral nervous system, most notably in sympathetic neurons and sensory neurons of the dorsal root ganglion.

Each author group independently performed a genome-wide scan, identifying a region of linkage on chromosome 2q. This region contains five genes encoding alpha subunits of voltage-gated sodium channels, two of which authors identified as being previously implicated in nociception (*SCN9A* and *SCN3A*). Bioinformatic analysis and/or functional candidate analysis pointed to *SCN9A* as the probable candidate gene. Sequence analysis led to identification of nonsense mutations in *SCN9A* in all patients, absent from all control chromosomes but present in clinically normal, heterozygous relatives. Goldberg et al. also identified two families of compound heterozygotes in which each mutation also appeared to result in a loss-of-function. Notably, Goldberg et al. identified a total of nine CIP families from seven different countries in their study. Table 1 provides further details on each study.

Cox et al. and Ahmad et al. evaluated the functional consequences of the nonsense mutations *in vitro*. Both groups performed patch-clamp analysis on cells transfected with wild type or one of the mutant $Na_v1.7$, and in both cases the mutant channel showed no difference in activity over background current, supporting the probable loss-of-function hypothesis.

Ahmad et al. also performed transient transfection of the mutant $Na_v1.7$ cDNA in a cell line endogenously expressing voltage-gated sodium channels. These transfected cells also demonstrated no difference over background, suggesting that the truncated protein is neither functional as an ion channel by itself nor responsible for compensatory effects on expression of other voltage-gated sodium channels. Ahmad et al. also examined the expression pattern of $Na_v1.7$ in rodents and primates in order to determine whether differential expression may explain the lethal phenotype in $Na_v1.7$-deficient mice. $Na_v1.7$ mRNA was expressed in both rat and mouse in the paraventricular hypothalamic nucleus and the supraoptic nucleus but was absent in both monkey and human tissue. Expression in the pituitary and adrenal glands was also notably present in both rodents species but absent in humans. The authors conclude that the differential expression of $Na_v1.7$ between rodents and humans may explain the lethal phenotype observed in mice, and that the channel may serve a slightly different function in the two species, despite its high degree of similarity.

Some subtle differences exist between the authors' reports. For example Goldberg et al. report the presence of anosmia or hyposmia (absence or reduction in smell) in their patients. It is unclear whether patients were assessed for anosmia in the other studies. Cox et al. report

Table 1. Comparison of findings from three papers describing CIP cases, published within several months

Study	Ethnic group	Patient identifier	cDNA genotype	Exon	Protein mutation	Comments
Cox et al.	Pakistan	Family 1	2691G→A	15	W897X	
		Family 2	2298delT	13	I767X	
		Family 3	1376C→G	10	S459X	
		Index[a]	ND	15	W897X	Index case deceased prior to assessment
Goldberg et al.	France	CIP-8	2488C→T	15	R830X	
	Argentina	CIP-10	5067G→A	26	W1689X	Families used to define CIP locus
	Canada	CIP-14	984C→A	8	Y328X	
	Switzerland	CIP-26	829C→T	6	R277X	
	United States	CIP-32	4462C→T	24	R1488X	
	Switzerland	CIP-33	829C→T	6	R277X	
	Italy	CIP-38	3600delT	19	F1200LfsX33	
	USA	CIP-05	2076_2077insT	13	E693X	
		CIP-05	4366-7_10delGTTT	Int. 23–24	Splice junction mutation	
	UK	CIP-102	3703_3713del	19	I1235LfsX2	
		CIP-102	4975A→T	26	K1659X	
Ahmad et al.	Canada[b] (Newfoundland)	N/A	984C→A	8	Y328X	

CIP, congenital indifference to pain.
[a]DNA was not available from the index case, however his mother tested positive for W897X.
[b]Also known as CIP-14, assessed by Goldberg et al.

normal proprioception despite 'being *ungainly* in gross motor movements', and it is not clear exactly what this means. Most interestingly, three of the four patients described by Ahmad et al. report sensation of pain from adolescence onward (the fourth patient was assessed at 3 years of age). Goldberg et al. does not report this finding in this family. Whether this represents true nociception or a learned behavior is not known. Re-examination of these individuals would be ideal; however, the patients declined consent for further study. Notably, Cox et al. report that older affected individuals understood the circumstances normally resulting in pain and would 'act as if in pain' in appropriate circumstances (for example, a soccer game).

All of the observed mutations appear to result in loss-of-function of $Na_v1.7$. Two patients assessed by Goldberg et al. were compound heterozygotes. One of these families was a compound heterozygote for two different mutations leading to protein truncation, while the second demonstrated a truncating mutation and a splice junction mutation that remained functionally unassessed.

In summary, all of the CIP patients assessed are homozygous or compound heterozygous for nonsense mutations at various locations throughout *SCN9A*, resulting in an early stop codon. Both Cox et al. and Ahmad et al. performed functional studies to demonstrate that no functional protein is produced, as demonstrated by patch-clamp studies of the mutant $Na_v1.7$. Published within the span of several months, these independent studies highlight that this phenotype occurs in several different populations from around the world. The number of different mutations seen suggest that most mutations that occur are private and/or occur in consanguineous families, and are therefore identical by descent. Identification of the locus involved in CIP in these patients validates *SCN9A* for genetic testing in patients presenting with similar findings. Most importantly, these studies establish $Na_v1.7$ as a critical component of nociception and highlight the potential for this protein in development of pharmacological analgesic agents. Fascinating to both the clinician and the geneticist, these patients furthermore underline the important adaptive purpose served by nociception.