Research Paper

Genetic Mediation of Neuropathic Phantom Limb Pain

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1. **Introduction**

This is a review of the genetic mediation of neuropathic pain, with a focus on phantom limb pain. Neuropathic pain is different from nociceptive pain. Nociceptive pain is the perception of physiological pain - damage to the body as communicated by the nerves. Neuropathic pain is, loosely, nerve damage. Phantom limb pain is a particular type of nerve damage. Phantom limb pain can occur after amputation of the arm, leg, penis, etc. It occurs most frequently after arm or limb amputation.

Pain involves genes. Some people have a genetic predisposition to feel no pain at all. In answer to the obvious question, it's a bad thing. They usually die young from infection and damage to their bodies. Congenital Indifference to Pain is, as the name suggests, genetic. The gene *TRKA* is involved in extreme form of this pathology, Congenital Insensitivity to Pain With Anhidrosis (Yotsumoto et al, 1999). The question at hand in this literature review is another genetic mediation, the genetic mediation of phantom limb pain. A study of 2500 amputee veterans re-

ported that 22% did not report phantom limb pain (Sherman et al, 1984). This variability implies some genetic mediation.

This review concerns the genetic mediation of neuropathic pain in general and the specific case of phantom limb pain. Neuropathic pain is less studied than nociceptive pain, but there has been interesting research using microarrays, lab rats, and PCR. The genetic mediation of phantom limb pain is sparsely studied. The main findings in this review are primarily from a mouse model of phantom limb pain—autotomy. This model has its limitations and contradictions and it will be criticized.

2. Genetic Mediation of Neuropathic Pain

As mentioned, neuropathic pain is colloquially known as nerve damage. Acute pain is adaptive, like the burning sensation that causes you to pull your hand away from a flame. Chronic neuropathic pain, on the other hand, is largely maladaptive. It is better viewed as a debilitating disease. There is no external stimulus that a person feeling neuropathic pain needs to respond to, they simply suffer.

One of the crucial sites for neuropathic pain is the dorsal root ganglia (DRG), a spot in the spinal cord where the peripheral nervous system hooks up to the thalamus and brain. Using gene chips, scientists have been able to observe the gene transcription that goes on in the DRG. The DRG must produce proteins to build or do anything, and the blueprint for those proteins is DNA. When DNA is transcribed it is copied over and over, so what's floating around in the cell is not DNA itself but copies, like mRNA. The mRNA can be detected and measured, and that tells us what genes are being transcribed. This process is greatly spread up by microarrays. Microarrays

are manufactured gene chips that store a copy of all the known DNA for a particular strain of rat, mouse, or human. Velder, Liu et al used Affymetrix gene chips to identify the genes involved in neuropathic allodynia (hypersensitivity) for Harlan and Holtzman rats (2003). They induced injury to the sciatic nerve on one side and compared the genes being regulated on that side to the genes being regulated on the other uninjured side. They found 8 and 27 genes that were upregulated for the Harlan and Holtzman rats, respectively. 101 and 88 genes were downregulated, respectively. This paper is concerned primarily with phantom limb pain, so it will limit its discussion of these results. One of the notable upregulated genes is *Bzrp*, notable because it's also implicated in phantom limb pain. Velder et al confirmed their results using PCR techniques as well. Wang et al performed similar microarray analysis on Sprague-Dawley rats (2002). A parallel study by Beltramo et al found upregulation of *MC4* and *POMC*, involved in the melanocortin system (2003). They suggested that melanocortin antagonists are analgesic and agonists are hyperalgesic, which opens another venue of research.

Most research, however, is focused on opiods and trying out new opiod drugs. Opoids like morphine have been used for acute pain since the Civil War. Their use for chronic neuropathic pain is controversial given the addictive properties of opiods. Subjects also habituate, requiring higher doses over time. Opiods, however, remain the best analgesic available. The body has three major classes of opioid receptors: μ, κ and δ. The μ receptor (the μ represents morphine) is the most important. The *Oprm* gene in mice encodes the murine μ receptor, and it is located near chromosome 10. Jeffrey Mogil et al have found differences in opiod efficacy on mice with polymorphisms of this gene, or promoter region nearby. His team also identified *Htr1b* as a candidate gene which affects opiod efficacy. *Htr1b* encodes for the serotonin 1B receptor. The effectiveness of opiods is genetically mediated—they simply do not work for some people, and some people

don't seem to need them.

