Heritability of responses to painful stimuli in women: a classical twin study

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There is as yet no conclusive evidence for the heritability of pain sensitivity in humans. We performed a classical twin study to evaluate the relative contributions of genetic and environmental factors on responses to painful stimuli in women. Ninety-eight pairs of twins, 51 monozygotic (MZ) and 47 dizygotic (DZ), were recruited from the TwinsUK adult registry held at St Thomas’ Hospital, London. The correlation of quantitative sensory testing scores for the different responses to painful stimuli were compared between the MZ and DZ twin pairs and structural equation modelling was used to provide an estimate of the heritability. Statistically significant genetic components (varying between 22 and 55%) were seen for the responses to the majority of painful stimuli including: heat pain threshold (HPT), the pain rating during induction of a thermal burn, the secondary areas of punctate hyperalgesia and brush evoked allodynia following the induction of a 45°C thermal burn, and the pain ratings during the iontophoresis of adenosine triphosphate (ATP) and acid. The area of skin flare following thermal burn induction did not have a significant genetic component; rather common environmental factors provided the greatest contribution (65%). In our experiment neither shared genetic nor environmental features were significant in determining the extent of thermal sensitisation.

In summary we show that sensitivity to a variety of experimental thermal, mechanical and chemical pain-producing stimuli has a genetic contribution. Our study demonstrates the importance of genetic factors in determining human experimental pain sensitivity, and opens the way for its use as a phenotype in gene discovery. Since experimental pain sensitivity is known to be a predictor for pathological pain, our data imply that genetic factors have an important aetiological contribution towards clinical pain states.

Keywords: allodynia; nociception; genetics; human pain models; hyperalgesia

Abbreviations: HPT = heat pain threshold; NRS = numerical rating scale; VAS = visual analogue scale


Introduction

Human pain sensitivity is a complex phenotype and shows large inter-individual variation. However, accumulating evidence suggests that part of this variation might have a familial basis (Violon and Giurgea, 1984; Edwards et al., 1985). For example, in a study of children attending a paediatric rheumatology clinic and their parents, Schanberg et al. (2001) found that children with higher pain ratings and poorer health status tended to have parents who were more likely to seek treatment for their own pain, or to report pain interfering with normal recreational activities. More recently, Bruehl and Chung (2006) have reported that a parental history of chronic pain is associated with enhanced pain sensitivity and implicate differences in endogenous opioid functions. A poor tolerance of pain among family members of subjects who themselves report pain has also been demonstrated in other settings, including among relatives of patients undergoing thoracic surgery (Bachiocco et al., 1993).

One difficulty in interpreting studies of familial aggregation lies in dissecting out the genetic factors from the role of the shared environment. The contribution of genetic factors to pain sensitivity is increasingly well recognized in animal studies. For example, Mogil and colleagues have demonstrated strong differences between 11 inbred mouse strains on 12 measures of nociception (Mogil et al., 1999).

In humans, the most informative approach to assessing the relative importance of genetic factors over the shared environment is through the study of twins. Monozygotic (MZ) twins are genetically identical, whereas dizygotic
(DZ) twins share only 50% of their segregating genes. If both types of twin are assumed to share their familial environment to the same extend, greater similarity for a particular response to a painful stimuli within MZ twins, when compared to DZ twins, can be attributed to genetic factors.

Twin studies have, in the past, been used to study a number of painful conditions, and many have been found to have a statistically significant heritable component. Examples of pain-related traits with a heritability of 40% or above include low-back pain and neck pain (MacGregor et al., 2004), carpal tunnel syndrome (Hakim et al., 2002), migraine (Larsson et al., 1995), osteoarthritis of the hip (Page et al., 2003), pelvic pain (Zondervan et al., 2005) and gastro-oesophageal reflux disease (Mohammed et al., 2003). Among these traits it is difficult to determine whether genetic variation in pain experiences is attributable to the presence or severity of diseases themselves, or reflect an inherent variability in pain processing between individual. This question can be addressed more precisely by studying the genetic basis of variation of responses to experimental pain stimuli in healthy human subjects, using methods that allow precise control of the intensity, location and duration of the applied pain-producing stimulus.

