
Author pre-print manuscript

Published in final form: Bagwath Persad LA, Kamerman PR, Wadley AL. Predictors of cold and pressure pain tolerance in healthy South African adults. *Pain Medicine* [Epub ahead of press], 2017. DOI: 10.1093/pm/pnw291, PMID: 28082523

Predictors of cold and pressure pain tolerance in healthy South African adults

Authors: Leeana Aarthi Bagwath Persad MSc, Peter Rowland Kamerman PhD, Antonia Louise Wadley PhD

Brain Function Research Group, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Correspondence: Antonia Wadley
School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Rd, Parktown, Johannesburg 2193
Email: antonia.wadley@wits.ac.za
Tel: +27 (0)11 717 2152

Email addresses of all Authors:

LP leeana.bagwathpersad@students.wits.ac.za

ALW antonia.wadley@wits.ac.za

PRK peter.kamerman@wits.ac.za

Running head: Predictors of pain tolerance in Africans

Funding sources: Funding was received from the Medical Faculty Research Endowment Fund of the University of the Witwatersrand, Medical Research Council of South Africa and the National Research Foundation Rated Researchers Programme.

Conflict of interest disclosure: The authors declare they have no conflicts of interest.

Abstract

Background: Studies on relationships between sex, ethnicity and pain largely have emanated from the US and Europe. We compared cold (CPT) and pressure pain tolerance (PPT) in male and female South Africans of African and European ancestry and assessed whether psychosocial factors (including pain beliefs) predicted differences in pain tolerance.

Methods: We recruited 106 (62 female) students of African ancestry and 106 (55 female) of European ancestry and subjected them to a cold-pressor test and pressure algometry. Socioeconomic status (SES), pain catastrophizing, depression, anxiety and pain beliefs were assessed as predictors of differences in pain tolerance.

Results: CPT was lower in students of African compared to European ancestry (for both sexes), and PPT was lower in female than male students (for both ethnicities). Men were less accepting of men expressing pain than were women, with males of African ancestry being least accepting. Multivariate analysis identified that being of African ancestry, and particularly a female of African ancestry predicted lower CPT. Anxiety was of borderline interest. Sex was the only significant predictor of PPT on multivariate analysis (PPT females < males) and catastrophizing was of borderline interest. Female sex and African ancestry were important predictors of acceptance of expression of pain in males. SES was a variable of interest.

Conclusions: Despite a different cultural and social background from US and European cohorts, we saw similar patterns of sex and ethnic differences in CPT and PPT in an African cohort. Traditional psychosocial predictors of pain sensitivity were identified as being of interest but were not strongly associated.

Keywords: cold; pressure; pain; tolerance; experimental; African

Introduction

Sex differences in clinical and experimental pain conditions have been well studied (for review: [1]). Clinically, pain is more prevalent in females who are also likely to experience pain more severely [2, 3], and females also have higher pain sensitivity under some experimental conditions such as lower tolerance to cold pain and pressure pain [1]. A recent systematic review suggested that there is little evidence for the effect of biological and physiological factors on these sex differences, but that psychosocial factors may play a role [4].

Differences in pain sensitivity clinically and experimentally have also been reported between ethnicities, but there are far fewer studies published compared to sex differences in pain sensitivity. In general, compared to people of European descent, African Americans tend to report greater pain sensitivity and disability. For example, African Americans with chronic pain report greater pain severity and disability [5, 6] and African Americans have displayed lower tolerance, higher pain intensity and unpleasantness to several experimental pain stimuli [7, 8]. Psychosocial factors such as socioeconomic status, hypervigilance and acculturation may contribute to differences in pain sensitivity between ethnicities [7, 9, 10].

Importantly, ethnicity and sex interact. For example, differences in pain catastrophizing between Americans of African and European ancestry mediated the sex differences seen in cold pain tolerance [8]. As psychosocial influences on pain perception and behaviour vary across cultures and countries, the effect these influences have on pain experience and expression cannot necessarily be generalised. So whilst *sex* differences may have been well studied in US and European populations, these differences require confirmation in other cohorts including from Africa. Indeed, there is a dearth of data on pain sensitivity, psychosocial modulators of pain sensitivity, and pain beliefs in populations of African ancestry from Africa (we are unaware of any published studies on populations from sub-Saharan Africa).

Whilst several psychosocial factors and their impact on pain both clinically and experimentally have been well studied, beliefs regarding the appropriateness of reporting and expression of pain have not. What studies there are, suggest that beliefs about expression of pain may influence pain sensitivity between sexes and ethnicities. For example, Israeli females believed that females had lower pain sensitivity than did men, but females from the US, UK and Libya expressed the opposite belief [11-13]. And, in a study comparing pain beliefs and pain tolerance in Indian and US university students, Indian students were less accepting about others expressing pain and had greater cold pain tolerance than their American counterparts [14]. The association between beliefs about expression of pain and pain tolerance has not been assessed for more than one pain stimulus before. Furthermore, neither pain beliefs nor their effect on pain tolerance have been assessed in an African population.

Having experimental pain data for an African population would provide a basis for greater understanding of geo-ethnic variation in pain sensitivity and beliefs. Therefore we investigated: i) pain tolerance to thermal and mechanical stimuli, ii) beliefs about pain expression, and iii) psychosocial factors that may influence pain sensitivity in a South African cohort of African ancestry. To establish whether there are sex differences in the measured variables we compared responses in males and females. Lastly, we compared the results to a cohort of males and females of European ancestry. We hypothesised that there would be no difference in pain tolerance to either cold or pressure stimuli between the sexes or ethnicities. Furthermore, we hypothesised that worse socioeconomic status, maladaptive psychological parameters (pain catastrophizing, depression, and anxiety), and negative pain beliefs would not predict lower pain tolerance.

Materials and Methods

Participants

The study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (Clearance certificate: M120648). We recruited healthy students of self-identified African and European ancestry from the student population at the University of the Witwatersrand, Johannesburg between September 2012 and June 2013. All participants were at least in their second year of study at university level, and were not subordinate to the investigators. The University is an English medium institution, and competence in English was assumed. Participants were excluded if they were pregnant, were taking medication for high blood pressure, were currently in pain, had a chronic pain condition, or had used analgesics within four hours of the interventions. We aimed to recruit at least 41 individuals into each ethnicity and sex group in order to detect an effect size of 0.57 for pain tolerance [15, 16].

