Research Paper

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Genome-wide association analysis of pain severity in dysmenorrhea identifies association at chromosome 1p13.2, near the nerve growth factor locus

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Abstract

Dysmenorrhea is a common chronic pelvic pain syndrome affecting women of childbearing potential. Family studies suggest that genetic background influences the severity of dysmenorrhea, but genetic predisposition and molecular mechanisms underlying dysmenorrhea are not understood. In this study, we conduct the first genome-wide association study to identify genetic factors associated with dysmenorrhea pain severity. A cohort of females of European descent (n = 11,891) aged 18 to 45 years rated their average dysmenorrhea pain severity. We used a linear regression model adjusting for age and body mass index, identifying one genome-wide significant ($P < 5 \times 10^{-8}$) association (rs7523086, $P = 4.1 \times 10^{-14}$, effect size 0.1 [95% confidence interval, 0.074–0.126]). This single nucleotide polymorphism is colocalising with *NGF*, encoding nerve growth factor. The presence of one risk allele corresponds to a predicted 0.1-point increase in pain intensity on a 4-point ordinal pain scale. The putative effects on NGF function and/or expression remain unknown. However, genetic variation colocalises with active epigenetic marks in fat and ovary tissues, and expression levels in aorta tissue of a noncoding RNA flanking *NGF* correlate. Participants reporting extreme dysmenorrhea pain were more likely to report being positive for endometriosis, polycystic ovarian syndrome, depression, and other psychiatric disorders. Our results indicate that dysmenorrhea pain severity is partly genetically determined. NGF already has an established role in chronic pain disorders, and our findings suggest that NGF may be an important mediator for gynaecological/pelvic pain in the viscera.

Keywords: Genome-wide association study, Dysmenorrhea pain severity, NGF locus

1. Introduction

Dysmenorrhea or painful menstruation is the most common gynaecological condition for women of reproductive age.[18] The prevalence of dysmenorrhea is unclear with studies performed in different populations reporting between 16% and 91%.[46] Despite its potentially high prevalence, dysmenorrhea remains underdiagnosed and undertreated,[13,18,73] impacting quality of life and productivity,[5] with considerable negative economic consequences (due to, eg, absenteeism from work).[45]

Classified as a chronic pelvic pain syndrome,[41] dysmenorrhea can be either primary or secondary based on the presence of underlying pathophysiology.[73] Primary dysmenorrhea (PD) consists of painful cramping in the lower abdomen accompanying menstruation, in the absence of any clear pathology.[37] Secondary dysmenorrhea is caused by existing pelvic conditions, the most common being endometriosis.[18,43] Dysmenorrheic pain exhibits features typical of visceral pain including poor localization with referral to and sensitization of somatic structures, in conjunction with nonspecific motor responses and strong autonomic and affective components.

Dysmenorrhea affects 16% to 91% of women of reproductive age,[46] but estimates are compounded by the lack of standardised definitions and diagnostic methodology.[16,42,71,73] The rates of PD are believed to be greatly underestimated, as few affected women seek medical diagnosis[45,95]; however, between 2% and 28% report their dysmenorrheic pain as either severe or distressing.[5,12,26,46]

Dysmenorrheic pain is of uterine origin, and predominantly a function of increased production of prostaglandins (inflammatory mediators) released by necrotic cells shed during menstruation, and it is these prostaglandins that sensitise nerve endings.[44] Although inhibition of prostaglandin-producing cyclooxygenase enzymes by nonsteroidal anti-inflammatory drugs are the most commonly prescribed pharmacological treatment for dysmenorrhea,[36,97] approximately 15% of women do not respond or have adverse effects to nonsteroidal

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anti-inflammatory drugs.[14] Oral contraceptives are used as a second-line therapy with some success. Primary dysmenorrhea has a high cooccurrence rate with other chronic pain conditions, such as irritable bowel syndrome,[4] fibromyalgia[78,96] and migraine,[56] and PD has been hypothesised to sensitise women to other pain syndromes.[9,85,90]

Risk factors for PD severity include earlier age at menarche and heavier menstrual flow,[38,82] younger age,[61,75] nulliparity,[48,82] and depression.[3,25] Women using oral contraceptives generally report less severe dysmenorrhea.[48,61,82] Other factors including smoking[61,69] and higher body mass index (BMI),[47] alcohol consumption,[38,69] education, employment,[64] marital status,[61] and physical activity[38,82] have been investigated, with inconclusive results. A positive family history also increases the risk of PD,[46,70] with studies reporting odds ratios between 3.8 (95% confidence interval 2.2–6.9) and 20.7 (95% confidence interval, 11.5–37.4),[83,89] suggesting a role for genetic factors on the development of dysmenorrhea.

To better understand variation in dysmenorrhea pain severity and identify genetic predisposition factors, we conducted a large genome-wide association study (GWAS) of self-reported dysmenorrhea pain in research participants from the 23andMe cohort,[28] which revealed a novel association at the nerve growth factor gene locus.

2. Methods

2.1. Description of the study cohort

Participants in our cohort were drawn from the customer base of 23andMe, Inc., a personal genetics company. Research participants provided informed consent and answered research questions online, under a protocol approved by the external AAHRPP-accredited institutional review board, Ethical and Independent Review Services (E&I). We designed a single survey to capture variance in dysmenorrhea pain severity on a 4-point scale (Supplementary Note, available online at http://links.lww.com/PAIN/A320) and deployed it to female research participants who had previously completed a range of health and lifestyle surveys. There were no restrictions applied relating to the presence of primary or secondary dysmenorrhea conditions. The dysmenorrhea question was devised to capture the full spectrum of research participant assessment of their pain levels before first childbirth, as parity has previously been suggested to reduce individual's reporting of dysmenorrhea pain severity level.[48,82] An upper age restriction of 45 years was applied to control for any possible confounding effect from poor recall of pain severity experienced by postmenopausal women. To minimise changes over time, we asked participants to recall their pain on average.

2.2. Statistical genome-wide association study analysis

Individuals were included in analysis based on selection for having >97% European ancestry. Further to this, a maximal set of unrelated individuals were chosen. This was determined by the methodology pipeline of 23andMe, described in detail in the Supplementary Note (available online at http://links.lww.com/PAIN/A320). We performed the genome-wide test using data from question 1 for dysmenorrhea pain severity using linear regression of the ordinal response against genotype, assuming an additive model for allelic effects, and adjusting for age and 5 principal components of the genotype data matrix. We applied genomic control inflation corrections to the individual GWAS result set ($\lambda = 1.024$). The dysmenorrhea pain severity phenotype (Q1) was captured as an ordered variable with the following possible values scored as $0 > 1 > 2 > 3$ ("not painful" > "little painful" > "moderately painful" > "extremely painful"), respectively, excluding responders selecting "I'm not sure."