Studies of experimental pain are free from the confound of disease progression, but can nonetheless be highly relevant to clinically relevant pain states. In the last decade or so a series of reports have appeared which have studied the predictors of persistent pain states and these consistently find that sensitivity to experimental pain is a significant determinant. The best studied cases consist of widespread changes in the nervous system’s sensitivity to noxious stimuli. A significant number of patients develop chronic pain after various forms of surgery and in some cases it has been demonstrated that the degree of acute post-operative pain or pre-operative pain predicts the development of chronic pain (e.g. Kroner et al., 1992; Callesen et al., 1999, Nikolajsen et al., 2006). This was found to be the case in series of 150 patients undergoing laparoscopic cholecystectomy, although in this case the chronic pain was not predicted by pre-operative sensitivity in a cold pressor test (Bisgaard et al., 2005). A link between experimental pain sensitivity and chronic pain was also reported by Tegeder et al. (2006) who found that a haplotype of the GCH1 gene was significantly associated both with reduced chronic pain following low-back surgery and reduced experimental pain sensitivity. Together, these data suggest that understanding the factors that determine experimental pain sensitivity in normal healthy people will be of value in predicting clinically relevant post-operative pain and perhaps in understanding some chronic pain states.

Here we present the results of an extensive classical twin study of experimental pain in female twins and report that several forms of acute pain sensitivity show statistically significant heritability.

**Methods**

The local ethics committee approved all procedures, and all subjects provided informed consent.

The experiment was conducted on 100 pairs of female Caucasian twin volunteers (52 pairs MZ, 48 pairs DZ) aged 19 to 76 years. The volunteers were recruited randomly for the study from the TwinsUK adult registry held at St Thomas’ Hospital, London. This is a cohort of twins that has been constructed by recruitment of volunteers from the general population via successive local and national media campaigns (Spector and Williams, 2006).

Random sampling was conducted in a stratified manner to achieve a balance in the number of MZ and DZ twin pairs and to achieve a similar proportion of twins in 10-year age bands.

Telephone screening was performed to determine subjects’ willingness to participate. During the screening, the twins were asked about any existing medical conditions and current analgesic use. Twins were not selected for inclusion in the study if they described suffering chronic pain, were regular users of analgesia or answered positively to a direct questioning about having any underlying illness which could affect their responses to painful stimuli (arthritis, malignancy, diabetes or neurological conditions). Approximately 70% of those contacted were both willing and able to participate in the study.

The zygosity of the twin pairs was determined by a standardized questionnaire (Cederlof et al., 1961), which has been shown to be over 95% accurate. If the zygosity was either uncertain or disputed following the questionnaire, then DNA analysis of short tandem
repeat polymorphisms was performed (AmpF1 STR Profiler kit; Applied Biosystems, Foster City, CA). This DNA analysis was performed in 52 (53%) of the twin pairs, with only one twin pair changing assignment after genotyping.

In the analysis, the similarity in responses to painful stimuli in both MZ and DZ twin pairs was estimated through the intraclass correlation coefficient (R). In the classical twin study model, the influence of the shared family environment is assumed to be equal in both MZ and DZ twin pairs. A greater correlation within MZ twin pairs compared with that for DZ twin pairs suggests a genetic influence on the response to the painful stimulus under consideration.

The potential genetic and environmental contributions on the responses to individual painful stimuli were further explored through variance components modelling (Neale and Cardon, 1992). This approach considers the variance of a response to a stimulus in a population to be the sum of both genetic and environmental variance components. Genetic variance can be partitioned into additive (A) and non-additive (dominance (D)) variance components. In twins, environmental variance has contributions from a component that is shared among families (common environment (C)) and from a random environmental component, which includes experimental error (E) (Falcozer, 1989).

