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Research Paper

Genome-wide Polygenic Burden of Rare Deleterious Variants in Sudden Unexpected Death in Epilepsy

Costin Leu [a,1], Simona Balestrini [a,b,c,1], Bridget Maher [a,b], Laura Hernández-Hernández [a,b], Padhraig Gormley [d,e,f,g], Eija Hämäläinen [h], Kristin Heggeli [a], Natasha Schoeler [a], Jan Novy [i], Joseph Willis [a], Vincent Plagnol [j], Rachael Ellis [k,l], Eleanor Reavey [k,l], Mary O'Regan [k], William O. Pickrell [m], Rhys H. Thomas [m], Seo-Kyung Chung [m], Norman Delanty [n], Jacinta M. McMahon [o], Stephen Malone [p], Lynette G. Sadleir [q], Samuel F. Berkovic [o], Lina Nashef [r], Sameer M. Zuberi [k,s], Mark I. Rees [m], Gianpiero L. Cavalleri [t], Josemir W. Sander [a,b], Elaine Hughes [u], J. Helen Cross [v,w], Ingrid E. Scheffer [o,x,y], Aarno Palotie [d,e,f,g,h,z], Sanjay M. Sisodiya [a,b,*]

[a] NIHR University College London Hospitals Biomedical Research Centre, Department of Clinical and Experimental Epilepsy, UCL Institute of Neurology, London, UK
[b] The Epilepsy Society, Chalfont-St-Peter, Bucks, UK
[c] Neuroscience Department, Polytechnic University of Marche, Ancona, Italy
[d] Analytic and Translational Genetics Unit, Department of Medicine, Massachusetts General Hospital, Boston, MA, USA
[e] Program in Medical and Population Genetics, The Broad Institute of MIT and Harvard, Cambridge, MA, USA
[f] The Stanley Center for Psychiatric Research, The Broad Institute of MIT and Harvard, Cambridge, MA, USA
[g] Psychiatric & Neurodevelopmental Genetics Unit, Department of Psychiatry, Massachusetts General Hospital, Boston, MA, USA
[h] Institute for Molecular Medicine Finland, University of Helsinki, Helsinki, Finland
[i] Department of Clinical Neurosciences, Centre Hospitalier Universitaire Vaudois (CHUV) and University of Lausanne, Lausanne, Switzerland
[j] University College London Genetics Institute, London, UK
[k] Paediatric Neurosciences Research Group, Royal Hospital for Sick Children, Glasgow, UK
[l] West of Scotland Genetic Services, Southern General Hospital, Glasgow, UK
[m] Wales Epilepsy Research Network, Institute of Life Science, College of Medicine, Swansea University, Swansea, UK
[n] Department of Neurology, Beaumont Hospital, Dublin, Ireland
[o] Departments of Medicine and Neurology, University of Melbourne, Austin Health, Melbourne, Australia
[p] Department of Neurosciences, Lady Cilento Children's Hospital, Brisbane, Queensland, Australia
[q] Department of Paediatrics, School of Medicine and Health Sciences, University of Otago, Wellington, New Zealand
[r] Department of Neurology, King's College Hospital, London, UK
[s] School of Medicine, University of Glasgow, Glasgow, UK
[t] Molecular and Cellular Therapeutics Department, Royal College of Surgeons in Ireland, Dublin, Ireland
[u] Children's Neurosciences, Evelina Children's Hospital at Guys and St Thomas' NHS Foundation Trust, Kings Health Partners Academic Health Science Centre, London, UK
[v] UCL Institute of Child Health, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK
[w] Young Epilepsy, Lingfield, UK
[x] Department of Paediatrics, University of Melbourne, Royal Children's Hospital, Parkville, Australia
[y] Florey Institute of Neuroscience and Mental Health, Melbourne, Australia
[z] Department of Neurology, Massachusetts General Hospital, Boston, MA, USA

