Pandemrix-induced narcolepsy is associated with genes related to immunity and neuronal survival

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A B S T R A C T

Background: The incidence of narcolepsy rose sharply after the swine influenza A (H1N1) vaccination campaign with Pandemrix. Narcolepsy is an immune-related disorder with excessive daytime sleepiness. The most frequent form is strongly associated with HLA-DQB1*06:02, but only a minority of carriers develop narcolepsy. We aimed to identify genetic markers that predispose to Pandemrix-induced narcolepsy.

Methods: We tested for genome-wide and candidate gene associations in 42 narcolepsy cases and 4981 controls. Genotyping was performed on Illumina arrays, HLA alleles were imputed using SNP2HLA, and single nucleotide polymorphisms were imputed using the haplotype reference consortium panel. The genome-wide significance threshold was \( p < 5 \times 10^{-8} \), and the nominal threshold was \( p < 0.05 \). Results were replicated in 32 cases and 7125 controls. Chromatin data was used for functional annotation.

Findings: Carrying HLA-DQB1*06:02 was significantly associated with narcolepsy, odds ratio (OR) 39.4 [95% confidence interval (CI) 11.3, 137], \( p = 7.9 \times 10^{-15} \). After adjustment for HLA, GDNF-AS1 (rs62360233) was significantly associated, OR = 8.7 [95% CI 4.2, 17.5], \( p = 2.6 \times 10^{-9} \), and this was replicated, OR = 3.4 [95% CI 1.2, 9.6], \( p = 0.022 \). Functional analysis revealed variants in high LD with rs62360233 that might explain the detected association. The candidate immune-gene locus TRAJ (rs1154155) was nominally associated in both the discovery and replication cohorts, meta-analysis OR = 2.0 [95% CI 1.4, 2.8], \( p = 0.0002 \).

Interpretation: We found a novel association between Pandemrix-induced narcolepsy and the non-coding RNA gene GDNF-AS1, which has been shown to regulate expression of the essential neurotrophic factor GDNF. Changes in regulation of GDNF have been associated with neurodegenerative diseases. This finding may increase the understanding of disease mechanisms underlying narcolepsy. Associations between Pandemrix-induced narcolepsy and immune-related genes were replicated.

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1. Introduction

Pandemrix was a monovalent AS03-adjuvanted swine flu vaccine approved by the authorities in Europe in September 2009, when the influenza A (H1N1) pandemic had been officially declared [1]. Pandemrix was used in several European countries, among them the UK, but the highest overall vaccine coverage was obtained in Finland, Ireland, Norway, and Sweden. In Sweden, 61% (5.8 million) of the inhabitants were vaccinated with Pandemrix in a nation-wide campaign, and the coverage was especially high in risk populations such as children [1]. During 2010 and 2011, an unexpected rise in the incidence of narcolepsy was observed in Sweden and other countries that used the AS03-adjuvanted vaccine Pandemrix [1]. One year after the vaccination campaign an increased incidence of up to 15 per 100,000 per year was reported in Sweden and Finland, compared with the previously reported incidence of 1 per 100,000 person per year [2]. In children and...
Research in context

Evidence before this study

Narcolepsy is a severe immune-related disorder characterized by an inability to control sleep and wakefulness. The number of young diagnosed with narcolepsy rose sharply following immunization with the swine flu influenza A (H1N1) pdm09 vaccine Pandemrix 2009–2010. Spontaneous and Pandemrix-induced narcolepsy was known to be strongly associated with HLA-DQB1*06:02. A weaker association between immune-related T-cell receptor alpha joining (TRAJ) genes and both spontaneous and Pandemrix-induced narcolepsy had also been seen.

Added value of this study

Since only 0.02% of carriers of HLA-DQB1*06:02 developed narcolepsy after vaccination with Pandemrix, we sought to determine whether other genetic factors contribute to the risk. As expected, we detected a strong association between Pandemrix-induced narcolepsy and HLA-DQB1*06:02. After correction for this HLA-haplotype, narcolepsy was significantly associated with GDNF-AS1. This gene may regulate the expression of GDNF, which is a neurotrophic factor essential for the maintenance and survival of neurons. The candidate immune gene locus, TRAJ, was also associated with narcolepsy to a lower extent.