Given a set of data on phenotypic variances and covariances from MZ and DZ twins, structural equation modelling provides a method for assessing which combination of genetic and environmental variance components best explain the patterns that are observed. Five potential models can be examined containing the components ACE, ADE, AE, CE and E. Models composed of the components DE are not considered biologically plausible; the model ADCE is over-specified and cannot be estimated using twin data alone. The significance of individual variance components is assessed by dropping parameters sequentially from sets of nested models: ACE→AE→E; ACE→CE→E; and ADE→AE→E. In choosing between models, variance components are excluded in the selection process if there is no significant deterioration in model fit (as assessed by the chi-squared statistic) after the component is dropped. The E component represents random error and as such is retained in all of the models.

**Thermal burn protocol**

The volar surface of the right forearm was inspected for possible confounds (e.g. cuts, bruises, burns or skin irritation, etc.) and then a 32 mm² probe connected to a servo-controlled peltier device (TSA-II, Medoc, Israel) was placed approximately equidistant between the elbow and wrist and secured with a fabric-covered elastic band. A manual sphygmomanometer cuff was wrapped over the probe and inflated to a pressure of 20 mmHg to standardize the contact pressure of the probe on the skin and prevent it from moving. The baseline heat pain threshold (HPT) represents the temperature at which the sensation evoked by a thermal stimulus changes from feeling ‘hot’ to feeling ‘painful’. This was measured by slowly heating the probe up from an adaptation temperature of 32°C at a rate of 0.5°C s⁻¹ until the subject perceived the stimulus as changing from hot to painful and stopped the experiment by pressing a button, at which point the temperature (the HPT) was logged and the probe temperature then quickly returned to 32°C. All subjects were given standardized instructions before having a ‘practice run’ at measuring HPT. A further three measurements were taken, with the skin held at adaptation for 5 s between each reading, and an average of the three readings used as the HPT.

The probe was kept at the same site and used to create a mild thermal burn injury. The probe was heated from adaptation to 45°C at a rate of 0.5°C s⁻¹ and maintained at this temperature for 330 s. As soon as the probe reached 45°C, and again after 120 and 210 s, subjects were asked to verbally rate how painful the burn was on a numerical rating scale (NRS, 0–10) using whole numbers, with 0 being defined as ‘no pain’ and 10 as ‘the worst pain you can imagine’. These three pain ratings were added together to provide a total rating of the pain during burn induction (out of 30).

The thermode was removed at the end of the heating and the burn site marked on the skin with a marker pen. An acetate template was used to mark dots at 1 cm increments along eight spokes radiating out from the primary burn area.

Sensory testing was performed 15 min following the removal of the thermode, which we found to be the time of peak response (data not shown). The extent of the skin flare (reddening of the unheated skin surrounding the primary burn site) at each spoke was measured by eye to the nearest 0.5 cm. The points on adjacent spokes were connected to form a triangular segment. The total area was calculated by the summation of all the eight triangular segments. Finally the primary burn area (32 mm²) was subtracted from this figure to give the area of secondary change. This technique was used for the area of flare, brush evoked allodynia and punctate hyperalgesia.

The degree of brush evoked allodynia (the alteration of the sensation evoked by brushing the skin with a soft brush, from an innocuous sensation to a painful one) was assessed with a No. 2 sable paintbrush (Justbrushes, UK). The brush stimulus was four 1 cm brush strokes applied perpendicularly to each point along the spoke at a frequency of ~0.5 Hz. The brush was initially applied to the outermost point of each spoke, to serve as a reference point for normal sensation, then applied to each point in turn working towards the 1° burn area until a change from a ‘soft tickly’ to a ‘scratchy or prickly’ sensation was reported.

The degree of punctate hyperalgesia (an increased sensation of pain following the perpendicular application of a mechanical force to the skin) was assessed with a 10 g von Frey filament (Bailey Instruments, UK). The testing procedure was the same as for brush evoked allodynia, but this time the von Frey stimulus was applied only once to each point with pressure being maintained for ~1 s. The sensation change described by subjects was a shift from ‘a prodding sensation’ to a ‘sharp prickling’.

