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Neurobiological basis for pain vulnerability: why me?

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Abstract: Not everyone is equally likely to develop chronic pain. This review sets out to discuss the underlying neurobiological reasons that might make some of us unfortunate patients, while others are spared. We will show that genetic factors are likely to play a role, but that their exact identity has proven difficult to determine and remains, to a large extent, elusive. We will also discuss how the environment can impact how likely we are to develop chronic pain, and summarize early tentative evidence for how this might come about biologically. Finally, we will touch briefly on potential implications for the concept of personalised medicine.

A great deal of mechanistic work conducted in pain research is concerned with answering the question of why a certain stimulus should be perceived as painful or how the nervous system in a chronic pain state differs from the healthy norm. In these kinds of studies, individual differences in perception are usually seen as a nuisance – a distraction from any "real signal". And indeed, there is a large amount of noise to contend with: pain experiences are extremely variable, both in health and disease.

In the following, we are going to make this variability the focus of our review. We will outline the evidence for its existence, discuss the various biological mechanisms that might underpin it, and finally, deliberate on potential implications such variability might have for the concept of personalised medicine.

Pain experience is variable

Like many biological phenomena, pain perception differs widely between individuals, be they humans [59; 61], rats [76] or mice [55; 56]. Pain is a subjective experience that can be reported upon by an individual, but not yet measured objectively in that person. This subjectivity is likely to be the result of a variety of factors, many of which are unrelated to differences in pain perception per se. For instance, a person's mental or motivational state on the day of testing can introduce variation as can their level of attention. Nonetheless, quantitative measures reveal a degree of spread in pain responsiveness that suggests underlying biological differences in nociception itself.

For instance, the normal range for heat pain thresholds encompasses anything from 35 to 50°C, while mechanical pain thresholds range from almost 0 to 250mN [73]. This is in contrast to non-painful measures that are just as sensitive to state variance, but have much tighter population distributions, for instance warmth and mechanical detection thresholds have 95% confidence intervals of 33-36°C and a few milli-Newtons, respectively.

The same variability can be observed in a clinical context, where pain and pathology are often only weakly correlated. The arthritis field provides some good examples of this. The degree of osteoarthritis in a joint is quantified with the help of X-rays which are scored according to the so-called Kellgren/Lawrence scale (K/L). A score of zero indicates that the X-ray ostensibly looks normal, while a score of 4 indicates a maximum degree of bone erosion and damage. Clearly, a degree of correlation exists between the amount of damage and the degree pain experienced, but it is a surprisingly weak correlation. Some patients with a K/L of 0 still experience pain, with mean visual analogue scale (VAS) scores of 36+/-30 in one study [5]. Conversely, patients who have very minor to no pain can still have visible signs of osteoarthritis according to K/L, e.g. in that same study, people with VAS scores of less than 10 had an average K/L score of 1.49. These surprisingly weak or even absent correlations between levels of pain and the different pathological features of osteoarthritis have been identified time and again [41] [10].

Finally, resolution of pathology does not always go hand in hand with cessation of pain. Perhaps the most recent, striking illustration of this is the dawning realisation that while anti-TNFα treatment has provided an almost magical cure for the inflammatory symptoms of rheumatoid arthritis, it frequently does not eliminate the pain which patients experience [1; 2].

What mechanisms underlie our divergent pain perceptions and varying vulnerability for pathological or long-lasting pain? On a macro-level, individual differences have to be either driven by genetic or environmental factors. In pain research, we have excellent epidemiological work drawing clear links

to both our genes and our life experiences. It is the biological details of these connections that remain hazy, as shall be outlined in the following sections.

<u>Genetic mechanisms underpinning variability</u>

Classically, the genetics of pain has been mostly studied in the context of rare familial mutations leading to gain- or loss-of-function [12], such as extreme pain disorders [25; 35; 50], erythromelalgia [85; 86] or congenital insensitivity to pain [16; 17; 60]. This kind of work has yielded interesting information regarding the importance of various ion channels (e.g. *SCN9A*), receptors (e.g. *NTRK1*), growth (e.g. *NGF*) and transcription factors (e.g. *PRDM12*). However, in many instances, the observed phenotypes are a result of disruption of normal sensory fibre development and are unlikely to explain vulnerability to chronic pain in the wider population. One notable exception is *SCN9A*, which is a potentially promising drug target, though the success of early-stage trials has been limited in terms of analgesic efficacy [14].