Measurements

Patients completed questionnaires to assess psychosocial factors (depression, anxiety, pain catastrophizing), socioeconomic status, and pain beliefs before taking part in cold-pressor and pressure pain tests.

Psychosocial factors

The Pain Catastrophizing Scale (PCS) was used to assess catastrophic thinking related to pain [17]. The PCS was administered before exposure to the experimental pain stimuli to determine trait catastrophizing, a person's general tendency to catastrophize. Participants rated each of the 13 items on the questionnaire on a 5-point Likert scale, ranging from 0 "not at all" to 4 "all the time". The total score is 52 with a higher score indicating a greater tendency to catastrophize. PCS scores > 30 indicate a clinically significant level of catastrophizing [18].

Anxiety and depression were assessed using the 25-item Hopkins Symptom Checklist (HSCL-25) [19]. Participants rated the extent to which they had experienced 25 symptoms within the last week, scoring each item on a 4-point Likert scale ranging from 1 "not at all" to 4 "extremely". Mean scores for the 10-item anxiety subscale and the 15-item depression subscale items were calculated (mean subscale scores > 1.75 indicate clinically relevant levels of anxiety or depression [20]).

Socioeconomic status
No validated, quantifiable measure of socioeconomic status (SES) exists for the South African context. Core measurement domains of socioeconomic status (SES) include education, income and occupation [21] and previously used measures in South Africa have assessed ownership of certain assets [22]. Therefore we measured education, income and occupation in a similar manner to a previous study of pain and SES in the US [10] and also asked about ownership of culturally relevant assets. Because the cohort consisted of full-time students we used parental/guardian education level, employment status, and ownership of material household items as proxies for participants' socioeconomic status.

Pain beliefs
The Appropriate Pain Behavior Questionnaire (APBQ) was used to assess pain beliefs [14]. The questionnaire is a 14-item questionnaire that measures beliefs about the appropriateness of expressing pain in the presence of others. Assessed pain behaviors included: crying, grimacing, talking about the pain or holding the painful site. There are two components to the APBQ: the APBQ-Male (APBQ-M), which assesses how appropriate individuals find men expressing pain to be and the APBQ-Female (APBQ-F), which assesses how appropriate individuals find women expressing pain to be.

Eight items on the ABPQ express a positive attitude to pain expression, and 6 express a negative attitude towards expressing pain. Participants rated the extent to which they agreed with each of the 14 statements by scoring them on a 7-point Likert scale, ranging from 1 "strongly disagree" to 7 "strongly agree". To facilitate interpretation of APBQ scores, we calculated the difference between the mean score of the eight statements that assess whether it is appropriate to express pain behaviors and the mean score of the six statements that assess whether it is inappropriate to express pain behaviors. The final score has a bounded range -6 to +6, with negative values indicating a bias towards pain expression being considered inappropriate and positive values indicating a bias towards pain expression being considered appropriate. We interpreted a score close to zero as indicating an individual had a neutral view of pain expression. Results of the ABPQ were assessed both as an outcome measure in the univariate analysis and also as a dependent variable in the multivariate analysis.

Experimental pain procedures

Following completion of the questionnaires, participants underwent a cold-pressor test to assess cold-pain tolerance (CPT), and then a pressure pain test to assess pressure-pain tolerance (PPT). The order of the interventions was kept constant.

The cold-pressor test involved participants submerging their dominant hand in a bath of ~5˚C water. The procedure ended when participants could no longer tolerate the cold or they reached a 300 second cut-off (participants were not informed about the cut-off before the intervention). Time in seconds was recorded from when participants submerged their dominant hand until they reached tolerance or the cut-off time was reached. On completion of the task, participants immediately recorded the intensity of their pain at tolerance or the cut-off time on a 100mm visual analog scale (VAS) anchored at "no pain" and "the worst pain imaginable".

Once participants had indicated that they had regained sensation and had no residual pain in their dominant hand, PPT was assessed in their non-dominant hand. Pressure was applied to the nail bed of the index finger using a pressure algometer with a 10mm^2 probe (Algometer, Somedic AB, Sweden). The test was terminated when participants were unable to tolerate the pressure, or a cut-off pressure of 1500kPa was reached. The pressure being applied, and the intensity of the pain experienced at tolerance or cut-off pressure, was recorded immediately after the intervention.

Statistical analysis

Continuous parametric data are presented as mean (standard deviation, SD) and non-parametric data as median (inter-quartile range, IQR). A Fisher's exact test was used to analyse each of the SES components (education, employment and household assets) and those reaching the time and pressure cutoffs in the cold and pressure tests. A Kruskal-Wallis test was used to compare psychosocial variables, pain beliefs and pain tolerance and intensities for the cold and pain tests. If participants did not complete a questionnaire fully, their score was excluded. For all univariate analyses, significant findings in omnibus tests were followed by specific *post-hoc* comparisons, such that comparisons were performed between ethnicity-matched sex groups (European ancestry males vs European ancestry females, and African ancestry males vs African ancestry females) and sex-matched ethnicity groups (African ancestry males vs European ancestry males, and African ancestry females vs European ancestry females). This pattern of comparisons was used in the univariate analysis to reduce the possible confounding influence interaction between ethnicity and sex may have had on the interpretation of findings. In the multivariate analysis we allowed for interaction between ethnicity and sex. As we were performing four comparisons for each posthoc analysis, we used a Bonferroni correction for multiple comparisons. As such, a p value <0.0125 was deemed significant.

When performing multivariate analyses, the data did not conform to the underlying assumptions of multiple linear regression (i.e., linearity, normality of residuals, homoskedasticity), despite attempts to transform the data, and therefore regression tree analysis was performed. Given the likelihood for complex interactions between sex, ethnicity and psychosocial factors, it is not surprising that the data did not conform to a global linear model. Regression tree analysis is a nonparametric recursive partitioning technique, which involves the repeated partitioning of the data into smaller bits that are more manageable from the perspective of modelling in the presence of interaction. These multiple smaller models are used to build the global model. The regression tree analysis was implemented to model predictors of pain intensity, pain tolerance, and pain behavior. Single trees were generated for each response variable, and the robustness (stability) of each model was confirmed using a bootstrap random forest approach. For the random forest analyses, each model was repeated four times; varying the number of bootstrap samples (500 or 2000) and the number used to seed the modeling process. In all models, the number of randomly preselected predictor variables for each split in the tree was set at three, and the calculation of variable importance assumed possible correlation between predictors. Variables were judged to be informative if their importance value was above the absolute value of the lowest negative-scoring variable. Data analysis was completed using GraphPad Prism 6 (GraphPad, California), and R version 3.1 [23] using the party package [24-27].