To evaluate the thermal hyperalgesia (an increased sensation of pain following the application of a thermal stimulus to the skin, represented by a thermal stimulus being perceived as shifting from ‘hot’ to ‘painful’ at a lower temperature) at the burn site, the HPT was re-measured on the same part of the arm as before. Subjects were instructed to close their eyes during the sensory testing to prevent any visual clues, e.g. skin flare, influencing their perception.

**Variability and reliability of thermal burn protocol**

The thermal burn protocol utilized in this study is milder than the most commonly utilized protocol, so the variability and reliability of this protocol was formally assessed. Our milder
thermal burn protocol was performed on 10 individuals, on two separate occasions separated by 2 weeks, with two different investigators. The variability and reliability were calculated using the methods described by Varrone et al. (2000). Briefly the variability of measurements was computed as the numerical difference between the measurements from each of the two testing sessions, expressed as a percentage of the mean value of measurements from both testing sessions. The reliability of the measures was assessed relative to the between- and within-subject variance by the intraclass coefficient correlation, calculated using the formula below.

\[
\text{reliability} = \frac{s_b^2 - s_s^2}{s_b^2 + (n-1)s_s^2}
\]

Where \(s_b^2\) is the mean sum of the square between subjects, \(s_s^2\) is the mean sum of the square within subjects and \(n\) is the number of within-subject measurements (in this study, \(n = 2\)).

**Iontophoresis**

Iontophoresis is a technique which uses an electric current to drive polar chemicals through the skin, where they can interact with their receptors. This is particularly useful in pain research, because many chemicals are able to be passed through the skin in this manner, producing pain when interacting with their receptors. We used the iontophoresis technique on the volar surface of the volunteer’s left forearms. An iontophoresis chamber was fixed to the skin with a ring of double-sided tape, filled with 200 \(\mu\)l of solution which was driven into the skin by a small 0.3 mA DC current for 4 min. A different site on the forearm was used for each of the solutions 10% saline, 10 mM adenosine triphosphate (ATP) and pH2 hydrochloric acid (HCl). For the saline and ATP solutions the chamber was connected to the negative terminal of the iontophoresor (Phoresor II, Motion Control, USA) with the positive terminal connected to a disposable electrode [Ag/AgCl Resting ECG Electrodes (Medicotest UK Ltd)] adhered to the thenar prominence of the left palm. The polarity was reversed for the HCl due to the positive charge of this molecule. During the 4 min subjects provided a pain rating every 20 s on an electronic visual analogue scale (VAS) using the computer programme. The VAS consisted of a grey bar, the left-hand side defined as ‘no pain’ and the right-hand as ‘the worst pain imaginable’. The software converts the location the bar was clicked into a value between 0 and 100 where 0 represent no pain. At the end of the 4 min all of the pain ratings are added together to provide the total pain during iontophoresis.

Finally a handheld iontophoresis chamber was attached to the positive terminal of the iontophoresor filled with a small wad of cotton wool soaked in 200 \(\mu\)l of histamine solution (1% made up in distilled water with 2.5% methyl cellulose). The chamber was held against the skin and the iontophoresor run for 20 s at 0.5 mA. The chamber was removed at the end of the 20-s period and the subject rated how much itching they were experiencing every 20 s for the next 4 min using the same VAS as before, but this time the left-hand side was defined as ‘no itching’ and the right-hand side as ‘the worst itching you can imagine’. At the end of the 4 min all of the values were added together to form the total itch following the histamine iontophoresis. Subjects with asthma, or those describing a history of severe allergic reaction, were excluded from the histamine iontophoresis to prevent any possible adverse reactions.

To minimize any potential differences in the testing procedure between subjects, the testing conditions were standardized as far as possible, including the information given to each subject regarding the test. The twins were tested separately and not aware of each others responses or ratings.