Implications of all the available evidence for practice

Variation in genes related to immunity and neuronal survival may interact to increase the susceptibility to Pandemrix-induced narcolepsy. This finding may also increase the understanding of disease mechanisms underlying spontaneous narcolepsy.

adolescents, it is estimated that the risk of developing narcolepsy was 1/18400 vaccinations, and among carriers of the major histocompatibility complex protein HLA-DQB1*06:02 the risk was 1/4500.

Narcolepsy is a chronic rapid eye movement disorder (REM) sleep disorder with excessive daytime sleepiness [3]. Two disease categories can be distinguished. Narcolepsy type 1 (NT1) is likely caused by an autoimmune-mediated destruction of hypocretin-producing neurons in the lateral hypothalamus. NT1 is almost always associated with cataplexy, which manifests as muscular weakness provoked by emotional stimuli. In narcolepsy type 2 (NT2), which is infrequent, there is no hypocretin deficiency or cataplexy. The great majority of narcolepsy cases associated with Pandemrix appears to be NT1, although it is unclear whether some cases may constitute NT2. >98% of NT1 cases carry HLA-DQB1*06:02, and current knowledge suggests that carrying this HLA type is a necessary prerequisite for the development of Pandemrix-associated NT1. However, in a series of 522 patients with narcolepsy and cataplexy from different countries, 9 patients (1.7%) with low levels of hypocretin in cerebrospinal fluid (CSF) were DQB1*06:02 negative [4], suggesting that this HLA type may not be required in all cases. Since DQB1*06:02 is present in approximately 30% of Swedish and Finnish populations [5], non-HLA factors, potentially genetic, may play a role in the development of the disorder. A non-HLA factor that has been associated with spontaneous narcolepsy is the locus for immune-related T-cell receptor alpha joining (TRAJ) genes [6]. These genes encode joining segments of the T cell receptor alpha chain that are important for the recognition of antigens. The highest association with spontaneous narcolepsy was obtained for the single nucleotide polymorphism (SNP) rs1154155 that is in almost complete linkage disequilibrium (LD) with rs12587781 in Caucasians ($r^2 = 0.96$) [6]. The TRAJ locus, represented by rs12587781, showed a nominal association with Pandemrix-induced narcolepsy in a candidate gene study, odds ratio (OR) 1.7, $p = 0.033$ [5]. No association with Pandemrix-induced narcolepsy was detected for the other tested non-HLA candidates: CSH, TNSF4, and the P2RY11/DNMT1 region [5].

Mass vaccinations of the population are essential for the prevention of contagious diseases, and any suspected genetic vulnerability to a serious vaccine related safety concern needs to be scrutinised. In this genome-wide association study (GWAS) we aimed to identify novel genetic markers for Pandemrix-associated narcolepsy. We also aimed to assess whether previous associations with DQB1*06:02 and the TRAJ locus could be replicated.

2. Methods

2.1. Ethical statement

The study was approved by the regional ethical review boards in Uppsala and Stockholm (2010/231 in Uppsala; 2007/644-31 and 2011/463-32 in Stockholm). Written informed consent was obtained from all participants.

2.2. Sample description

The basis for case recruitment was through nation-wide spontaneous adverse drug reaction reports sent from health care professionals to the Swedish Medical Products Agency (MPA) up until November 2017. In addition, we recruited patients not previously reported to the MPA from the department of Neurology at Uppsala University Hospital, Sweden. Each patient was required to be at least 18 years of age at the time of recruitment and able to give informed consent. Case definition for narcolepsy was according to the International Classification of Sleep Disorders – Third Edition [7].

The first report of Pandemrix-associated narcolepsy was received by the MPA in February 2010. In total, we identified 142 patients with Pandemrix-associated narcolepsy and who were at least 18 years at the time of recruitment start. We were unable to recruit 60 (patient declined participation ($n = 12$), patient did not complete study ($n = 10$), patient was not possible to contact ($n = 17$), reporter was not possible to contact ($n = 20$), the reporter thought the patient should not be contacted ($n = 1$)). We further did not try to recruit one patient reported to the MPA as the report stated that onset was five years following vaccination.

