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

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ABSTRACT

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], $p = 7.9 \times 10^{-9}$. 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.

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

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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: CTSH, TNFSF4, 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 (69%) 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.

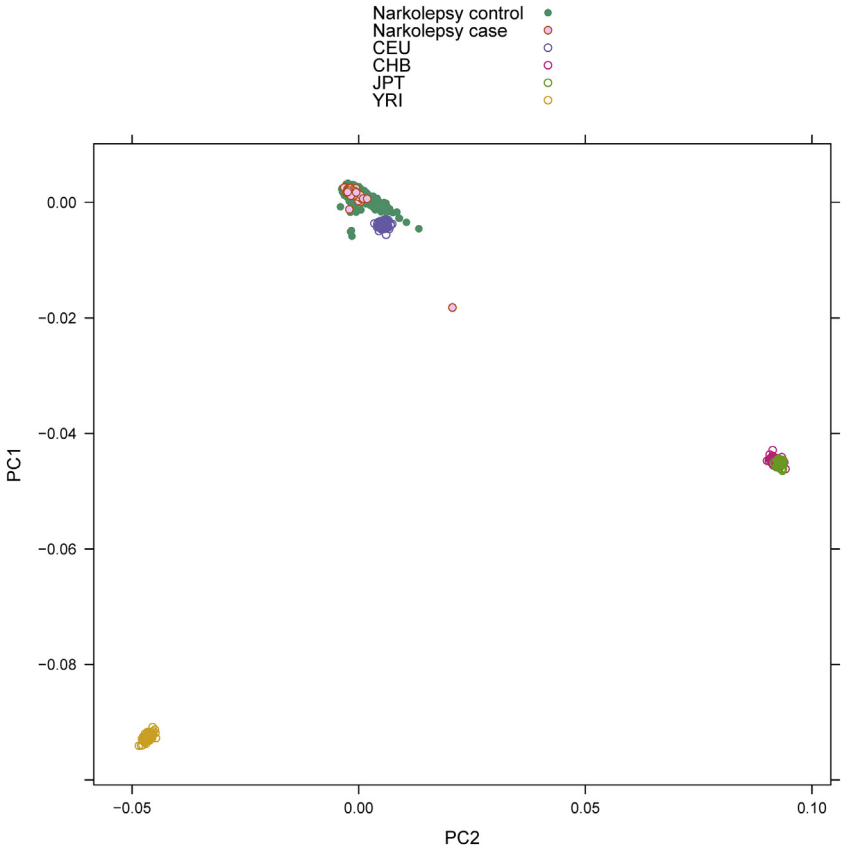


Fig. 1. Analysis of principal components 1 and 2 (PC 1 and PC2) for cases ($n = 42$) and controls ($n = 4891$) in the discovery cohort. Comparison is made with Utah residents with Northern and Western European ancestry from the CEPH collection (CEU), Han Chinese in Beijing, China (CHB), Japanese in Tokyo,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