Demonstrations that pain is heritable in the wider population come mostly from twin studies [58], and there is little doubt that experimental pain perception [61], specific pain conditions [24], as well as persistent pain in the general population [49] are explained by genetics to quite a significant degree. Heritability estimates range from 25-60% depending on the study and condition. Candidate gene and genome-wide association (GWAS) studies have duly been carried out in an attempt to identify the disease-associated single nucleotide polymorphisms (SNPs) [53; 89]. The results have been somewhat underwhelming, with SNPs accounting for only a tiny proportion of the variance in phenotype and many candidates failing to replicate. To a large extent, this is due to the field being plagued by small sample sizes, and hence low power. Inadequate phenotyping is also a common issue and difficult to address, given the heterogeneity in aetiologies and symptoms displayed by chronic pain patients. There have been a several larger GWAS examining osteoarthritis, but, with the exception of one small sub-cohort, did not include explicit measures of pain [4; 15; 79]. Indeed, the word pain is mentioned a grand total of five times in all three papers.

Perhaps the only highly powered GWAS the pain field can currently boast of, therefore, is a recent meta-analysis of work in the migraine field [29]. The study analysed data from 59,574 migraineurs and 316,078 control subjects, identifying 44 disease-associated SNPs. Interestingly, 30% of loci were associated with vascular function, while only 2 were in close proximity to neuronal channels and 6 were associated with nitric oxide signalling. From the viewpoint of basic science, these findings are very interesting, potentially giving a boost to the vascular hypothesis of migraine which has somewhat fallen out of fashion in recent years [64; 74]. However, the results also reveal an uncomfortable truth about genetic variation: even experiments with very large sample sizes that should be able to detect the majority of SNPs associated with a given phenotype rarely explain even a fraction of heritability. In this particular case, the odds ratios of individual loci ranged between 0.89 and 1.11, and all together, could not account for more than 10-20% of the genetic variance observed. Bigger studies may reveal more SNPs, but of course, statistically they are likely to have even smaller effect sizes.

So what is driving the remaining 80% that we attribute to genetics? This "missing heritability" is a well-known and much discussed phenomenon among geneticists e.g. [90]. Some people hope that the study of rare variants (with a minor allelic frequency of less than 0.5%) may be the answer [27],

but so far the data are not looking particularly promising. Certainly, in the pain field, such efforts are in their infancy with only one whole exome sequencing study having been performed so far [83]. It did not reveal any single variant that was significantly associated, at a genome wide level, with pain perception. It is important to realise that statistical testing necessarily presents quite a high bar: frequently each study will test the association of each of more than 15,000 genes with pain, and therefore any association has to be extremely strong to pass correction for such large multiple testing. Another option is that missing heritability can be explained by gene x gene interactions (also known as epistasis) or gene x environment interactions. This seems more likely, and indeed there is evidence for gene x environment effects in the pain field [54]. Unfortunately, the presence of such interactions will make the underlying genetic contributions much harder to identify [71; 80].

In summary, the simple point is that in pain studies, as in many other areas of biomedicine, the effect size of common genetic variants is nearly always small. To even detect these influences requires huge sample sizes that will not be easy to collect, even with international collaborations, since many pain phenotypes require expert diagnosis. Whether the effort will be worthwhile is still unclear. A genetic variant – especially in a gene having a vital function – may not confer much variability on a trait. But knowing that the gene is important may allow a dissection of pathways that can be exploited pharmacologically to great effect. So in this sense the jury is still out on the value of the whole GWAS effort.

Environmental mechanisms underpinning variability

From the previous section, it should be clear that our genes contribute to pain perception and vulnerability, even though the precise role of individual variants remains largely elusive. But what about the environment? It clearly also plays a very important role. Known risk factors for chronic pain conditions include age, gender, certain personality traits, such as a tendency to catastrophize, psychological stress and a prior history of pain [26; 39]. Of these, the latter two are likely to driven to a significant degree by environmental influences. Prospective studies of post-surgical pain show that the level of pain prior to surgery is one of the best predictors of chronic post-operative pain [39]. Additionally, past physical injuries can be linked to the later development of chronic pain, for instance in the case of osteoarthritis [13] or neck pain following whiplash [6].

