Microdeletion in a FAAH pseudogene identified in a patient with high anandamide concentrations and pain insensitivity

Abdella M. Habib1,2, Andrei L. Okorokov4, Matthew N. Hill3, Jose T. Bras4,5, Man-Cheung Lee1,6,7, Shengnan Li1, Samuel J. Gossage1, Marie van Drimmelen8, Maria Morena3, Henry Houlden5, Juan D. Ramirez9, David L. H. Bennett9, Devjit Srivastava10,* and James J. Cox1,*

1Molecular Nociception Group, Wolfson Institute for Biomedical Research, University College London, London, UK, 2College of Medicine, Member of Qatar Health Cluster, Qatar University, Doha, Qatar, 3Hotchkiss Brain Institute, Departments of Cell Biology and Anatomy and Psychiatry, University of Calgary, Calgary, AB, Canada, 4UK Dementia Research Institute at UCL, London, UK, 5Department of Molecular Neuroscience, Institute of Neurology, University College London, London, UK, 6University Division of Anaesthesia, University of Cambridge, Addenbrooke’s Hospital, Hills Road, Cambridge, UK, 7Department of Anesthesia and Perioperative Care, University of California, San Francisco, San Francisco, CA, USA, 8Department of Pathology, Raigmore Hospital, Inverness, UK, 9Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, UK and 10Department of Anaesthesia, Raigmore Hospital, Inverness, UK

*Corresponding authors. E-mails: dev.srivastava@nhs.net, j.j.cox@ucl.ac.uk

Summary

The study of rare families with inherited pain insensitivity can identify new human-validated analgesic drug targets. Here, a 66-yr-old female presented with nil requirement for postoperative analgesia after a normally painful orthopaedic hand surgery (trapeziectomy). Further investigations revealed a lifelong history of painless injuries, such as frequent cuts and burns, which were observed to heal quickly. We report the causative mutations for this new pain insensitivity disorder: the co-inheritance of (i) a microdeletion in dorsal root ganglia and brain-expressed pseudogene, FAAH-OUT, which we cloned from the fatty-acid amide hydrolase (FAAH) chromosomal region; and (ii) a common functional single-nucleotide polymorphism in FAAH conferring reduced expression and activity. Circulating concentrations of anandamide and related fatty-acid amides (palmitoylethanolamide and oleoylethanolamine) that are all normally degraded by FAAH were significantly elevated in peripheral blood compared with normal control carriers of the hypomorphic single-nucleotide polymorphism. The genetic findings and elevated circulating fatty-acid amides are consistent with a phenotype resulting from enhanced endocannabinoid signalling and a loss of function of FAAH. Our results highlight previously unknown complexity at the FAAH genomic locus involving the expression of FAAH-OUT, a novel pseudogene and long non-coding RNA. These data suggest new routes to develop FAAH-based analgesia by targeting of FAAH-OUT, which could significantly improve the treatment of postoperative pain and potentially chronic pain and anxiety disorders.

Keywords: anandamide; anxiolytic; endocannabinoids; pain insensitivity; postoperative analgesia
Fatty-acid amide hydrolase (FAAH) is the major catabolic enzyme for a range of bioactive lipids called fatty-acid amides (FAAs).\(^1\)\(^2\) These FAAs include N-acyl ethanolamines, such as anandamide (AEA), that act as endogenous ligands for cannabinoid receptors (i.e. endocannabinoids). Other substrates of FAAH include palmitoylethanolamide (PEA), oleoylthanolamine (OEA), and N-acyl-taurines. 2-Arachidonoylglycerol (2-AG) is another related endocannabinoid and FAAH, but is metabolised mostly by monoacylglycerol lipase (MAGL). AEA has roles in nociception, fear-extinction memory, anxiety, and depression.\(^3\)\(^4\) FAAH knockout mice have elevated brain concentrations of AEA, display an analgesic phenotype in response to acute thermal stimuli, and show reduced pain in formalin and carrageenan inflammatory models.\(^5\)\(^6\) FAAH is therefore an attractive drug target for treating pain, anxiety, and depression, although recent clinical trials with FAAH inhibitors were unsuccessful.\(^7\)\(^8\)

The human FAAH gene contains a commonly carried hypomorphic single-nucleotide polymorphism (SNP) (C385A; rs524420; C allele frequency 74%, A 26%) that significantly reduces the activity of the FAAH enzyme. Genetic association studies have investigated the link between this and other FAAH SNPs and pain sensitivity.\(^9\)\(^10\)\(^11\) Notably, homozygous carriers of the hypomorphic SNP (A allele) in a cohort of women undergoing breast cancer surgery were less sensitive to cold pain and had a reduced need for postoperative analgesia.\(^10\) Furthermore, a mouse knock-in model of the human SNP showed that both the mouse and human SNP carriers display enhanced fear-extinction learning and decreased anxiety-linked behaviours.\(^13\) Here, we describe a pain-insensitive patient with a non-anxious disposition presenting with a novel genetic disorder associated with loss of function of FAAH.