Results

Demographic characteristics

Two-hundred and twelve students were recruited and gave informed consent. One-hundred and six participants identified themselves as being of European ancestry (55 female) and 106 participants identified themselves as being of African ancestry (61 female). The four groups (African ancestry males, European ancestry males, African ancestry females and European ancestry females) were similar with regards to age [Mean (SD) of whole cohort: 20.5 years (2.0); Kruskal Wallis between groups: p = 0.42] and years of education [Median (range) 14 (13 - 21); Kruskal Wallis between groups: p = 0.26]. Following posthoc analysis, body mass index was greater in European ancestry males than European ancestry females (24.3 vs 22.8 kg/m^2; Kruskal Wallis; p=0.001) but was similar between the female groups and African ancestry females and males (Kruskal Wallis; p > 0.0125). Regarding socioeconomic status: there was lower ownership of assets by African ancestry students, who were less likely to own a car, washing machine or microwave oven. There was no difference between the groups in terms of their parents' employment status, however, and the difference in education status between the groups' parents, did not survive Bonferroni correction (Table 1).

Univariate analysis

Table 2 shows pain tolerance and pain intensity data for cold-pressor and pressure-pain tests across the ancestry and sex groups and number of individuals in each group who reached the cutoff limit.

Cold pressor test results

Cold pain tolerance differed significantly between the groups, such that female participants had a lower cold pain tolerance than their ancestry-matched male counterparts (Kruskal Wallis test p=<0.01; Post hoc Dunn's tests: AAF < AAM and EAF < EAM), and participants of African ancestry had a lower cold pain tolerance than their sex-matched counterparts of European ancestry (Kruskal Wallis tests p<0.0001; Post hoc Dunn's tests: AAM < EAM and AAF < EAF). Significantly fewer females of African ancestry reached the time cutoff for the cold pain test than females of European ancestry and males of African ancestry (Fisher's exact tests, p<0.0001 for both). There was no difference between the females and males of European ancestry or between the two male groups (Fisher's exact, p>0.05 for both). Despite differences in how long the groups could tolerate the cold water for, all the groups reported similar pain intensity at tolerance (Kruskal Wallis tests; p>0.05).

Pressure test results

For the mechanical stimulus, females had a significantly lower pressure-pain tolerance than their ancestry-matched male counterparts (Kruskal Wallis tests p<0.0001; Post hoc Dunn's tests: AAF < AAM and EAF < EAM). However, within each sex, there were no significant differences between the groups of African and European ancestry (Kruskal Wallis tests; p > 0.05). Despite the sex differences in pressure pain tolerance, all groups reported similar pain intensities at tolerance (Kruskal Wallis; p = 0.46). Very few individuals reached the pressure cutoff, and there were no differences between the groups in the number of people reach the cutoff (Chi squared; p = 0.08).

Pain beliefs

Table 3 shows the results of the APBQ assessment on attitudes towards the expression of pain by males (APBQ-M) and females (APBQ-F). Scores from the APBQ-F showed that on average all groups, (irrespective of sex or ancestry) were accepting of females expressing pain. Scores from the APBQ-M however, showed that males of African ancestry found it less appropriate for males to express pain behaviors than did females of African ancestry and males of European ancestry. Although the scores of males of African ancestry were significantly lower than those of the other groups, the median ABPQ-M score for males of African ancestry still was positive, but close to zero.

Pain catastrophizing, anxiety and depression

Table 4 shows the results for psychological variables (pain catastrophizing, anxiety and depression) in the four groups. Females of African ancestry had higher catastrophizing scores than males of African ancestry and females of European ancestry. There were no differences in catastrophizing scores between the male groups or between females and males of European ancestry. Whilst on average, none of the groups had clinically relevant levels of pain catastrophizing [18], 19% of females of African ancestry, compared with 4% of females of European ancestry, 9% of males of African ancestry, and 2% of males of European ancestry had catastrophizing scores greater than 30, which are considered clinically significant [18].

Female participants had higher scores for the anxiety and depression subscales of the HSCL-25 than their ethnicity-matched male counterparts. There were no differences between the sex-matched groups on the anxiety subscale, nor between the female groups on the depression subscale but males of African ancestry had greater depression scores than the males of European ancestry. Forty-nine percent of females of African ancestry and 40% of females of European ancestry had HSCL-25 anxiety subscale scores greater than 1.75, which are deemed clinically significant [20], compared with 12% of males of African ancestry and 8% of males of European ancestry. Similarly, 54% of females of African ancestry and 34% of females of European ancestry had HSCL-25 depression subscale scores greater than 1.75, compared with 19% of males of African ancestry and 2% of males of European ancestry.

Multivariate analysis

Multivariate regression tree and random forest analysis were used to identify predictors of pain sensitivity and pain beliefs. Variables were included in the model if they were found to be significant on univariate analysis (i.e., predictors of cold-pain tolerance, pressure pain tolerance, APBQ-M).

Figure 1 shows the regression tree analyses for predictors of cold and pressure pain tolerance, and scores for the male appropriate pain behaviour questionnaire. For cold-pain tolerance, regression tree analysis identified being of African ancestry, and particularly being a female of African ancestry predicted lower cold pain tolerance. The importance of these two factors was confirmed by random forest analysis. Additionally, anxiety was identified as a variable of interest but the association was borderline (Supplementary Figure 1). Regression tree analysis identified female sex as the only significant predictor of pressure-pain tolerance, with females having lower pressure-pain tolerance than did men. This finding was confirmed by the random forest analysis and catastrophizing was identified as a variable of interest although the association was borderline (Supplementary Figure 2). Regression tree analysis identified that sex and self-identified ancestry were important predictors of APBQ-M score, such that being female predicted greater APBQ-M scores, and amongst males, being of African ancestry predicted significantly lower APBQ-M scores than did being of European ancestry. Random forest analysis supported these findings and also identified ownership of assets as a variable of interest (Supplementary Figure 3).

