Study to identify the genetic variations associated with phantom limb pain

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Ethical review	Approved WMO
Status	Will not start
Health condition type	Bone disorders (excl congenital and fractures)
Study type	Observational invasive

Summary

ID

NL-OMON43785

Source
ToetsingOnline

Brief title
Phantom Pain Genetics study

Condition

- Bone disorders (excl congenital and fractures)
- Vascular disorders NEC

Synonym
Pain after amputation

Research involving
Human

Sponsors and support

Primary sponsor: Leids Universitair Medisch Centrum
Source(s) of monetary or material Support: Walter Reed centrum USA

Intervention

Keyword: genetics, phantom pain

Outcome measures

Primary outcome

The primary goals of this study are to:

i. Identify genetic variations responsible for PLP across the whole genome

More than 90% of SNPs are found in introns or intergenic regions and most of them are probably of little functional consequence. However, SNPs in promoter regions can alter the affinity of DNA binding proteins and modify the level of gene expression. Other SNPs in exon / intron boundaries result in intron retention or exon skipping, thus profoundly changing the structure of the resulting protein. It is also possible that any genomic region where the SNP is located can function as an RNA interference element. The existence of SNPs in any portion of genomic DNA can be meaningful for the phenotype because of the complicated dynamics of DNA structure and gene expression. Genome wide association study along with multi-layer designed follow up study has been applied to clinical pain research. The advantage of this approach is that no specific functional genetic hypotheses are required prior to undertaking analysis.

ii. Identify the gene expression profiles between individuals with and without PLP using microarray technology.

It is suggested that analyzing gene expression profile will have clinical applications to neurological diseases in humans. Recently published studies have shown that there are specific gene expression profiles in the peripheral

blood of patients with various types of neurological diseases. The working hypothesis is that differences in gene expression profiles after major limb amputation produce a unique phenotype through molecular interactions. Characteristics of PLP such as presence and severity of phantom pain may be modulated by changes in gene expression over the time in the post-amputation period. We will test the hypothesis that the gene expression profiles are different between PLP and non-PLP amputee patients.

Secondary outcome

The secondary goal of this study is to:

Identify predictive factors for the prognosis of PLP among the interactions between genetic variations, gene expressions, protein levels and other physiologic variables from the integrative genomic-phenomic analyses.

The analyzed data from primary goals will contribute to the analysis for secondary goal. This integrative genomic-phenomic analysis can generate hypothesis for underlying pathways suggesting how gene expression changes in the serum can be linked to neurological diseases such as PLP. Phenotypic data will be determined from elements of the patient questionnaire used as potential covariates to classify PLP. The tertiary goal of this study is to:

Verify candidate SNPs that are associated with PLP severity or duration in a group of non-Caucasian subjects.

After identifying SNPs that are associated with PLP severity or duration in an ethnically homogenous sample, we will determine whether these findings can be generalized across different ethnic populations by comparing our genomic and proteomic findings from Caucasian and non-Caucasian subjects. The DNA and RNA

analysis will be the same for both groups, and we will use principal component analysis to determine if SNPs identified in the Caucasian group are similar for non-Caucasian subjects. While previous research has suggested that frequency of genomic markers vary among different ethnic groups, there has not been any evidence to suggest an association between PLP and ethnicity. We therefore hypothesize that there will be no difference between PLP associated genes in the Caucasian group and the non-Caucasian group.

Study description

Background summary

BACKGROUND AND SIGNIFICANCE

Literature Review. Almost immediately after the loss of a limb, 90-95% of all patients with major limb amputations experience a vivid phantom limb sensation, such as warmth, cold, itching, pressure, and sense of position (1). When the sensations become intense enough to define as painful, they are referred to as phantom limb pain (PLP). PLP occurs in 80-90% of amputees and usually appears immediately following awakening from anesthesia, but may be delayed up to a few days or weeks in 25% of patients. Onset is not affected by the limb amputated or site of amputation (2). In most cases the phantom is present for a few days or weeks, then gradually fades from consciousness. However, 30-70% of amputees have pain persisting for years or decades, with pain persisting greater than six months becoming more difficult to treat (2, 3). The causes of PLP and non-painful phantom phenomena are not known; both peripheral and central processes have been implicated (4). Some patients are able to recall a phantom limb at will after its disappearance by intense concentration or rubbing the residual limb (5). Memories of the limb*s posture and form prior to amputation often survive in the phantom (6, 7). The study principal investigator observed a patient injured by an improvised explosive device (IED) who was holding his rifle prior to the injury. This patient reported his amputated index finger stuck in the firing position on the trigger, giving him cramping pain for six weeks afterwards. After a period of several weeks, a patient*s phantom limb sometimes fades from consciousness and disappears completely. Additionally, in approximately 50% of cases, and especially in upper limb amputees, the arm progressively becomes shorter until the subject is left with just the phantom hand alone, dangling from the stump,