The experiment protocol is outlined as a flowchart in Fig. 1.

**Results**

**Subjects**

Of the 100 twin pairs tested, 98 pairs (51 pairs of MZ and 47 pairs of DZ) were able to complete the experiment successfully. There were two twin pairs who did not complete the experiment because they had a very low HPT (<40°C) and were excluded along with their co-twin, as it was felt a sustained 330-s thermal stimulus 5°C supra-threshold would be too painful.

**Thermal burn induction**

During creation of the thermal burn subjects described an initial sharp stinging sensation which gave way to a more generalized and less severe burning sensation. As the burn progressed the intensity of the pain began to rise with the peak pain being reported in the last reading taken 4 min after the start of burn creation. The mean pain during burn induction was 14.13 (SE = 0.49) (maximum possible = 30).

The thermal burn elicited significant areas of secondary changes (i.e. changes in skin sensitivity surrounding the burn site); skin flare, punctuate hyperalgesia and brush allodynia were present in the secondary area of 96, 89 and
38% subjects, respectively. In those subjects who developed secondary changes the mean areas for skin flare, punctuate hyperalgesia and brush allodynia were 18.69, 12.63 and 6.17 cm², respectively. The thermal hyperalgesia which developed at the burn site was modest, but highly statistically significant (1.04°C, P < 0.01).

Variability and reliability of thermal burn protocol

The thermal burn protocol was shown to be reliable in an investigator-independent manner. For the area of flare evoked by the thermal burn the variability was 25% and the reliability was 0.91. For the area of punctate hyperalgesia evoked by the thermal burn the variability was 40% and the reliability was 0.49. For the area of brush evoked allodynia the variability was 42% and the reliability was 0.89.

Iontophoresis

The iontophoresis of ATP and acid elicited modest pain while the iontophoresis of histamine produced a classical flare and wheal response accompanied by a sensation of itch. Subjects likened the sensation during the acid and ATP iontophoresis to a stinging nettle sting.

Due to a technical problem one twin pair was unable to receive any of the iontophoresis stimuli, and because of the exclusion criteria highlighted earlier, only 154 (79%) of subjects received the histamine iontophoresis.

Relationships between variables

The extent to which responses to pain sensitivity testing were related within individual subjects was also examined. Figure 2 lists the pairwise correlations for each of these variables among the study participants. As expected, the higher the HPT the lower the pain rating during burn induction (r = −0.338, P < 0.0001). This relationship was also true for the pain rating during iontophoresis of saline, ATP and acid. The ratings of pain during acid, ATP and saline iontophoresis and itch following histamine iontophoresis were all correlated. The area of skin flare following thermal burn induction, however, appeared independent of both the area of punctate hyperalgesia and brush evoked allodynia (r = 0.09 and r = 0.06, respectively).

The HPT became higher with increasing age, whereas the area of skin flare became smaller (r = 0.18 and r = −0.23, respectively). The pain ratings during iontophoresis of ATP and acid also showed a statistically significant negative correlation with age (r = −0.23 and r = −0.17).

Heritability

The intra-pair correlations for both MZ and DZ twin pairs were calculated for each of the variables (Table 1). With the exception of primary thermal hyperalgesia there was statistically significant correlation within the MZ twin pairs for all variables. For all of the variables the correlations within MZ twin pairs were higher than those within DZ twin pairs, again with the exception of primary
thermal hyperalgesia. Table 1 also contains the ‘best fit’ results for each variable in variance components analysis. A model combining both additive genetic and unique environmental effects (AE) was found to be the best fit for most of the variables, with heritability estimates of between 22 and 55%.

The best fit model for the area of skin flare following the thermal burn was found to be one combining common and unique environmental effects (CE), with the role of common environment estimated to be 65% (CI 52–75%).

In our experiment the development of primary thermal hyperalgesia could not be satisfactorily explained by any of the models, so it appears that neither shared genetic nor environmental features are significant in determining the extent of thermal sensitisation.