Discussion

We assessed i) pain tolerance to thermal and mechanical stimuli, ii) beliefs about pain expression, and iii) psychosocial factors that may influence pain sensitivity in a South African cohort of African ancestry. As sex and ethnicity both affect pain tolerance in clinical and experimental conditions, we assessed sex differences in these variables and also compared the results to a cohort of males and females of European descent. This was the first assessment of factors affecting pain tolerance and intensity of experimental pain in a sub-Saharan African population and the first time more than one stimuli has been assessed in any African population. Our study was necessary because cultural and social factors are reportedly important in influencing pain tolerance [1, 8, 10]. These factors vary between populations and the available data are primarily from the US and Europe, with almost no African, and certainly no sub-Saharan African, data. We hypothesised that, in contrast to previous data, there would be no difference between students of African or European ancestry for tolerance to either cold or pressure stimuli, which was true for tolerance to the pressure test but there was a difference between students of African and European ancestry students for tolerance to the cold test. Males of African ancestry were the least accepting of males expressing pain, but pain beliefs did not explain differences in cold pain tolerance. Anxiety identified as a variable of interest in predicting cold pain tolerance, however. Conversely, sex differences were demonstrated for pressure-pain tolerance but not for cold. We had hypothesised that there would be no differences for either test. Women were more accepting of pain expression than men, but neither pain beliefs nor psychosocial factors explained differences in pressure-pain tolerance. This was the first characterisation of beliefs about the appropriateness of pain expression in an African cohort and the first assessment of these beliefs in relation to pressure pain tolerance anywhere.

Our univariate analyses showed that for both ethnicity groups, male participants had greater cold-pain tolerance than did females, and within each sex, individuals of European ancestry had a greater cold-pain tolerance than their counterparts of African ancestry (Table 2). Both these findings are consistent with those reported by other investigators [1, 28], including those comparing cold-pain tolerance in African Americans to Europeans [7, 29, 30]. Between sex and ethnicity, multivariate analysis demonstrated that ethnicity was the most important of these predictors, followed by sex, such that females of African ancestry had the lowest cold-pain tolerance, followed by males of African ancestry (Figure 1 and Supplementary Figure 1). Anxiety was identified as a variable of interest for predicting cold pain tolerance on random forest analysis but the association was borderline.

There also were similarities between our findings and those of investigators assessing pressure-pain tolerance in European and US cohorts [1], such that we found a significant sex-effect, with males tolerating higher pressures than did females in univariate (Table 2) and multivariate analyses (Figure 1 and Supplementary Figure 2). Moreover, we found no association between ethnicity and pressure-pain tolerance. We are unaware of other studies comparing pressure-pain tolerance between different ethnicities, but studies comparing thresholds for pressure-pain in Europeans and African Americans also reported no ethnicity differences in sensitivity to mechanical stimuli [29, 30].

Differences in cold-pain and pressure-pain tolerance between the groups in our study were not accompanied by significant differences in the pain intensity reported when tolerance was reached. It is not clear whether groups with lower cold- and/or pressure-pain tolerance reached their maximum pain intensity sooner than did those with greater tolerance levels, or whether those with greater tolerance levels merely tolerated the pain intensity for longer once the tolerance intensity had been reached. A limitation of our study was that we did not measure pain intensity at regular intervals during the pain tests, including documenting pain threshold, as these data may have highlighted which of these scenarios was the case. We could also have measured pain intensity at set pressures in the pressure test. Future studies should add these additional pain measures for a more comprehensive understanding of responses to experimental stimuli.

For tolerance differences between males and females, sex-related differences in the physico-mechanical properties of the hand and fingers may have contributed to the differences we observed, but we do not feel such physico-mechanical factors would have contributed significantly to ethnicity differences within each sex. Elsewhere, a study investigating cold-pain tolerance between groups of European ancestry and African Americans also reported no difference in cold-pain intensity at tolerance, despite the African American cohort having lower cold-pain tolerance [30]. Differences in pain intensity reported at tolerance to a mechanical stimulus have been mixed [1].

Socioeconomic status has been found to be an important factor in pain sensitivity [10]. There are several components used in the assessment of socioeconomic status including education, occupation and material wealth and the weighting of each component is debatable [21]. We only found differences in ownership of assets between the groups in our study (Table 1). Ownership of assets was only identified as a borderline variable of interest for predicting APBQ-M score but we recruited from a student population from a single tertiary-education institution and so the cohort may well have been too homogeneous.

Pain catastrophizing is greater in African Americans than it is in Europeans, and is associated with reduced cold-pain tolerance in African Americans compared to subjects of European ancestry [8, 31, 32]. We found no such ethnicity-dependent differences in cold-pain tolerance despite participants of African ancestry having greater levels of pain catastrophizing than did participants of European ancestry, with females of African ancestry having the highest pain catastrophizing scores (Table 4). Neither of the papers assessing trait pain catastrophizing in

US students of African and European ancestry divided their cohort by both sex and ethnicity so sex and ethnicity-dependent comparisons cannot be made [8, 31]. However, mean catastrophizing scores of our entire cohort [15.6 SD (10.4)] were similar to one cohort of US students [15.9 (8.2)] [31], but much lower than another (means of individual sex and ethnicity groups ranged from 21-28) [8]. It is not clear whether healthy young South Africans tend to catastrophize less than those in North America, or whether high catastrophizers did not volunteer to take part in our study [33]. Whilst our lack of association between catastrophizing and cold pain tolerance is consistent with the results from a Danish study [33] the catastrophizing score of these Danish students was so low (median PCS score 8) the analysis may have been unable to detect an association. We did find catastrophizing a variable of interest in predicting PPT, however, the association was borderline. Catastrophizing has associated with PPT in Canadians presenting for physiotherapy for neck pain [34] and also had a borderline association with PPT in healthy Swiss students [35]. We also measured the burden of depression and anxiety symptoms (Table 4). Whilst the HSCL-25 is not a clinical diagnosis, scores >1.75 on the subscales of the HSCL-25 suggest clinically relevant levels of anxiety and/or depression [20]. Over 40% of females had relevant levels of anxiety. Additionally, a third of the females of European ancestry and half of the females of African ancestry had relevant levels of depression. In comparison, clinically relevant anxiety and depression scores in the males of African and European ancestry were less than 19% and 11%, respectively. Whilst depression and anxiety are frequently associated with clinical pain conditions [34], their effect on tolerance to short-lived experimental pain tests and contribution to sex differences in experimental pain are less convincing [4, 35], however, anxiety was a borderline variable of interest for predicting cold pain tolerance.