3. Results

3.1. Dysmenorrhea cohort

We observed a relatively normal distribution in participant responses across the 4 choices for dysmenorrheic pain severity (**Table 1**, top row). To further define our cohort demographically, we collated phenotypic data for common conditions that have previously been suggested as modulatory factors for dysmenorrhea-related conditions or dysmenorrhea pain severity levels (phenotype ascertainment and logic for defining cophenotype status is described in Supplementary Note, available online at http://links.lww.com/PAIN/A320).

Table 1

Cohort characteristics of female research participants from 23andMe reporting dysmenorrhea pain severity.

	Pain severity question answer options				Total (n)
	Not painful (n)	Little painful (n)	Moderately painful (n)	Extremely painful (n)	
Responders (% total)	1785 (15.0)	3478 (29.2)	4208 (35.4)	2420 (20.4)	11,891
Age range					
18-29 y (% total)	492 (12.4)	1114 (28.1)	1522 (38.4)	839 (21.1)	3967 (33.4)
30-45 y (% total)	1293 (16.3)	2364 (29.8)	2686 (33.9)	1581 (20.0)	7924 (66.6)
Cophenotype					
Endometriosis (positive/negative [% positive])	38/1176 (3.1)	77/2276 (3.3)	181/2659 (6.4)	315/1309 (19.4)	611/7411 (7.6)
Uterine fibroids (positive/negative [% positive])	38/613 (5.8)	78/1138 (6.4)	128/1307 (8.9)	135/761 (15.1)	379/3819 (9.0)
PCOS (positive/negative [% positive])	87/1140 (7.1)	167/2240 (6.9)	208/2762 (7.0)	204/1492 (12.0)	666/7634 (8.0)
Depression (positive/negative [% positive])	500/1218 (29.1)	1179/2166 (35.2)	1594/2442 (39.5)	1087/1240 (46.7)	4360/7066 (38.2)
Some psychiatric disease (positive/negative [% positive])	729/1020 (41.7)	1654/1756 (48.5)	2212/1933 (53.4)	1401/980 (58.8)	5996/5689 (51.3)
Some hormonal oral contraceptive (positive/negative [% positive])	522/773 (40.3)	899/1631 (35.5)	987/2153 (31.4)	586/1233 (32.2)	2994/5790 (34.1)

Unrelated European female participants recorded their pain severity level as 1 of 4 answer options; n, number of responders; responder number for each answer options were broken down into age range, and whether responder self-reported as positive or negative, and % positive for a listed cophenotype.
PCOS, polycystic ovarian syndrome.

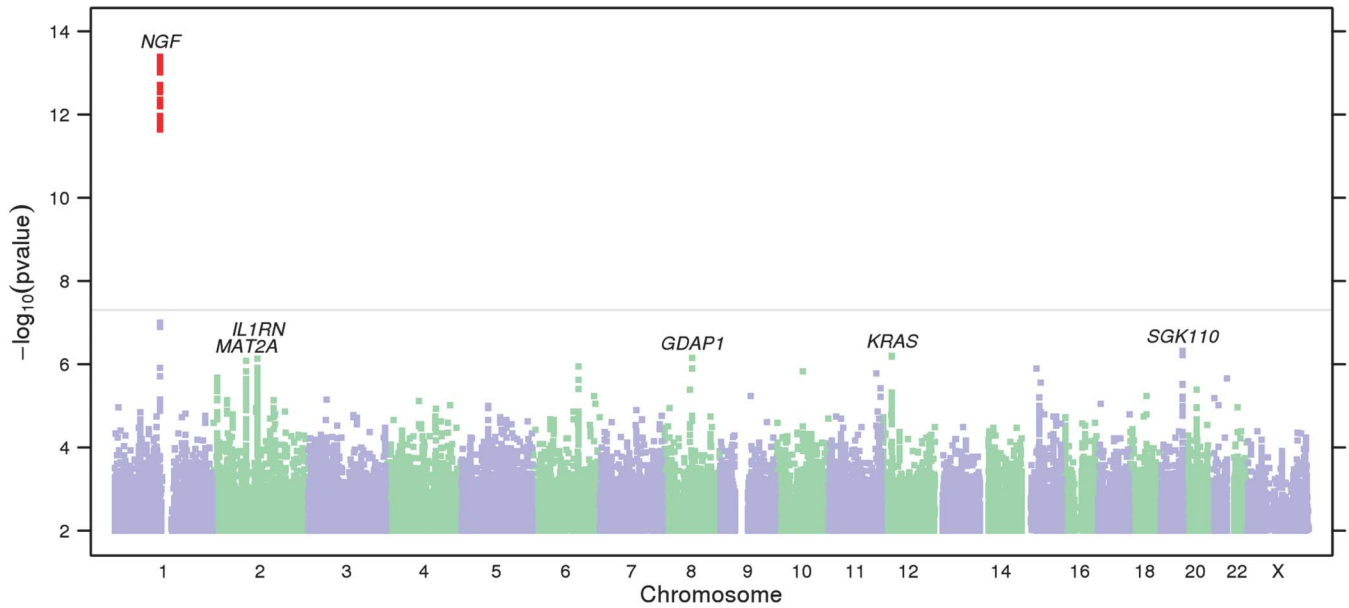

Figure 1. Manhattan plot of genome-wide significant loci for dysmenorrhea pain severity. The gray line corresponds to $P = 5 \times 10^{-8}$, and results above this threshold are shown in red. Gene labels are annotated as the nearby genes to the significant SNPs.

For a large proportion of our cohort, we were able to assign positive or negative status for endometriosis (n = 8022), uterine fibroids (n = 4198), polycystic ovarian syndrome (PCOS, n = 8300), depression (n = 11,426), any other psychiatric disease (excluding depression, n = 11,685), or having been prescribed hormonal oral contraceptive use (n = 8784) (**Table 1**).

