

Narcolepsy risk loci are enriched in immune cells and suggest autoimmune modulation of the T cell receptor repertoire

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Abstract

Type 1 narcolepsy (T1N) is a neurological condition, in which the death of hypocretin-producing neurons in the lateral hypothalamus leads to excessive daytime sleepiness and symptoms of abnormal Rapid Eye Movement (REM) sleep. Known triggers for narcolepsy are influenza-A infection and associated immunization during the 2009 H1N1 influenza pandemic. Here, we genotyped all remaining consented narcolepsy cases worldwide and assembled this with the existing genotyped individuals. We used this multi-ethnic sample in genome wide association study (GWAS) to dissect disease mechanisms and interactions with environmental triggers (5,339 cases and 20,518 controls). Overall, we found significant associations with HLA (2 GWA significant subloci) and 11 other loci. Six of these other loci have been previously reported (*TRA*, *TRB*, *CTSH*, *IFNAR1*, *ZNF365* and *P2RY11*) and five are new (*PRF1*, *CD207*, *SIRPG*, *IL27* and *ZFAND2A*). Strikingly, in vaccination-related cases GWA significant effects were found in *HLA*, *TRA*, and in a novel variant near *SIRPBI*. Furthermore, *IFNAR1* associated polymorphisms regulated dendritic cell response to influenza-A infection in vitro (p-value = 1.92×10^{-25}). A partitioned heritability analysis indicated specific enrichment of functional elements active in cytotoxic and helper T cells. Furthermore, functional analysis showed the genetic variants in *TRA* and *TRB* loci act as remarkable strong chain usage QTLs for *TRAJ**24 (p-value = 0.0017), *TRAJ**28 (p-value = 1.36×10^{-10}) and *TRBV**4-2 (p-value = 3.71×10^{-117}). This was further validated in TCR sequencing of 60 narcolepsy cases and 60 DQB1*06:02 positive controls, where chain usage effects were further accentuated. Together these findings show that the autoimmune component in narcolepsy is defined by antigen presentation, mediated through specific T cell receptor chains, and modulated by influenza-A as a critical trigger.

1 Main Text

2 Type 1 narcolepsy (T1N) is a sleep disorder that affects 1/3,000 individuals across ethnic
3 groups¹⁻³. Onset is typically in childhood through early adulthood. Symptoms are caused by the
4 destruction of hypocretin/orexin neurons, a small neuronal subpopulation of the hypothalamus⁴.
5 Although the disease is considered autoimmune, the exact mechanism leading to hypocretin cell
6 death is still unclear. Indeed, T1N is strongly associated with alleles encoding the heterodimer
7 DQ0602 haplotype (HLA-DQA1*01:02~DQB1*06:02, 97% vs. 25%) across ethnic groups^{5,6}.
8 Other loci previously associated with the disease include T cell receptor (TCR) loci alpha (*TRA*)
9 and beta (*TRB*), receptors of HLA-peptide presentations, and other autoimmune associated
10 genes (*CTSH*, *P2RY11*, *ZNF365*, *IFNAR1* and *TNFSF4*)⁷⁻¹⁰.

11
12 Triggers of T1N point to the immune system, including influenza and Streptococcus Pyogenes
13 infections^{9,11,12}, as well as immunization with Pandemrix®, an influenza-A vaccine developed
14 specifically against the H1N1 “swine flu” strain¹³⁻²⁰ suggest a strong environmental modifier of
15 disease risk for narcolepsy. Increased T1N incidence following the Pandemrix® vaccination was
16 first seen in Northern Europe¹³⁻²⁰ with 8-fold increase in incidence in (0.79/100,000 to
17 6.3/100,000) in children. The specificity was striking, as increased T1N was later detected in all
18 countries where Pandemrix® was used, whereas countries using other pH1N1 vaccine brands
19 did not detect vaccination-associated increases in incidence¹³⁻²².

20
21 Despite the genetic and epidemiological evidence for T1N being an immune-system mediated
22 disease, only a few genetic risk factors have been found or characterized so far. Furthermore, the
23 functional consequence of these variants has remained unstudied. Therefore, we examine and
24 characterize genetic factors for T1N across multiple ethnic groups in a sample three times larger
25 than earlier studies finding novel mechanisms how these variants affect RNA expression and T
26 cell receptor chain usage. Our novel findings show that the autoimmune component in narcolepsy
27 is defined by antigen presentation, mediated through specific T cell receptor chains, and
28 modulated by influenza-A as a critical trigger.

1

2 **Results**

3 **GWAS discovers five novel risk loci for narcolepsy.** To discover novel narcolepsy loci, we first
 4 meta-analyzed a large multiethnic cohort of 5,339 T1N cases and 20,518 controls consisting of
 5 samples from nine independent cohorts across three ethnic groups. In addition to the strongest
 6 associations in the HLA locus (minimum p-value < 10^{-216}), we discovered additional 228 genome-
 7 wide significant SNPs with no evidence of genomic inflation²³ ($\lambda=1.06$) (meta-analysis p-value <
 8 5×10^{-8} ; **Fig. 1**). These results confirmed six out of eight previously identified loci (*TRA*, *TRB*,
 9 *CTSH*, *IFNAR1*, *ZNF365* and *P2RY11*), and identified five novel loci near *CD207*, *SIRPG*, *IL27*,
 10 *ZFAND2A* and *PRF1* (**Fig. 1**, **Table 1**, **Supplementary Figs. 1-2**). Further fine-mapping
 11 suggested more than one signal in *TRB*, *ZNF365*, *TRA*, *SIRPG* and *IFNAR1* loci (Supplementary
 12 information). Furthermore, a GCTA gene based test²⁴ showed association with three known
 13 autoimmune or inflammatory disease genes with *GPR25*^{25,26}, *C1ORF106*²⁷ and *PD-1*^{28,29},
 14 suggesting that additional variants remain to be discovered using larger sample sizes (see
 15 **Supplementary Tables 1-3**) doubling the number of variants in T1N.

16

17 Next we examined the genetic architecture of T1N by calculating the narrow sense heritability
 18 explained by the typed variants. GCTA estimated the observed scale heritability to be $h^2_{\text{SNP}}[\text{ci}]$
 19 $=0.403$ [0.015]³⁰ and the population heritability to be $h^2_{\text{SNP}}[\text{ci}] = 0.231$ [0.0088] assuming a
 20 prevalence estimate of 0.03%^{1,2}. One third of observed heritability was mediated by genetic
 21 variation within the extended MHC region and similar to other pediatric autoimmune diseases³¹.

22 **Narcolepsy shares variants with autoimmune diseases.** We next examined genome-wide
 23 shared genetic correlation with other traits excluding variants at the extended HLA locus.³² Note
 24 that we performed this analysis using samples of Whites as reflecting the genetic makeup of the
 25 population for which public data is available. The strongest correlations were seen between T1N
 26 and autoimmune diseases (Wilcox signed rank p-value = 0.031). Of all autoimmune traits

1 examined using LD Score Regression³³, the shared heritability was largest with type-1 diabetes
2 (T1D) ($r_g=0.3261$ (se=0.1015), p-value = 0.0013).

3

4 We next examined whether genome wide significant T1N associations are shared with other
5 autoimmune diseases, suggesting shared mechanisms at single loci. Significant associations in
6 T1N were compared with autoimmune disease associations using published studies and GWAS
7 central³⁷⁻³⁹. Most notably, co-localization of signals using coloc analysis⁴⁰ was found at *IL27*
8 between T1N and both ankylosing spondylitis (posterior probability [pp] = 0.96) and Crohn's
9 disease (pp=0.93).

10

11 We also discovered strong overlap between T1N and T1D at *CTSH* pp=0.998 and *SIRPG*
12 pp=0.999, as well as evidence for partial sharing at *IL27* pp=0.71, while signals were independent
13 for *P2RY11* (pp=0.02). T1D is also the only autoimmune trait besides narcolepsy where any
14 association was seen near the TRA locus, although the T1D signal (rs7145202, beta = 0.1, p-
15 value = 4×10^{-6})⁴¹ is independent from the narcolepsy signal ($r^2 < 0.5$) and located ~100 kb
16 upstream of the TRA loci per se. While previous studies have shown either a small increase or no
17 increased risk for autoimmune diseases in T1N patients,³⁴⁻³⁶ we found statistical evidence of
18 global genetic correlation between T1N and other autoimmune diseases and co-localization of
19 individual associations.

20

21 **Genetics of vaccination-triggered narcolepsy.** We have previously shown that both influenza
22 infections and, in rare cases, immunization with Pandemrix® can trigger narcolepsy^{13,18,19,42,43}.

23 The baseline for narcolepsy in unvaccinated vs. Pandemrix® vaccinated individuals was
24 0.7/100,000 vs. 9/100,000 person years with on average 10-fold increase in risk^{13,18,19,42-44}. We
25 therefore recruited Pandemrix® vaccination-related narcolepsy cases in five countries and
26 examined the genetic load for narcolepsy (**Table 2**). All Pandemrix® vaccination cases were
27 carriers also for HLA-DQB1*06:02. Weighted genetic risk score (GRS) excluding HLA showed a
28 strong association in Pandemrix® vaccination related narcolepsy in each sub cohort (p<0.01 for

1 all cohorts) and with combined vaccination related narcolepsy sample (p-value = 7.96×10^{-10}).
2 (Table 2, and Supplementary Table 8 and Supplementary Figs. 3-4).