Like previous reports, we found both sex and culture affected how appropriate expressing pain was seen to be. Whilst all groups were equally accepting of females expressing pain, differences were seen in how appropriate it was perceived for men to express pain. Firstly, there was a sex difference, but only between students of African ancestry. Whilst, other studies comprising European, Japanese and Indian participants, have found females generally to be more accepting than males of men expressing pain [14, 36], females of African ancestry fitted this pattern but there was no difference between females and males of European ancestry. Secondly, we saw an ethnicity difference. In contrast to lower ABPQ scores given by Indian women compared to American women of European ancestry, South African females of African and European ancestry were similarly accepting of pain expression in males [14]. Additionally, like other non-European groups, males of African ancestry were less accepting than males of European ancestry, of men expressing pain [14, 36]. However, to put this in context, males of African ancestry had a score close to zero, indicating a neutral opinion about expressing pain. The advantage of using our version of the scoring system for the ABPQ was that we could pick up this subtlety. Indeed, as all groups were relatively accepting of pain expression, we may not have had the range of scores to pick up an association between pain beliefs and pain sensitivity as seen in the comparison of Indians and Americans [14]. Another limitation of our study was that we only considered sex differences and not gender differences. This would have allowed a more nuanced evaluation of social learning about pain between cultures and gender role expectations about pain have affected pain tolerance elsewhere [13, 37]. Furthermore, the experimenter in this study was female and self-identified as Indian. She was thus ethnicity-neutral to the participants but her sex may have influenced the pain intensity and tolerance scores of participants. That is, male participants may have displayed greater pain tolerance due to the female experimenter's presence [38] and although we saw no differences in pain intensity between all the groups, participants of African ancestry may have reported higher pain intensity ratings [28].

In conclusion, in the first assessment of pain tolerance to two painful stimuli in an African population, we found students of African ancestry had lower cold-pain tolerance than did students of European ancestry, and male students had greater pressure-pain tolerance than did female students. We hypothesized that because of the different social and cultural background in South Africa, we would see different results compared to studies from the US, however our results were the same, suggesting similar contributing factors. Psychosocial factors were identified as variables of interest in predicting cold and pressure pain tolerance and perceptions about the appropriateness of expressing pain. These results require replication in a larger, more socially heterogeneous cohort. In addition to the higher levels of depression and anxiety found in females of African ancestry, we also found high levels of catastrophizing in females of African ancestry too. Should this level of catastrophizing form part of a profile of fear avoidant beliefs, it may predict future risk of chronic pain [39]. If this scenario was the case, non-pharmacological interventions aimed at reducing catastrophic thinking and increasing self-efficacy in females of African ancestry in particular would be warranted.

Acknowledgments
We thank the students who participated in the study. Additionally, AW is grateful to the Hillel Friedland Trust for Fellowship funding.

Author contributions
LP designed the project, collected the data, completed part of the data analysis, discussed the results with PK and AW and wrote the first draft of the manuscript.
PK helped design the project, completed the rest of the data analysis, assisted with data interpretation and reviewed the manuscript.
AW helped design the project, interpreted the data and reviewed and edited the manuscript.
All authors approved the final version of the manuscript.

References

1. Racine M, Tousignant-Laflamme Y, Kloda LA, Dion D, Dupuis G and Choiniere M. A systematic literature review of 10 years of research on sex/gender and experimental pain perception - part 1: are there really differences between women and men? *Pain* 2012;**153**:602-18.

2. Ruau D, Liu LY, Clark JD, Angst MS and Butte AJ. Sex differences in reported pain across 11,000 patients captured in electronic medical records. *The Journal of Pain* 2012;**13**:228-34.

3. Tighe PJ, Riley JL, 3rd and Fillingim RB. Sex differences in the incidence of severe pain events following surgery: a review of 333,000 pain scores. *Pain Medicine* 2014;**15**:1390-404.

4. Racine M, Tousignant-Laflamme Y, Kloda LA, Dion D, Dupuis G and Choiniere M. A systematic literature review of 10 years of research on sex/gender and pain perception - part 2: do biopsychosocial factors alter pain sensitivity differently in women and men? *Pain* 2012;**153**:619-35.

5. Green CR, Baker TA, Sato Y, Washington TL and Smith EM. Race and chronic pain: A comparative study of young black and white Americans presenting for management. *The Journal of Pain* 2003;**4**:176-83.

6. McCracken LM, Matthews AK, Tang TS and Cuba SL. A comparison of blacks and whites seeking treatment for chronic pain. *The Clinical Journal of Pain* 2001;**17**:249-55.

7. Campbell CM, Edwards RR and Fillingim RB. Ethnic differences in responses to multiple experimental pain stimuli. *Pain* 2005;**113**:20-6.

8. Forsythe LP, Thorn B, Day M and Shelby G. Race and sex differences in primary appraisals, catastrophizing, and experimental pain outcomes. *The Journal of Pain* 2011;**12**:563-72.

9. Chan MY, Hamamura T and Janschewitz K. Ethnic differences in physical pain sensitivity: role of acculturation. *Pain* 2013;**154**:119-23.

10. Green CR and Hart-Johnson T. The association between race and neighborhood socioeconomic status in younger Black and White adults with chronic pain. *The Journal of Pain* 2012;**13**:176-86.

11. Alabas OA, Tashani OA and Johnson MI. Effects of ethnicity and gender role expectations of pain on experimental pain: a cross-cultural study. *European Journal of Pain* 2013;**17**:776-86.

12. Defrin R, Shramm L and Eli I. Gender role expectations of pain is associated with pain tolerance limit but not with pain threshold. *Pain* 2009;**145**:230-6.

13. Robinson ME, Riley JL, 3rd, Myers CD, Papas RK, Wise EA, Waxenberg LB and Fillingim RB. Gender role expectations of pain: relationship to sex differences in pain. *The Journal of Pain* 2001;**2**:251-7.

14. Nayak S, Shiflett SC, Eshun S and Levine FM. Culture and gender effects in pain beliefs and the prediction of pain tolerance. *Cross-Cultural Research* 2000;**34**:135-151.

15. Lan L and Lian Z. Application of statistical power analysis – How to determine the right sample size in human health, comfort and productivity research. *Building and Environment* 2010;**45**:1202-1213.

16. Riley JL, 3rd, Robinson ME, Wise EA, Myers CD and Fillingim RB. Sex differences in the perception of noxious experimental stimuli: a meta-analysis. *Pain* 1998;**74**:181-7.

17. Quartana PJ, Campbell CM and Edwards RR. Pain catastrophizing: a critical review. *Expert Review of Neurotherapeutics* 2009;**9**:745-58.

18. Sullivan MJL. The Pain Castrophizing Scale User Manual. 2009;**Available from: http://sullivan-painresearch.mcgill.ca/pdf/pcs/PCSManual_English.pdf**.