The prevalence of these conditions recorded by other studies accords well with that observed in our cohort. The use of self-reported phenotype is validated by demonstrating the comorbidity profile for clinically diagnosed dysmenorrhea matches that previously reported in the literature. Overall, we observed a 7.6% incidence of endometriosis, which has been reported to affect between 5% and 10% of women of reproductive age.[30] The prevalence of uterine fibroids was 9.0% in our cohort, within the 4.5% to 17.8% range reported in the general population for women aged 30 to 39 years.[35] For PCOS, we observed an incidence of 8.0%, which is in keeping with the 4% to 8% prevalence estimates in women of reproductive age.[7,8]

For depression, the incidence of women receiving a formal diagnosis of major depressive disorder is approximately 21%.[57] Depression linked to premenstrual syndrome is more prevalent, with a rate between 20% and 40%.[66] In our study, depression

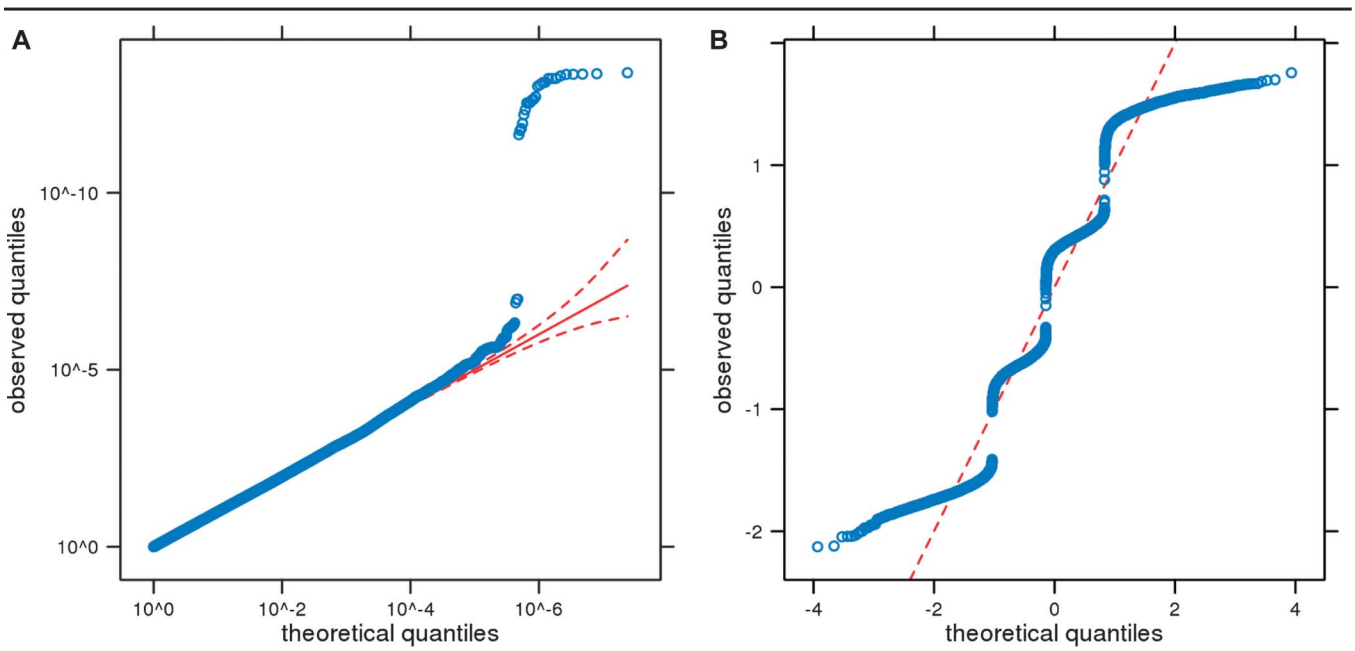

Figure 2. Quantile–quantile plots. (A) Observed *P* values vs theoretical *P* values under the null hypothesis of no association, plotted on a log scale. The solid red line is shown with a slope of 1, and dashed red lines represent a 95% confidence envelope under the assumption that the test results are independent. (B) Q-Q plot of residuals from the null model vs a normal distribution.

Table 2

Genome-wide significant index single nucleotide polymorphisms (SNPs) for dysmenorrhea pain severity variation.

SNP	Region	Chr	Position	SNP quality	Alleles (A/B)	BAF	Effect size for B allele (95% CI)	*P*	Gene context
rs7523086	1p13.2	chr1	115,823,387	0.9992	A/G	0.621	0.1 (0.074 to 0.126)	4.1×10^{-14}	TSPAN2—[]-NGF
rs201074342	19q13.42	chr19	56,051,311	0.6848	D/I	0.896	−0.138 (−0.192 to −0.085)	4.8×10^{-7}	SBK2-[]SGK110
rs7137301	12p12.1	chr12	25,450,816	0.8888	A/T	0.453	0.068 (0.041 to 0.095)	6.4×10^{-7}	KRAS–[]—IFLTD1
rs189805869	8q21.11	chr8	75,435,311	0.6948	A/G	0.007	−0.409 (−0.571 to −0.247)	7.1×10^{-7}	GDAP1—[]—PI15
rs315934	2q13	chr2	113,883,706	0.9837	C/T	0.796	0.078 (0.047 to 0.109)	7.4×10^{-7}	IL1F10–[]-IL1RN
rs186838326	2p11.2	chr2	85,715,143	0.87	C/T	0.059	0.193 (0.116 to 0.270)	8.4×10^{-7}	SH2D6–[]–MAT2A

Region, cytogenetic band; chr, chromosome; position, build 37 map position of the SNP; SNP quality is average r^2 from imputation; alleles A and B are assigned based on their alphabetical order; BAF, B allele frequency across all study participants; effect size, magnitude of effect for the B allele; CI, confidence interval; *P*, λ adjusted significance level; gene context, gene(s) spanning or flanking (<1 Mb away from) the index SNP: brackets indicate the position of the SNP, and dashes indicate distance to a flanking gene (-, >1 kb, >10 kb, >100 kb).

was observed in 38.2%, perhaps suggesting that not all responders positive for depression receive a formal major depressive disorder diagnosis, but do experience some mood suppression, irritability, and lethargy that typify premenstrual syndrome, which has depressive features.

We were also able to determine the collective incidence of other psychiatric disorders, having separated out depression for separate analysis because of its strong links to pain perception reported in the literature. The prevalence for women developing the various psychiatric disorders captured in our cohort are as follows: anxiety (30.5%),[58] posttraumatic stress disorder (10.4%),[33] attention-deficit disorder/attention-deficit hyperactivity disorder (4.9%),[51] panic attacks (3.8%),[50] phobias (3.5%),[94] obsessive-compulsive disorder (3.1%),[58] schizophrenia (2.7%),[60] bipolar disorder/manic depression (1.3%),[80] and eating disorders, including anorexia (0.9%) and bulimia (0.5%),[40] autism (0.5%) and Asperger syndrome (~0.07%),[27] which are not necessarily mutually exclusive, but collectively total 62.5%. These psychiatric disorders were captured in our cohort using self-reporting surveys with varying diagnostic stringency; a positive status was given for surveys phrased to ask whether a particular condition was diagnosed by a medical professional, in addition to surveys that were phrased to capture what conditions the responder had experienced (Supplementary Note, available online at http://links.lww.com/PAIN/A320). Clearly, self-reporting surveys may incur some level of false reporting that could confound accurate phenotyping. Overall, 51.3% of our dysmenorrhea cohort self-reported being positive for one or more of these psychiatric conditions, reflecting the high incidence of psychiatric diseases when assessed in aggregate.