From the 81 patients that completed the study, we collected clinical data (demographics, medical history, drug treatment history, laboratory data, and ancestry) through interviews using a standardized questionnaire, and by obtaining and reviewing medical records. Each case was adjudicated by a specialist in sleep medicine and child psychiatry, and by a specialist in clinical pharmacology. Based on this evaluation, a total of seven cases were excluded for the following reasons: did not fulfill diagnostic criteria ($n = 4$), onset before vaccination ($n = 1$), bone marrow transplantation ($n = 1$), differential diagnosis of sleep apnea not excluded ($n = 1$).

Of the 74 cases that passed adjudication, the first recruited 42 cases were defined as the discovery cohort, and the last recruited 32 cases as the replication cohort.

We compared the cases in the discovery cohort with 4891 population controls from the Swedish Twin Registry [8], all non-related individuals of predominantly Swedish origin, and born between 1911 and 1958. The replication controls were 176 Pandemrix-vaccinated without a self-reported diagnosis of narcolepsy born to predominantly Swedish (68%) or Finnish (13%) parents 1974–1999, and nearly 7000 unrelated individuals from the Swedish Twin Registry born to Swedish parents 1992–2005. In total, we had 6990 replication controls for rs62360233, and 7125 replication controls for rs1154155.
2.3. Power calculation

Given a genome-wide significance level of $p < 5 \times 10^{-8}$ and using an additive genetic model, our sample size was powered to detect common genetic variants with effect sizes of clinical utility [9]. In the genome-wide analyses, we had approximately 80% power to detect an OR of 4 for variants with a minor allele frequency (MAF) of 40%, and 80% power to detect an OR of 5 for variants with a MAF of 20%.

2.4. Genotyping of the discovery cohort

Deoxyribonucleic acid (DNA) was extracted from peripheral venous blood. Cases were genotyped with the Illumina Infinium OmniExpressExome 1 M array, and controls with the Illumina HumanOmniExpress 700 K array at SNP array. All were genotyped at the Department of Medical Sciences, SNP&SEQ Technology Platform. Genotype calls were generated using the Genome Studio software from Illumina and the Genome Reference Consortium human assembly GRCh37.

Genotyping quality control (QC) and data management was performed using PLINK v1.9 [10]. The resulting merged data included 600 kSNPs post QC. Imputation was performed using the Sanger imputation server [11]. The pipeline with Eagle2 (v2.0.5) prephasing [12] and positional Burrows-Wheeler transform (PBWT) imputation [13] were used with the haplotype reference consortium panel as reference (v1.1) [11]. The total number of SNPs after imputation and QC was 8.6 million. With the exception of one case, the discovery cohort was within the European cluster according to genetic principal component analysis (PCA) (Fig. 1).

![Fig. 1. Analysis of principal components 1 and 2 (PC 1 and PC2) for cases (n = 42) and controls (n = 4891) in the discovery cohort. Comparison is made with Utah residents with Northern and Western European ancestry from the CEPH collection (CEU), Han Chinese in Beijing, China (CHB), Japanese in Tokyo, Japan (JPT), and Yoruba people in Ibadan, Nigeria (YRI).](image)

| Table 1 Characteristics of the discovery and replication cases. |
|------------------|------------------|
|                  | Discovery        | Replication |
| n total          | 42               | 32           |
| n narcolepsy type 1 [proportion] | 37 [0.88] | 30 [0.94] |
| Time to onset (months, mean [range]) | 5.00 [1–17] | 10.42 [1–48] |
| Age at onset (years, mean;median [range]) | 22.71;19 | 19.06;15 |
| Gender (n male [proportion male]) | 22 [0.52] | 11 [0.34] |
| Time to first health-care related contact (months, mean [range]) | 19.02 [2–63] | 33.90 [2–78] |
| Daytime sleepiness (n [proportion]) | 42 [1] | 32 [1] |
| Cataplexy (n [proportion]) | 36 [0.86] | 29 [0.91] |
| MSLT positive (n [proportion]) | 37 [0.88] | 29 [0.91] |
| Low cerebrospinal hypocretin (n [proportion]) | 12 [0.29] | 13 [0.41] |
| Normal cerebrospinal hypocretin (n [proportion]) | 1 [0.02] | 3 [0.09] |
| Cerebrospinal hypocretin not measured (n [proportion]) | 29 [0.69] | 16 [0.50] |
| HLA-DQB1*06:02 carrier (n [proportion]) | 39 [0.93] | N/A |
| Disrupted night time sleep (n [proportion]) | 22 [0.52] | 21 [0.66] |
| Hypnagogic hallucinations (n [proportion]) | 18 [0.43] | 17 [0.53] |
| Sleep paralysis (n [proportion]) | 19 [0.45] | 15 [0.47] |
| Unexpected weight gain (n [proportion]) | 6 [0.14] | 13 [0.41] |
| Behavioral or emotional problems (n [proportion]) | 2 [0.05] | 8 [0.25] |
| Other sleep abnormalities† | 3 [0.07] | 3 [0.09] |
| Ethnicity |                  |
| Swedish (n [proportion]) | 37 [0.88] | 27 [0.84] |
| Finnish (n [proportion]) | 0 | 0 |
| Other European (n [proportion]) | 4 [0.10] | 4 [0.13] |
| Other (n [proportion]) | 1 [0.02] | 1 [0.03] |