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ABSTRACT

Sudden unexpected death in epilepsy (SUDEP) represents the most severe degree of the spectrum of epilepsy severity and is the commonest cause of epilepsy-related premature mortality. The precise pathophysiology and the genetic architecture of SUDEP remain elusive. Aiming to elucidate the genetic basis of SUDEP, we analysed rare, protein-changing variants from whole-exome sequences of 18 people who died of SUDEP, 87 living people with epilepsy and 1479 non-epilepsy disease controls. Association analysis revealed a significantly increased genome-wide polygenic burden per individual in the SUDEP cohort when compared to epilepsy ($P = 5.7 \times 10^{-3}$) and non-epilepsy disease controls ($P = 1.2 \times 10^{-3}$). The polygenic burden was driven both by the number of variants per individual, and over-representation of variants likely to be deleterious in the SUDEP cohort. As determined by this study, more than a thousand genes contribute to the observed polygenic burden within the framework of this study. Subsequent gene-based association analysis revealed five possible candidate

Abbreviations: AED, anti-epileptic drug; MAF, minor allele frequency; *n*, number; QC, quality control; SUDEP, sudden unexpected death in epilepsy; WES, whole-exome sequencing.

* Corresponding author at: Department of Clinical and Experimental Epilepsy, NIHR University College London Hospitals Biomedical Research Centre, UCL Institute of Neurology, 33 Queen Square, London WC1N 3BG, UK.
E-mail address: s.sisodiya@ucl.ac.uk (S.M. Sisodiya).

[1] Joint first author.

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Burden

genes significantly associated with SUDEP or epilepsy, but no one single gene emerges as common to the SUDEP cases. Our findings provide further evidence for a genetic susceptibility to SUDEP, and suggest an extensive polygenic contribution to SUDEP causation. Thus, an overall increased burden of deleterious variants in a highly polygenic background might be important in rendering a given individual more susceptible to SUDEP. Our findings suggest that exome sequencing in people with epilepsy might eventually contribute to generating SUDEP risk estimates, promoting stratified medicine in epilepsy, with the eventual aim of reducing an individual patient's risk of SUDEP.

1. Introduction

Sudden unexpected death in epilepsy (SUDEP) is the commonest cause of epilepsy-related premature mortality (Walczak et al., 2001). The incidence of SUDEP varies from about 1/1000 patient-years in population-based studies (Thurman et al., 2014) up to 6.5/1000 patient-years in cohorts of people with drug-resistant epilepsy unsuitable for surgery (Bell et al., 2010). The precise pathophysiology of SUDEP is unknown: mechanisms may be specific to an individual or shared across individuals, or both. General principles aimed at reducing SUDEP risk, such as seizure control (Ryvlin et al., 2013), should be considered for everyone with epilepsy. The reasons for the effectiveness of such measures, and other preventative strategies (Ryvlin et al., 2013), are not known. Better understanding of the underlying causes of SUDEP is required to establish and target improved preventative strategies.

The cause of SUDEP is likely to be multifactorial, involving underlying genetic susceptibility related to individual epilepsy syndrome (Sakauchi et al., 2011) (of which Dravet Syndrome is the most recognised), brain functional and pathological characteristics (Lhatoo et al., 2010; Bozorgi et al., 2013), uncontrolled generalised tonic–clonic seizures, and the circumstances in which death occurs (e.g. prone position) (Liebenthal et al., 2015). Whilst evidence for genetically-driven mechanisms in SUDEP is provided by familial cases (Hindocha et al., 2008; Kawamata et al., 2010), and animal models (Goldman et al., 2009; Qi et al., 2014; Wagnon et al., 2015), the genetic architecture remains elusive. Substantial genetic heterogeneity is implicated by diverse putative pathophysiologic mechanisms underlying SUDEP (Glasscock et al., 2007; Klassen et al., 2014; Massey et al., 2014).

To elucidate the genetic basis and architecture of SUDEP, we used an unbiased sequencing approach based on whole-exome sequencing data. We examined overall burden and over-representation of deleterious variants in people who died of SUDEP compared to living people with epilepsy and non-epilepsy disease controls.