For our dysmenorrhea cohort, 34.1% self-reported as currently taking hormonal contraceptive. According to the US National Health Statistics,[22] between 2011 and 2013, approximately 16% of women aged 15 to 44 years took oral contraceptive, 7.2% were on long-acting reversible contraceptives (contraceptive implants and intrauterine devices), 4.4% used the Depo-Provera contraceptive ring/patch, totalling 27.6% of women who may be taking some hormonal oral contraceptive. The higher percentage of hormonal contraceptive consumption in our dysmenorrhea cohort could be due to that it has been used as way to treat pain.

We observed that when compared with the lower 3 pain severity groups, those reporting "extremely painful" dysmenorrhea were significantly enriched for endometriosis ($P \leq 2.2 \times 10^{-16}$), uterine fibroids ($P = 5.5 \times 10^{-13}$), PCOS ($P = 4.0 \times 10^{-12}$), and also depression and any psychiatric condition (both $P \leq 2.2 \times 10^{-16}$ all using the Pearson χ^2 test). Oral contraceptive use was less common in those reporting the most severe pain, compared with those not reporting any pain ($P = 5.8 \times 10^{-7}$) (**Table 1**).

3.2. Results from genome-wide association study on dysmenorrhea pain severity

To identify common genetic factors that correlate with dysmenorrhea pain severity, we analysed participant data from a total of 11,892 unrelated females of European descent. After quality controls, a total of 11,942,402 single nucleotide polymorphisms (SNPs) were analysed by linear regression adjusting for BMI, age, and principal components, and then adjusted for a genomic inflation factor of 1.024. Analysis revealed one genome-wide significant (GWS) association ($P < 5 \times 10^{-8}$) at a region on chromosome 1p13.2 (**Fig. 1** for the Manhattan plot, **Fig. 2** for the quantile–quantile plots, **Table 2** for covariate results from GWAS analysis and **Table 3** for a list of top associations). The GWS association colocalises with *NGF*, the gene encoding nerve growth factor (rs7523086, $P = 4.1 \times 10^{-14}$) (**Fig. 3**). The index SNP rs7523086 is a common polymorphism (G/A, MAF A allele: 0.328, 1000 Genomes CEU population), and the effect size of 0.1 (95% confidence interval, 0.074-0.126), means that the presence of one risk allele corresponds to a predicted increase of 0.1 points in a 4-point ordinal scale for increasing pain intensity. Furthermore, there were 5 loci with suggestive levels of association ($P < 1 \times 10^{-7}$) (**Table 3**).

3.3. Functional annotation of genetic association at 1p13.2

We investigated the functional significance of the GWS association at 1q13.2 by using the Web-based computational tool HaploReg, which facilitates identification of tagging SNPs

Table 3

Results from covariates after fitting null model for dysmenorrhea pain severity.

	Estimate	SE	*t*	Pr (>|*t*|)
Age	−0.01149	0.00134	−8.6	1.2×10^{-17}
BMI	0.01121	0.00142	7.9	3.8×10^{-15}
pc.0	0.01644	0.00896	1.8	0.067
pc.1	−0.01082	0.00895	−1.2	0.23
pc.2	−0.00136	0.00895	−0.2	0.88
pc.3	0.00499	0.0089	0.6	0.58
pc.4	0.00173	0.00888	0.2	0.85

Principal coordinates have been standardized, so the effect sizes for those are given in units of ordinal pain per SD (denoted by the "Estimate" column), whereas for age and body mass index (BMI), the effect estimates are in units of ordinal pain per year and per BMI unit, respectively. The SE of the effect estimates are given by the "SE" column, whereas the significance of each term in the linear regression model is given by column "Pr (>|*t*|)."