MSLT = multiple sleep latency test that measures how quickly a person falls asleep in a quiet environment during the day. HLA = human leukocyte antigen.

† Among the discovery cases, two patients reported nightmares and one periodic limb movements. Among the replication cases, three patients reported nightmares.
Table 2
Top genome-wide associations with Pandemrix-associated narcolepsy.

| CHR | SNP       | BP       | Minor allele | N   | OR     | LRS   | USI   | P   | GTPS | MAF cases | MAF controls | Gene |
|-----|-----------|----------|--------------|-----|--------|-------|-------|-----|------|-----------|--------------|------|

For Table 2, the top GWAS results based on 8.6 million SNPs after imputation in 42 cases versus all 4891 population controls. All results were adjusted for genetic principal components 1–4. The threshold for statistical significance was \( p < 5 \times 10^{-8} \). Base pair positions are according to Genome Reference Consortium human assembly GRCh37.

GWAS = genome-wide association study, CHR = chromosome, SNP = single nucleotide polymorphism, BP = base pair, N = number, GTPS = Guanosine-5'-triphosphates, MAF = minor allele frequency, OR [95% CI] = odds ratio with 95% confidence interval, P = p-value.

Fig. 2. Manhattan plot of the genome-wide association analysis. All analyses were made on 42 cases of Pandemrix-associated narcolepsy vs 4891 population controls with 8.6 million SNPs after imputation, adjusted by sex and genetic principal components 1–4. The red line shows the threshold for genome-wide significance of \( 5 \times 10^{-8} \). A) Main analysis. The top SNP is located in the human leukocyte antigen (HLA) region on chromosome 6 position 32,213,150 according to Genome Reference Consortium human assembly GRCh37. B) Adjustment for HLA-DQB1*06:02. The top SNP was rs62360233 on chromosome 5, located near glial cell line-derived neurotrophic factor (GDNF) anti-sense 1 (AS1), GDNF-AS1 (OR = 8.6 [95% CI 4.2, 17.5], \( p = 2.6 \times 10^{-10} \)). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
10 min. Genotyping was performed with a sequencing primer (5′-biotin-GATGAGATTTGGGT-3′) according to manufacturer’s recommended conditions for all reagents. The PCR typed for rs1154155 in the candidate gene locus [14]. The SNP rs62360233 was imputed using 1000-Genomes data at the Department of Medical Sciences, SNP&SEQ Technology Platform were genotyped using the Illumina Infinium PsychArray-24 BeadChip at the Department of Medical Sciences, SNP&SEQ Technology Platform, Waltham, USA). The 6814 controls from the Swedish Twin Registry (phase 3, version 5) [15].

The 6000–6040 Roadmap Epigenomics project [18–20]. Chromatin state was based on the 7500 Fast Real-Time PCR System (Thermo Fisher Scientific, Waltham, USA) at the Department of Medical Sciences, Clinical Pharmacology. We used the TaqMan® SNP Genotyping Assay kit C_3141474_10 containing primers and probes for allele discrimination (Thermo Fisher Scientific, Waltham, USA). The 6949 additional controls from the Swedish Twin Registry were genotyped using the Illumina Infinium PsychArray-24 BeadChip at the Department of Medical Sciences, SNP&SEQ Technology Platform [14]. The SNP rs1154155 was imputed using 1000-Genomes data (phase 3, version 5) [15].