3. Genetic Mediation of Phantom Limb Pain

Is phantom limb pain genetically mediated at all? Neuropathic pain, as discussed, is often genetically mediated. Furthermore, the fact that 550 out of 2500 veterans did not report phantom limb pain implies that genetic polymorphisms could be involved. This is by no means evidence, and it does not mean that any particular gene *causes* phantom limb pain. Many environmental factors could account for the variation in phantom limb pain. Some of the variation may, however, be attributed to genetic factors. This question has not been adequately studied in human subjects. The majority of research has been done on mice, specifically *Mus musculus*, and even this research in inconclusive and contradictory at times.

The broadest hint of genetic mediation is when multiple family members display a trait. Congenital Insensitivity to Pain, for example, has been found in multiple members of Saudi (Karkashan 2002) and Japanese (Yotsumoto et al 1999) families. Unfortunately, the study of phantom limb pain is largely restricted to mouse families. As a further complication of mouse studies, mice do not report phantom limb pain. Researchers must use the indirect measure of how much of their denervated limb they chew off (autotomy). This section will first discuss the validity of the mouse model and autotomy, and then discuss the results of the studies under review.

3.1 Validity of Mouse Model

The ideal way to study genetic mediation of phantom limb pain is to clone hundreds of hu-

mans, manipulate their genes, and amputate their limbs. Then scientists could document the results of various changes in the genome on phantom limb pain and compare these results to cloned controls. This is wholly unethical. In order to not be Nazis, scientists study our distant cousin– the mouse. Mice and humans shared a common ancestor around 75 million years ago. "A full 99 percent of a mouse's genes have counterparts in humans, including genes that cause mice to have tails. In fact, researchers said they have identified only 300 genes that are unique to either creature." (Wired, 2002). The genes are very much the same, just reshuffled into different locations. The publication of the human and mouse genome makes it possible to match mouse genes to their human counterparts. It is important to note that while 99 percent of our genes may be the same, subtle differences in the genome (location and content) can lead to drastically different protein synthesis, and proteins—not DNA—build the organism. Mice can also be inbred within an inch of their lives – breeding brother with sister until every mouse is homozygous for every trait. These mice are virtual clones and they have been giving birth to genetically identical progeny for decades now. Mice also breed very fast so you can get a sample of 200 identical subjects very quickly. This type of experimental control is impossible with humans or even apes.

Mouse studies are more reliable than human studies, but less valid. The degree of experimental control is high, but the results can't necessarily be applied to humans. Despite this limitation, mouse studies are currently the best way to study phantom limb pain. Twin and family studies are popular ways of studying genetic influence, but twins or family members who both have amputations are rare. It is possible to perform controlled studies of amputees, or simply studies with large samples (as in Sierra Leone). However, people in a population—even when grouped into similar demographics— have massively heterozygous and diverse genes. It is impossible to make causal or even correlational statements about genetic influence with confidence. This is why

all the studies cited here regarding the genetic influence on phantom limb pain come from mice.

3.2 Validity of Autotomy as a Model of Phantom Limb Pain

Autotomy is not phantom limb pain. Autotomy is a reaction which an animal has to pain – basically chewing off its own leg. Researchers usually induce autotomy by cutting the sciatic and saphenous nerves in the leg, effectively denervating the limb. The same nerves would be cut if the limb was amputated, but then there could be no measure of phantom limb pain. Leaving the limb allows researchers to count the number of toes chewed off, etc.

Autotomy and phantom limb pain are not one and the same, but there are numerous correlations. Autotomous mice have significantly higher levels of corticosterone than non-autotomous mice, indicating that they feel the high stress levels associated with chronic pain. The same drugs that treat chronic pain also decrease autotomy. "The relationship between autotomy and pain is strengthened by the finding that self-mutilation following deafferentation in animals is blocked by pharmacological agents which reduce chronic pain in humans. As mentioned above, guanethidine treatments cause a significant decrease in autotomy in mice and rats" (Coderre et al 1986).

3.3 Experimental Results

Using artificial selection, Devor and Raber were able to breed two mouse populations—one which displayed high levels of autotomy, and one which displayed low levels of autotomy (Devor & Raber 1990). In order to track the gene they crossed the high (HA) and low (LA) strains. In this case the F1 hybrid showed low autotomy, meaning that the gene causing high autotomy is most likely recessive. They the backcrossed F1 hybrids with the HA line. If the trait is recessive,

mendellian inheritance would predict 50% high autotomy. This was indeed the case. "Taken together, the data suggest that autotomy is inherited as a single-gene autosomal recessive trait" (Devor & Raber).

Jeffery Mogil et al have measured autotomy levels of existing mouse strains, without slectively breeding for the trait. He and his team evaluated 11 inbred strains of mice (genetically identical) and found different levels of autotomy. Some strains like C57BL displayed high autotomy, chewing off many of their toes (presumambly because they are in such pain). Strains like AKR, C58 and A chew off little or none of their foot, implying that they feel little pain in the phantom. The environments of these mice are highly controlled, so these differences are attributable to genetic influence.