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

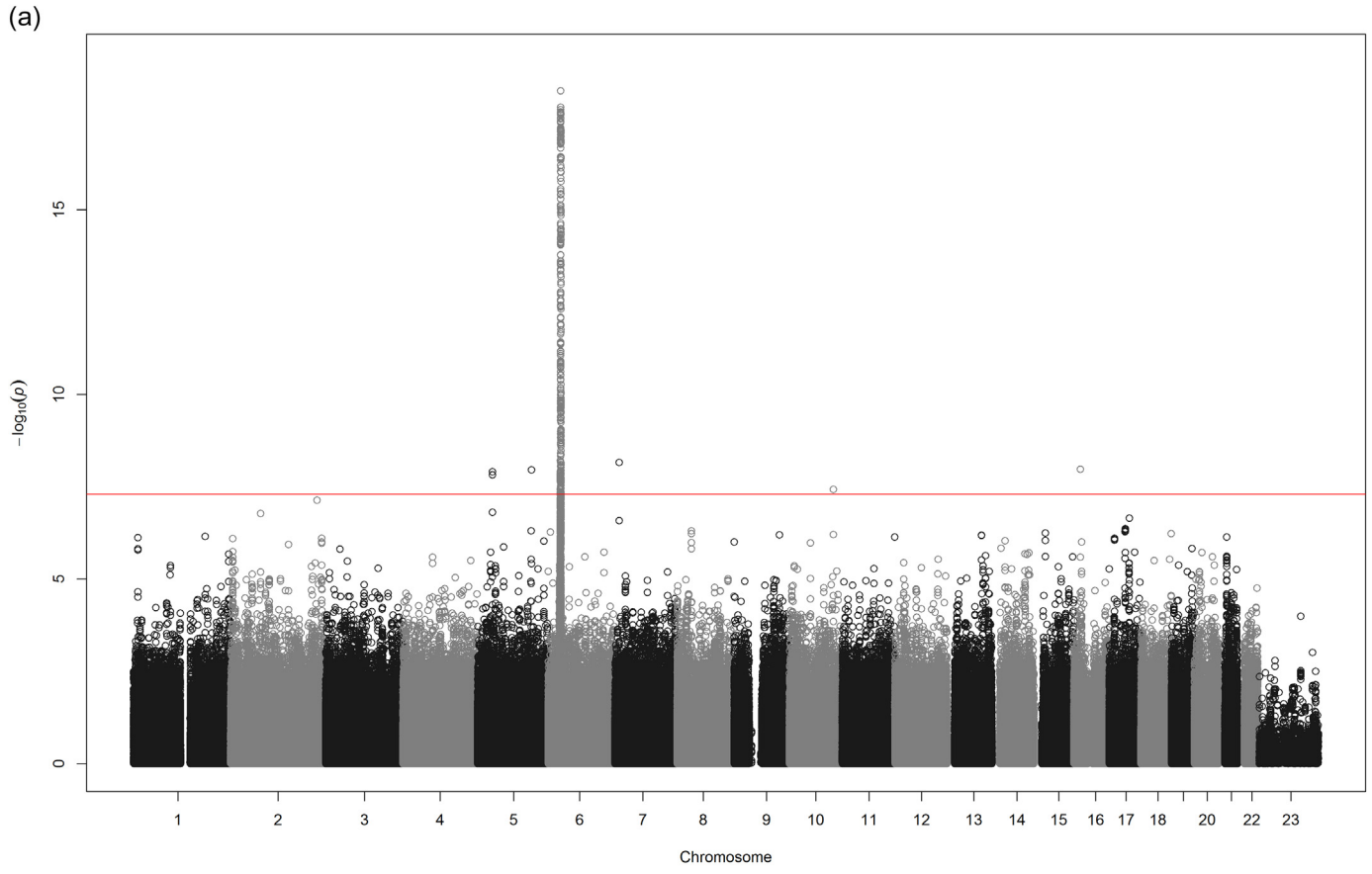
Deoxyribonucleic acid (DNA) was extracted from peripheral venous blood. Cases were genotyped with the Illumina Infinium OmniExpressExome 1 M array, and controls with the Illumina HumanOmniExpress 700 K array at SNP array. All were genotyped at the Department of Medical Sciences, SNP&SEQ Technology Platform. 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).