2.5. Genotyping of the replication cohort

DNA was extracted from peripheral venous blood or saliva. Thirty-two cases and 176 Pandemrix-exposed controls were genotyped for rs62360233 in GDNF-AS1 by pyrosequencing with PyroMark Q48 Autoprep System (Qiagen, Hilden, Germany) at the Department of Medical Sciences, Clinical Pharmacology. We used a 5′-biotylated forward primer/molecule chain reaction (PCR) primer (5′-biotin-GATCAGATTGTGGGTG GGTGACA-3′) and a reverse primer (5′-AGTTCCAAGTAAGAAGCGG CAG-3′). The assay was performed in a 25 μL reaction volume with the manufacturer’s recommended concentrations for all reagents. The PCR conditions were initial denaturation at 95 °C for 15 min, followed by 45 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s, with a final extension step at 72 °C for 10 min. Genotyping was performed with a sequencing primer (5′-AAGTAAGAAGCGGACAGGTG-3′) according to manufacturer’s recommended protocol. The 6814 controls from the Swedish Twin Registry were genotyped using the Illumina Infinium PsychArray-24 BeadChip at the Department of Medical Sciences, SNP&SEQ Technology Platform [14]. The SNP rs62360233 was imputed using 1000-Genomes data (phase 3, version 5) [15].

Thirty-two cases and 176 Pandemrix-exposed controls were genotyped for rs1154155 in the candidate gene locus TRA on the 7500 Fast Real-Time PCR System (Thermo Fisher Scientific, Waltham, USA) at the Department of Medical Sciences, Clinical Pharmacology. We used the TaqMan® SNP Genotyping Assay kit C_3141474_10 containing primers and probes for allele discrimination (Thermo Fisher Scientific, Waltham, USA). The 6949 additional controls from the Swedish Twin Registry were genotyped using the Illumina Infinium PsychArray-24 BeadChip at the Department of Medical Sciences, SNP&SEQ Technology Platform [14]. The SNP rs1154155 was imputed using 1000-Genomes data (phase 3, version 5) [15].

2.6. HLA allele imputation

HLA allele imputation of the discovery cohort to first and second field resolution of 180 classical HLA alleles, amino acid residues, and individual SNPs was performed on the non-imputed merged and quality-controlled genome-wide data using the software SNP2HLA with a reference panel of 5225 individuals [16]. The threshold for significance in this analysis was adjusted to 2.78 × 10^-4 (Bonferroni correction).

2.7. Statistical analyses

Logistic regression on a genome wide level was performed using PLINK v1.9 [10]. All genome-wide analyses were adjusted for the first four principal components. SNP effects were modelled as additive and the conventional genome-wide significance threshold p < 5 × 10^-8 was used to correct for multiple testing [17]. HLA effects were modelled as both additive and dominant. Differences in allele frequency between cases and controls were expressed as ORs with 95% CIs, and results visualized as Manhattan plots. In the candidate gene analysis, the significance level was set to 0.05. A meta-analysis of associations with rs1154155 in the discovery and replication cohorts was performed using a fixed effects model.

2.8. Functional analysis

Functional annotations were obtained by intersecting the top GWAS SNPs and SNPs in high LD in five European populations (LDlink) with transcription factor binding sites reported from the ENCODE project and with chromatin state models from the Roadmap Epigenomics project [18–20]. Chromatin state was based
on deoxyribonuclease (DNase) I hypersensitive clusters, regions with histone modifications H3K4me3 and H3K27ac indicating active regulatory regions. We used annotations in brain-derived tissues (Roadmap epigenome identifiers: E053-054,067-074,081-082), astrocyte cell line (E125), muscle-derived tissue (E089-090,107-108), and primary cultures of human skeletal muscle (E120-121).

2.9. Role of the funding source

The study sponsors played no role in study design, collection, analysis, and interpretation of data, the writing of the report or the decision to submit the paper for publication. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.
2.10. Data statement

Access to data on the 74 cases and 176 treated controls can be obtained by collaborating with Swedegene. Access to data on the population controls can be obtained after application to the Swedish Twin Registry.