As indicated earlier age accounted for <4% of the variance for each of the responses to painful stimuli. The variance component estimates were not influenced by including age in the models.

To summarize the results from the heritability experiment, it was found that the area of punctate hyperalgesia, HPT, pain during burn induction, itch after histamine iontophoresis, pain during acid and ATP iontophoresis and finally area of brush evoked allodynia all had statistically significant heritable components. Common environment only appeared to play a significant role in determining the area of skin flare following thermal burn induction.

**Reproducibility**

To reinforce our findings, we performed a replication study for the HPT testing on another 160 twin pairs (74 MZ and 86 DZ). The original study found HPT had a significant heritable component of 53% (0.34–0.68), and we obtained a significant heritable component of 45% (0.26–0.61) in the replication study group.

**Discussion**

Here we show a formal demonstration of a heritable component to human pain sensitivity. We examined several different aspects of pain sensitivity, with virtually all of them demonstrating a statistically significant genetic component, with shared environment (such as family influences) contributing little. The only exception to this was the area of skin flare following thermal burn induction. Skin flares are known to vary with some environmental influences such as season, in both healthy volunteers and atopic patients (Magerl et al., 1990; Tupker et al., 1995).

We observed different levels of heritability for different pain phenotypes. This is perhaps not surprising since not all pains are the same. But some pain phenotypes are likely to share underlying mechanisms and therefore genetic influences.

It is worth noting that there are many different models for evoking different types of experimental pain and that within each model there is tremendous variation as to the exact protocols used by different research groups. Examples of human pain models include applying noxious chemicals (e.g. Helme and McKernan, 1985; Koltzenburg et al., 1992; Wasner et al., 2004), electrical stimulation (Dowman, 1991), UVB-induced skin inflammation (Soter, 1990) and hot or cold stimulation creating thermal or freeze burns (Kilo et al., 1994; Pedersen and Kehlet, 1998). Due to the lack of standardized experimental pain models, we used techniques which are common to those employed in the field of pain research (McMahon and Koltzenburg, 2005). Our protocols are typical of those utilized by other groups and have been shown to be appropriate measures, except for the thermal burn protocol. It was important to take into account the need for our models to be well tolerated by our large number of volunteers drawn from a finite registry, so a milder protocol was used for the thermal burn induction (45°C for 330s) compared to many others.

### Table 1 The results from maximum likelihood modeling for all of the pain measures examined in the heritability experiment

<table>
<thead>
<tr>
<th>Transformation</th>
<th>RMZ</th>
<th>RDZ</th>
<th>Model</th>
<th>A</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Punctate hyperalgesia area</td>
<td>Log</td>
<td>0.63 (0.43–0.77)</td>
<td>0.23 (–0.06–0.49)</td>
<td>AE</td>
<td>0.55 (0.33–0.77)</td>
</tr>
<tr>
<td>Heat pain threshold</td>
<td>Identify</td>
<td>0.57 (0.35–0.73)</td>
<td>0.3 (0.02–0.54)</td>
<td>AE</td>
<td>0.53 (0.34–0.68)</td>
</tr>
<tr>
<td>Pain during burn induction</td>
<td>Identify</td>
<td>0.36 (0.09–0.58)</td>
<td>0.08 (–0.21–0.36)</td>
<td>AE</td>
<td>0.34 (0.08–0.55)</td>
</tr>
<tr>
<td>Itch after histamine iontophoresis</td>
<td>Log</td>
<td>0.39 (0.10–0.62)</td>
<td>0.05 (–0.29–0.38)</td>
<td>AE</td>
<td>0.35 (0.11–0.54)</td>
</tr>
<tr>
<td>Pain during acid iontophoresis</td>
<td>Log</td>
<td>0.33 (0.06–0.56)</td>
<td>0.11 (–0.19–0.39)</td>
<td>AE</td>
<td>0.31 (0.07–0.52)</td>
</tr>
<tr>
<td>Pain during ATP iontophoresis</td>
<td>Log</td>
<td>0.29 (0.02–0.52)</td>
<td>–0.08 (–0.36–0.22)</td>
<td>AE</td>
<td>0.22 (0.00–0.45)</td>
</tr>
<tr>
<td>Brush evoked allodynia area</td>
<td>Threshold</td>
<td>0.50 (0.50–0.70)</td>
<td>0.34 (0.17–0.55)</td>
<td>AE</td>
<td>0.25 (0.00–0.62)</td>
</tr>
<tr>
<td>Primary thermal hyperalgesia</td>
<td>Identify</td>
<td>0.19 (–0.09–0.44)</td>
<td>0.16 (–0.13–0.43)</td>
<td>E</td>
<td>–</td>
</tr>
<tr>
<td>Skin flare area</td>
<td>Identify</td>
<td>0.72 (0.56–0.83)</td>
<td>0.55 (0.31–0.72)</td>
<td>CE</td>
<td>–</td>
</tr>
</tbody>
</table>

