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Variations in inflammation-related genes may be associated with childhood febrile seizure susceptibility

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ABSTRACT

Purpose: To investigate whether genetic variants in inflammation-related genes are associated with increased risk of childhood-onset febrile seizures.
Method: Tagging single nucleotide polymorphisms (SNPs) from 19 inflammation-related candidate genes were identified and genotyped on the Sequenom platform in a sample of Caucasian childhood-onset febrile seizures cases ($n = 98$) compared to ethnicity, age and gender matched febrile controls presenting without seizures ($n = 123$). Tests for allelic association were carried out using PLINK. SNPs generating empirical *P*-values ($P < 0.05$) were analysed in an expanded Caucasian control sample ($n = 2692$) from the 1958 Birth Cohort.
Results: Six SNPs generated empirical pointwise significance values $P < 0.05$ in the febrile seizures case–control analysis in the *P2X7R* (purinergic receptor P2X7), *TLR4* (toll-like receptor 4), *IL6R* (interleukin 6 receptor) and *PTGER3* (prostaglandin E receptor 3, subtype EP3) genes. The most significant result was for missense SNP rs208294 in *P2X7R* ($P = 0.009$); this novel association was supported in the expanded case–control analysis using the 1958 Birth Cohort (pointwise $P = 0.009$, OR = 0.63, familywise $P = 0.039$).
Conclusion: Genetic variants in inflammation-related genes, specifically purinergic receptor *P2X7*, may be involved in susceptibility to childhood-onset febrile seizures.

1. Introduction

Febrile seizures (FS) affect 2–5% children under 5 years of age and are usually associated with a viral infection not directly affecting the brain but producing a sudden rise in body temperature. An association exists with hippocampal sclerosis (HS), an important cause of temporal lobe epilepsy (TLE), although whether HS is a cause or consequence of FS has been somewhat controversial.[1] Epidemiological and recent prospective analyses of prolonged FS and febrile status epilepticus do suggest that such seizures can lead to TLE.[2] Furthermore, persisting memory impairments have now been reported in children after prolonged FS.[3]

Substantial evidence implicates immune and inflammatory processes in the aetiopathogenesis of FS and HS. Interleukin-1 (IL-1) is a key pro-inflammatory cytokine and 'endogenous pyrogen'. IL-1 has a pivotal influence in the host response to infection and production of fever. Cytokine genes including IL-1 are up-regulated in experimental seizures[4] and IL-1β enhances seizure activity,[5,6] whilst the naturally occurring IL-1 receptor antagonist (IL-1Ra) has been shown to be powerfully anticonvulsant.[7] Thus, altered regulation of either the production or biological effects of IL-1 may be a critical determinant of susceptibility to FS. It is possible

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for example that reduced production of IL-1Ra might promote fever and a tendency to develop FS. Clinical studies suggest plasma and cerebrospinal fluid (CSF) cytokine concentrations change in FS,[8,9] and that specific IL-1 gene polymorphisms may increase the risk of FS.[10]

Innate immune responses may both combat infectious organisms and drive pathological inflammation, with inflammasome complexes, including the NLRP-3 inflammasome, being a central component of these processes via regulation of IL-1β and IL-18.[11] The purinergic P2X7 receptor (P2X7R), a plasma membrane receptor for extracellular ATP, is involved in an apparently inflammasome dependent non-classical pathway of IL-1β maturation, also involving caspase-1 activation.[12] TLR4 is a toll-like receptor which recognises bacterial lipopolysaccharide and is important in innate immune system activation, with TLR4 signalling also linked to inflammasome activation.[13] In terms of genes relevant to thermoregulation and IL-1 pathways, 3 of the most pertinent and 'inflammation sensitive' are cyclooxygenase-2 (COX-2) and membrane associated prostaglandin E2 synthase (mPGES-1), which are key PGE2 synthesising enzymes, whilst EP3 is a PGE2 receptor expressed within the CNS.

In this study, we chose to conduct an exploratory association study of SNPs from nineteen IL-1 and other inflammation-related candidate genes described above in a sample of Caucasian childhood-onset FS cases compared to ethnicity, age and gender matched febrile controls presenting without seizures. SNPs showing evidence for association were further investigated in an expanded ethnically matched control sample from the Wellcome Trust Case Control Consortium 1958 Birth Cohort (1958BC).[14]

2. Methods

Appropriate local research ethics committee and research governance approvals were obtained for this study. We undertook a prospective case–control association study of 157 FS cases and 163 ethnicity and age-matched febrile controls presenting to Alder Hey Children's Hospital, Liverpool between April 2006 and July 2009, and to Booth Hall Hospital, Manchester, or Manchester Royal Infirmary between August 2007 and July 2009.