3
4 Similarly to GRS evidenced shared signal, we found GWA significant signal with HLA-
5 DQB1*06:02, *TRA* rs1154155 and a variant between *SIRPB1-SIRPG* locus (rs76958425, OR=
6 2.49 [1.82 - 3.41], p-value = 1.12×10^{-8} , Table 2) not present in regular cases (rs76958425, p-
7 value=0.15, beta = -0.0694, OR=0.93). The overall association of GRS and two shared loci
8 indicate that vaccination related narcolepsy is fundamentally the same disorder as idiopathic T1N.

9
10 **Functional analyses highlight effects on immune cells.** Analysis using GARFIELD⁴⁵ showed
11 the variants with p-value<0.00001 have a 5.9-fold enrichment for missense variants and 5.3 fold
12 enrichment for 5'UTRs (**Fig.2, Supplementary Figs 5-7**). Further, many associated variants in
13 Table 1 are in tight linkage with non-synonymous substitutions in the corresponding genes, such
14 as variants in *CTSH* (rs2289702 G11R), *TRA* (rs1483979, F8L), *PRF1* (rs35947132, A91V),
15 *SIRPG* (rs6043409, V263A), *CD207* (rs13383830, N288D and rs57302492, K313I, $r^2 = 1$) and
16 *IL27* (rs181206 L119P) as well as variants marking different HLA-alleles.

17
18 We confirmed that variants within *CTSH* are also important in the predisposition of T1N. Among
19 immune cells, *CTSH* is only expressed in Class II positive antigen presenting cells (B cells,
20 dendritic cells and monocytes), and is known to process antigen for HLA presentation, thus
21 furthering a role for HLA-DQ presentation in T1N. Of note, we also observed a sub threshold
22 association with another cathepsin gene, *CTSC* (rs3888798, C allele frequency =0.06), OR =
23 1.276 [1.169-1.394] p-value = 5.8×10^{-8}), which was not associated with vaccination related
24 narcolepsy (rs3888798, OR=0.76, p-value= 0.336).

25
26 In *PRF1*, the leading variant rs35947132 causes an amino acid change A91V that acts as a
27 hypomorph and disrupts cytotoxicity of the immunological HLA class I synapse^{46,47}. This
28 relatively rare variant (allele frequency 0.03 in Whites) has been shown to prevent perforin, a

1 protein expressed only by natural killer (NK) and cytotoxic (CD8⁺) T cells, to form functional
2 complexes, thus preventing cytotoxic cells from destroying target cells^{46,48}. These findings
3 indicate direct involvement of cytotoxic T cells, most likely CD8⁺ T cells, in hypocretin cell
4 destruction.

5
6 In addition, we discovered associations is in signal-regulatory protein gamma *SIRPG* (rs6110697,
7 V263A) a receptor-type transmembrane glycoprotein known to interact with *CD47*, an anti-
8 autophagy signal for the immune system that has shown success in cancer immunotherapy⁵⁹.
9 Although V263 is conserved in all SIRP family members, it is also located within an alternate
10 exon. Unlike other members of the SIRP family, *SIRPG* is almost exclusively expressed in CD4⁺
11 and CD8⁺ T cells. Furthermore, the SNP is also a strong eQTL in thymus and whole blood⁶⁰.
12 Interestingly, vaccination-associated cases displayed an additional GWAS significant association
13 with rs76958425, a strong QTL for *SIRPB1*, another SIRP family member known to interact with
14 *CD47*. This association is not present in the overall narcolepsy sample (rs76958425, beta = -
15 0.0694138, OR=0.93, p=0.15). *SIRPB1* is mostly expressed in antigen presenting cells and has
16 been shown to modulate neuronal killing in Alzheimer's disease⁶¹, suggesting it could also be
17 important for hypocretin-cell survival, though it may play a role in the modulation of T cell
18 population survival.

19
20 One of the strongest novel factors associated with narcolepsy is rs2409487 in the *IFNAR1* gene,
21 a gene mediating interferon α/β inhibition of virus replication type 1 interferon response
22 associated with T1N. We observed that this SNP is a strong eQTL for *IFNAR1* expression in
23 various tissues in GTEx⁶⁸. In addition, a different lead variant (e.g. rs2284553) has been
24 associated with other autoimmune diseases. *IFNAR1* controls dendritic cell responses to viral
25 infections, notably influenza A⁶⁹. We therefore examined *IFNAR1* expression in DC following
26 H1N1 infection (PR8 delta NS1) finding that our predisposing SNP (rs2409487) is a major eQTL
27 for this effect (p-value = 1.92×10^{-25} , beta =0.140), and in perfect LD with the leading variant for the
28 signal (rs6517159, D'=1, r²=0.995, coloc pp = 0.964 **Supplementary Fig. 8**). The findings

suggest that rs2409487 in *INFAR1* mediates predisposition to T1N by modulating response to Influenza-A infection.

Overlap of risk with cell-type specific chromatin regions. We examined whether associations with narcolepsy were enriched genome-wide on specific enhancer elements using stratified LD score regression on Epigenome Roadmap cell type specific annotations (n=216 cell types)⁷¹. Partitioned heritability by functional categories enriched in the hematopoietic cell lines (**Supplementary Fig. 2b and 2c, Supplementary Fig 8.**). Consistent with our model, association was driven by CD4⁺ T cells, with leading effects in CD3+ primary H3K27ac, CD4+/CD25-/IL17- PMA&ionomycin stimulated primary H3K4me1, and CD4+/CD25- primary H3K4me1 (each enriched over 35-fold in predicted heritability per SNP). Additional effects were seen in Th17 CD4⁺ T cells and CD8⁺ T cells, confirming the importance of these cell types in narcolepsy. Importantly, no enrichment was seen in neuronal cell types. While immune cells have been suggested to play a role in the predisposition to T1N⁷², these novel findings show that the effects are specific to both helper and cytotoxic T cells, and that individual variants genome-wide are substantially enriched in specific T cell lineages predisposing to T1N.

Risk variants in T cell receptor loci modulate $\alpha\beta$ T cell receptor repertoire. T1N is the only autoimmune disease with significant association in HLA and T cell receptor (TCR) loci (TRA and TRB). TCR molecules are formed through VDJ somatic recombination at the genomic level, a process that allows for substantial TCR sequence diversity. The recombinant T cell clones are later subjected to negative and positive selection in the thymus in order to optimize pathogen responses while avoiding auto-reactivity. As a consequence, most of TCR binding diversity is ensured by selection in the context of specific HLA molecules. TCR α and β chains heterodimerize to form biologically functional molecules that recognize peptides presented by the Major histocompatibility complex (MHC) encoded by the highly variable classical HLA genes. On one hand, T1N is associated with the DQB1*06:02 allele of the MHC class II β subunit and the highly linked DQA1*01:02 allele of MHC class II α subunit. On the other hand, T1N is strongly

associated with TCR α and β chains. Notably, this association is also seen in cases with vaccination-triggered narcolepsy (**Table 2**). This suggests that T1N is directly linked with autoimmunity that is mediated by T-cell activation.

Clearly the strong association of T1N with the HLA locus will affect the presented epitope and the TCR repertoire⁷³, however how does the association with TRA and TRB affect the TCR repertoire? In the TRB region, association peaks over 32 SNPs (from hg19 chr7:142025523-142248636) over a 22kb segment. The association signal within TRA locus spans over several J genes over 18 kb, with 5 SNPs (rs1154155, rs1483979, rs3764159, rs3764160) in perfect LD across ethnic groups. Among TRA SNPs, a SNP changing F8L in the peptide recognizing groove of CDR3 region of TRA J24 is an obvious candidate defining two J24 alleles we denote as J24*01 and J24*02 respectively. We next examined the effects of these SNPs on T-cell receptor V or J gene chain usage using RNA sequencing in 895 individuals⁷³. Strikingly, rs1154155 with TRA J28 expression in total RNA sequencing from blood (p -value= 1.36×10^{-10} , $\beta = -0.212$, **Fig. 3**) with the same lead variants that associated with narcolepsy and posterior probability for shared variant was $pp=0.958$ suggesting that rs1154155 in T1N predisposition mediates its action through effects on TRA J28 repertoire (See supplementary **Table S5** for all rs1154155 effects). J24 usage is also among the top associations for rs1154155 effects, although in this case correlation is opposite and the associated SNP increases usage (p -value=0.0017, $\beta=0.104$, $pp=0.54$). Associations with multiple target variants within the same haplotype have been defined with complex traits with both regulatory and non-coding effects before and are likely to have a role in T1N predisposition⁷⁴.

To further investigate the mechanism of the TRA variants specifically on CD4⁺ T cells, which are the most likely causal cell type because of their interactions with DQB1*0602, we performed T cell receptor sequencing of CD4⁺ memory T cells in 40 individuals with T1N and 61 DQ0602 matched controls (**Fig. 3**). Although we found no significantly over-represented T cell clones, we discovered a similar effect of rs1154155 on J28 usage in CD4⁺ in T1N and healthy controls (β

1 = -0.32, p-value<0.001, **Fig. 3**). Furthermore, the effect was stronger in individuals with T1N that
 2 had significantly lower expression level of TRA J28 than healthy controls (beta = - 0.20, p-value =
 3 0.027). Similarly, the effect of rs1154155 on J24 usage was also similar population cohort (beta =
 4 0.33, p-value<0.001). We also confirmed that these effects were *cis* mediated, and the ratio of
 5 J24*01 (F) over J24:02(L) was only 0.4 in heterozygotes, indicating lower allele specific
 6 expression with F-narcolepsy associated alleles, with similar effects in other T cell subpopulations
 7 (**Supplementary Fig. 10**). The findings suggest that the predisposition to T1N is mediated either
 8 by decreasing usage of TRA J28, or by increasing TCR recognition through J24*01, although in
 9 this case the effect would be mitigated by decreased expression of this allele.