Stress on a purely psychological level may also be important, with prospective cohort studies indicating that individuals that score highly on anxiety measures, display depression or poor sleep are more likely to develop chronic widespread pain [33]. Indeed, there is evidence that the psychological consequences of a physical injury are what ultimately increases our risk for chronic pain in many cases [6]. Mechanistically, these processes may be mediated, at least in part, by dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis [48], which also is prominently associated with neonatal pain-related stress ([81]. There is only a very weak direct link [44], if any [32], between premature birth and adult chronic pain. However, indirectly, preterm babies are more likely to show altered brain development and stress responses, which in turn will make them more vulnerable.

While the association between pain and environmental experience is therefore quite well supported by epidemiological evidence, its underlying biology is currently still only poorly understood. Moreover, the various explanations that are on offer have emerged from quite different levels of

study. There is work examining epigenetic mechanisms [18; 20], studies of priming [3; 69] or early-life injury [45; 82], and finally a significant body of literature on the reorganisation of cortical and top-down modulatory circuitry [8; 62]. We have previously discussed some of these efforts in another review [21]. Here, we will therefore focus on some of the newer developments, particularly those relating to epigenetics.

Epigenetics is the study of stable molecular modifications such as histone modifications and DNA methylation that are faithfully transmitted to daughter cells upon mitosis. Epigenetic mechanisms are crucial for normal development, where they play a central role in cell differentiation [57; 70]. As a consequence, they are highly cell-type specific [34; 75] and should ideally be studied in pure cell populations. The number of epigenomic profiles of increasing specificity has exploded over the past several years, not least due to large-scale efforts such as ENCODE and Blueprint. This has furthered our understanding of nervous system epigenomes significantly. Just to give a few examples, we now know that DNA methylation in cortical neurons is quite unique compared to other cell types [40]. The brain is not only enriched for hydroxymethylation, which has been known for a long time [65], but neurons further show methylation in a non-cytosine-guanine (CH) context [42; 84]. The latter modification is typical of embryonic stem cells [43], but absent in other differentiated cell types [88]. In the human brain, less than two percent of all CH sites are methylated (compared to more than 80% of cytosine guanine sites), but their appearance coincides with key stages of synaptic development during childhood and adolescence [42].

A second crucial body of knowledge has been emerging from the study of histone modifications, where it has become clear over the years that their presence is indicative of underlying transcriptional states [23; 72]. There is still a lot of discussion around whether a specific histone mark leads to transcription or the other way around [22; 36]. But regardless of the direction of causality, we can now obtain a glimpse of how the DNA sequence is used in different neuronal cell types at a previously unprecedented level of detail, e.g. [51; 52]. This is particularly useful when trying to identify or characterise putative regulatory regions, such as enhancers, e.g. [37; 46], which previously had to be identified by much more laborious means [31]. These initial screens can then feed into functional follow-up work, e.g. using reporter assays or genetic modification. This remains a necessary and important step, especially given that we do not yet know to what extent the epigenetic and transcriptional processes that have been observed translate into function [30; 78].

How many of these promising new approaches have been imported into pain research? Research to date is in its infancy, not least because it is technically very challenging to obtain pure nociceptive cell populations at sufficient numbers. Most of the work has either centred on pharmacological studies, using compounds which interfere with processes like histone acetylation, e.g. [20; 87], or has employed the use of mixed tissue – making the interpretation of its results very difficult [28; 47]. We were ourselves involved in an early effort to link epigenetic signatures in human back to pain perception [11]. The study examined identical twins discordant for heat pain thresholds and found the latter to be correlated with methylation levels at the TrpA1 promoter. Like many of the early epigenome wide association studies (EWAS) in the field of neuroscience, the work had to rely on whole blood, given the difficulties in accessing human neural tissue. However, we were able to obtain human skin and correlate the blood-derived changes in methylation to more disease relevant TrpA1 expression patterns.