19. Derogatis LR, Lipman RS, Rickels K, Uhlenhuth EH and Covi L. The Hopkins Symptom Checklist (HSCL): a self-report symptom inventory. *Behavioral Science* 1974;**19**:1-15.

20. Nettelbladt P, Hansson L, Stefansson CG, Borgquist L and Nordstrom G. Test characteristics of the Hopkins Symptom Check List-25 (HSCL-25) in Sweden, using the Present State Examination (PSE-9) as a caseness criterion. *Social Psychiatry and Psychiatric Epidemiology* 1993;**28**:130-3.

21. Shavers VL. Measurement of socioeconomic status in health disparities research. *JAMA: Journal of the National Medical Association* 2007;**99**:1013-23.

22. Barbarin OA and Khomo N. Indicators of economic status and social capital in South African townships. *Childhood* 1997;**4**:193-222.

23. Team CR. R: A language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria* 2015;**http://www.R-project.org/**.

---

24. Hothorn T, Buhlmann P, Dudoit S, Molinaro A and Van der Laan MJ. Survival ensembles. *Biostatistics* 2006;**7**:355-373.

25. Hothorn T, Hornik K and Zeileis A. Unbiased recursive partitioning: a conditional inference framework. *Journal of Computational and Graphical Statistics* 2006;**15**:651-674.

26. Strobl C, Boulesteix AL, Kneib T, Augustin T and Zeileis A. Conditional variable importance for random forests. *BMC Bioinformatics* 2008;**9**:307.

27. Strobl C, Boulesteix AL, Zeileis A and Hothorn T. Bias in random forest variable importance measures: illustrations, sources and a solution. *BMC Bioinformatics* 2007;**8**:25.

28. Weisse CS, Foster KK and Fisher EA. The influence of experimenter gender and race on pain reporting: does racial or gender concordance matter? *Pain Medicine* 2005;**6**:80-7.

29. Rahim-Williams FB, Riley JL, 3rd, Herrera D, Campbell CM, Hastie BA and Fillingim RB. Ethnic identity predicts experimental pain sensitivity in African Americans and Hispanics. *Pain* 2007;**129**:177-84.

30. Riley JL, 3rd, Cruz-Almeida Y, Glover TL, King CD, Goodin BR, Sibille KT, Bartley EJ, Herbert MS, Sotolongo A, Fessler BJ, Redden DT, Staud R, Bradley LA and Fillingim RB. Age and race effects on pain sensitivity and modulation among middle-aged and older adults. *The Journal of Pain* 2014;**15**:272-82.

31. Fabian LA, McGuire L, Goodin BR and Edwards RR. Ethnicity, catastrophizing, and qualities of the pain experience. *Pain Medicine* 2011;**12**:314-21.

32. Meints SM and Hirsh AT. In Vivo praying and catastrophizing mediate the race differences in experimental pain sensitivity. *The Journal of Pain* 2015;**16**:491-7.

33. Kristiansen FL, Olesen AE, Brock C, Gazerani P, Petrini L, Mogil JS and Drewes AM. The role of pain catastrophizing in experimental pain perception. *Pain Practice* 2014;**14**:E136-45.

34. Walton DM, Levesque L, Payne M and Schick J. Clinical pressure pain threshold testing in neck pain: comparing protocols, responsiveness, and association with psychological variables. *Physical Therapy* 2014;**94**:827-37.

35. Neziri AY, Scaramozzino P, Andersen OK, Dickenson AH, Arendt-Nielsen L and Curatolo M. Reference values of mechanical and thermal pain tests in a pain-free population. *European Journal of Pain* 2011;**15**:376-83.

36. McWilliams LA, Goodwin RD and Cox BJ. Depression and anxiety associated with three pain conditions: results from a nationally representative sample. *Pain* 2004;**111**:77-83.

37. Kulkarni B, Bentley DE, Elliott R, Julyan PJ, Boger E, Watson A, Boyle Y, El-Deredy W and Jones AK. Arthritic pain is processed in brain areas concerned with emotions and fear. *Arthritis and Rheumatism* 2007;**56**:1345-54.

38. Hobara M. Beliefs about appropriate pain behavior: cross-cultural and sex differences between Japanese and Euro-Americans. *European Journal of Pain* 2005;**9**:389-93.

39. Robinson ME, Gagnon CM, Riley JL, 3rd and Price DD. Altering gender role expectations: effects on pain tolerance, pain threshold, and pain ratings. *The Journal of Pain* 2003;**4**:284-8.

40. Kallai I, Barke A and Voss U. The effects of experimenter characteristics on pain reports in women and men. *Pain* 2004;**112**:142-7.

41. Westman AE, Boersma K, Leppert J and Linton SJ. Fear-avoidance beliefs, catastrophizing, and distress: a longitudinal subgroup analysis on patients with musculoskeletal pain. *The Clinical Journal of Pain* 2011;**27**:567-77.

Table 1. Indicators of socioeconomic status

	Male		Female		Omnibus Fisher's exact test p-value	Outcome of *post hoc* 2x2 Fisher's exact tests
	African ancestry count (%)	European ancestry count (%)	African ancestry count (%)	European ancestry count (%)		
PARENT/GUARDIAN'S EDUCATION LEVEL *(sample size)**	*41*	*49*	*63*	*56*		
None	0 (0)	0 (0)	0 (0)	0 (0)	0.03	Unable to detect
Primary school	5 (12)	0 (0)	2 (3)	0 (0)		
High school	8 (20)	8 (16)	8 (13)	6 (11)		
Tertiary level	28 (68)	41 (84)	53 (84)	50 (89)		
PARENT/GUARDIAN'S EMPLOYMENT STATUS *(sample size)**	*44*	*50*	*64*	*56*		
Income	38 (86)	49 (98)	56 (88)	54 (96)	0.05	
No income	6 (14)	1 (2)	8 (12)	2 (4)		
HOUSEHOLD ASSETS *(sample size)**	43	50	64	56		
Refrigerator						
Yes	41 (95)	50 (100)	64 (100)	55 (98)	0.12	
No	2 (5)	0 (0)	0 (0)	1 (2)		
Television						
Yes	39 (91)	49 (98)	62 (97)	53 (95)	0.40	
No	4 (9)	1 (2)	2 (3)	3 (5)		
Home telephone						
Yes	27 (63)	47 (94)	40 (63)	50 (89)	< 0.01	AAM < EAM AAF < EAF
No	16 (37)	3 (6)	24 (37)	6 (11)		
Car						
Yes	28 (65)	50 (100)	50 (78)	55 (98)	< 0.01	AAM < EAM AAF < EAF
No	15 (35)	0 (0)	14 (22)	1 (2)		
Washing machine						
Yes	29 (67)	50 (100)	53 (83)	55 (98)	<0.01	AAM < EAM