10
 11 Within the TRB region, rs1108955 was the leading variant for TRBV4-2, TRBV3-1 and TRBV2
 12 expression (Supplementary Table 6). While it has been observed that individual variants can
 13 affect multiple target genes ⁷⁵, the strongest evidence was seen with TRBV4-2. The leading T1N
 14 variant was in perfect LD with the lead variant for TRBV4-2 expression, and the association of
 15 same variants for eQTLs in TRB expression for TRBV4-2, TRBV3-1 and TRBV2 pp>0.95 with
 16 strongest evidence for TRBV4-2 usage pp=0.99 (**Supplementary Fig. 11**).

17
 18 We finally examined whether usage of specific TRAJ, TRAV, TRBJ or TRBV genes in CD4⁺ T
 19 cells was associated with seasonal influenza vaccination (12 cases versus 5 cases) or with
 20 narcolepsy case/control status (59 narcolepsy cases versus 47 DQ0602 controls). Unique T cell
 21 receptor gene usage was not associated with influenza vaccination (**Appendix 1, Table 1-16**).
 22 However, we did see a statistically significant difference between narcolepsy and controls with
 23 *TRBJ1-3*01* usage (p=0.0012, beta=0.00425). Similarly, although TRAJ28 was the second most
 24 significantly associating clone between narcolepsy and control with both protective and
 25 predisposing clones the association was not statistically significant (p<0.0001, corrected p=1,
 26 Appendix 1. Table 23 and Table 24). These findings are in line with usage effects seen with
 27 narcolepsy risk variants.

To summarize, the finding that specific TRA and TRB variants associate with narcolepsy suggests specificity for the autoimmune pathology through the T cell receptors. The co-localization of signal at the population sample with expression suggests a direct effect on the specific usage of TRAJ28 expression coding effect on TRAJ24 (F8L) variation as well as TRBV4-2 gene expression. This was also seen specifically in T cell receptor sequencing in CD4+ T cells and is stronger in patients ($p < 0.05$) suggesting for direct causal effect for disease pathophysiology through expression and autoantigen recognition.

Multi-loci association of narcolepsy within the HLA region. The strongest association in narcolepsy is within the HLA locus. Strikingly, T1N is one of the few diseases where nearly all affected individuals carry at least one copy of exactly the same HLA allele, DQB1*06:02^{5,6}. To fine map this association, we imputed HLA haplotypes using HIBAG⁷⁶ and HLA IMP:02⁷⁷. We then performed ethnic specific HLA association and combined them using fixed effects meta-analysis. As expected^{5,6}, the strongest association was with the *DQA1*01:02~DQB1*06:02* (DQ0602) haplotype.

To look for additional independent signal, we performed conditional analysis using stepwise forward regression. We detected (1) a strong protective effect of *DQA1*01:01* and *DQA1*01:03* alleles (OR=0.30, $p\text{-value} < 10^{-15}$ and OR =0.30, $p\text{-value} < 10^{-20}$, respectively) with combined protective OR=0.41, $p\text{-value} < 10^{-40}$; (2) predisposing effects for *DQB1*03:01* and *DQA1*01:02* across ethnic groups as shown before^{5,6,78,79} (OR=1.36, $p\text{-value} < 5 \times 10^{-8}$ and OR=1.68 $p\text{-value} < 5 \times 10^{-8}$, respectively) (**Supplementary table 7**). The protective effects of *DQA1*01:01* and *DQA1*01:03* have been suggested to be mediated via heterodimerization with DQB1*06:02, indirectly reducing *cis* encoded *DQA1*01:02/DQB1*06:02* (DQ0602) heterodimer availability^{5,79}.

Controlling for both *DQB1* and *DQA1* effects, a strong protective association was seen with *DPB1*04:02* allele ($p\text{-value} < 10^{-20}$) whereas smaller predisposing effect was found with *DPB1*05:01* allele, a mostly Asian subtype ($p\text{-value} < 10^{-3}$). Finally, after adjusting for the DQ and

1 DP effects significant associations were seen at HLA class I with *A*11:01*, *B*51:01*, *B*35:01* and
2 *B*35:03* and with *A*03:01* (p-value <0.01, Supplementary table 7). These findings confirm and
3 extend results of two previously publications^{6,81}, with effects of *B*51:01* likely secondary to LD
4 with *A*11:01* in whites.

5

Discussion

In this study, we explored genetic risk for narcolepsy and potential disease mechanisms of identified genetic risk factors. The strongest associations were seen with the HLA region. In addition, we confirmed six previously described risk loci (*TRA*, *TRB*, *CTSH*, *IFNAR1*, *ZNF365* and *P2YR11*) and discovered five novel associations in *PRF1*, *CD207*, *SIRPG*, *IL27* and *ZFAND2A*. Analysis of functional consequences of these loci in a multi-ethnic sample discovered remarkable association with immune loci evidenced by individual associations and partitioned heritability enrichment. A notable example is the effect of both missense and regulatory variants in the *TRA* and *TRB* regions that had a substantial effect on the T cell receptor chain usage. All these findings strongly suggest specific risk factors in genes controlling immune reactions.

Two loci in addition to the HLA region were implicated in vaccination-associated narcolepsy (*TRA*, *SIRPB1*). Findings indicate that although genetic factors predisposing to regular and vaccine-triggered narcolepsy are largely shared, there are slight differences. These findings may reflect a primary role for genetic factors in immune response per se versus infection and immune response in other cases. A detailed analysis of the loci where the leading variants for T1N are located suggests both antigen presentation and recognition. Indeed, the majority of variants have effects in antigen presenting cells (*HLA*, *CTSH*), e.g. dendritic cells (*IFNAR1*, *CD207*), T cells (*TRA*, *TRB*, *P2YR11*, *SIRPG*), e.g. T helper cells (*HLA-DQ*, *HLA-DP*, *IL27*), and cytotoxic T cells (*HLA-A*, *PRF1*), sketching a remarkably narrow disease pathway (**Fig. 4**). Accordingly, a direct effect of *TRA* and *TRB* associations with T cell receptor expression was seen; *TRA* lead variant was an eQTL for *TRAJ28* and *TRAJ24* expression whereas strongest eQTL effect for *TRB* lead variant was seen with *TRBV4-2*. The effect was accentuated in T1N cases, suggesting for the first time that specific T cell receptor chains such as *TRAJ24*, *TRAJ28* and *TRBV4-2* are strong risk factors for narcolepsy and potentially causal factors recognizing and binding the autoantigen. This association is unique to T1N and has not to our knowledge been seen with other autoimmune diseases.

1 In addition, a strong functional connection with Influenza A infection in dendritic cells was found at
2 *IFNAR1*, furthering the role of this virus as a common trigger for the disease. We also discovered
3 associations with *ZNF365* and *ZFAND2A*, ubiquitously expressed transcription factors with, in the
4 case of *ZNF365*, strong known associations with other autoimmune diseases^{82,83}. The
5 *ZFAND2A* association (also called Arsenite-inducible RNA-associated protein AIRAP) is unique
6 to narcolepsy, and was opposite in post vaccination cases, an effect that could suggest
7 differential effects on influenza infection and immune response modulation. The *ZFAND2A*
8 associated SNP, is in perfect linkage disequilibrium ($r^2=1$) with a very large number of SNPs over
9 a 250 kb region that encompasses and regulates many genes. Of possible interest in this region
10 is *GPR146*, a gene highly enriched in unstimulated macrophages and dendritic cells, whose
11 reduced expression is associated with the $INF\gamma$ response and suppresses HCMV replication in
12 infected dendritic cells⁸⁴. We were able to examine for the effects of these variants in post
13 Pandemrix® cases. *TRA* association was particularly strong, suggesting involvement of T cell
14 receptor oligoclonality in autoantigen recognition.

15
16 Based on these observations, we propose that narcolepsy is the result of an autoimmune process
17 triggered primarily by influenza-A on an HLA-DQA1*01:02~DQB1*06:02 (DQ0602) background.
18 The involvement of influenza-A is likely to explain why the genetic associations we found are
19 universal. Indeed, influenza is one of few viruses that act worldwide on a seasonal basis. The
20 universal association is especially clear for DQ0602 as it is found with different HLA-DRB1
21 alleles, DRB1*15:01 in White (Europe and USA) and Asians (China, Korea, Japan and India), but
22 DRB1*15:03 or DRB1*11:01 in Blacks (confusion of ancestral continent of origin and sample
23 location?)^{5,6}. The primacy of DQ0602 over DRB1*15:01 is also demonstrated by the fact
24 DRB1*15:01~DQA1*01:03~DQB1*06:01 haplotype is not associated with narcolepsy in China
25 and by the fact additional DQ effects are mostly mediated by DQA1 alleles that interact in trans
26 with DQB1*06:02. In contrast to narcolepsy, other autoimmune diseases commonly have
27 different HLA associations or disease presentations across countries, and resulting HLA
28 associations are more complex. Type 1 diabetes, for example, is well known to be primarily

1 associated with HLA-DQ in Whites whereas DRB1*04:05 specific effects are evident in Japan
2 where the disease is rare^{83,85,86 77}.