2. Methods

The study was approved by the relevant institutional review boards, accredited regional/national biobanks or international cohorts with ethical frameworks. Details of the difficult issue of sample collection for SUDEP research are given in Supplementary Method 1.

2.1. Study Design

We used whole-exome sequencing (WES) data from 18 people with epilepsy who died of SUDEP and two control cohorts: a group of 87 living people with epilepsy, which we termed 'epilepsy controls', and 1479 non-epilepsy 'disease control' samples. To ensure data homogeneity, a joint calling strategy, and stringent variant and individual-level quality control (QC) were applied for all WES datasets (Fig. 1 and Supplementary Methods 5–8). Only individuals of white European ancestry were included in subsequent analyses (Supplementary Method 6.2 and Supplementary Fig. 1). We tested the genome-wide burden of rare (or novel) deleterious variants in the SUDEP cohort against both control cohorts separately. Supported by the findings of the genome-wide burden analysis, we sought to identify candidate genes for SUDEP using gene-based association analyses. The study analytic design is outlined in the Supplementary Fig. 2.

2.2. Study Participants

The 18 DNA samples from people who had died of SUDEP sometime after DNA donation were selected from DNA archives at the National Hospital for Neurology and Neurosurgery, London ($n = 8$), the Epilepsy Research Centre, Melbourne ($n = 5$), the Royal College of Surgeons in Ireland, Dublin ($n = 2$), the Institute of Life Science, Swansea ($n = 2$), and the Royal Hospital for Sick Children, Glasgow ($n = 1$). The cause of death was classified into definite, probable, or near-SUDEP, according to the most recent proposed system: definite SUDEP required post mortem examination, without an identified toxicological or anatomical cause of death (Nashef et al., 2012). Details of SUDEP cases are given in Supplementary Table 1.

Epilepsy controls ($n = 87$) were patients from the National Hospital for Neurology and Neurosurgery, London ($n = 71$) and the Epilepsy Research Centre, Melbourne ($n = 16$), who had had whole-exome sequencing for other projects and were alive at the time of selection. These controls remain at risk of SUDEP. We applied previous incidence data from a comparable group of people with chronic epilepsy, reporting a SUDEP incidence of 5.9/1000 patient-years (Nashef et al., 1995), to the number of years that our cohort of epilepsy control subjects have already lived with epilepsy (summed minimum epilepsy duration = 2563 years). This suggests that 15/87 would have been expected to have succumbed to SUDEP, whilst, in fact, none have. Thus, the epilepsy control group is enriched with those at lower risk. For all epilepsy cases, we reviewed epilepsy diagnosis (Berg et al., 2010), age at onset of first seizure, presence of intellectual disability (Supplementary Method 2), anti-epileptic drug (AED) treatment, and presence of convulsive or nocturnal seizures over the 12-month period prior to death or latest follow-up. Details of the statistical analyses applied are provided in the Supplementary Method 3.

WES data of disease control samples (pre-QC, $n = 3{,}263$; post-QC, $n = 1{,}479$; Supplementary Fig. 2) were obtained from the University College London exomes consortium (UCL-exomes, detailed in the Supplementary Method 4). The disease control samples had no diagnosis of epilepsy or cardiac disease.

2.3. Whole-exome Sequencing

All epilepsy samples were sequenced using either Agilent's SureSelect Human All Exon V1 (38 Mb, $n = 42$) and SureSelect Human All Exon V5 (50 Mb, $n = 56$) or Illumina's Nextera Rapid Capture Exome kit (37 Mb, $n = 16$). For the disease control samples, NimbleGen's SeqCap EZ and Illumina's TruSeq Exome capture technology were also used. Sequencing was performed on Illumina HiSeq2500 or GAIIx sequencing systems.

We used a multi-sample joint calling strategy across all SUDEP cases, epilepsy and disease control samples to mitigate problems caused by the heterogeneity of sequence capture kits. One major confound in case–control variant burden analyses can arise when either single-sample calling, or multi-sample calling in different batches, is used to generate the variant calls. Standard practice in single-sample calling is