The intra-pair correlations are shown for both MZ and DZ twin pairs with 95% confidence intervals in parentheses. If a transformation was performed to achieve a normal distribution it is listed in the transformation column (identity indicates no transformation was performed). The column labelled A lists the relative contribution of additive genetic effects and the column labelled C lists the relative contribution of common environment in the best fitting model, as decimal values where 1.00 is equal to 100%. The abbreviations used in the table are: A = Additive Genetic; C = Common Environment; D = Dominant Genetic; E = Unique Environment; Model = Best fitting Model.
(typically 47°C for 420 s) Pedersen and Kehlet (1998). Our thermal burn protocol was shown to be a reliable measure.

The major outcome measure here is the subjective report of pain. The use of VAS ratings for this purpose has been formally validated (Price et al., 1983). Moreover, it appears that the variability in pain reporting between people arises from physiological rather than purely psychological processes, since brain activation patterns studied by fMRI are highly correlated with pain reporting across subjects (Coghill et al., 2003).

The intra-subject correlations between the different pain measures highlighted some interesting interactions. The lack of a significant correlation between the area of skin flare following thermal burn induction, and the areas of punctate hyperalgesia and brush evoked allodynia is supportive of other findings in the literature (Schulte et al., 2004). This suggests that the neuronal mechanisms behind neurogenic flare formation are not directly linked to those responsible for hyperalgesia and allodynia formation in human subjects following a thermal burn injury.

The negative correlation of age and HPT was an interesting finding also seen in other studies (Scudds and Scudds, 1999; Gibson and Farrell, 2004) and a possible mechanism for this is differential age-related changes in HPT served by Aδ compared to C-fibres (Chakour et al., 1996). The decrease in the area of skin flare following thermal burn induction with increasing age has previously been shown using topical application of capsaicin (Helme and McKernan, 1985), and may represent lower primary sensory neurone neuropeptide levels with age.

Potential limitations of the study merit further consideration. The sample was confined to healthy female twins and the result cannot necessarily be extrapolated to males or to individuals experiencing clinical pain. The study sample were healthy volunteers selected from the TwinsUK registry, shown to be representative of the healthy UK adult population for a range of anthropometric, disease-related and lifestyle variables (Andrew et al., 2001). Twins were selected to be included in the present study at random and individual twins were approached independently of their co-twin, without knowledge of the hypothesis. Although it is possible that factors relating to their willingness to particulate and volunteer to be involved in a study of pain perception may have introduced subtle bias, this is unlikely to have a major effect in the twin design.

The sample size of 100 MZ and 100 DZ twins was selected to have a power to detect a heritability of ~30% for a continuous trait (Neale et al., 1994). However, for variables with point estimates of heritability that are <50%, the confidence intervals around A in the AE models in these data are relatively wide, limiting inference on the precise size of the genetic contribution. In all the models except for primary thermal hyperalgesia and skin flare, the higher correlation in MZ when compared to DZ twins infers genetic factors are the most plausible explanation of the data, although a larger sample size would have been needed to formally exclude a small influence of the common environment.