Since the early days of EWAS in blood, neuroscience is now starting to move on to more ambitious technical projects, utilizing NeuN sorted nuclei from post-mortem material, see e.g. the PsychENCODE project [68]. This move towards more cell-type specificity will be crucial in advancing the field. We have therefore also tried to adopt a more differentiated approach, at least in the context of a pre-clinical model of persistent pain. We examined the histone mark H3k4me1, which is associated with enhancers, in mouse spinal cord microglia after partial sciatic nerve ligation. A small number of loci displayed increased H3k4me1 binding as a result of the injury. The affected regions were in close proximity to transcriptionally regulated genes, and the altered binding appeared to persist over a period of 28 days, a time at which transcription had largely reverted back to normal. We therefore posited that these stretches of DNA might function as latent enhancers, a type of regulatory region first described in macrophages [38; 63]. This work is still very much in its initial stages. There are many outstanding questions relating to how exactly the increase in H3k4me1 binding relates to enhancer function and regulation of pain-relevant genes. Technically, the experiments remain very challenging due to low cell numbers and lack of *in vivo* tools that allow for the study of causality.

Implications for personalised medicine

The previous two sections should have made it clear that the extent to which we experience pain throughout our lives is dependent on both genetic and environmental factors. The field has started to identify pain relevant genes and mechanisms which may affect how the environment impacts their function. However, since each gene is likely to contribute only little to the consequent phenotype, it is difficult to predict clinical outcomes on the basis of individual genetics.

Worse still is the predictive power of environmental influences. It has been an open secret in epidemiology for nearly 30 years that shared environment, i.e. the measurable influence of parenting and other common factors on a child, explains very little of the variance inherent in behavioural traits [67; 77]. It is the non-shared environmental components, i.e. the unsystematic, stochastic sources of variance, that crop up in the vast majority of epidemiological models, e.g. [66]. In practice, this means that the influence of environmental factors is only apparent at population level (where it certainly plays a very important role), while the specific outcome for an individual is mostly down to complex genetics and pure chance.

Despite this, valiant, and in some case, successful efforts have been made to allocate patients to certain treatment groups. So far, the use of genetics has worked best for individuals with rare familial disorders, where the exact location of a mutation can sometimes be used to predict the correct treatment response [25]. Another approach that may be promising is to disregard genetics, and instead focus on improved diagnosis, using quantitatively determined sensory phenotypes to stratify patients into subgroups [7; 9; 19].

With the exception of monogenic disease, however, these promising results are still very far removed from what the general public imagines and hopes "personalised medicine" to be. The dream is to draw up an individual treatment profile based on a person's genetics and case history. However, for the reasons outlined above, most complex cases of chronic pain are unlikely to ever be conclusively traceable to one specific mutation or injury event – let alone one that then leads to "personalised treatment". Just like life itself, some aspects of chronic pain may end up remaining irreducibly complex and ultimately unpredictable.

Figure legends:

Figure 1: Pain experience is variable in health and disease
Pain perception varies widely in the healthy population, e.g. the normal heat and mechanical pain thresholds encompass a wide range. Equally, in disease, the degree of pain and the extent of underlying pathology is not as highly correlated as one might expect. Data on the right represent mean VAS (visual analogue scale) and K/L (Kellgren/Lawrence) scores derived from [5]. The K/L scale is used to clinically grade the radiographic degree of a pateint's osteoarthritis.

Figure 2: The genetics of pain
Genetic variants that could be linked to chronic pain conditions have been revealed by the study of rare familial mutations (left) or association studies in the general population (right). See [53; 89] for review.

Figure 3: Environmental influences on pain
There are many risk factors for developing chronic pain, some of which, such as prior history of pain, are likely to have an environmental component. Left: Data depict the regression coefficients of pre-operative chronic pain levels with post-operative outcomes across 30 different surgical procedures (+ 95% confidence intervals, adapted from [26]). Right: Possible mechanisms remain unclear, but evidence to date points at a variety of different processes, many of which are likely to occur in tandem. TFs: transcription factors. H3: H3 histone. k4: lysine residue 4. m: methyl group.

Figure 4: Model of genetic and environmental contributions to chronic pain
Research to date suggests that genetics contribute about 40% of a patients pain phenotype, while the environment contributes the rest. However, there are many complex interaction effects of as yet unknown size, and non-shared environment plays a much larger role than shared environmental components. This has implications for personalized medicine. Note that the size of the pie slices shown here are merely abstract representations of probable effect size, as opposed to definitive results based on empirical data.

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