3

4 Other autoimmune diseases, unlike narcolepsy, are also associated with a plethora of
5 autoantibodies and known autoantigen targets. For example Insulin, GAD, IA-2 and ZNT8 are
6 involved in T1D and β -cell antigen targeting, suggest that these other diseases involve multiple B
7 and T cell mechanisms and antigens, likely explaining the weaker and more complex HLA effects
8 and a lack of association with any specific TCR polymorphisms. It is our hypothesis that the
9 strong effects of TCR polymorphisms in narcolepsy likely represent the fact autoimmunity in this
10 disease is oligoclonal and limited to one or a few hypocretin cell antigen epitopes. These epitopes
11 may bind DQ0602 specifically and involve a few $\alpha\beta$ TCR receptors containing TRAJ24, TRAJ28 or
12 TRBV4-2 (**Fig 4**). Other groups have suggested involvement of TRIB2, prostaglandins and
13 HCRTR2⁸⁷⁻⁹¹. However, these associations have not been universal. Systematic studies of T-cell
14 reactivity with TCR identification in the context of DQ0602 and flu or autoantigen epitopes are
15 ongoing in various laboratories to address this issue.

16

17 In this study, perforin, a gene of critical importance to NK and CD8⁺ T cell cytotoxicity was
18 strongly protective of narcolepsy, whether or not it was triggered by vaccination. In the context of
19 compound null heterozygotes of the perforin gene, A91V has been associated with late onset
20 hemophagocytic lymphohistiocytosis (HLH) type 2⁴⁹, a recessive disorder associated *PRF1* null
21 alleles. HLH type 2 is characterized by excessive T cell activation that may involve abnormal
22 reactivity to viral pathogens⁵⁰ or decreased CD8⁺ T cytotoxic pruning of dendritic cells⁵¹.
23 Interestingly, *Prf1* knock-out mice do not develop the syndrome unless infected with viruses such
24 as murine lymphocytic chorio-meningitis virus or murine cytomegalovirus, a phenomenon
25 involving CD8⁺ T cells and increased IFN γ ⁵⁰. Other perforin-damaging mutations have also been
26 anecdotally associated with susceptibility to multiple sclerosis⁵² and T1D⁵³. Importantly, the allele
27 associated with narcolepsy impairs cytotoxicity and cell killing, suggesting that the effect of the
28 variant on cytotoxicity may be targeting hypocretin cells directly.

1

2 Although it is conceivable NK cells could be involved, the most likely explanation is involvement
3 of CD8⁺ T cell in hypocretin cell killing in collaboration with CD4⁺ T cells or microglia. This was
4 also supported by CTSC association, an enzyme of critical importance to cytotoxic CD8⁺
5 activation of pro-granzymes⁵⁸. Bernard-Valnet et al.⁹² used transgenic mice with expression of a
6 neoantigen in hypocretin neurons, and found that infusion of CD8⁺ T cell targeting the neoantigen
7 were able to cause hypocretin cell destruction while infusion of neoantigen-specific CD4⁺ T cell
8 alone was insufficient, although CD4⁺ T cells migrated closely to the target neurons. These earlier
9 experiments together with genetic association with PRF1 variants suggest a direct role of CD8⁺ T
10 cells in hypocretin cell destruction. CD8⁺ mediation of cell killing has also been suggested by
11 observation of a CD8 T cell infiltrate in a paraneoplastic anti-Ma2 encephalitis case with
12 symptomatic hypocretin cell destruction⁹³.

13

14 In summary, although the culprit autoantigen has not been identified, genetic data indicate
15 autoimmunity in T1N with strongest genetic overlap with T1D, another organ-specific autoimmune
16 disease suggesting shared pathophysiology. A particularity of the disease is involvement of
17 polymorphisms such as in IFNAR1 that regulate response to influenza-A infection, a result that
18 complement epidemiological studies indicating seasonality of disease onset⁴² and increased
19 incidence that has occurred following vaccination with Pandemrix® in Europe^{13,18,19}. Other
20 genetic factors implicate dendritic processing of antigens, presentation by DQ0602 to CD4⁺ T
21 cells and subsequent cell killing of hypocretin neurons by CD8⁺ cells, with likely involvement of
22 only a few autoantigen epitopes and a restricted number of T-cell receptors. The lack of
23 detectable autoantibodies has made objective demonstration of autoimmunity challenging, but will
24 likely made the eventual discovery of the culprit T cell antigen even more informative to our
25 understanding of T cell immunity in the brain.

26

Methods

Study subjects: 5,339 unrelated individuals with type 1 narcolepsy^{8,9}, and 20,518 ethnicity-matched controls were included in the study. In addition, 245 individuals with vaccination related narcolepsy and 18862 controls were recruited in Finland (N=76 cases and 2796 controls), Sweden (N=39 and 4894 controls), Norway (N=82 cases and 429 controls), and United Kingdom and Ireland (N=48 cases and 10743 controls)^{13,16,94,95}. All cases had documented immunization with Pandemrix®. All cases had narcolepsy with clear-cut cataplexy and were *DQB1*06:02* positive, or had narcolepsy with documented low hypocretin-1 in the cerebrospinal fluid. Informed consent in accordance with governing institutions was obtained from all subjects. The research protocol was approved by IRB Panels on Medical Human Subjects at Stanford University, and by respective IRB panels in each country providing samples for the study.

Genotyping: Subjects were genotyped using Affymetrix Affy 5.0, Affy 6.0⁸, Affymetrix Axiom CHB1⁹, Affymetrix Axiom EUR, Axiom EAS, Axiom LAT, Axiom AFR, Axiom PMRA and Human Core Exome chip platforms. Genotypes were called with Affypipe⁹⁶, Affymetrix genotyping console or Genome Studio. Markers with genotyping quality (call rate < 0.95) or deviation from Hardy-Weinberg equilibrium (p-value<10⁻⁶) were discarded from further analysis. Samples were checked for relatedness with filtering based on proportion of identity-by-descent using cut off >0.2 in PLINK 1.9 PI_HAT score⁸⁸. One pair of related individuals was removed. If related individuals were a case and a control, cases were retained in the analysis. Three first principal components within each cohort were visualized and outliers were removed. **Supplementary Table 1** shows for each cohort N QCed original genotypes, N for those passing the QC and N for individuals removed during QC.

Imputation: We imputed samples by prephasing cases and controls together using SHAPEIT v2.2⁸⁹ and imputed with IMPUTE2 v2.3.2^{97,98} and 1000 genomes phase 1v3 build37 (hg19) in 5Mb chunks across autosomes. For variants having both imputed and genotyped values, the

genotyped values were kept except for those individuals where the genotype was missing. In this case imputed values were kept.

Analysis: Analyses for all data sets were performed at Stanford University except for the Finnish and Swedish vaccination related cases and European Narcolepsy Network samples, which were analyzed by respective study teams using exactly the same analysis. Genome-wide association analysis was first performed in each case control group separately using SNPTEST v.2.5.2⁹⁹. We used linear regression implemented in SNPTEST method score adjusting for ten first principal components in order to adjust for cohort specific population stratification. Standard post imputation quality control was done: Variants with info score <0.7 and minor allele frequency (MAF) <0.01 were removed from the analysis. Signals specific for one genotyping platform only and variants in each locus with heterogeneity p-value<10⁻²⁰ were removed. We used fixed effects model implemented in METAv1.7 with inverse-variance method based on a fixed-effects model for combining the association results¹⁰⁰. In total 12,600,187 markers across the studies were included in the final case control meta-analysis. Significance level for statistically significant association was set to genome-wide significance (p-value<5*10⁻⁸) controlling for multiple testing. Overall test statistics showed no genomic inflation. GCTA was used for heritability and gene based tests¹⁰¹. Coloc analysis was done using coloc package in R version 3.4.2 (2017-09-28)⁴⁰, Manhattan and QQ-plots were created with QQman or FUMA⁹⁷. Shared heritability was estimated using LD score regression³².

Typing and imputation of HLA variants: High resolution HLA imputation in 4-digit resolution (2-field, amino acid level) for HLA A, B, C, DRB1, DQA1, DQB1, DPA1 and DPB1 was performed using HLA*IMP:02 as implemented in Affymetrix HLA or the HIBAG package in R version 3.1.2 (2014-10-31). HIBAG is an HLA imputation tool that uses attribute bootstrap aggregation of several classifiers (SNPs) to select groups of SNPs that predict HLA type and allows the use of own HLA reference panels⁷⁶. Reference HLA types were used from published imputation models and for Asian and Blacks obtained with Sirona sequencing¹⁰² in ethnic specific populations

N=500 Blacks, N=2,000 Whites and N=368 Asians. Imputation accuracy was further verified by Luminex HLA typing in a subset of samples and accuracy was over 95% for all ethnic groups and common alleles with > 5% frequency in population. For all alleles the accuracies were for Whites: 0.98 in HLA-A, 0.97 in HLA-B, 0.98 in HLA-C, 0.96 in HLA-DRB1, 1.00 in HLA-DQA1, 1.00 in HLA-DQB1, 1.00 in HLA-DPA1, and 0.92 in HLA-DPB1 and for Asian for alleles where typing was also available 0.95 for HLA-DRB1, 0.94 for HLA-DQA1, and 0.98 for HLA-DQB1.