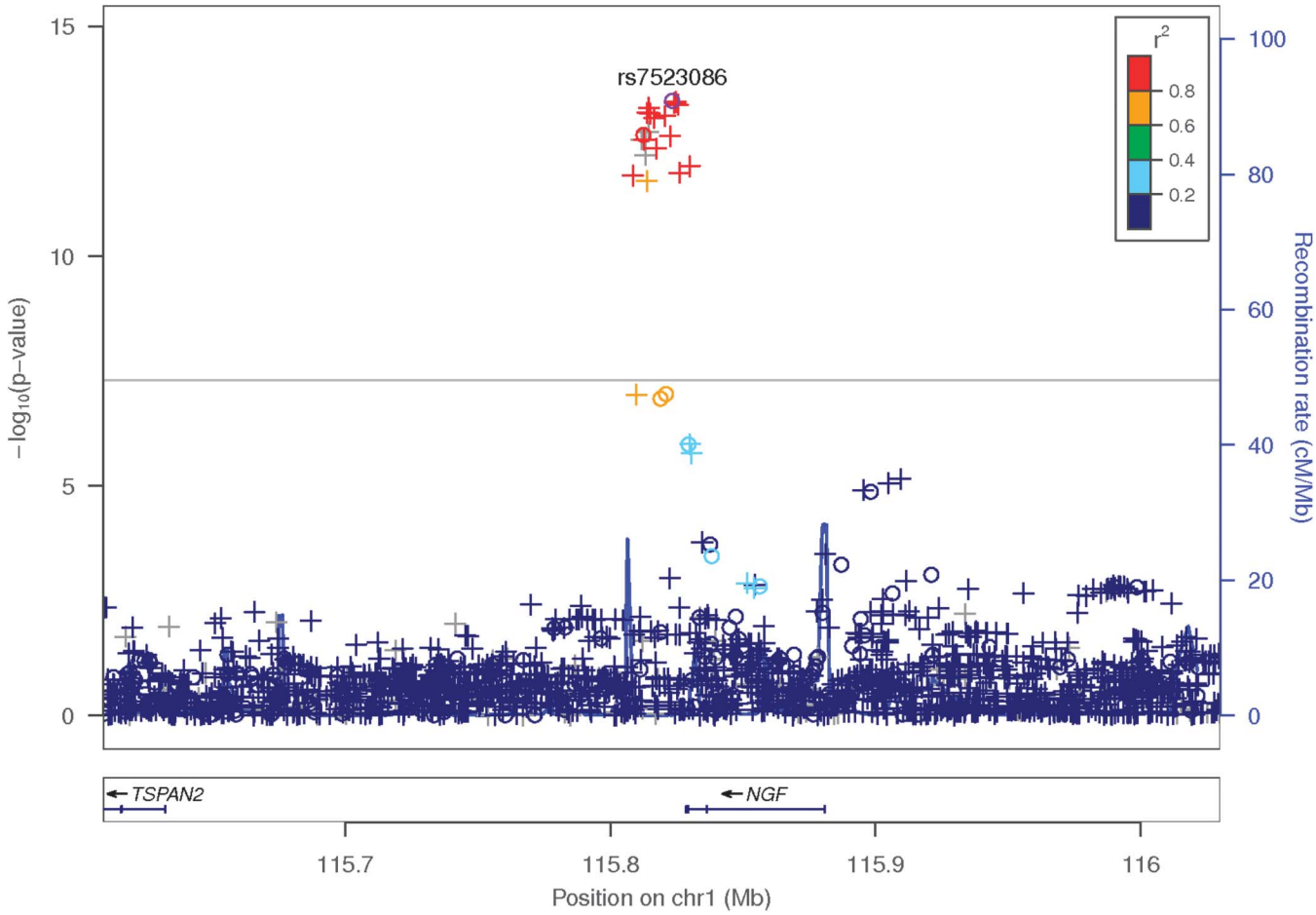

Figure 3. Regional plot depicting genome-wide significant association with dysmenorrhea pain severity at chromosome 1p13.2. Colour symbols indicate linkage disequilibrium with the index single nucleotide polymorphism, which is labeled and coloured purple. Open circles indicate imputed variants, filled circles indicate partially genotyped variants, and filled squares indicate fully genotyped variants. Plots were generated using LocusZoom.[74] Results are in NCBI Build 37 coordinates.

in high linkage disequilibrium, and exploration of chromatin states, conservations, and transcription factor regulatory motif alterations.[91,92]

We identified 23 variants in high linkage disequilibrium ($r^2 > 0.8$) with the GWS index SNP rs7523086, which cover an approximate 23-kb region 5′ to *NGF*, with one variant situated in an *NGF* intron. Approximately half of the tagged variants colocalise with regulatory histone modifications captured in different cell types. Annotation with ChromHMM status[29] is one useful approach that broadly assesses evidence for epigenetic activity at a particular genetic locus. For 1p13.2, we observed enrichment for active marks that denote the presence of promoter and enhancer elements specifically in fat and ovary tissues, both of which are highly relevant tissues for the pathophysiology of dysmenorrhea (**Fig. 4**). Altogether, this suggests that our nominated variants colocalise with a particular regulatory domain that may control gene expression. Moreover, our nominated SNPs and colocalising marks of histone activation span a region that encompass the last intron of *NGF*, and the predicted transcriptional start site of a noncoding RNA, called *RP4-663N10.1*. This noncoding RNA produces a 2,610-bp transcript from 2 exons read in the forward orientation. The entire *NGF* locus sits within the single intron of *RP4-663N10.1* and is transcribed in the opposite, antisense direction.

Inspection of *RP4-663N10.1* expression across the range of tissues captured by GTEx[15,20,21] demonstrated highest expression in adipose, aorta, and various gynaecological tissues, including the uterus, fallopian tube, and cervix, with no/little expression detected in brain tissues or peripheral blood leukocytes. This expression profile closely mirrors that observed for *NGF*, although *RP4-663N10.1* expression levels are vastly smaller in magnitude compared with that for *NGF* (**Fig. 5**). Interestingly, many of the pain severity variants in high linkage with the index SNP were identified as expression quantitative loci (eQTL) for *RP4-663N10.1* (**Fig. 5**). Results from the GTEx Project, which examines genotypic correlation with tissue-specific gene expression levels in samples from healthy male and female donors,[15,20,21] indicated the minor allele of the variant rs6328 ($r^2 = 0.85$ with dysmenorrhea index SNP rs7523086) is significantly correlated with increased *RP4-663N10.1* expression in aorta tissue ($P = 1.8 \times 10^{-7}$, effect size 0.34). This suggests that increased dysmenorrhea pain severity (denoted by the major allele for the index SNP rs7523086) may be correlated with decreased *RP4-663N10.1* expression; however, there was no direct correlation detected between SNP genotype and *NGF* gene expression levels and correlations in this tissue may not be generalizable. At present, how all these contribute to a functional mechanism that might impact on dysmenorrhea pain severity levels is not clear.