a phenomenon that has been termed *telescoping* (8, 9). Ultimately, PLP is remarkably difficult to treat, with several reports of failed drug trials in the clinical literature (3, 4).
Human Subjects Justification. A more general problem in new analgesic development is that many drugs that have appeared to relieve pain in rats have failed in humans. Some researchers believe technical improvements in animal models of pain will lead to a better understanding of the etiology of pain in humans. However, there may be interspecies differences in the neurochemical mechanisms of pain, and a more fundamental solution may require the integration of human data into the early drug development triage process. Clinical genetic association studies are one such approach. For example, a polymorphism that decreases the function of a molecule was associated with reduced pain in patients exposed to similar injuries. It might be reasonable to expect that a drug inhibiting that molecule would prevent, or even relieve, pain in humans.

Genetic variations: We believe that identifying single nucleotide polymorphisms (SNPs) between patients with phantom limb pain and those without may indicate why human pain perception is highly variable from one individual to the next. SNPs are DNA sequence variations that are usually of no consequence; however, some SNPs may predispose individuals to certain diseases or alter the intended effect of a particular drug. There is previous research that suggests a link between phantom limb pain and differences in the genetic code. A group of researchers led by Ze*ev Seltzer examined amputees in the Israeli army and found several SNPs that appear to have a correlation with the presence or absence of phantom pain (Seltzer, 2008 personal communication). Our proposed study expands upon that pilot investigation and includes more thorough data collection on the nature and characteristics of the subjects* phantom pain.

The Affymetrix Whole Genome Human SNP Microarray will be used to identify all SNPs between the two study groups (patients with phantom limb pain and patients who have little to no experience with phantom limb pain). This study will further examine whether identifiable SNPs play a role in the perception of painful phantom limb sensations.

Gene expression: Many underlying causes for neuropathic pain involve changes in mRNA levels because of altered gene expression or transcript stability (10, 11). RNA is pertinent to our genetic association study because mRNA transcripts can vary widely according to changing genetic conditions (such as the emergence of PLP), unlike the genome itself which is relatively fixed in comparison. By extracting and quantifying RNA, we will create a gene expression profile. Recently published articles have shown that there are specific gene expression profiles in the peripheral blood of patients with various types of neurological diseases, including dementia, Parkinson*s, and other behavioral conditions (12-14).

BDNF analysis: The protein brain-derived neurotrophic factor (BDNF) plays a key role in the modulation of pain nociception (15) and therefore may

be implicated in phantom limb pain. After peripheral nerve injury, BDNF expression is dramatically increased in pain receptors of the brainstem. This upregulation signals neurons that peripheral nerve damage has occurred (16-18). Previous research has shown that continuous signaling between BDNF and its neuronal receptor is necessary and sufficient to maintain the sensation of neuropathic pain (16, 19). Blocking this pathway in mice inhibits the development of allodynia, a condition in which a painful response occurs to a normally innocuous stimulus (19). Conversely, inducing BDNF release from microglia promotes the development of allodynia and contributes to long term synaptic enhancement of the pain pathways (20). The reinforcement of chronic pain by BDNF in the central nervous system suggests to us that BDNF may be relevant to the presence of phantom limb pain. We expect that BDNF levels will be markedly higher in subjects with phantom limb pain than in those without phantom limb pain. By studying the levels of BDNF in the serum of both subject groups, we hope to confirm this hypothesis and elucidate the specific role that BDNF plays in phantom pain signaling.
PLP in non-Caucasians: There is no data at this time to suggest any differences in the potential development of phantom limb pain based on gender or race/ethnicity. However, ethnicity does affect genotype frequency and haplotype pattern significantly. By comparing SNPs, gene expression and BDNF across different ethnic populations, we hope to determine whether there is a relationship between PLP associated genes and ethnicity. This comparison will allow us to generalize our findings for non-Caucasian populations.