However, strangely enough, Seltzer et al attempted to replicate this work and found the exact opposite results (2001). In their study C57BL had the lowest score for autotomy while A had a high score for autotomy. This direct contradiction of the Mogil study is puzzling to both researchers. For the purposes of this paper, I will discuss the research by Seltzer et al in depth because they performed the full analysis to identify the genes involved in phantom limb pain. The results, however, remain questionable until they are replicated.

In order to compare, Seltzer bred A and C57BL (referred to as B) lines to produce hybrids: AXB-BXA. Using a hybrid line makes it possible to compare and contrast the effects of alleles inherited from different parents. Seltzer et al used a database of 395 markers to make a Quantitative Trait Locus (QTL) map of the AXB-BXA genome. They found "a very strong contrast in frequency of autotomy between the two groups of RI lines which inherited the A versus the B progenitor allele for marker D15Mit28 ... This marker is located on chromosome 15 at a position 43.7 centimorgans (cM) from the telomere" (Seltzer et al). Several genes within 2 cM of D15Mit28 are

candidates that could explain some of the autotomy variance between A and B strains. I have paraphrased their descriptions from Seltzer's study:

- ***Pva*** : This gene encodes the protein parvalbumin, a natural neuroprotectant against input excitotoxity triggered by NMDA-mediated injury discharge emitted by damaged sensory fibers (Dubner 1991).
- ***Bzrp*** : This is a benzodiazepine receptor whose expression increases following neural injury (Raghavendra et al 2000) and inescapable stress (Lehmann et al 1999). The drug diazepam (brand name: Valium) binds to these receptors and can reduce autotomy in mice.
- ***Emo2*** : This QTL is associated with anxiety, fear and depression (Turri et al 1999).
- ***Gal3*** : This the receptor for galanin, a neuropeptide connected to autotomy (Ji et al 1999) and nociceptive processing (Kerr et al 2000) of peripheral nerve injury.
- ***Il2rb*** : This gene may be involved because it is receptor for the interleukin 2 receptor β. Xu et al, 1997 performed a knockdown of interleukins—that is, prevented their transcription—and found that this increased autotomy.

These results show that neuropathic pain is more complex than acute pain, which can usually be treated adequately with opiods. During autotomy the severed nerves often branch out randomly trying to reconnect and form a neuroma, a malformed tangle of nerves. This neuroma can often emit excitotoxic signals, which may be soothed by *Pva*. Perhaps a novel analgesic could have similar effects. *Bzrp* demonstrates an interesting link between inescapable stress and phantom limb pain and points to Valium as a relevant analgesic. *Bzrp* is also indicated in the microarray studies cited in the neuropathy section, meaning it is implicated in neuropathic pain in general. *Emo2*

shows that phantom limb pain may be linked to generalized anxiety and stress … though these could be coincidental. Galanin and interleukins also point to new venues of research. These genes listed, however, are only best guesses based on the general region that was implicated. There are many genes on that region of chromosome 15, and further knock-down, knockout, etc studies would be needed to make any conclusions. Furthermore, these results must be taken with a grain of salt until the striking contradiction with the Mogil data is resolved. One of the studies needs to be replicated, and often.

4. Conclusion

Both phantom limb pain and neuropathic pain are new venues of study. They both should be studied so that someday we may alleviate peoples' suffering. Thanks to modern body armor fatal casualties have dropped in Iraq. Today the greatest number of casualties in Iraq are limb wounds, which are frequently amputated. Anywhere from 70-90% of these soldiers may be experiencing phantom limb pain. Modern microarray research has shown that the genetic factors mediating neuropathic pain are much more complicated than opiods alone can address. In phantom limb pain factors as diverse as galanin and excitotoxicity caused by NMDA are implicated. The research on the mouse model is still unreliable as it seems to resist replication. The only cure for this is more studies. The validity of the mouse model is limited as well. At the time of this writing I could find no corresponding studies of phantom limb pain in humans, probably because the experimental design remains difficult. The subject is, however, worthy of study.

