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

Abdella M. Habib¹,², Andrei L. Okorokov¹, 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 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). 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). FAAH plays roles in nociception, fear-extinction memory, anxiety, and depression. 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. FAAH is therefore an attractive drug target for treating pain, anxiety, and depression, although recent clinical trials with FAAH inhibitors were unsuccessful.

The human FAAH gene contains a commonly carried hypomorphic single-nucleotide polymorphism (SNP) (C385A; rs324420; 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. 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. 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. 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 interposition and extensor pollicis longus realignment after a diagnosis of bilateral pantrapezial osteoarthritis. There was significant deformity and deterioration in the use of the right thumb, which was reported as painless before operation. The pre-assessment note classed her as ASA physical status 1, but her examination was remarkable for multiple varicose veins and dental procedures for which she has never required analgesia. She also reports of having some degree of pain insensitivity, but not to the same extent as her. She does not take any medication for pain.

For the surgery, she received general anaesthesia with an ultrasound-guided axillary nerve block. She received fentanyl 50 μg i.v., propofol 200 mg i.v., ondansetron 4 mg i.v. intraoperatively, and levobupivacaine 0.25% (20 ml) for the axillary nerve block. After operation, her pain intensity score was 0/10 until the next day when she was discharged home. The only postoperative analgesic she received in hospital was paracetamol 1 g i.v. in the PACU on the day of her surgery. She also received cyclizine 50 mg i.v. twice. Extraordinarily, she required no postoperative analgesics other than paracetamol. The only paracetamol 2 g orally on Postoperative days 1 and 2, reporting that she was encouraged to take the paracetamol, but that she did not ask for any analgesics. She was also administered a single dose of morphine sulphate 10 mg orally on the first postoperative evening that caused severe nausea and vomiting for 2 days. After operation, her pain intensity scores were 0/10 throughout except for one score of 1/10 on the first postoperative evening. Her past surgical history was notable for multiple varicose vein and dental procedures for which she has never required analgesia.

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...
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 and the hypomorphic FAAH SNP, and displayed a full hypoalgesic phenotype. Neither the unaffected mother nor daughter carries the microdeletion. (c–f) Circulating anandamide (AEA), palmitoylethanolamide (PEA), oleoylethanolamine (OEA), and 2-arachidonoylglycerol (2-AG) concentrations. 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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).\textsuperscript{26} Alternatively, FAAH-OUT may have an epigenetic role in regulating FAAH transcription, or the deletion removes a critical transcriptional regulatory element.\textsuperscript{25,27} Future work will help us to understand whether targeting FAAH-OUT by viral shRNA or gene editing techniques is an effective analgesic/antiolyltic 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.

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**Declaration of interest**

The authors declare that they have no conflicts of interest.

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**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**


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