No	14 (33)	0 (0)	11 (17)	1 (2)		AAF < EAF
Microwave						
Yes	35 (81)	50 (100)	59 (92)	55 (98)	< 0.01	AAM < EAM
No	8 (19)	0 (0)	5 (8)	1 (2)		

* Incomplete data

Post hoc 2x2 Fisher's exact tests were performed only if $p < 0.05$ for the omnibus comparison

AAM: African ancestry male; AAF: African ancestry female; EAM: European ancestry male; EAF: European ancestry female

Table 2. Pain tolerance, intensity and numbers reaching cutoff data for cold and pressure-pain tests

	Males				Females			
	African ancestry (*n = 44*)		European ancestry (*n = 51*)		African ancestry (*n = 62*)		European ancestry (*n = 55*)	
	Median (IQR)	**No (%) reaching test cutoff**	**Median (IQR)**	**No (%) reaching test cutoff**	**Median (IQR)**	**No (%) reaching test cutoff**	**Median (IQR)**	**No (%) reaching test cutoff**
Cold pain tolerance (s)	57 (41-300)	17 (39)	300 (70-300)	29 (57)	41(29-63)	4 (6)	111 (39-300)	22 (40)
Cold pain intensity (VAS: 0 to 100)	54 (37-75)	-	58 (36-69)	-	62 (43-72)	-	52 (27-68)	-
Pressure pain tolerance (kPa)	976 (710-1101)	1 (2)	915 (792-1134)	3 (6)	685 (545-859)	0 (0)	655 (557-810)	0 (0)
Pressure pain intensity (VAS: 0 to 100)	58 (41-71)	-	55 (40-69)	-	52 (34-66)	-	51 (35-68)	-

Differences in pain tolerance and intensity analysed with a Kruskal Wallis. Numbers reaching test cutoff analysed with a Fisher's exact test. Results presented in the text.

Table 3. Attitudes towards females (APBQ-F) and males (APBQ-M) expressing pain*

	Males†		Females‡		KW statistic	p-value	Outcome of *post hoc* Dunn's tests
	African ancestry *(n = 44)*	European ancestry *(n = 52)*	African ancestry *(n = 64)*	European ancestry *(n = 56)*			
APBQ-F	3.6 (2.8 - 4.7)	3.9 (2.3 - 5.3)	3.2 (1.8 - 4.7)	3.6 (2.8 - 4.7)	4.34	0.23	
APBQ-M	0.4 (-1.5 - 2.4)	2.2 (0.8 - 4.1)	3.2 (1.6 - 4.3)	3.1 (1.6 - 4.8)	35.2	≤ 0.01	BM < WM BM < BF

Data are presented as median (IQR)

APBQ: Appropriate Pain Behavior Questionnaire

KW: Kruskal-Wallis test. *Post hoc* analyses were performed only if $p < 0.05$ for KW

*APBQ scores range from -6 to +6. Negative values indicate pain expression is perceived as inappropriate. Positive values indicate pain expression is perceived as appropriate.

†Missing APBQ-F data from 1 African ancestry male and 9 European ancestry males

‡Missing APBQ-M data from 7 African ancestry females and 5 European ancestry females

Table 4. Sex and race-dependent differences in pain catastrophizing, anxiety, and depression

	Males		Females		KW statistic	p-value	Outcome of *post hoc* Dunn's tests
	African ancestry *(n = 44)*	European ancestry* *(n = 52)*	African ancestry[†] *(n = 64)*	European ancestry *(n = 56)*			
PCS score	15 (13-30)	11 (7-18)	22 (10-22)	12 (5-18)	28.91	≤ 0.01	AAM < AAF EAF < AAF
HSCL-25 anxiety score	1.35 (1.10-1.60)	1.40 (1.30-1.50)	1.70 (1.40-2.20)	1.60 (1.40-2.06)	29.83	≤ 0.01	AAM < AAF EAM < EAF
HSCL-25 depression score	1.30 (1.15-1.67)	1.20 (1.07-1.33)	1.70 (1.40-2.13)	1.47 (1.27-1.87)	50.81	≤ 0.01	AAM < AAF EAM < EAF

Data presented as median (IQR)
KW: Kruskal-Wallis test. *Post hoc* analyses were performed only if $p < 0.05$ for KW
PCS: Pain Catastrophizing Scale. Scores > 30 indicate a clinically significant level of pain catastrophizing[34]
HSCL-25: 25-item Hopkins Symptom Checklist. Subscale scores > 1.75 indicate a clinically relevant level of anxiety or depression[20]
AAM: African ancestry male; AAF: African ancestry female; EAM: European ancestry male; EAF: European ancestry female
*Missing HSCL-25 data for 1 individual † Missing PCS data for 2 individuals