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]
n narcolepsy type 2 [proportion]	5 [0.22]	2 [0.06]
Time to onset (months, mean [range])	5.00 [1–17]	10.42 [1–48]
Age at onset (years, mean;median [range])	22.71;19 [14–50]	19.06;15 [11–45]
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.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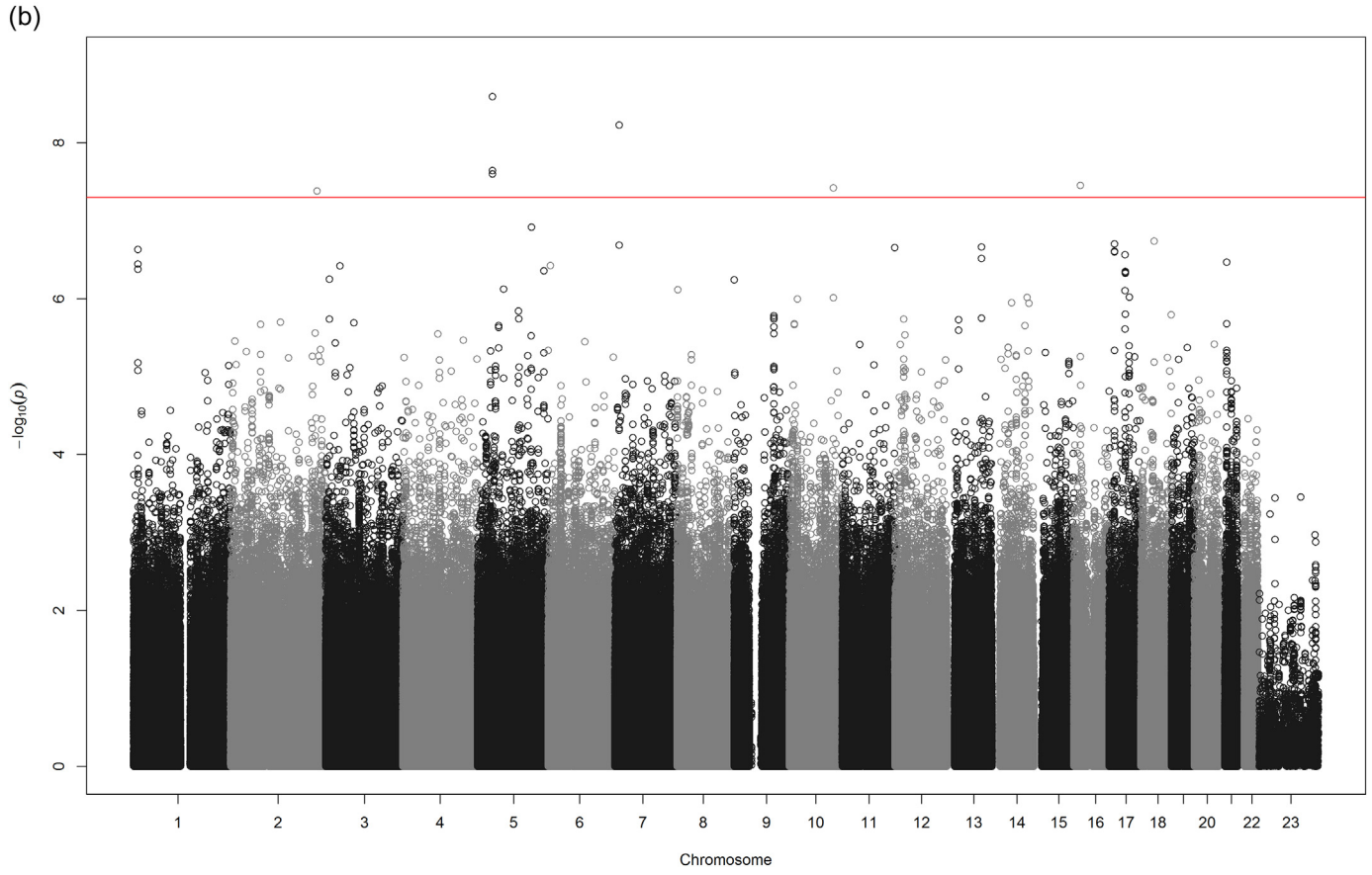
Chromosome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Chromosome

Table 2
Top genome-wide associations with Pandemrix-associated narcolepsy.