**Case report**

A 66-yr-old Caucasian female presented to Raigmore Hospital in Inverness, Scotland for orthopaedic surgery, specifically a trapeziectomy with ligament reconstruction and tendon realignment after a recent road traffic accident. She had been diagnosed with osteoarthritis of the left shoulder (trapeziectomy with ligament reconstruction and tendon realignment, she scored 0/29, classified as mild.\(^15\) She reported long-standing memory lapses (e.g. frequently forgetting words mid-sentence and placement of keys). She also reported never panicking, not even in dangerous or fearful situations, such as in a recent road traffic accident.

After the painless trapeziectomy surgery and a history of ‘painless operations’, she was referred to and further investigated by pain genetics teams from University College London and the University of Oxford at age 67 yr. Ethical approval was granted from both institutions, and written consent taken from the patient, her two children, and mother. On clinical examination, she had multiple scars around the arms and on the back of her hands. Quantitative sensory testing (Supplementary Fig. S3) demonstrated hyposensitivity to noxious heat both in the hands and feet (see Supplementary data for further clinical details).

**Genetic tests identify a microdeletion downstream of FAAH**

Genomic DNA was isolated from the patient, her two children, and her mother for exome sequencing. After filtering of variants, four candidate mutations in the patient and her son were identified, but none were considered likely to be causal for the phenotype (see Supplementary data). We broadened our genetic analyses and searched for cytogenetic copy number changes across the genome using the CytoScan™ HD Array (Thermo Fisher Scientific, UK). This identified an ~8 kb heterozygous microdeletion on Chromosome 1 that began ~4.7 kb downstream from the 3′ end of FAAH (Fig 1a; Supplementary Fig. S4). Polymerase chain reaction and

\[^{1}\] S. P. Scherrer et al., Cell 159, 944–957 (2014).


sequencing analyses confirmed that the patient co-inherited the microdeletion and FAAH hypomorphic SNP allele (rs324420) (Fig 1b). Her unaffected mother and daughter did not carry the microdeletion, but her son, who also has some pain-sensitivity deficits, was heterozygous for the microdeletion (Supplementary Fig. S5), but did not carry the hypomorphic SNP allele. One Colombian male (HG01353) (pain phenotype unknown) out of 5008 alleles screened in the 1000 Genomes Project also carries a similar-sized microdeletion (esv3585936 in Supplementary Table S1), but is homozygous wild type for FAAH SNP rs324420.

Given the extraordinary phenotype in the patient and the vicinity of the microdeletion to FAAH, we investigated how the microdeletion could be pathogenic. Molecular cloning experiments (see Supplementary data) identified novel 50 exons of an expressed FAAH pseudogene, herein called FAAH-OUT (2.845 kb cDNA; KU950306), that mapped to within the microdeletion (Fig 1a). Tissue expression analyses showed FAAH-OUT to be expressed in a wide range of tissues, including fetal and adult brain, and in dorsal root ganglia (DRG; Supplementary Fig. S6). FAAH-OUT likely encodes a long non-coding RNA (Supplementary Fig. S7). We considered that the microdeletion may negate the normal function of FAAH through a reduction in neural expression of FAAH-OUT or through loss of a critical genomic regulatory element for FAAH, and hence, obtained blood samples to measure FAAH-regulated lipids.

**Elevated FAA concentrations in blood**

To determine the effects of carrying both the microdeletion in FAAH-OUT and the hypomorphic FAAH SNP, we measured the circulating FAA concentrations from blood samples from the patient and four controls, two of which were heterozygous carriers of the SNP. Circulating concentrations of AEA, PEA, OEA, and 2-AG were measured by mass spectrometry from blood samples obtained from the patient and four unrelated normal controls. AEA, PEA, and OEA are substrates for FAAH; 2-AG is not. Controls A and B are homozygous wild type for the hypomorphic SNP; Controls C and D are heterozygous carriers. Average values for the controls were AEA (1.2 pmol ml⁻¹), PEA (43.4 pmol ml⁻¹), OEA (5.1 pmol ml⁻¹), and 2-AG (42.2 pmol ml⁻¹), which is consistent with previous data using a similar measurement protocol. Average values for the patient (two measurements) were AEA (2.0 pmol ml⁻¹), PEA (113.1 pmol ml⁻¹), OEA (17.3 pmol ml⁻¹), and 2-AG (45 pmol ml⁻¹).