Analysis of HLA variants: HLA effects in narcolepsy were analyzed as described before⁶. We examined altogether variation from 23,410 individuals with 9,789 Asians, 13,621 Whites. In each ethnicity HLA alleles were analyzed using additive model under logistic regression adjusting for 10 first population specific principal components to adjust for local population stratification. We identify independent associations using conditional analysis (stepwise forward regression in each cohort). Fixed effects meta-analysis was used to combine associations using Plink 1.9¹⁰³ and R version 3.2.2. We considered alleles sustaining Bonferroni correction for correction of number of alleles with minor allele frequency over 2% (N=110 HLA alleles) significant resulting in Bonferroni cut-off $p=0.00045$.

Analysis of expression quantitative trait loci (eQTL): We used tissue specific summary statistics from the GTEx consortium and from Westra et al. to examine total blood specific effects of associating variants on gene expression^{75,104}. Furthermore, we examined how the genetic variants modulated T cell and antigen presenting (dendritic cell and monocyte) gene expression by RNA sequencing and RNA expression. To examine environment specific triggers for eQTLs we challenged the dendritic cells on influenza-A infection, or stimulated them with interferon or LPS^{105,106}. Finally, we identify short range (cis) SNPs and trans HLA alleles association with TCR V and J usage estimated from total peripheral blood RNA sequencing as described before⁷³.

T cell receptor RNA sequencing in matched narcolepsy case control data set and in population cohorts: We performed RNA sequencing in 895 individuals with total blood RNA sequencing and

1 in T cells from 60 individuals with narcolepsy and 60 healthy individuals from using total CD4+ T
2 cells, CD4+ T memory and CD8+ T cell populations. We used fastqc to infer quality and trimmed
3 low quality reads. We then performed barcode demultiplexing, after which local blast was used to
4 align and extract CDR3s. Linear regression was fit for TRA usage ~ Genotype adjusting for age
5 and gender, RNA sequencing lane and case/control status as covariates. We also analyzed
6 separately coding consequences for each TRAJ24 containing productive CDR3 fragment as one
7 of the most significantly associating SNPs was a coding SNP (rs1483979) was changing an
8 amino acid Leucine to Phenylalanine. These 'LQF' and 'FQF' were extracted and their
9 frequencies were computed. Ratio of FQF/(LQF+FQF) was further computed across all the
10 samples.

11

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Table 1 Genome-wide significant associations observed in T1N across ethnic groups.

Closest Gene	chr	rsid	pos	non-coded allele	coded allele	p-value	coded af	OR [CI lower - upper]	beta	se
CD207 (Langerin)	2	rs13383830	71058306	T	C	2.65E-09	0.078	1.285 [1.184-1.396]	0.251	0.042
ZFAND2	7	rs75674288	1195322	A	C	4.05E-08	0.913	0.778 [0.711-0.851]	-0.251	0.046
TRB	7	rs1008599	142038782	A	G	6.63E-12	0.332	0.813 [0.767-0.862]	-0.207	0.03
ZNF365	10	rs4237304	64407845	C	T	8.40E-10	0.824	1.233 [1.154-1.319]	0.21	0.034
PRF1	10	rs35947132	72360387	G	A	1.40E-09	0.04	0.570 [0.475-0.684]	-0.562	0.093
TRA	14	rs1154155	23002684	T	G	1.48E-73	0.255	1.643 [1.559-1.733]	0.497	0.027
CTSH	15	rs34593439	79234957	G	A	1.44E-08	0.09	1.246 [1.154-1.345]	0.22	0.039
IL27	16	rs200840505	28539396	GTGTGTA	G	4.70E-08	0.281	0.849 [0.801-0.901]	-0.163	0.03
P2YR11	19	rs34849604	10229098	T	TG	4.26E-09	0.537	1.232 [1.148-1.323]	0.209	0.036
SIRPG	20	rs6110697	1615661	T	C	1.83E-10	0.74	1.206 [1.138-1.28]	0.188	0.03
IFNAR1	21	rs2409487	34684958	C	T	1.23E-15	0.754	1.214 [1.158-1.273]	0.194	0.024

Leading SNP of loci associated with T1N at a genome wide significant level ($p\text{-value} < 5 \times 10^{-8}$). Heterogeneity p-value is calculated between the nine cohorts in this study. Altogether 228 variants were significantly associated with T1N. Associations tested using SNPtest, and META with fixed effects test statistics are shown^{99,107}. Positions are shown for genome build human genome build 37 (GRCh37/hg19).

1 **Table 2 |** Locus specific (from Table 1) and Genome-Wide significant associations observed in vaccination-triggered T1N cases.

Closest Gene	chr	rsid	pos	non-coded allele	coded allele	p-value	OR	beta	se	p-value heterogeneity
CD207 (Langerin)	2	rs13383830	71058306	T	C	0.757	1.106 [0.584 - 2.097]	0.101	0.326	0.421
ZFAND2	7	rs75674288	1195322	A	C	4.92E-04	3.189 [1.66- 6.122]	1.160	0.333	0.673
TRB	7	rs1008599	142038782	A	G	0.099	0.798 [0.61 - 1.043]	-0.226	0.137	0.822
ZNF365	10	rs4237304	64407845	C	T	0.410	1.116 [0.85 - 1.450]	0.110	0.133	0.948
PRF1	10	rs35947132	72360387	G	A	4.48E-04	0 [0 - inf]	-10.879	50.123	1*
TRA	14	rs1154155	23002684	T	G	1.58E-13	2.531 [1.978 - 3.239]	0.929	0.126	0.365
CTSH	15	rs34593439	79234957	G	A	0.074	1.418 [0.967 - 2.079]	0.349	0.195	0.614
IL27	16	rs200840505	28539396	GTGTGTA	G	0.318	0.834 [0.583 - 1.191]	-0.182	0.182	1**
P2YR11	19	rs34849604	10229098	T	TG	0.024	1.515 [1.057 - 2.171]	0.415	0.184	1**
SIRPG	20	rs6110697	1615661	T	C	0.050	1.336 [1.00- 1.785]	0.290	0.148	0.737
IFNAR1	21	rs2409487	34684958	C	T	0.720	1.077 [0.719 - 1.614]	0.074	0.206	0.964
SIRPB1- SIRPG	20	rs76958425	1602668	C	T	1.12E-08	2.491 [1.821 - 3.408]	0.913	0.16	0.078

2

3 Association with vaccination related narcolepsy is shown for loci having genome wide significant association with T1N or those loci being

4 genome-wide significant with vaccination related narcolepsy. Associations tested using SNPtest or Chisq test (Irish) with meta-analysis

5 using META with fixed effects test statistics are shown^{99,107}. Positions are shown for genome build human genome build 37

- 1 (GRCh37/hg19). * SNP imputed in Finnish cohort only ** SNP imputed in Norwegian cohort only. For cohort specific association see
- 2 Supplementary Table 8.

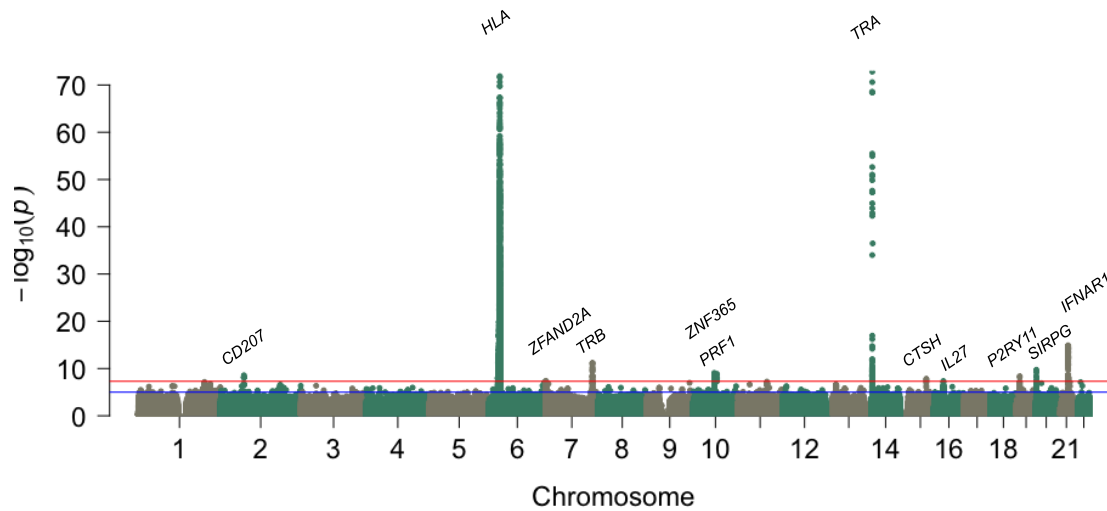


Fig. 1. Multi ethnic genetic analysis of type 1 narcolepsy. Multi-ethnic analysis conducted in 5,339 cases and 20,518 controls reveals genome-wide significant associations in 11 loci plus HLA. The x-axis shows genomic location by chromosome and the y-axis shows $-\log_{10}$ p-values. Red horizontal line indicates genome-wide significant p-value threshold of 5×10^{-8} . P-values smaller than 10^{-75} were set to 10^{-75} (HLA locus has many SNPs with p-value $< 10^{-216}$).