CHR	SNP	BP	Minor allele	N	OR	L95	U95	P	GTPS	MAF cases	MAF controls	Gene
6	6:32213150	32,213,150	A	4933	8.008	5.063	12.67	5.959×10^{-19}	A/G	0.595	0.164	
6	6:32249987	32,249,987	T	4933	7.688	4.875	12.12	1.669×10^{-18}	T/C	0.583	0.161	
6	6:32295419	32,295,419	A	4933	7.688	4.875	12.12	1.681×10^{-18}	A/C	0.583	0.161	C6orf10
6	6:32519108	32,519,108	G	4933	7.701	4.877	12.16	1.948×10^{-18}	G/C	0.607	0.166	
6	6:32522434	32,522,434	T	4933	7.639	4.843	12.05	2.233×10^{-18}	T/G	0.607	0.167	HLA-DRB6
6	6:32364667	32,364,667	A	4933	7.623	4.835	12.02	2.249×10^{-18}	A/G	0.571	0.158	BTNL2
6	6:32505551	32,505,551	A	4933	7.64	4.843	12.05	2.297×10^{-18}	A/G	0.607	0.167	
6	6:32522401	32,522,401	G	4933	7.639	4.842	12.05	2.353×10^{-18}	G/A	0.607	0.167	HLA-DRB6
6	6:32501122	32,501,122	G	4933	7.62	4.831	12.02	2.451×10^{-18}	G/T	0.607	0.167	
6	6:32246919	32,246,919	A	4933	7.601	4.821	11.98	2.492×10^{-18}	A/G	0.583	0.163	
6	6:32247115	32,247,115	T	4933	7.601	4.821	11.98	2.492×10^{-18}	T/A	0.583	0.163	
6	6:32255454	32,255,454	A	4933	7.601	4.821	11.98	2.492×10^{-18}	A/G	0.583	0.163	
6	rs6457535	32,262,406	A	4933	7.601	4.821	11.98	2.492×10^{-18}	A/G	0.583	0.163	C6orf10
6	6:32522389	32,522,389	T	4933	7.612	4.826	12.01	2.518×10^{-18}	T/G	0.607	0.167	HLA-DRB6
6	6:32577385	32,577,385	T	4933	7.671	4.855	12.12	2.546×10^{-18}	T/C	0.595	0.163	
6	6:32324055	32,324,055	A	4933	7.603	4.82	11.99	2.685×10^{-18}	A/C	0.583	0.163	C6orf10
6	6:32329598	32,329,598	A	4933	7.603	4.82	11.99	2.685×10^{-18}	A/G	0.583	0.163	C6orf10
6	6:32295965	32,295,965	T	4933	7.56	4.794	11.92	3.193×10^{-18}	T/C	0.583	0.164	C6orf10
6	6:32409058	32,409,058	T	4933	7.586	4.806	11.97	3.227×10^{-18}	T/C	0.571	0.158	HLA-DRA
6	6:32321272	32,321,272	C	4933	7.561	4.794	11.93	3.306×10^{-18}	C/A	0.583	0.164	C6orf10
6	6:32220397	32,220,397	G	4933	7.511	4.767	11.83	3.505×10^{-18}	G/A	0.583	0.164	
6	6:32413051	32,413,051	A	4933	7.506	4.761	11.83	3.956×10^{-18}	A/G	0.571	0.159	
6	6:32413545	32,413,545	G	4933	7.506	4.761	11.83	3.956×10^{-18}	G/A	0.571	0.159	
6	6:32410987	32,410,987	A	4933	7.506	4.761	11.83	4.022×10^{-18}	A/G	0.571	0.159	HLA-DRA
6	6:32392906	32,392,906	C	4933	7.531	4.773	11.88	4.148×10^{-18}	C/A	0.571	0.16	
6	6:32392981	32,392,981	C	4933	7.531	4.773	11.88	4.148×10^{-18}	C/T	0.571	0.16	
6	6:32393235	32,393,235	G	4933	7.531	4.773	11.88	4.148×10^{-18}	G/C	0.571	0.16	
6	6:32406579	32,406,579	T	4933	7.531	4.772	11.89	4.228×10^{-18}	T/C	0.571	0.16	
6	rs111911331	32,493,298	G	4933	7.531	4.771	11.89	4.365×10^{-18}	G/T	0.607	0.169	HLA-DRB5
6	6:32421227	32,421,227	A	4933	7.371	4.683	11.6	6.131×10^{-18}	A/G	0.583	0.16	
6	6:32422125	32,422,125	G	4933	7.371	4.683	11.6	6.131×10^{-18}	G/A	0.583	0.16	
6	6:32423915	32,423,915	C	4933	7.371	4.683	11.6	6.131×10^{-18}	C/G	0.583	0.16	
6	6:32367017	32,367,017	G	4933	7.286	4.639	11.44	6.589×10^{-18}	G/A	0.571	0.16	BTNL2
6	6:32577531	32,577,531	T	4933	7.351	4.669	11.57	7.021×10^{-18}	T/G	0.595	0.165	
6	6:32580411	32,580,411	A	4933	7.35	4.669	11.57	7.094×10^{-18}	A/G	0.595	0.165	
6	6:32580637	32,580,637	C	4933	7.35	4.669	11.57	7.094×10^{-18}	C/T	0.595	0.165	
6	6:32581333	32,581,333	G	4933	7.35	4.669	11.57	7.094×10^{-18}	G/A	0.595	0.165	
6	6:32581515	32,581,515	G	4933	7.35	4.669	11.57	7.094×10^{-18}	G/A	0.595	0.165	
6	6:32549259	32,549,259	G	4933	7.351	4.669	11.57	7.157×10^{-18}	G/A	0.595	0.165	HLA-DRB1
6	6:32549501	32,549,501	A	4933	7.351	4.669	11.57	7.157×10^{-18}	A/T	0.631	0.276	HLA-DRB1
6	6:32549501	32,549,501	A	4933	7.351	4.669	11.57	7.157×10^{-18}	A/T	0.631	0.165	HLA-DRB1
6	6:32549501	32,549,501	A	4933	7.351	4.669	11.57	7.157×10^{-18}	A/C	0.595	0.276	HLA-DRB1
6	6:32549501	32,549,501	A	4933	7.351	4.669	11.57	7.157×10^{-18}	A/C	0.595	0.165	HLA-DRB1
6	6:32561794	32,561,794	C	4933	7.351	4.669	11.57	7.157×10^{-18}	C/G	0.595	0.165	
6	6:32566011	32,566,011	C	4933	7.351	4.669	11.57	7.157×10^{-18}	C/A	0.595	0.165	
6	6:32572461	32,572,461	G	4933	7.351	4.669	11.57	7.157×10^{-18}	G/T	0.595	0.165	
6	6:32575369	32,575,369	G	4933	7.351	4.669	11.57	7.157×10^{-18}	G/T	0.595	0.165	
6	6:32575544	32,575,544	A	4933	7.351	4.669	11.57	7.157×10^{-18}	A/T	0.595	0.165	
6	6:32575575	32,575,575	T	4933	7.351	4.669	11.57	7.157×10^{-18}	T/A	0.595	0.165	
6	6:32578449	32,578,449	T	4933	7.351	4.669	11.57	7.157×10^{-18}	T/C	0.595	0.165	
6	6:32578885	32,578,885	T	4933	7.351	4.669	11.57	7.157×10^{-18}	T/C	0.595	0.165	
6	rs9270022	32,553,142	C	4933	7.351	4.669	11.57	7.157×10^{-18}	C/T	0.595	0.165	HLA-DRB1
6	rs9270101	32,554,607	T	4933	7.351	4.669	11.57	7.157×10^{-18}	T/C	0.595	0.165	HLA-DRB1
6	6:32573991	32,573,991	T	4933	7.351	4.668	11.58	7.294×10^{-18}	T/G	0.595	0.165	
6	rs9270090	32,554,386	G	4933	7.445	4.714	11.76	7.319×10^{-18}	G/T	0.607	0.171	HLA-DRB1
6	6:32503930	32,503,930	C	4933	7.314	4.649	11.51	7.644×10^{-18}	C/T	0.595	0.165	
6	6:32509417	32,509,417	A	4933	7.314	4.649	11.51	7.644×10^{-18}	A/T	0.595	0.165	
6	6:32511698	32,511,698	A	4933	7.314	4.649	11.51	7.644×10^{-18}	A/G	0.595	0.165	
6	6:32521484	32,521,484	G	4933	7.314	4.649	11.51	7.644×10^{-18}	G/A	0.595	0.165	HLA-DRB6
6	6:32503613	32,503,613	A	4933	7.314	4.648	11.51	7.83×10^{-18}	A/G	0.595	0.165	
6	6:32589842	32,589,842	G	4933	7.32	4.651	11.52	7.83×10^{-18}	G/A	0.595	0.166	
6	rs34195497	32,526,670	G	4933	7.314	4.648	11.51	7.83×10^{-18}	G/A	0.595	0.165	HLA-DRB6
6	6:32574060	32,574,060	A	4933	7.275	4.626	11.44	8.497×10^{-18}	A/C	0.595	0.166	