In healthy human subjects quantitative sensory testing (QST) provides a measure of baseline values to simple pain tests. The importance of QST in humans has been recognised in the last few years in a number of studies that have demonstrated that such measures are good predictors of the development of clinically relevant pain in several settings (see ‘Introduction’ section). Our findings of a significant genetic influence on the responses to various experimental pain stimuli are likely therefore to have relevance to more complex pathological and clinically relevant pain states. Pain is one of the most prevalent symptoms patients experience following surgery (Chiaretti and Langer, 2005), and identification of the genetic components determining pain sensitivity could be used to identify patients at risk of high levels of post-operative pain allowing appropriate use of pre-emptive analgesia. Our findings also suggest that some clinical trials studying post-operative pain could benefit from QST measures in selecting the most appropriate patients.

Turkat published a pair of studies which suggested that family pain models can exert an influence on pain behaviour in both healthy and diseased individuals (Turkat, 1982; Turkat and Noskin, 1983). This work could represent familial learning behaviour, or it could be due to biological variations in response to an identical pathology between subjects e.g. pain sensitivity.

There has been a previous attempt by our group to evaluate the heritability of pain sensitivity using a classical twin study approach. The study used a dolorimeter to measure pressure pain threshold (PPT) on the foreheads of 609 healthy female–female twin pairs. The most influential determinant of variation in pain reporting was the shared environment of the twins with genetic factors only accounting for 10% (MacGregor et al., 1997). The difference between the present findings might be accounted for by methodological differences in the two study designs. In the earlier study the twins were not blinded to their co-twins responses and competition between twins may have acted to mask a genetic effect. Alternatively the finding may reflect aspects of the pain response that have not been captured in the present study, that are more greatly influenced by the shared family environment, for example learned patterns of behaviour. We are aware of only one other study of experimental pain in twins (Ullrich et al., 2007) which used a cold pressor test in only 15 twin pairs and was mainly focussed on a comparison of pain and fatigue in chronic fatigue syndrome.

There is evidence in various fields of the literature for several genes exerting a possible effect on pain sensitivity, and these may well be contributing to the results seen in our experiment. In Mogil’s studies of inbred mouse strains,
the largest differences observed between species were seen between AKR and C57BL/6 mice, which were attributed to functional differences in primary afferent nociceptors. The gene which encodes for calcitonin gene-related polypeptide (CGRP) is the Calca gene, and linkage mapping revealed this to be a likely candidate gene for the strain difference in heat pain sensitivity (Mogil et al., 2005).

Another potential candidate for pain sensitivity is the gene encoding catecholine-O-methyltransferase (COMT); an enzyme with a range of biological features including regulating catecholamine and enkephalin levels (Mannisto and Kaakkola, 1999). Variations of the COMT gene have been linked with differing pain sensitivity and variable propensity to developing temporomandibular joint disorder (TMD), a painful condition (Diatchenko et al., 2007). One particular polymorphism in the COMT gene (val158met) may account for some of the role of COMT in shaping pain sensitivity (Zubieta et al., 2003). Other studies have implicated the vanilloid receptor subtype 1 gene (TRPV1) and the δ opioid receptor subtype 1 gene (OPRD1) (Kim et al., 2004), and, more recently, the mu-opioid receptor (OPRM1) gene (Fillingham et al., 2005) and the GTP cyclohydrolase (GCH1) gene (Tegeder et al., 2006). In conjunction with these studies, our research contributes to the evidence for heritability of sensitivity to painful stimuli.

In conclusion, our study is one of the first formal demonstrations of the importance of genetic factors in determining human pain sensitivity. These results will provide insight into the pain responses that are worth pursuing in the search for specific genes (or sets of genes) that might explain this putatively large genetic contribution to pain perception.

Acknowledgements

The project was funded by a project grant from the Arthritis Research Campaign. TwinsUK registry is supported by the Wellcome Trust.

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