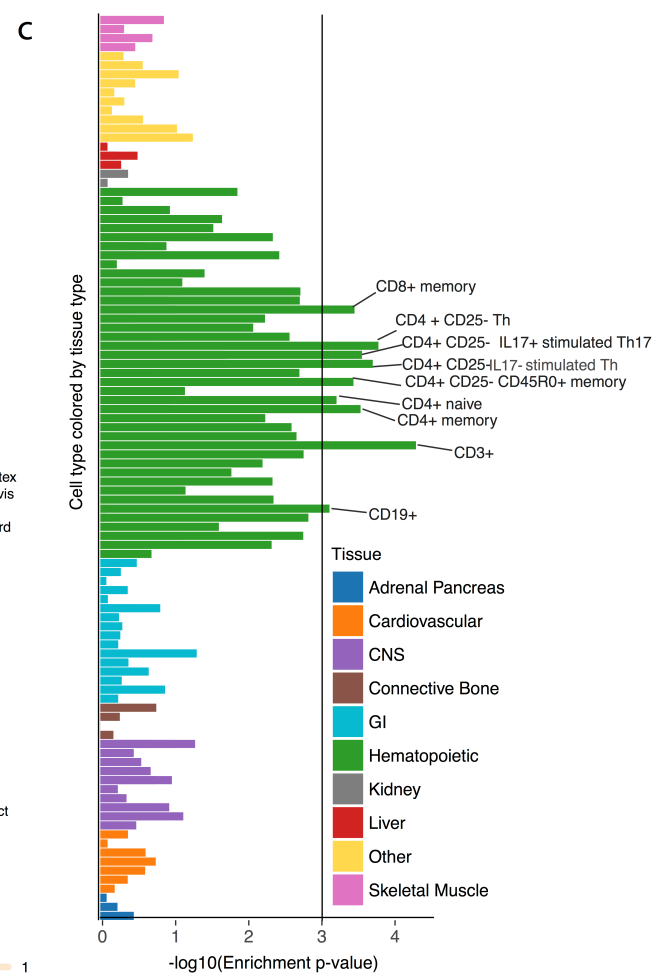
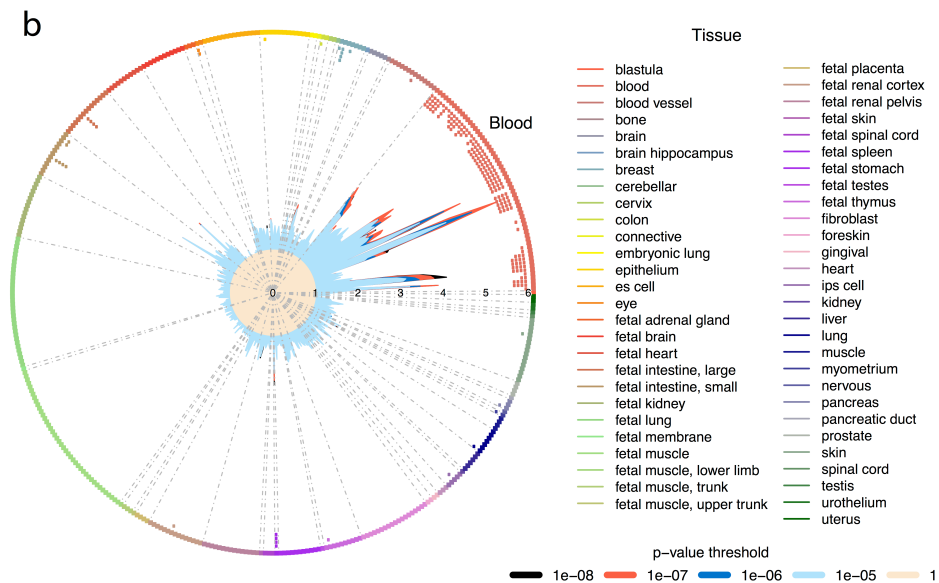
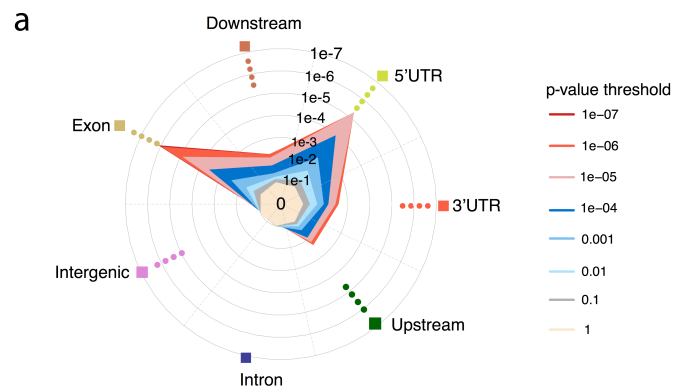


Fig. 2 Narcolepsy risk variants are enriched in immune cells and for missense variants.

a) GARFIELD analysis of narcolepsy associated variants shows a 6 fold enrichment for exon variants and a 5.2 fold enrichment in 5'UTRs located variants. b) overall enrichment in DNA hypersensitivity regions is seen specifically in circulating hematopoietic (blood) cells c) Epigenome roadmap data shows that the majority of narcolepsy heritability is enriched in hematopoietic cell lineages, with changes most pronounced in immune cells notably T helper and cytotoxic cells. Statistically significant enrichment is marked with a line corresponding to an Benjamin Hochberg enrichment p-value = 0.001.

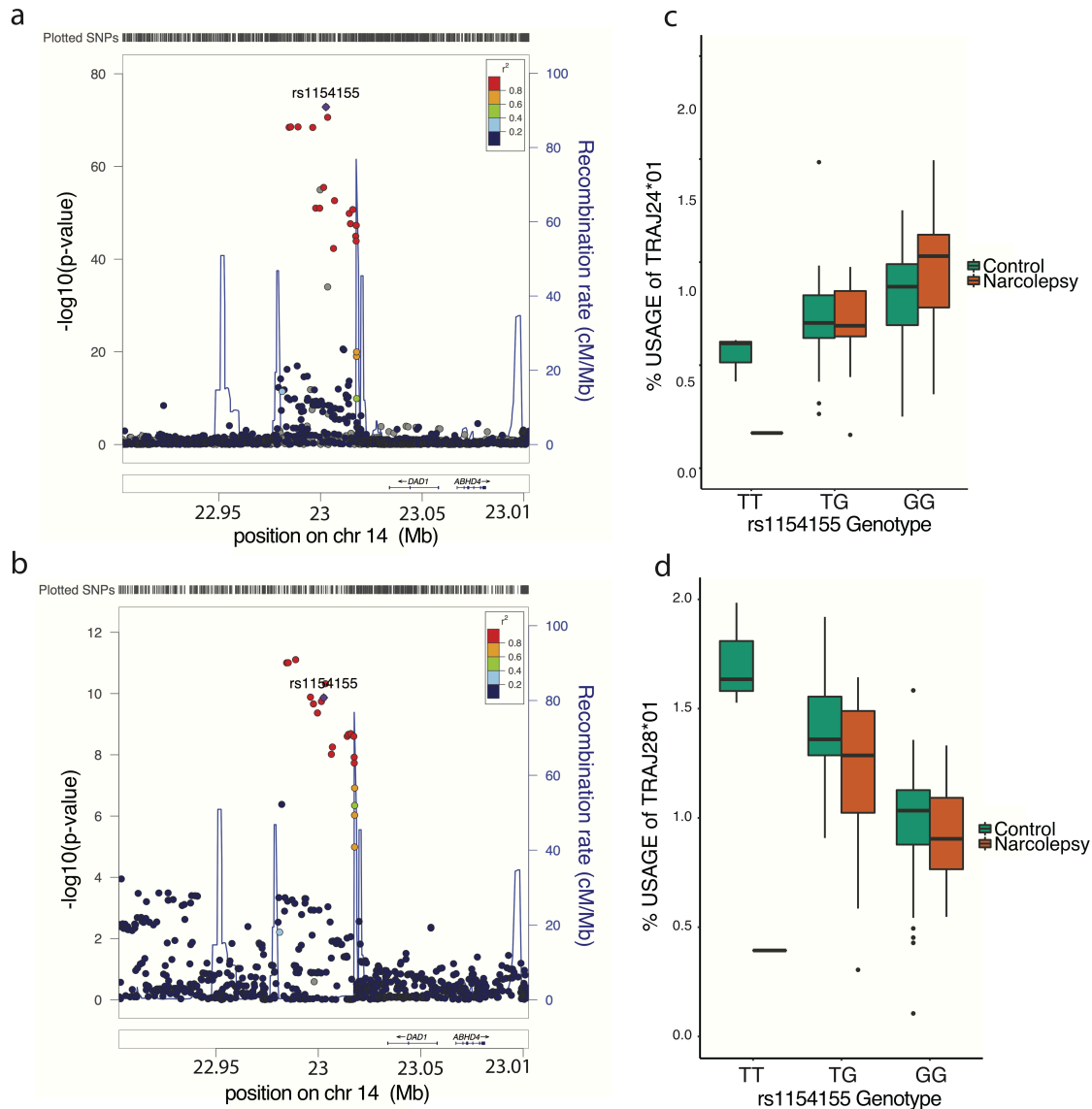


Fig. 3. TRA lead variant rs1154155 is associated with repertoire usage of TRAJ24 and

TRAJ28 genes. (a) T1N association with TRA. T1N association with T cell receptor alpha chain

locus spans a region that contains 5 SNPs with almost perfect LD (rs1154155, rs1483979,

rs3764159, rs3764160) and high LD over 18kb. **(b)** Usage of TRAJ28*01 in 895 individuals

shows similar association with T1N lead variant rs1154155 with posterior probability of 0.958

between narcolepsy and TRAJ28 usage. T cell receptor sequencing in CD4⁺ T memory cells in

60 type-1 narcolepsy patients and matched controls confirmed the effect of rs1154155 on usage

1 of both (c) TRAJ24*01 and (d) TRAJ28*01 with higher effect seen in the type-1 narcolepsy
2 cases.