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 5×10^{-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], $p = 2.6 \times 10^{-9}$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Top 30 imputed human leukocyte antigen (HLA) alleles at second field resolution adjusted for sex and genetic principal components 1–4.

CHR	Allele	BP	Minor allele	N	OR	L95	U95	p-value	Substitution	MAF case	MAF control
6	HLA_DRB1_1501	32,660,042	P	4933	7.227	4.594	11.37	1.179×10^{-17}	P/A	0.56	0.158
6	HLA_DQB1_0602	32,739,039	P	4933	7.116	4.526	11.19	1.94×10^{-17}	P/A	0.548	0.153
6	HLA_DQA1_0102	32,716,284	P	4933	5.816	3.693	9.162	3.073×10^{-14}	P/A	0.631	0.226
6	HLA_C_0702	31,346,171	P	4933	2.943	1.88	4.608	2.362×10^{-6}	P/A	0.357	0.157
6	HLA_B_0702	31,431,272	P	4933	2.918	1.857	4.586	3.408×10^{-6}	P/A	0.345	0.151
6	HLA_A_0301	30,019,970	P	4933	2.356	1.477	3.757	3.197×10^{-4}	P/A	0.31	0.165
6	HLA_A_0202	30,019,970	P	4933	31.33	3.124	314.2	3.409×10^{-2}	P/A	0.012	0
6	HLA_C_0304	31,346,171	P	4933	0.2214	0.0697	0.7032	0.01056	P/A	0.036	0.148
6	HLA_DQA1_0501	32,716,284	P	4933	0.3962	0.1903	0.8252	0.01338	P/A	0.095	0.208
6	HLA_A_0101	30,019,970	P	4933	0.2774	0.1003	0.7673	0.0135	P/A	0.048	0.143
6	HLA_DQA1_0101	32,716,284	P	4933	0.2306	0.0719	0.7399	0.01364	P/A	0.036	0.136
6	HLA_DQB1_0302	32,739,039	P	4933	0.236	0.07445	0.7478	0.01414	P/A	0.036	0.143
6	HLA_DQB1_0501	32,739,039	P	4933	0.1921	0.04698	0.7856	0.02169	P/A	0.024	0.114
6	HLA_DRB1_0301	32,660,042	P	4933	0.2578	0.08104	0.8204	0.02172	P/A	0.036	0.125
6	HLA_B_1501	31,431,272	P	4933	0.1968	0.04841	0.8003	0.02314	P/A	0.024	0.117
6	HLA_DRB1_1601	32,660,042	P	4933	4.906	1.147	20.98	0.03196	P/A	0.024	0.005
6	HLA_B_0801	31,431,272	P	4933	0.2841	0.08955	0.9013	0.03265	P/A	0.036	0.117
6	HLA_DRB1_0101	32,660,042	P	4933	0.123	0.01711	0.8843	0.03733	P/A	0.012	0.094
6	HLA_DQB1_0603	32,739,039	P	4933	0.1218	0.01654	0.8971	0.03878	P/A	0.012	0.086
6	HLA_A_6601	30,019,970	P	4933	8.716	1.112	68.33	0.03933	P/A	0.012	0.002
6	HLA_DQB1_0201	32,739,039	P	4933	0.3498	0.1275	0.9598	0.04138	P/A	0.048	0.125
6	HLA_DQB1_0502	32,739,039	P	4933	4.209	1.024	17.3	0.04626	P/A	0.036	0.006
6	HLA_B_4101	31,431,272	P	4933	7.991	1	63.85	0.04998	P/A	0.012	0.001
6	HLA_B_1302	31,431,272	P	4933	3.108	0.97	9.959	0.05629	P/A	0.036	0.011
6	HLA_B_4001	31,431,272	P	4933	0.2663	0.06537	1.085	0.06479	P/A	0.024	0.088
6	HLA_C_0701	31,346,171	P	4933	0.4574	0.1994	1.049	0.0648	P/A	0.071	0.145
6	HLA_C_0303	31,346,171	P	4933	0.1649	0.02292	1.186	0.07341	P/A	0.012	0.071
6	HLA_DQA1_0301	32,716,284	P	4933	0.578	0.3065	1.09	0.09032	P/A	0.131	0.22
6	HLA_DRB1_0402	32,660,042	P	4933	5.651	0.7325	43.59	0.09665	P/A	0.012	0.002