**Discussion**

The endocannabinoid system is an important physiological system that performs a wide array of homeostatic functions and is important for pain perception. FAAH is a critical
enzyme for the breakdown of a range of bioactive lipids (including the endocannabinoid AEA and related FAAs and N-acyl-taurines) with diverse physiological roles. Mouse modelling of FAAH loss of function mutations and pharmacological inhibition studies have shown a range of phenotypes, including hypoalgesia, accelerated skin wound healing, enhanced fear-extinction memory, reduced anxiety, and short-term memory deficits.6,13,18–21 Furthermore, human hypomorphic FAAH SNPs are associated with a reduced need for postoperative analgesia, increased postoperative nausea and vomiting induced by opioids, and decreased anxiety-linked behaviours.10,13,16,22–24

Here, we report a new human genetic disorder in a patient with hypoalgesia, altered fear and memory symptoms, and a non-anxious disposition. This disorder is attributable to co-inheritance of a microdeletion in a novel pseudogene and a known FAAH hypomorphic SNP. The microdeletion is flanked by repeated sequences that likely predispose the region to genomic rearrangements, as seen in other genomic disorders.25 Consequently, there are likely to be additional similar individuals in the general population. The likelihood that this disorder has been under-reported is highlighted by the fact that the patient was diagnosed at age 66 yr despite a recurrent history of painless injuries. Lipid profiling in peripheral blood showed significant increases in AEA, OEA, and PEA, which could be further exaggerated in the brain and DRG. Further work is needed to understand which FAA is the major contributor to the painless phenotype.

The microdeletion removes the promoter and first two exons of FAAH-OUT, but how this disrupts the function of FAAH is still to be elucidated. A hypothesis is that the FAAH-OUT transcript normally functions as a decoy for microRNAs as a result of the high sequence homology, and protects FAAH mRNA from degradation (Supplementary Fig. S7).26 Alternatively, FAAH-OUT may have an epigenetic role in regulating FAAH transcription, or the deletion removes a critical transcriptional regulatory element.25,27 Future work will help us to understand whether targeting FAAH-OUT by viral shRNA or gene editing techniques is an effective analgesic/anxiolytic drug development strategy.

This patient provides new insights into the role of the endocannabinoid system in analgesia and more specifically on the FAAH genomic locus, and highlights the importance of the adjacent, previously uncharacterised FAAH-OUT gene to pain sensation. Given the previous failure of FAAH-inhibitor analgesic drug trials, this report has significance, as it provides a new route to developing FAAH-related analgesia through targeting of FAAH-OUT.

Authors’ contributions
Clinical work: HH, JDR, DLHB, DS.
Molecular genetics: AMH, ALO, MCL, SL, SJG, JJC.
Exome sequencing data analyses: JTB.
Bioinformatics: AMH, ALO, JTB, MCL, JJC.
Blood preparation and anandamide analyses: MNH, MvD, MM.
Research design: DS, JJC.
Wrote the manuscript with help from all authors: JJC.
Approved the final manuscript: all authors.

Supplementary material
Supplementary material is available at British Journal of Anaesthesia online.

Acknowledgements
The authors would like to thank the family for participation in this study, and the volunteers for donating blood samples for analyses. The authors thank John N. Wood for helpful discussions and advice throughout this project. The authors also thank Patrick Fox, Hamish Hay, and Louise Reid (Raigmore Hospital, Inverness, UK); Iain Jones (Southern General Hospital, Glasgow, Scotland); and Judith Singleton (Leith Walk Surgery, Edinburgh, UK) for help with blood sampling, and Sylvie Rose (formerly Addenbrooke’s Hospital, Cambridge, UK) for help and advice regarding the cytogenetics analyses. The authors thank the Southern Alberta Mass Spectrometry Centre, located in and supported by the Cumming School of Medicine, University of Calgary, for their services in targeted liquid chromatography–tandem mass spectrometry.

Declaration of interest
The authors declare that they have no conflicts of interest.

Funding
Medical Research Council (Career Development Award G1100340 to JJC); Wellcome Trust (200183/Z/15/Z to JJC, 0956982/11/Z and 202747/Z/16/Z to DLHB); Alzheimer’s Society (research fellowship to JTB), University of Cambridge Academic Foundation Programme (to MCL); Molecular Nociception Group (to MCL); National Institutes of Health (Bethesda, MD, USA) Ruth L. Kirschstein Institutional National Research Service Award (to MCL); Wellcome Trust funded London Pain Consortium (to JDR); Colciencias through a Francisco Jose de Caldas Scholarship (LASPAU, Harvard University) (to JDR); Canadian Institutes of Health Research (CHHR, to MNH); CIHR (postdoctoral funding to MM).

Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.bja.2019.02.019.

References