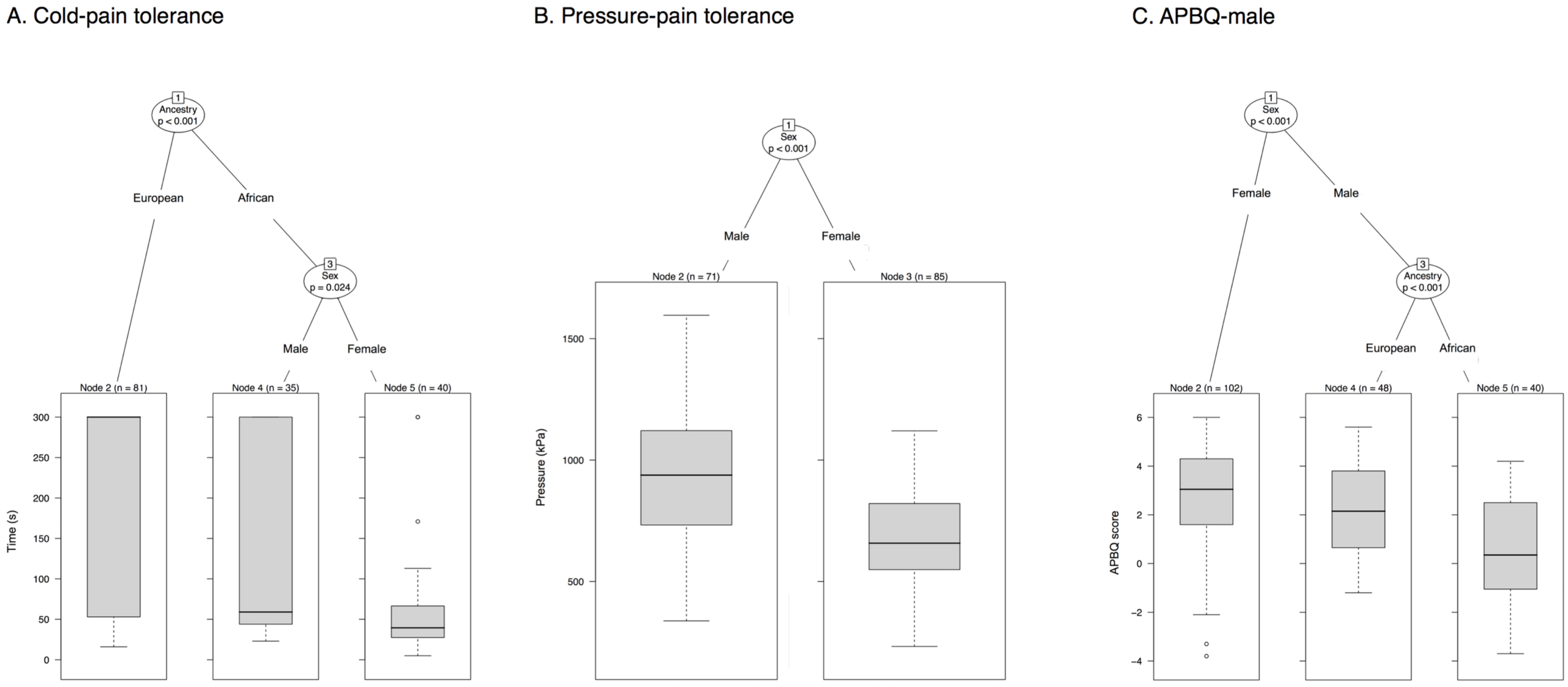
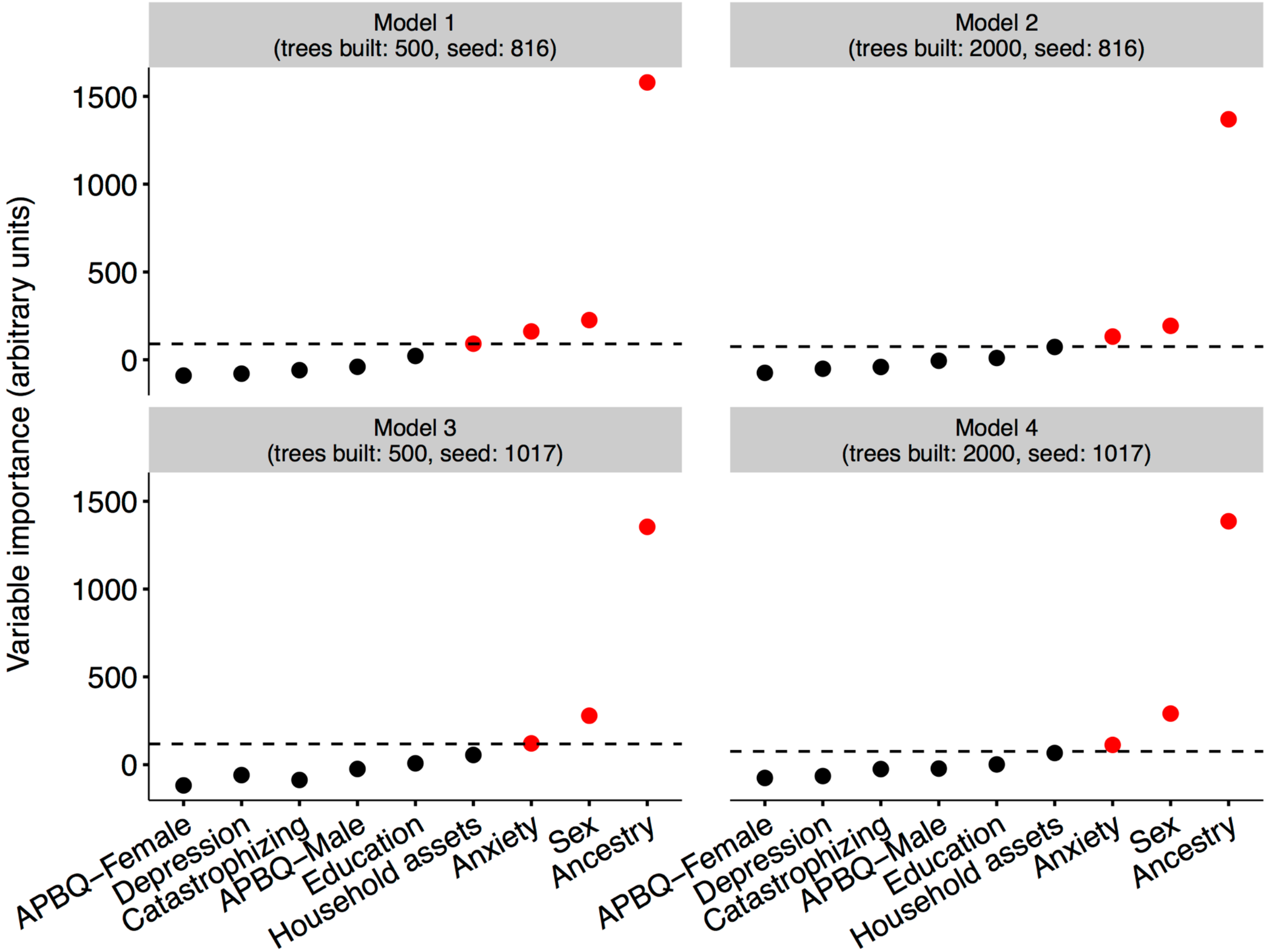


Figure 1. Regression tree plot for A) cold pain tolerance B) pressure-pain tolerance and C) APBQ-M scores. Levels (nodes) are hierarchically arranged, with the top level being the most informative predictor. The lower panels show the box-and-whisker plots (median, IQR and range) of time to tolerance (seconds) in each branch.



Supplementary Figure 1. Random forests plots of predictors of cold-pain tolerance under four different modelling conditions. Predictors above the dashed line (red points) are informative variables for predicting cold-pain tolerance.