MILITARY RELEVANCE

Almost immediately after the loss of a limb, 90-95% of all patients experience a vivid phantom. Case reports suggest that the incidence may be higher following traumatic limb loss than after a planned surgical amputation of a non-painful limb. The presence of phantom limb pain not only impairs recovery by delaying rehabilitation but also causes continued discomfort and associated depression. Over 800 amputees have entered the military care system since the beginning of the Iraq conflict (personal communication, Mr. Giovani Ortega). By current estimates, 85-90% will at some time experience PLP (21). Understanding the genetic component of PLP may help in predicting which patients will experience PLP and which amputees will respond to the various treatment options available. Furthermore, the discovery of a genetic predisposition or link to PLP may guide researchers in developing new treatments for PLP. Because of the large number of amputee patients in the military healthcare system, optimizing care and finding new, potentially more effective treatments is of great military relevance.

Study objective

The primary goals of this study are to:
i. Identify genetic variations responsible for PLP across the whole genome
More than 90% of SNPs are found in introns or intergenic regions and most of

them are probably of little functional consequence. However, SNPs in promoter regions can alter the affinity of DNA binding proteins and modify the level of gene expression. Other SNPs in exon / intron boundaries result in intron retention or exon skipping, thus profoundly changing the structure of the resulting protein. It is also possible that any genomic region where the SNP is located can function as an RNA interference element. The existence of SNPs in any portion of genomic DNA can be meaningful for the phenotype because of the complicated dynamics of DNA structure and gene expression. Genome wide association study along with multi-layer designed follow up study has been applied to clinical pain research. The advantage of this approach is that no specific functional genetic hypotheses are required prior to undertaking analysis.
ii. Identify the gene expression profiles between individuals with and without PLP using microarray technology.
It is suggested that analyzing gene expression profile will have clinical applications to neurological diseases in humans. Recently published studies have shown that there are specific gene expression profiles in the peripheral blood of patients with various types of neurological diseases. The working hypothesis is that differences in gene expression profiles after major limb amputation produce a unique phenotype through molecular interactions. Characteristics of PLP such as presence and severity of phantom pain may be modulated by changes in gene expression over the time in the post-amputation period. We will test the hypothesis that the gene expression profiles are different between PLP and non-PLP amputee patients.

The secondary goal of this study is to:
Identify predictive factors for the prognosis of PLP among the interactions between genetic variations, gene expressions, protein levels and other physiologic variables from the integrative genomic-phenomic analyses.
The analyzed data from primary goals will contribute to the analysis for secondary goal. This integrative genomic-phenomic analysis can generate hypothesis for underlying pathways suggesting how gene expression changes in the serum can be linked to neurological diseases such as PLP. Phenotypic data will be determined from elements of the patient questionnaire used as potential covariates to classify PLP. The tertiary goal of this study is to:
Verify candidate SNPs that are associated with PLP severity or duration in a group of non-Caucasian subjects.
After identifying SNPs that are associated with PLP severity or duration in an ethnically homogenous sample, we will determine whether these findings can be generalized across different ethnic populations by comparing our genomic and proteomic findings from Caucasian and non-Caucasian subjects. The DNA and RNA analysis will be the same for both groups, and we will use principal component analysis to determine if SNPs identified in the Caucasian group are similar for non-Caucasian subjects. While previous research has suggested that frequency of genomic markers vary among different ethnic groups, there has not been any evidence to suggest an association between PLP and ethnicity. We therefore hypothesize that there will be no difference between PLP associated genes in

the Caucasian group and the non-Caucasian group.

Study design

This observational, case-controlled, cross-sectional study will evaluate whether genetic differences correlate with phantom limb pain. There will be no blinding since both patient groups will need to report their level of PLP to the investigators. This will not affect genetic analysis. There will not be randomization, as the placement of subjects into groups will depend on degree of PLP.

Subjects will be males or females at least 18 years of age who have sustained a major limb amputation at least three months ago. PLP phenotype will be determined at screening with the inclusion/exclusion criteria screening form.