The effect is modelled per increase of one allele. The threshold for significance is 2.78×10^{-4}.
CHR = chromosome, BP = base pair, A = absent, P = present, N = number, GTPS = Guanosine-5′-triphosphates, MAF = minor allele frequency, OR [95% CI] = odds ratio with 95% confidence interval.

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′-biotinylated forward polymerase chain reaction (PCR) primer (5′-biotin-GATGAGATTTGGGT GGTGACA-3′) and a reverse primer (5'-AGTTCCCAAGTAAGAAAGCGA CAG-3′). The assay was performed in a 25μL 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'-AAGTAAGAAAGCGACAGGGT-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 *TRAJ* 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 allelic 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 $p < 5 \times 10^{-8}$ 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

Table 4
Top genome-wide associations with Pandemrix-associated narcolepsy after adjustment for HLA-DQB1*0602.

CHR	SNP	BP	Minor allele	N	OR	L95	U95	P	GTPS	MAF cases	MAF controls	Gene
5	rs62360233	37,923,854	A	4933	8.622	4.245	17.51	2.553×10^{-9}	A/C	0.131	0.022	GDNF-AS1
7	rs117922062	11,236,379	A	4933	11.44	5.033	25.98	5.916×10^{-9}	A/G	0.107	0.013	AC004160.1
5	rs62360232	37,923,343	A	4933	7.968	3.849	16.5	2.276×10^{-8}	A/C	0.119	0.021	GDNF-AS1
5	rs112179570	37,923,559	A	4933	11.56	4.888	27.35	2.517×10^{-8}	A/C	0.095	0.012	GDNF-AS1
16	rs148271597	16,159,192	G	4933	9.932	4.391	22.46	3.518×10^{-8}	G/A	0.095	0.012	ABCC1
10	rs190054430	113,200,488	A	4933	8.105	3.846	17.08	3.784×10^{-8}	A/C	0.119	0.021	Upstream of ANKS6
2	rs143074069	220,925,313	C	4933	10.36	4.492	23.89	4.167×10^{-8}	C/T	0.095	0.013	
5	rs11745282	137,904,077	T	4933	7.661	3.604	16.28	1.209×10^{-7}	T/C	0.131	0.019	HSPA9
18	rs144017419	33,664,926	A	4933	10.33	4.295	24.84	1.831×10^{-7}	A/G	0.083	0.013	
17	rs77105166	13,868,176	T	4933	8.426	3.774	18.81	1.984×10^{-7}	T/C	0.107	0.018	COX10-AS1
7	rs112261492	11,007,093	G	4933	11.39	4.548	28.51	2.053×10^{-7}	G/C	0.083	0.009	
13	rs9557123	88,188,711	A	4933	4.636	2.595	8.279	2.183×10^{-7}	A/G	0.202	0.057	MIR4500HG
11	rs145343222	134,614,198	G	4933	7.629	3.537	16.46	2.214×10^{-7}	G/A	0.107	0.019	AP01999.1
1	rs115845680	11,652,656	A	4933	9.555	4.061	22.48	2.34×10^{-7}	A/G	0.083	0.012	
17	rs79847507	13,857,009	T	4933	8.261	3.704	18.42	2.467×10^{-7}	T/C	0.107	0.018	COX10-AS1
17	rs76811567	13,868,894	A	4933	8.246	3.697	18.39	2.53×10^{-7}	A/G	0.107	0.018	COX10-AS1
17	rs118191398	40,787,514	T	4933	5.966	3.02	11.79	2.738×10^{-7}	T/C	0.143	0.033	
13	rs9554546	88,186,723	C	4933	4.541	2.544	8.105	3.061×10^{-7}	C/T	0.202	0.057	MIR4500HG
21	rs111758606	19,522,661	C	4933	7.765	3.532	17.07	3.4×10^{-7}	C/A	0.107	0.023	CHODL
1	rs138918965	11,621,557	T	4933	9.141	3.898	21.44	3.606×10^{-7}	T/C	0.083	0.013	
6	rs7765638	5,739,416	C	4933	4.943	2.668	9.156	3.768×10^{-7}	C/T	0.179	0.049	FARS2
3	rs145350225	36,725,558	T	4933	10.43	4.219	25.78	3.819×10^{-7}	T/G	0.083	0.012	
1	rs79576265	11,619,457	T	4933	8.984	3.838	21.03	4.212×10^{-7}	T/C	0.083	0.013	
5	rs76219007	169,685,187	G	4933	8.419	3.683	19.25	4.417×10^{-7}	G/A	0.107	0.016	LCP2