3. Results

3.1. Genome-wide analysis

Characteristics of the 42 cases in the discovery cohort are shown in Table 1. Pandemrix-induced narcolepsy was significantly associated with multiple SNPs in the HLA region on chromosome 6 (Fig. 2A and Table 2). After HLA allele imputation and using an additive model, the strongest association was with HLA-DRB1*15:01, OR 7.2 [95% confidence interval (CI) 4.6, 11.4], \(p = 1.2 \times 10^{-7}\), followed by HLA-DQB1*06:02, OR 7.1 [95% CI 4.5, 11.2], \(p = 1.9 \times 10^{-7}\) (Table 3). These HLA-types are on the same haplotype in almost all in Swedish individuals \((r^2 = 0.98)\) [21]. The proportion of carriers of HLA-DQB1*06:02 was 93% among cases, compared with 28% in the control population. The odds ratio increased to 39.4 [95% CI 11.3, 137], \(p = 7.9 \times 10^{-9}\) when carriage of HLA-DQB1*06:02 was compared between cases and controls using a dominant model.

After correction for HLA-DQB1*06:02, rs62360233 was associated with Pandemrix-induced narcolepsy on a genome-wide level, OR = 8.7 [95% CI 4.2, 17.5], \(p = 2.6 \times 10^{-9}\) (Fig. 2B and Table 4). rs62360233 was replicated in 32 adjudicated cases (Table 1), and 6990 population controls, and the minor allele was significantly more common among cases than controls, OR = 3.4 [95% CI 1.2–9.6], \(p = 0.022\).

3.2. Functional analysis

The top SNP rs62360233 is located on chromosome 5 in intron 2 of the gene for the glial cell line-derived neurotrophic factor antisense 1 (GDNF-AS1). This gene encodes the antisense RNA 1 for the glial cell-derived neurotrophic factor gene (GDNF). GDNF-AS1 is expressed in several tissues including the brain cortex, cerebellum, spinal cord, peripheral nerves, and skeletal muscle [22]. The associated SNP rs62360233 has in itself no functional annotation in ENCODE or Roadmap data [19,20]. Two variants in high LD with the top hit rs62360233 have annotations that might explain the detected association: rs75921262 and rs79455475 with LD 0.76 and 0.71, respectively. They are located in exon 4 of GDNF-AS1, and could therefore potentially affect the structure or function of the antisense RNA. In addition, rs75921262 is located in a DNase 1 hypersensitive site in normal human astrocyte and human skeletal muscle myoblast derived cell lines. Furthermore, rs75921262 is in an annotated regulatory element in several muscle tissues including muscle satellite cultured cells, skeletal muscle, psoas muscle, and fetal muscle trunk.

3.3. Candidate gene analysis

The candidate locus TRAJ was in our study represented by rs1154155. Forty-two adjudicated cases (Table 1) were compared with 4891 population controls in the discovery cohort. The minor allele of rs1154155 was significantly more common among cases than controls, OR = 2.1 [95% CI 1.3, 3.3], \(p = 0.0036\). In the replication in 32 adjudicated cases (Table 1) and 7125 controls, rs1154155 was more common among cases, OR 1.8 [95% CI 1.0, 3.2], \(p = 0.04\). A meta-analysis of the two cohorts showed a significant association between Pandemrix-induced narcolepsy and the TRAJ locus, meta-analysis OR = 2.0 [95% CI 1.4, 2.8], \(p = 0.0002\) (Fig. 3).

4. Discussion

This study confirmed a strong association between HLA-DQB1*06:02 and Pandemrix-associated narcolepsy. We estimate that the risk of narcolepsy after vaccination with Pandemrix was 49-fold increased in people carrying HLA-DQB1*06:02. While the vast majority of narcolepsy patients were carriers of this HLA type (93% compared with 28% among controls), 7% were not positive. This suggests that, although being a strong risk factor, HLA-DQB1*06:02 is neither necessary nor sufficient to explain the development of narcolepsy in all patients.