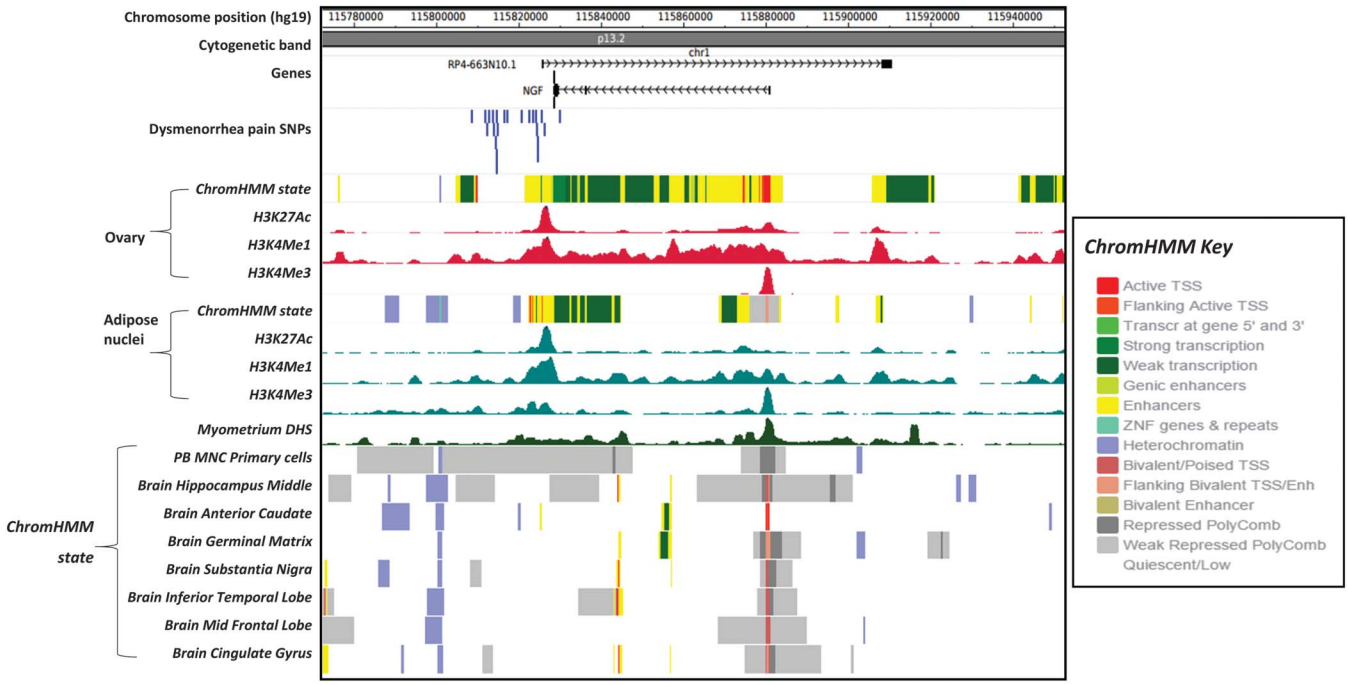

Figure 4. Regional plot depicting epigenetic annotations for genome-wide significant dysmenorrhea pain severity association at chr1p13.2. Chromosome and cytogenetic position (hg19/NCBI Build 37) and gene annotation are depicted (GENCODE v17), alongside dysmenorrhea pain SNPs in high linkage disequilibrium ($r^2 > 0.8$) with index association variant (each single nucleotide polymorphism [SNP] represented by one blue line). Further annotation of 1p13.2 with available regulatory information from ENCODE and the Roadmap Epigenomics Project indicate that the dysmenorrhea pain SNPs colocalise with epigenetic marks; marks captured in human ovary (pink peaks) and mesenchymal adipose nuclei (teal peaks), and DNase I hypersensitivity (DHS) mark in myometrium (black peaks); tissues that are relevant to dysmenorrhea pathophysiology. H3K27 acetylation (H3K27Ac), H3K4 monomethylation (H3K4Me1), and H3K4 trimethylation (H3K4Me3) ChIP-seq peaks indicate localised epigenetic activity. For comparison, the level of epigenetic activity inferred from histone modification state (ChromHMM) in peripheral blood mononuclear (PB MNC) cells and various brain tissues is provided (see ChromHMM key panel for further definition, image produced using WashU EpiGenome Browser).

4. Discussion

We describe the largest study to date capturing self-reported dysmenorrheic pain and findings from GWAS on pain severity. We identified one GWS association at 1q13.2 that colocalises with *NGF*, a neurotrophin linked to pain pathophysiology. Subjects carrying the risk allele reported more painful dysmenorrhea than did subjects without the risk allele, although the effect size was weak (increase of 0.1 points on a 4-point ordinal scale for increasing pain severity, explaining 0.48% of the observed variance).

We adjusted for 2 modulatory factors previously reported to impact on dysmenorrheic pain severity—age[61,75] and BMI.[38] Increased pain was correlated with younger age ($P = 1.2 \times 10^{-17}$) and lower BMI ($P = 3.8 \times 10^{-25}$). Effect estimates for age and BMI were only slightly stronger than the lead genetic variant, explaining 0.62% and 0.52% of the observed endpoint variance, respectively.

We observed enrichment for secondary dysmenorrheic conditions (including endometriosis, uterine fibroids, and PCOS) in our dysmenorrhea cohort. Endometriosis is a heritable disorder,[84] with a few common risk factors identified.[1,2,76,88] Analysis on surgically confirmed cases staged according to disease severity revealed that genetic factors account for a greater proportion of the risk of developing the most severe forms of endometriosis, which suggests that genetic factors may influence disease progression.[63,68] In secondary dysmenorrhea associated with endometriosis, pain is caused by endometrial tissue formed on extrauterine locations, and benign invasion of endometrial tissue into the myometrium in those with adenomyosis.[11] However, pain symptoms attributed to endometriosis occur in women without an endometriosis diagnosis, and for some individuals pain symptoms and severity correlate poorly with lesion characteristics.[32,81] Uterine fibroids are benign tumours of the uterus that are often asymptomatic, but when diagnosed, patients often present with dysmenorrhea, excessive menstrual bleeding, bloating, and less frequently chronic pelvic pain.[17] Evidence for genetic predisposition for uterine fibroids has been observed in both familial aggregation and twin studies.[54] Polycystic ovarian syndrome is a common, heterogeneous endocrine disorder characterised by irregular menses, hyperandrogenism, and polycystic ovaries.[79] Severe pain is not as common in PCOS; any pain experienced largely correlates with menstrual disturbances. Nevertheless, we also observed significant enrichment of PCOS in participants reporting extreme dysmenorrhea pain.

Depression is an important risk factor[46,83] and was a common comorbidity in our cohort, with significant enrichment in those reporting extreme pain. Our cohort reported similar incidence of psychiatric conditions as previously reported for women of reproductive age. Studies have demonstrated that genetic factors contribute to development of depression[19] and other common psychiatric conditions like anxiety[39]; however, whether these have any relevance to dysmenorrhea remains unknown.

Shared genetic pleiotropy between dysmenorrhea, painful gynaecological conditions, depression, and other related disorders is an important area for investigation to further understand these conditions. Current GWAS data for these conditions have not identified a signal at the NGF locus.[67,77] This might not be