References

1. Associated Press (2002). "Man or Mouse Genome?". *Wired News*. Retrieved 4 April 2004. URL=http://www.wired.com/news/medtech/0,1286,56723,00.html
2. Beltramo M, Campanella M, et al. "Gene expression profiling of melanocortin system in neuropathic rats supports a role in nociception". *Molecular Brain Research, Vol 118*, 111-118.
3. Coderre T, Grimes R, Melzack R (1986). "Deafferentation and Chronic Pain in Animals: an Evaluation of Evidence Suggesting Autotomy is Related to Pain". *Pain, Vol 26*, 61-84.
4. Devor M, Raber P (1990). "Heritability of Symptoms in an Experimental Model of Neuropathic Pain". *Pain, Vol 42*, 51-67.
5. Dijkstra, P; Geertzen, J et al. "Phantom Pain and Risk Factors: A Multivariate Analysis". *Journal of Pain and Symptom Management, Vol 24*, 578-585.
6. Dubner R (1991). "Pain research and clinical management." In: Bond MR, Charlton JE, Woolf CJ, editors. *Proceedings of the VIth world congress of pain, Vol. 6*, 263-276.
7. Ji RR, Zhang Q, Bedecs K, et al (1994). "Galanin antisense oligonucleotides reduce galanin levels in dorsal root ganglia and induce autotomy in rats after axotomy". *Proc Natl Acad Sci USA, Vol 91,* 12540-12543.
8. Karkashan, E (2002). Congenital Insensitivity to Pain in Four Related Saudi Families". *Pediatric Dermatology, Vol 19*, 333-335.
9. Kauppila T (1998). "Correlation between autotomy-behavior and current theories of neuropathic pain". *Neuroscience and Biobehavioral Reviews, Vol 23*, 111-129.
10. Kerr B, Cafferty W, Gupta Y, et al (2000). "Galanin knockout mice reveal nociceptive deficits following peripheral nerve injury". *Eur J Neurosci Vol 12*, 793-802.
11. Lehmann J, Weizman R, Pryce C, et al (1999). "Peripheral benzodiazepine receptors in cerebral cortex, but not in internal organs, are increased following inescapable stress and subsequent avoidance/escape shuttle-box testing". *Brain Res, Vol 851*, 141-147.
12. Mogil J, Sternberg W, Marek P, et al (1996). "The genetics of pain and pain inhibition". *Proceedings of the National Academy of Sciences, Vol 93*, 3048-3055.
13. Mogil J (1998). "The genetic mediation of individual differences in sensitivity to pain and its inhibition". *Proceedings of the National Academy of Sciences, Vol 96*, 7744-7751.

14. Mogil J, Wilson S, et al (1999). "Heritability of Nociception I: Responses of 11 Inbred Mouse Strains on 12 Measures of Nociception". *Pain, Vol 80*, 67-82.
15. Mogil J, Yu L, Basbaum A (2000). "Pain Genes?: Natural Variation and Transgenic Mutants". *Annual Review of Neuroscience, Vol 23*, 777-811.
16. Raghavendra Rao VL, Dogan A, et al (2000). "Traumatic brain injury leads to increased expression of peripheral-type benzodiazepine receptors, neuronal death, and activation of astrocytes and microglia in rat thalamus". *Exp Neurol, Vol 161*, 102-114.
17. Ramachandran, V.S.; Hirstein, W (1998). "The Perception of Phantom Limbs: The D.O. Hebb Lecture". *Brain, Vol 121,* 1603-1630.
18. Seltzer Z, Wu T, Max M, Diehl S (2001). "Mapping a Gene for Neuropathic Pain-Related Behavior Following Peripheral Neurectomy in the Mouse". *Pain, Vol 93,* 101-106.
19. Sherman R, Sherman C, Parker L (1984). "Chronic Phantom and Stump Pain Among American Veterans: Results of a Survey". *Pain, Vol 18*, 83-95.
20. Turri M, Talbot C, Radcliffe R, et al (1999). "High-resolution mapping of quantitative trait loci for emotionality in selected strains of mice". *Mamm Genome, Vol 10*, 1098-1101.
21. Valder C, Liu J, Song Y, Luo Z (2003). "Coupling Gene Chip Analyses and Rat Genetic Variances in Identifying Potential Targe Genes That May Contribute to Neuropathic Allodynia Development". *Journal of Neurochemistry, Vol 87*, 560-567.
22. Wang H, Sun H, et al (2002). "Chronic neuropathic pain is accompanied by global changes in gene expression and shares pathobiology with neurodegenerative diseases." *Neuroscience, Vol 114*, 529-546.
23. Xu XJ, Hao JX, et al (1997). "Nociceptive responses in interleukin-6-deficient mice to peripheral in¯ammation and peripheral nerve section". *Cytokine, Vol 9*, 1028-1033.
24. Yotsumoto S, Setoyama M, Hozumi H, et al (1999). "A Novel Point Mutation Effecting the *TRKA* Gene in a Family With Congenital Insensitivity to Pain With Anhidrosis". *Journal of Investigative Dermatology, Vol 112*, 810-817.
25. Zimmermann, M (2001). "Pathobiology of Neuropathic Pain". *European Journal of Pharmacology, Vol 429*, 23-37.