We detected a novel association between Pandemrix-associated narcolepsy and the non-coding RNA gene GDNF-AS1. This association was confirmed in a replication cohort. In general, antisense RNAs are transcribed to prevent translation of a complementary mRNA by base pairing to it and blocking translation [23]. It is plausible that this antisense RNA exerts an effect on the gene GDNF that is located head to head with GDNF-AS1. GDNF encodes GDNF, a potent neurotrophic factor that promotes neuronal survival [24]. Knockdown of GDNF-AS1 has been shown to increase GDNF mRNA in vitro [24]. There is thus experimental evidence that GDNF-AS1 regulates GDNF. Our intronic top hit in GDNF-AS1 appears to be non-functional, but exon 4 variants in high LD with our top hit could be functional by changing the RNA sequence [25]. The exon 4 variant rs75921262 also has annotations suggesting a gene regulatory effect on GDNF in skeletal muscle and skin. The neurotrophic factor GDNF is, however, predominantly produced by multiple cell types in the central and peripheral nervous system, and has a beneficial effect on several cells including sympathetic, parasympathetic, sensory, and motor neurons [26,27]. Neurotrophic factors have been

| Study       | Cases | Controls | Odds ratio [95% confidence interval] |
|-------------|-------|----------|-------------------------------------|
| Discovery   | 42    | 4891     | 2.10 [1.31, 3.38]                    |
| Replication | 32    | 7125     | 1.82 [1.03, 3.21]                    |
| Fixed effects model |       |          | 1.98 [1.38, 2.85]                    |

Fig. 3. Meta-analysis of T-cell receptor alpha joining (TRAJ). Results for the TRAJ variant rs1154155 from the discovery and replication cohorts, and meta-analysis using a fixed effects model.
demonstrated to activate neuronal repair genes under conditions of neurodegeneration [28]. Changes in regulation of GDNF have been associated with neurodegenerative diseases such as Alzheimer’s and Parkinson’s disease, and GDNF has received attention as a potential therapeutic agent for the treatment of several neurological diseases [29,30]. Based on the above, we speculate that genetic variants leading to a decrease in GDNF expression may increase the risk of narcolepsy through impaired neuronal survival in predisposed patients. We thus believe that genetic variation in GDNF-AS1 may play a role for the susceptibility to Pandemrix-associated narcolepsy.

The candidate genetic locus TRAJ was also associated with Pandemrix-induced narcolepsy on a nominal level. TRAJ has been associated with spontaneous narcolepsy in a study by Hallmayer et al. [6], and with Pandemrix-associated narcolepsy by Bomfim et al. [5]. The associations were described for two intergenic SNPs, rs1154155 and rs12587781, which are in near complete LD in Caucasians (r² = 0.96) [5,6]. These SNPs both have annotations suggesting a gene regulatory effect in T-cells, and are in high LD with several other SNPs within the TRAJ locus [25]. The TRAJ locus encodes joining segments of the T-cell receptor-αβ-heterodimer, a protein expressed by T lymphocytes [6]. The T-cell receptor interacts with both HLA class I (CD8 in cytotoxic T-cells) and HLA class II (CD4 in helper T-cells), including the DQβ1 heterodimer denoted DQ0602, encoded by DQB1*06:02. As hypothesized by Hallmayer et al., it is possible that rs1154155 tags a specific T-cell receptor-αβ receptor subtype that interacts with the HLA-haplotype that is associated with narcolepsy [6]. However, since rs1154155 was absent in about half of the patients, other factors are likely to be involved as well.

Some limitations of this study should be considered. Although we were able to recruit at total of 74 patients with Pandemrix-associated narcolepsy, the power to detect associated variants was limited. Another limitation is that this study lacks a control group patients with spontaneous narcolepsy. It was therefore not possible to determine whether there are differences in terms of genetic susceptibility between spontaneous and Pandemrix-associated narcolepsy.

5. Conclusion

We detected a novel association between Pandemrix-associated narcolepsy and the non-coding RNA gene GDNF-AS1. This gene potentially regulates the production of the neurotrophic factor GDNF that is important for neuronal survival. The finding should be investigated in further studies of Pandemrix-associated narcolepsy. We also confirmed a strong association between Pandemrix-induced narcolepsy and the HLA-DQB1*06:02:HLA-DRB1*15:01 haplotype. Furthermore, the candidate genetic locus TRAJ was nominally associated, suggesting that a specific T-cell receptor-αβ receptor interacts with the HLA-haplotype associated with narcolepsy. In summary, variation in genes related to immunity and neuronal survival may interact to increase the risk of Pandemrix-induced narcolepsy in certain individuals.

Conflict of interest

None of the others declare any conflicts of interest.

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