The study sample will be comprised of one thousand (1200) amputees (950 with a history of PLP; 250 with no history of PLP). Each participant*s one study visit will consist of the following: (1) a questionnaire to assess the nature of PLP, (2) a current medication listing, (3) a routine 30 mL blood draw from which DNA, RNA, and BDNF will be harvested, and (4) a DEXA scan to account for the effect of body fat composition on BDNF levels.

All data and blood samples will be de-identified. The de-identified patient questionnaire, medication listing, and DEXA scan results will remain at WRNMMC, while de-identified blood samples will be shipped to and analyzed by collaborators at the NIH. Volunteers recruited outside of military treatment facilities (MTFs) will not be listed on the master list since the only link to their data from blood tubes and CRF*s would be the consent form. This is discusse din more detail in sections 7 and 8.

Blood draws pose minimal risk to the subject, but participants undergoing a DEXA may be exposed to a small amount of radiation, the effects of which are too small to be determined. To minimize these risks, the blood draws and DEXA scans will be performed by experienced practitioners in a controlled setting.

Study burden and risks

Actions to Minimize Risks

Confidentiality Protection: Each subject will be identified throughout this study by coded identifiers (i.e. P1, P2, NP1, NP2) assigned by the lead site*s research team. All data collection forms will be pre-labeled with the subject*s code. Only the subject*s code (not the subject*s name) will appear on data collection forms and blood samples.

For subjects recruited outside of the military clinical setting, no link will

be established between the subject*s study code and the subject*s personal identifying information (i.e. name, age, etc). Signed consent forms will be stored in a portable locked box for the duration of the off-site recruitment. The locked box will be kept with a member of the study team during transport across state lines. Only members of the local site study team will have access to this box. At the local site, the signed consent forms will be stored in a locked filing cabinet that only members of the local study team will have access to.
For subjects recruited within the military clinical setting, a link between the subject*s code and personal identity will be kept in a concealed binder/folder in a locked filing cabinet housed in the an office supervised by the local PI for three years. Only the local PI, not the lead site PI, will have access to this linkage information. After three years, the link between the subject*s name and the study will be destroyed in a manner that is irreversible and that renders the data unreadable. For non-digital data, disposal will involve shredding of all documents. All data reported in publications will be aggregated, de-identified data.

Certificate of Confidentiality: N/A

Reporting Adverse Events

Please refer to performance site protocols for information about the reporting of adverse events or unanticipated problems. Each performance site will adhere to local guidelines and policies.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

A total of twelve hundred (1200) subjects with upper or lower extremity amputations of any level will be enrolled in this study. Nine hundred fifty (950) subjects will have a history of phantom limb pain (PLP) and 250 will have no history of PLP.

Exclusion criteria

Chronic PLP Group:
* Subjects under age 18.
* Less than three months lapsed since amputation
* Known uncontrolled systemic disease- known cancer not in remission, known on-going infection, lupus, kidney disease requiring dialysis, any other systemic disease which might affect ability to participate in this study*s blood draw
* Any condition or situation that, in the investigator's opinion, may put the subject at significant risk or confound the study results
* Experienced PLP for less than one month or less than 3 times/week
* Hemophilia or other chronic disease or medication regimen that would make a blood draw dangerous or inadvisable for the subject as determined by querying the subject;Non-Chronic PLP Group:
* Subjects under age 18.
* Less than three months lapsed since amputation
* Known uncontrolled systemic disease * known cancer not in remission, known on-going infection, lupus, kidney disease requiring dialysis, any other systemic disease which might affect ability to participate in this study*s blood draw
* Any condition or situation that, in the investigator*s opinion, may put the subject at significant risk or confound the study results

Study design

Design

Study type:	Observational invasive
Intervention model:	Other
Allocation:	Non-randomized controlled trial
Masking:	Open (masking not used)
Control:	Active
Primary purpose:	Diagnostic

Recruitment

NL

Recruitment status:	Will not start
Enrollment:	650
Type:	Actual

Ethics review

Approved WMO

Date:	18-11-2015
Application type:	First submission
Review commission:	METC Leiden-Den Haag-Delft (Leiden) metc-ldd@lumc.nl

Approved WMO

Date:	12-12-2016
Application type:	Amendment
Review commission:	METC Leiden-Den Haag-Delft (Leiden) metc-ldd@lumc.nl

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL54039.058.15
Other	WRNMMC IRB REVIEW OF 20429-24

Study results

Date completed: 17-06-2020

Actual enrolment: 0

Summary results
Trial never started