17	rs143637156	40,953,108	A	4933	6.758	3.218	14.19	4.475×10^{-7}	A/C	0.119	0.024	CNTD1
17	rs61755611	40,943,095	T	4933	6.749	3.214	14.17	4.569×10^{-7}	T/C	0.119	0.024	WNK4
17	rs74965130	40,915,503	G	4933	6.743	3.21	14.16	4.636×10^{-7}	G/A	0.119	0.025	Downstream of RAMP2
17	rs74717875	40,913,928	C	4933	6.733	3.206	14.14	4.742×10^{-7}	C/A	0.119	0.025	RAMP2
3	rs17815448	9,536,767	A	4933	11.64	4.451	30.46	5.638×10^{-7}	A/G	0.071	0.011	
9	rs112174060	290,271	G	4933	11.33	4.375	29.34	5.729×10^{-7}	G/T	0.071	0.01	DOCK8
5	rs111251540	67,047,050	A	4933	6.926	3.217	14.91	7.558×10^{-7}	A/G	0.107	0.02	AC112206.2/AC106798.1
8	rs35578989	1,998,978	A	4933	8.208	3.562	18.91	7.696×10^{-7}	A/C	0.095	0.02	MYOM2
17	rs77532561	40,583,610	C	4933	5.746	2.87	11.5	7.916×10^{-7}	C/T	0.131	0.033	
17	rs117272873	51,598,056	T	4933	6.55	3.089	13.89	9.552×10^{-7}	T/C	0.119	0.025	AC034268.2
14	rs79406488	89,748,080	G	4933	6.491	3.071	13.72	9.69×10^{-7}	G/C	0.119	0.026	FOXN3
10	rs192906116	113,123,314	G	4933	6.1	2.958	12.58	9.762×10^{-7}	G/C	0.119	0.024	
10	rs117590051	20,684,673	C	4933	8.11	3.505	18.77	1.009×10^{-6}	C/T	0.095	0.02	
14	rs118101874	50,019,584	A	4933	8.248	3.527	19.29	1.127×10^{-6}	A/G	0.083	0.013	
14	rs149839061	94,174,698	A	4933	7.662	3.372	17.41	1.155×10^{-6}	A/G	0.095	0.018	UNC79
5	rs558421089	105,281,087	G	4933	6.935	3.155	15.24	1.439×10^{-6}	G/A	0.095	0.022	
17	rs76475715	41,043,533	A	4933	6.663	3.072	14.45	1.577×10^{-6}	A/C	0.107	0.022	LINC00671
18	rs76289699	77,628,801	T	4933	6.512	3.029	14	1.61×10^{-6}	T/C	0.107	0.02	KCNG2
9	9:101568098	101,568,098	T	4933	7.491	3.287	17.07	1.652×10^{-6}	T/C	0.095	0.021	Upstream of ANKS6
9	rs10119228	101,563,029	T	4933	7.491	3.287	17.07	1.652×10^{-6}	T/C	0.095	0.021	Upstream of ANKS6
9	rs10125255	101,554,869	C	4933	7.491	3.287	17.07	1.652×10^{-6}	C/G	0.095	0.021	ANKS6
9	rs1555520	101,565,796	G	4933	7.491	3.287	17.07	1.652×10^{-6}	G/A	0.095	0.021	Upstream of ANKS6
9	rs4742742	101,557,017	G	4933	7.491	3.287	17.07	1.652×10^{-6}	G/C	0.095	0.021	ANKS6
9	rs4743277	101,557,529	T	4933	7.491	3.287	17.07	1.652×10^{-6}	T/C	0.095	0.021	ANKS6
9	rs4743280	101,560,558	G	4933	7.491	3.287	17.07	1.652×10^{-6}	G/T	0.095	0.021	Upstream of ANKS6
9	rs62561321	101,556,336	A	4933	7.491	3.287	17.07	1.652×10^{-6}	A/G	0.095	0.021	ANKS6
9	rs6415849	101,554,218	T	4933	7.491	3.287	17.07	1.652×10^{-6}	T/C	0.095	0.021	ANKS6
9	rs7039118	101,566,770	A	4933	7.491	3.287	17.07	1.652×10^{-6}	A/T	0.095	0.021	Upstream of ANKS6
9	rs754222	101,571,153	A	4933	7.491	3.287	17.07	1.652×10^{-6}	A/G	0.095	0.021	GALNT12
9	rs337583	101,500,689	A	4933	7.456	3.272	16.99	1.747×10^{-6}	A/C	0.095	0.021	ANKS6
9	rs337585	101,498,539	A	4933	7.456	3.272	16.99	1.747×10^{-6}	A/T	0.095	0.021	ANKS6
9	rs337586	101,498,021	T	4933	7.456	3.272	16.99	1.747×10^{-6}	T/C	0.095	0.021	ANKS6
9	rs337587	101,497,796	C	4933	7.456	3.272	16.99	1.747×10^{-6}	C/T	0.095	0.021	ANKS6
9	rs337588	101,497,501	T	4933	7.456	3.272	16.99	1.747×10^{-6}	T/C	0.095	0.021	ANKS6
9	rs371521	101,506,317	T	4933	7.456	3.272	16.99	1.747×10^{-6}	T/C	0.095	0.021	ANKS6
9	rs439347	101,496,346	A	4933	7.456	3.272	16.99	1.747×10^{-6}	A/G	0.095	0.021	ANKS6