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Peripheral response

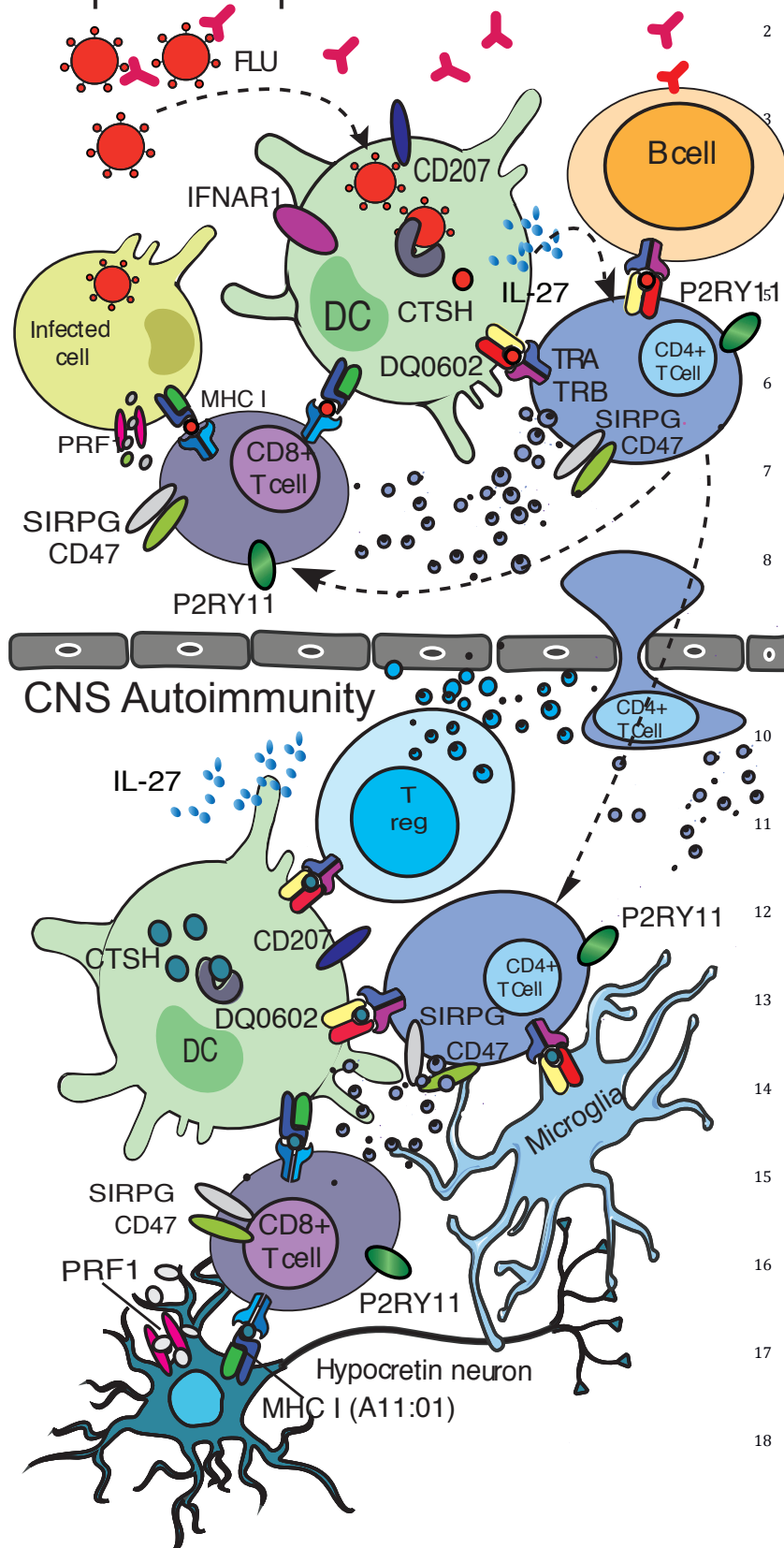


Fig. 4. Postulated disease mechanisms in autoimmune narcolepsy. 1) Peripheral response:

Influenza virions or vaccine protein debris are ingested by DCs facilitated by CD207; flu proteins are processed by cathepsins CTSH and CTSC for presentation by HLA molecules to specific TCR α/β bearing CD4⁺cells, initiating an immunological synapse and responses to influenza. Presentation by DC is modulated by IFNAR1 in the context of influenza infection. Cross presentation of influenza antigens processed via the MHC class I pathway in DCs is necessary to activate CD8⁺ cells that mature into cytotoxic lymphocytes (CTLs), initiating cell killing of viron infected cells. Activated CD4⁺ cells produce cytokines such as IFN γ , IL-2 and IL27 which augment cytotoxic activity of CTLs via perforin (PRF1). On the other hand, activated CD4⁺ cells interact with B-cells via the MHC class II pathway and initiate influenza-specific antibody production, class switching and somatic hypermutation. SIRPG and P2RY11 on activated T cells may also promote cell-cell adhesion and proliferation in this response. 2) CNS Autoimmunity:

Activated and primed specific CD4⁺ cells migrate to the CNS where they interact with microglia and resident DCs via DQ0602 bound to an influenza-mimic autoimmune-epitope (derived from hypocretin cells) initiating a secondary memory response. Hypocretin cell proteins are processed by cathepsins CTSH and CTSC for presentation by DQ0602 to specific TCR α/β bearing CD4⁺cells, initiating an immunological synapse and autoimmune responses. Chain usage for TRAJ24-2, TRAJ28, and TRBV4-2 is associated with narcolepsy risk and may be crucial for autoantigen recognition. Further, cross presentation by resident DCs and microglial cells activate specific CD8⁺cells via MHC class I binding of another hcr neuron-derived peptides. These primed cytotoxic CD8⁺ then kill hcr neurons after recognizing MHC class I (such as A*11:01, associated with narcolepsy independently of DQ0602) bound cognate hcr neuron derived peptide on hcr neurons. SIRPB1 on DC or microglia and SIRPG plus P2RY11 on activated T cells may also promote cell-cell adhesion and proliferation in this response. The role of ZFN365 and ZFAND2A is unknown.

REFERENCES

1. Hublin, C., Partinen, M., Kaprio, J., Koskenvuo, M. & Guilleminault, C. Epidemiology of narcolepsy. *Sleep* **17**, S7-12 (1994).
2. Silber, M.H., Krahn, L.E., Olson, E.J. & Pankratz, V.S. The epidemiology of narcolepsy in Olmsted County, Minnesota: a population-based study. *Sleep* **25**, 197-202 (2002).
3. Ohayon, M.M., Priest, R.G., Zulley, J., Smirne, S. & Paiva, T. Prevalence of narcolepsy symptomatology and diagnosis in the European general population. *Neurology* **58**, 1826-33 (2002).
4. Peyron, C. *et al.* A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. *Nat Med* **6**, 991-7 (2000).
5. Mignot, E. *et al.* Complex HLA-DR and -DQ interactions confer risk of narcolepsy-cataplexy in three ethnic groups. *Am J Hum Genet* **68**, 686-99 (2001).
6. Ollila, H.M. *et al.* HLA-DPB1 and HLA class I confer risk of and protection from narcolepsy. *Am J Hum Genet* **96**, 136-46 (2015).
7. Faraco, J. *et al.* ImmunoChip study implicates antigen presentation to T cells in narcolepsy. *PLoS Genet* **9**, e1003270 (2013).
8. Hallmayer, J. *et al.* Narcolepsy is strongly associated with the T-cell receptor alpha locus. *Nat Genet* **41**, 708-11 (2009).
9. Han, F. *et al.* Genome wide analysis of narcolepsy in China implicates novel immune loci and reveals changes in association prior to versus after the 2009 H1N1 influenza pandemic. *PLoS Genet* **9**, e1003880 (2013).
10. Kornum, B.R. *et al.* Common variants in P2RY11 are associated with narcolepsy. *Nat Genet* **43**, 66-71 (2011).
11. Ambati, A. *et al.* Increased beta-haemolytic group A streptococcal M6 serotype and streptodornase B-specific cellular immune responses in Swedish narcolepsy cases. *J Intern Med* **278**, 264-76 (2015).
12. Aran, A. *et al.* Elevated anti-streptococcal antibodies in patients with recent narcolepsy onset. *Sleep* **32**, 979-83 (2009).
13. O'Flanagan, D. *et al.* Investigation of an association between onset of narcolepsy and vaccination with pandemic influenza vaccine, Ireland April 2009-December 2010. *Euro Surveill* **19**, 15-25 (2014).
14. Persson, I. *et al.* Risks of neurological and immune-related diseases, including narcolepsy, after vaccination with Pandemrix: a population- and registry-based cohort study with over 2 years of follow-up. *J Intern Med* **275**, 172-90 (2014).
15. Dauvilliers, Y. *et al.* Increased risk of narcolepsy in children and adults after pandemic H1N1 vaccination in France. *Brain* **136**, 2486-96 (2013).
16. Heier, M.S. *et al.* Incidence of narcolepsy in Norwegian children and adolescents after vaccination against H1N1 influenza A. *Sleep Med* **14**, 867-71 (2013).
17. Miller, E. *et al.* Risk of narcolepsy in children and young people receiving AS03 adjuvanted pandemic A/H1N1 2009 influenza vaccine: retrospective analysis. *BMJ* **346**, f794 (2013).
18. Partinen, M. *et al.* Increased incidence and clinical picture of childhood narcolepsy following the 2009 H1N1 pandemic vaccination campaign in Finland. *PLoS One* **7**, e33723 (2012).
19. Nohynek, H. *et al.* AS03 adjuvanted AH1N1 vaccine associated with an abrupt increase in the incidence of childhood narcolepsy in Finland. *PLoS One* **7**, e33536 (2012).
20. Winstone, A.M. *et al.* Clinical features of narcolepsy in children vaccinated with AS03 adjuvanted pandemic A/H1N1 2009 influenza vaccine in England. *Dev Med Child Neurol* **56**, 1117-23 (2014).
21. Gadroen, K. *et al.* Patterns of spontaneous reports on narcolepsy following administration of pandemic influenza vaccine; a case series of individual case safety reports in Eudravigilance. *Vaccine* **34**, 4892-4897 (2016).
22. Montplaisir, J. *et al.* Risk of narcolepsy associated with inactivated adjuvanted (AS03) A/H1N1 (2009) pandemic influenza vaccine in Quebec. *PLoS One* **9**, e108489 (2014).
23. Zheng, G., Freidlin, B., Li, Z. & Gastwirth, J.L. Genomic control for association studies under various genetic models. *Biometrics* **61**, 186-92 (2005).
24. Bakshi, A. *et al.* Fast set-based association analysis using summary data from GWAS identifies novel gene loci for human complex traits. *Sci Rep* **6**, 32894 (2016).
25. International Genetics of Ankylosing Spondylitis, C. *et al.* Identification of multiple risk variants for ankylosing spondylitis through high-density genotyping of immune-related loci. *Nat Genet* **45**, 730-8 (2013).
26. Ricano-Ponce, I. *et al.* Refined mapping of autoimmune disease associated genetic variants with gene expression suggests an important role for non-coding RNAs. *J Autoimmun* **68**, 62-74 (2016).
27. Yan, J., Hedl, M. & Abraham, C. An inflammatory bowel disease-risk variant in INAVA decreases pattern recognition receptor-induced outcomes. *J Clin Invest* **127**, 2192-2205 (2017).