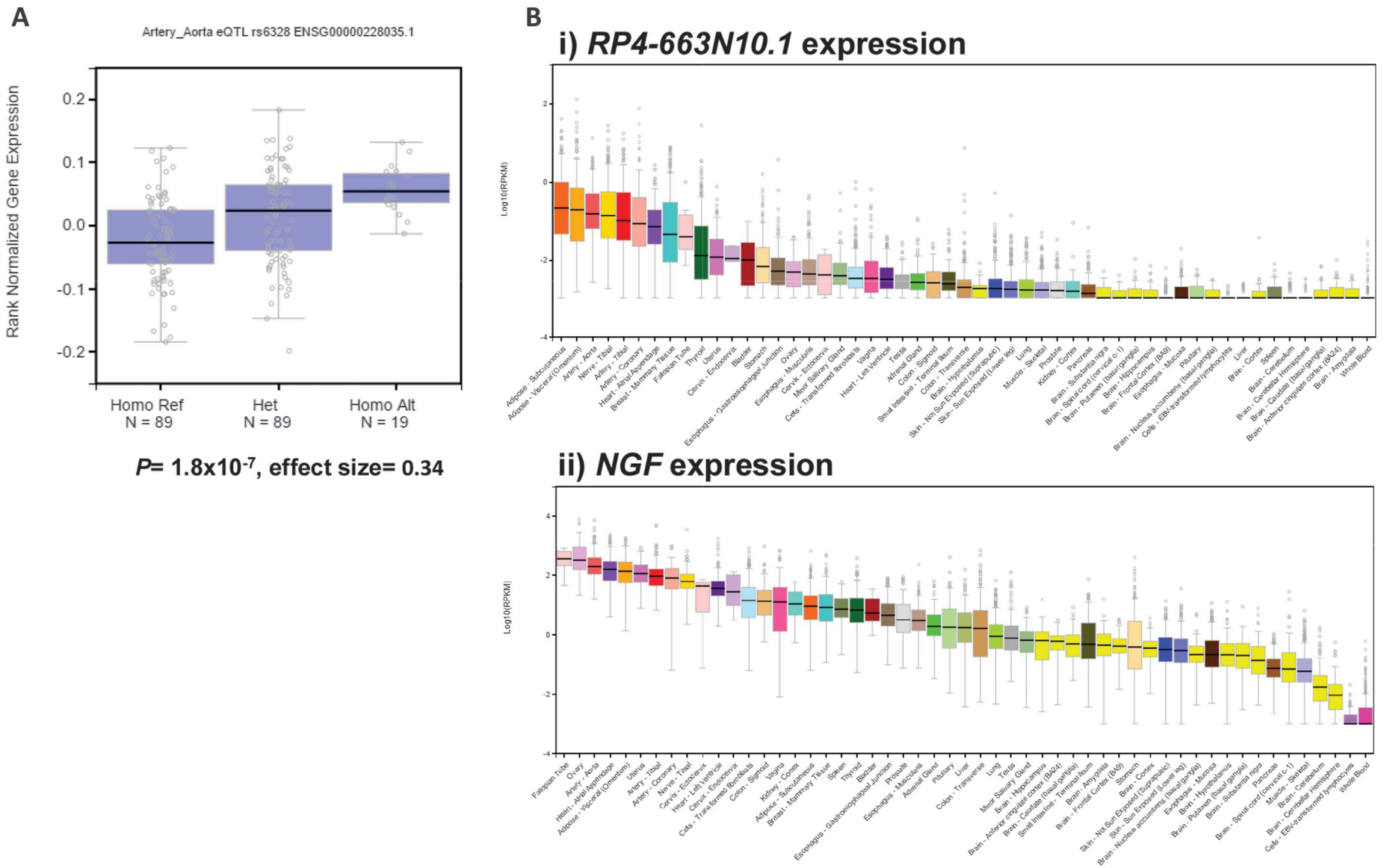

Figure 5. Expression profiling of noncoding RNA *RP4-663N10.1*. (A) Variant rs6328 is in high linkage disequilibrium with rs7523086 (the index association identified by dysmenorrhea pain severity GWAS) is an expression quantitative loci (eQTL) in aorta tissue for *RP4-663N10.1*, a noncoding RNA that flanks *NGF* at chromosome 1q13.2. (B) Expression profiles across 53 cell and tissue types for *RP4-663N10.1* (i) and *NGF* (ii) (images from GTeX Portal).

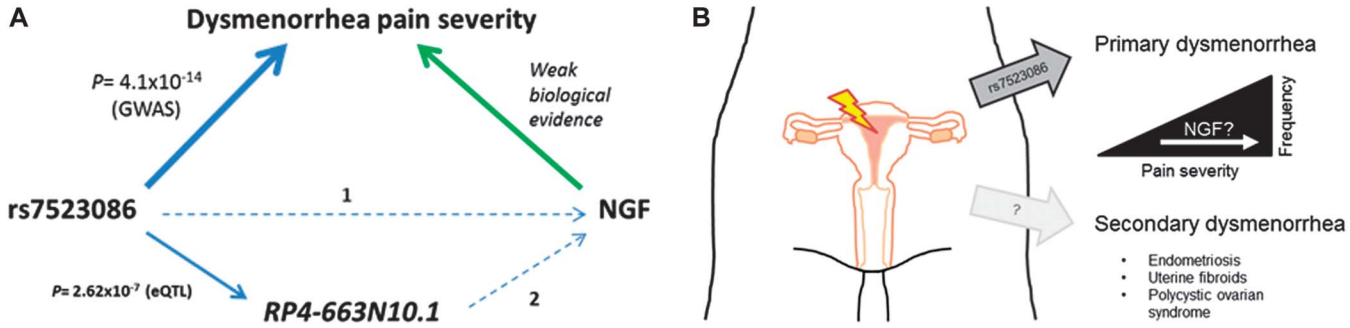

Figure 6. Hypothesised function mechanism for genetic link between dysmenorrhea pain and NGF. (A) Genome-wide association study identified a significant association between genetic variant rs7523086 and severity of pain in dysmenorrhea suggesting by biological evidence the involvement of NGF signalling pathways. Expression of a noncoding RNA (*RP4-663N10.1*) correlates with variant allelic status, but the link between the noncoding RNA (1), and between the genetic variant and NGF (2) remains to be established. (B) The association of rs7523086 with dysmenorrhea pain severity implicates a contribution to primary dysmenorrhea; however, it is unclear whether rs7523086 is important in pelvic disease pathogenesis (including endometriosis, uterine fibroids, or polycystic ovarian syndrome) or contributes to the severity of secondary dysmenorrhea associated with these conditions.

surprising as these genetic studies were designed to identify genetic factors that specifically influence risk of disease and not pain severity associated with disease.