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.

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), astrocytes cell line (E125), muscle-derived tissue (E089-090,100,107-108), and primary cultures of human skeletal muscle (E120-E121).

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.

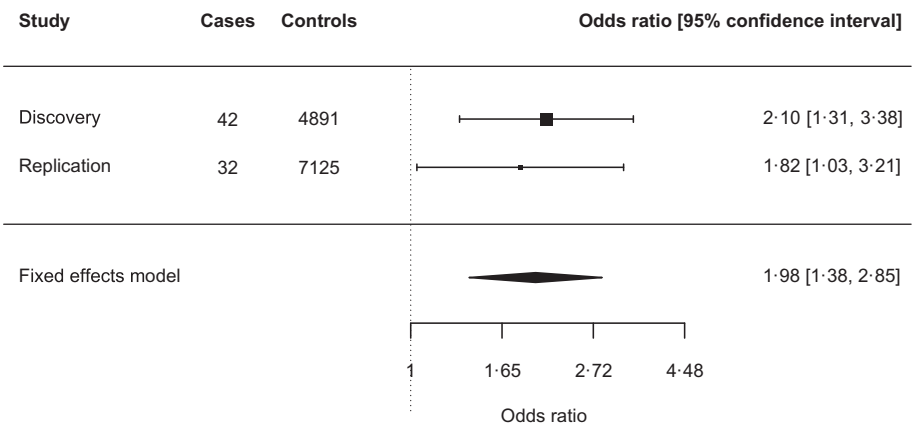

Fig. 3. Meta-analysis of T-cell receptor alpha joining (*TRAJ*). Results for the *TRAJ* variant rs1154155 from the discovery and replication cohorts, and meta-analysis using a fixed effects model.

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], $p = 1.2 \times 10^{-17}$, followed by *HLA-DQB1*06:02*, OR 7.1 [95% CI 4.5, 11.2], $p = 1.9 \times 10^{-17}$ (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 carriership 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 I 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

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 ($r^2 = 0.96$) [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 α-chain 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 DQαβ 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 susceptibility to Pandemrix-induced narcolepsy in certain individuals.

Conflict of interest

None of the others declare any conflicts of interest.

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