2.1. Cases and controls

Inclusion criteria for cases and controls were childhood febrile illness with peak recorded aural temperature >38 °C and written informed consent from a parent or guardian. Cases were additionally required to have had a seizure, classified as simple or complex, sporadic or familial as described below. Exclusion criteria for cases and controls were family history of epilepsy; history of neurological disease; history of any condition with potential to cause brain damage (e.g. birth injury); and non-Caucasian ethnic origin. In addition cases were excluded if there was evident cause of acute symptomatic seizure, either evident central nervous system (CNS) infection or metabolic disturbance, whilst controls were also excluded if there was evident CNS infection or metabolic disturbance. Cases with FS were initially compared to ethnicity, age and gender matched febrile controls presenting without seizures, as the optimal control sample. An expanded ethnically matched control sample from the Wellcome Trust Case Control Consortium 1958 Birth Cohort[14] was used for confirmatory analysis.

2.2. Febrile seizure classification

FS were classified as simple if all of the four following features were present: generalised tonic–clonic seizure activity without focal features, <15 min in duration, no recurrence within a 24 h period, and spontaneous resolution. FS were classified as complex if any of the following features were present: focal onset or focal features during the seizure, prolonged duration (15 min or longer), or recurrence within 24 h or within the same febrile illness.

2.3. Sample collection

Buccal swabs were collected from all participants, and DNA extracted as described previously.[15] DNA samples were assessed for quality using a commercially available assay (DNA OK). Additional ethnically matched unselected UK control data were obtained from the 1958 Birth Cohort,[14] genotyped on the Illumina 1.2M array.

2.4. Candidate gene and SNP selection

Nineteen genes were selected based on their putative role in the inflammatory response (Table 1). Two hundred and seventy-eight SNPs were identified across these genes (±10 kb flanking sequence)

Table 1
Summary of genes investigated.

Gene	Gene Name	Position (hg19)	Gene size (bp)	tSNPs[a]
CASP1	Caspase 1	Chr11:104896237–104905857	9620	7 (2)
IL18	Interleukin 18	Chr11:112013976–112034840	20,864	6
IL1A	Interleukin 1, beta proprotein	Chr2:113531492–113542971	11,479	4 (1)
IL1B	Interleukin 1, alpha proprotein	Chr2:113587337–113594356	7019	3
IL1R1	Interleukin 1 receptor, type I	Chr2:102759246–102796334	37,088	17
IL1R2	Interleukin 1 receptor, type II	Chr2:102608306–102644884	36,578	15 (5)
IL1RN	Interleukin 1 receptor antagonist	Chr2:113875470–113891593	16,123	11 (3)
IL33	Interleukin 33	Chr9:6215807–6257982	42,175	8 (1)
IL6	Interleukin 6	Chr7:22766798–22771620	4822	6 (1)
IL6R	Interleukin 6 receptor	Chr1:154377669–154440188	62,519	12 (2)
NLRP3	NLR family, pyrin domain containing 3	Chr1:247579475–247612406	32,931	23
P2X7R	Purinergic receptor P2X7	Chr12:121570678–121623858	53,180	35 (8)
PTGER3	Prostaglandin E receptor 3, subtype EP3	Chr1:71318036–71513491	195,455	69 (11)
PTGES	Prostaglandin E synthase	Chr9:132500612–132515344	14,732	8 (2)
PTGS2	Prostaglandin-endoperoxide synthase 2 precursor	Chr1:186640969–186649556	8587	6
TLR4	Toll-like receptor 4	Chr9:120466610–120479766	13,156	10
TNF	Tumour necrosis factor alpha	Chr6:31543350–31546112	2762	9
TNFRSF1A	Tumour necrosis factor receptor 1	Chr12:6437923–6451261	13,338	5
TNFRSF1B	Tumour necrosis factor receptor superfamily member 1B	Chr1:12227149–12267077	39,928	24 (2)

[a] Number of tagging SNPs for each gene (redundant SNPs for assay failure).