In our study, pain severity was scored on an ordinal scale, a standard approach used for assessing pain in a variety of disorders including dysmenorrhea.[55,86] Participant responses were normally distributed (**Table 1**), with 20.4% reporting extreme pain. A previous study capturing self-reported PD pain, reported that only 2% of participants experienced severe pain.[93] The greater numbers reporting extreme pain in our cohort are likely due to our broader selection criteria, which did not exclude more painful secondary dysmenorrhea conditions. Previous studies similar in design to ours reported extreme pain in 15% to 22% of participants.[71,72,83]

Defining the biological basis behind this genetic association will further progress our understanding of dysmenorrhea pain pathophysiology. Our GWAS has identified a GWS locus at *NGF*, where associated variants colocalise with epigenetic regulatory regions, and are eQTLs for expression of a non-coding RNA that flanks the *NGF* locus. Although there is no evidence for a direct correlation of tagging SNPs and *NGF* expression, NGF remains the most plausible functional target for this pain-related phenotype. One possible mechanism for how a noncoding RNA might exert a functional impact on expression levels of another neurotrophin has been delineated by Modarresi et al.[62] Brain-derived neurotrophic factor (BDNF) modulates pain signalling pathways at neuronal synapses.[59] Expression of the *BDNF* gene is upregulated upon repression of an endogenous antisense transcript *BDNF-AS*, situated near and transcribed in the opposite orientation to *BDNF* in mice and humans. Repression of *BDNF* by *BDNF-AS* is not due to a direct knockdown interaction between transcripts but rather *BDNF-AS* transcription modulates local chromatin structure, altering repressive chromatin marks at the *BDNF* promoter and stimulating transcription of *BDNF* in a locus-specific manner. It remains to be determined whether a similar mechanism of posttranscriptional control mediated by endogenous antisense transcripts operates for other neurotrophins, including *NGF.*

These observations provide a roadmap to confirming a functional mechanism (**Fig. 6**). Further studies are necessary to explore whether there is a direct correlation between risk variant allele status and *NGF* transcript/protein levels in a relevant tissue, such as endometrium, and some studies have begun to explore this link.[6,10] Exploring whether NGF plays a causal role in dysmenorrhea pain merits further investigation and could lead to the nomination of NGF as a dysmenorrhea-related pain biomarker.

The utility of assaying secreted NGF in fluids collected from tissues of interest has led to the suggestion that NGF could act as a clinical biomarker for chronic pain associated with inflammation,[52] such as that typical in osteoarthritis. Likewise, levels of NGF measured in peritoneal tissues and fluid samples from endometriosis patients are higher in those with severer pain, which suggests that NGF production locally may contribute to endometriosis-associated pelvic pain.[10,49] Despite the recent identification of *BDNF* Val66Met polymorphism (rs6265) as a risk factor for PD in a small cohort of Asians,[53] peritoneal BDNF levels were not elevated in patients with endometriosis[10] and we failed to observe significance of this variant in our dysmenorrhea pain GWAS ($P = 0.94$, effect = 0.0009 [−0.031 to 0.032], observed minor allele frequency = 18.6%).

NGF contributes to persistent pain states in adults acting as a peripheral mediator of inflammation leading to sensitization of pain pathways. In rodent models of cystitis and colitis, NGF causes reduced thresholds for activation of peripheral nociceptors to mechanical stimulation of visceral organs.[23,24,34] Continued peripheral nociceptor input has been shown to be required for the maintenance of chronic pain conditions, and it is likely that NGF acts as a sensitizing factor in such states. Although the aetiology of dysmenorrhea remains unclear, uterine afferent sensitivity to menstrual contraction is one mechanism in which NGF signalling could result in altered pain perception.

How neurotrophins, and specifically NGF, contribute to visceral pain during endometriosis is unclear, but mechanisms involving sensitization of afferent pathways exposed in the peritoneal cavity or de novo sensory neurite outgrowth may contribute.[6,10] Importantly, anti-NGF antibodies (such as fulranumab and tanezumab) and small-molecule TrkA inhibitors (including K252a) have proven effective in inhibiting increased peripheral mechanical sensitivity in models of other visceral inflammatory conditions, such as colitis and cystitis.[23,34] Tanezumab is effective in treating chronic visceral pain, reducing pain in women with interstitial cystitis/painful bladder syndrome, but not men with prostatitis/chronic pelvic pain syndrome, which implicates that NGF may contribute to chronic pain conditions in a sex-specific manner.[31,65]

The value of Web-based surveys capturing phenotypic information on common traits in a large recontactable, genotyped cohort, has been demonstrated by the identification of novel genetic associations,[28] and replication of findings previously identified in data sets compiled using stricter clinical diagnostic records.[87] In this study, we relied solely on participants' assessment of their dysmenorrhea pain severity, and although this pain phenotype is captured by self-report in the clinic, there is still an inherent unreliability as the survey used was neither standardised nor validated, and is subject to recall bias.[16] We acknowledge that the use of nonstandardised or validated self-reporting surveys may incur reporting error and anticipate a degree of bias of effect size estimates to the null, a caveat that should be considered when interpreting the effect size identified. The primary aim of GWAS is to discover new genetic predisposition factors. We demonstrate that a very large sample size was required to be informative when the trait in question is quantitative with a narrow dynamic range. Comparative dysmenorrhea studies currently lack sufficient sample sizes required to achieve power to replicate findings from this study (>3000 subjects would be required). Nevertheless, we encourage validation of these results in an independent cohort sufficiently detailed with phenotypic and clinical information, enabling further investigation to define the role of genetic predisposition factors on dysmenorrhea risk, classification, and comorbidities.

Further experimental validation exploring biological mechanisms are key to defining the role, if any, that NGF plays in dysmenorrhea pain severity (**Fig. 6**). If evidence is identified to support NGF as the causal gene, this opens the possibility for repurposing analgesics that target the NGF pathway for dysmenorrhea and related indications.

Conflict of interest statement

A. V. Jones, J. R.F. Hockley, C. Hyde, D. Gorman, J. Bilsland, G. McMurray, A. Sredic-Rhodes, P. J. Cox, and S. Scollen are current or past employees of and own stock or stock options in Pfizer, Inc. D. A. Hinds, Y. Hu, and N. A. Furlotte are current or past employees of and own stock or stock options in 23andMe, Inc.

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This publication is the work of the authors, and A. V. Jones and D. A. Hinds will serve as guarantors for the contents of this article.

Author contributions: A. V. Jones designed the study, analysed the data, and wrote the manuscript. J. R.F. Hockley analysed the data and wrote the manuscript. C. Hyde designed the study and analysed the data. D. Gorman, Y. Hu, N. A. Furlotte, and D. A. Hinds analysed the data. A. Sredic-Rhodes, J. Bilsland, and G. McMurray wrote the manuscript. S. Scollen and P. J. Cox designed and oversaw the study and wrote the manuscript. All authors reviewed the manuscript. P. J. Cox and S. Scollen share joint senior authorship.

URLs

HaploReg V4.1; http://www.broadinstitute.org/mammals/haploreg/haploreg.php.

GTeX; http://www.gtexportal.org/home/.

WashU EpiGenome Browser; http://epigenomegateway1.wustl.edu/browser/.

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Appendix A. Supplemental Digital Content

Supplemental Digital Content associated with this article can be found online at http://links.lww.com/PAIN/A320.

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