- 1 28. Altorok, N. *et al.* Genome-wide DNA methylation patterns in naive CD4+ T cells from patients with primary
2 Sjogren's syndrome. *Arthritis Rheumatol* **66**, 731-9 (2014).
- 3 29. Erickson, J.J. *et al.* Viral acute lower respiratory infections impair CD8+ T cells through PD-1. *J Clin Invest* **122**,
4 2967-82 (2012).
- 5 30. Yang, J., Lee, S.H., Goddard, M.E. & Visscher, P.M. GCTA: a tool for genome-wide complex trait analysis. *Am J*
6 *Hum Genet* **88**, 76-82 (2011).
- 7 31. Li, Y.R. *et al.* Genetic sharing and heritability of paediatric age of onset autoimmune diseases. *Nat Commun* **6**,
8 8442 (2015).
- 9 32. Zheng, J. *et al.* LD Hub: a centralized database and web interface to perform LD score regression that
10 maximizes the potential of summary level GWAS data for SNP heritability and genetic correlation
11 analysis. *Bioinformatics* **33**, 272-279 (2017).
- 12 33. Bulik-Sullivan, B. *et al.* An atlas of genetic correlations across human diseases and traits. *Nat Genet* **47**, 1236-
13 41 (2015).
- 14 34. Martinez-Orozco, F.J. *et al.* Narcolepsy with cataplexy and comorbid immunopathological diseases. *J Sleep Res*
15 **23**, 414-9 (2014).
- 16 35. Barateau, L. *et al.* Comorbidity between central disorders of hypersomnolence and immune-based disorders.
17 *Neurology* **88**, 93-100 (2017).
- 18 36. Martinez-Orozco, F.J., Vicario, J.L., De Andres, C., Fernandez-Arquero, M. & Peraita-Adrados, R. Comorbidity of
19 Narcolepsy Type 1 With Autoimmune Diseases and Other Immunopathological Disorders: A Case-
20 Control Study. *J Clin Med Res* **8**, 495-505 (2016).
- 21 37. Ellinghaus, D. *et al.* Analysis of five chronic inflammatory diseases identifies 27 new associations and
22 highlights disease-specific patterns at shared loci. *Nat Genet* **48**, 510-8 (2016).
- 23 38. Hrdlickova, B. *et al.* Expression profiles of long non-coding RNAs located in autoimmune disease-associated
24 regions reveal immune cell-type specificity. *Genome Med* **6**, 88 (2014).
- 25 39. Paternoster, L. *et al.* Multi-ancestry genome-wide association study of 21,000 cases and 95,000 controls
26 identifies new risk loci for atopic dermatitis. *Nat Genet* **47**, 1449-1456 (2015).
- 27 40. Plagnol, V., Smyth, D.J., Todd, J.A. & Clayton, D.G. Statistical independence of the colocalized association
28 signals for type 1 diabetes and RPS26 gene expression on chromosome 12q13. *Biostatistics* **10**, 327-34
29 (2009).
- 30 41. Teumer, A. *et al.* Genome-wide Association Studies Identify Genetic Loci Associated With Albuminuria in
31 Diabetes. *Diabetes* **65**, 803-17 (2016).
- 32 42. Han, F. *et al.* Narcolepsy onset is seasonal and increased following the 2009 H1N1 pandemic in China. *Ann*
33 *Neurol* **70**, 410-7 (2011).
- 34 43. Picchioni, D., Hope, C.R. & Harsh, J.R. A case-control study of the environmental risk factors for narcolepsy.
35 *Neuroepidemiology* **29**, 185-92 (2007).
- 36 44. Sarkanen, T., Alakuijala, A., Julkunen, I. & Partinen, M. Narcolepsy Associated with Pandemrix Vaccine. *Curr*
37 *Neurol Neurosci Rep* **18**, 43 (2018).
- 38 45. Iotchkova, V. *et al.* GARFIELD - GWAS Analysis of Regulatory or Functional Information Enrichment with LD
39 correction. *bioRxiv* (2016).
- 40 46. Martinez-Pomar, N., Lanio, N., Romo, N., Lopez-Botet, M. & Matamoros, N. Functional impact of A91V
41 mutation of the PRF1 perforin gene. *Hum Immunol* **74**, 14-7 (2013).
- 42 47. Voskoboinik, I. *et al.* Perforin activity and immune homeostasis: the common A91V polymorphism in
43 perforin results in both presynaptic and postsynaptic defects in function. *Blood* **110**, 1184-90 (2007).
- 44 48. Lowin, B., Beermann, F., Schmidt, A. & Tschopp, J. A null mutation in the perforin gene impairs cytolytic T
45 lymphocyte- and natural killer cell-mediated cytotoxicity. *Proc Natl Acad Sci U S A* **91**, 11571-5 (1994).
- 46 49. Clementi, R. *et al.* Adult onset and atypical presentation of hemophagocytic lymphohistiocytosis in siblings
47 carrying PRF1 mutations. *Blood* **100**, 2266-7 (2002).
- 48 50. Jordan, M.B., Hildeman, D., Kappler, J. & Marrack, P. An animal model of hemophagocytic lymphohistiocytosis
49 (HLH): CD8+ T cells and interferon gamma are essential for the disorder. *Blood* **104**, 735-43 (2004).
- 50 51. Terrell, C.E. & Jordan, M.B. Perforin deficiency impairs a critical immunoregulatory loop involving murine
51 CD8(+) T cells and dendritic cells. *Blood* **121**, 5184-91 (2013).
- 52 52. Cappellano, G. *et al.* Variations of the perforin gene in patients with multiple sclerosis. *Genes Immun* **9**, 438-
53 44 (2008).
- 54 53. Orilieri, E. *et al.* Variations of the perforin gene in patients with type 1 diabetes. *Diabetes* **57**, 1078-83 (2008).
- 55 54. Slots, J. Update on human cytomegalovirus in destructive periodontal disease. *Oral Microbiol Immunol* **19**,
56 217-23 (2004).
- 57 55. Slots, J. & Contreras, A. Herpesviruses: a unifying causative factor in periodontitis? *Oral Microbiol Immunol*
58 **15**, 277-80 (2000).
- 59 56. Noack, B. *et al.* Cathepsin C gene variants in aggressive periodontitis. *J Dent Res* **87**, 958-63 (2008).

- 1 57. Aswath, N. *et al.* Heterozygous Ile453Val codon mutation in exon 7, homozygous single nucleotide
2 polymorphisms in intron 2 and 5 of cathepsin C are associated with Haim-Munk syndrome. *Eur J Dent*
3 **8**, 79-84 (2014).
- 4 58. Perisic Nanut, M., Sabotic, J., Jewett, A. & Kos, J. Cysteine cathepsins as regulators of the cytotoxicity of NK and
5 T cells. *Front Immunol* **5**, 616 (2014).
- 6 59. Brooke, G., Holbrook, J.D., Brown, M.H. & Barclay, A.N. Human lymphocytes interact directly with CD47
7 through a novel member of the signal regulatory protein (SIRP) family. *J Immunol* **173**, 2562-70
8 (2004).
- 9 60. Gabrielsen, I.S. *et al.* Genetic risk variants for autoimmune diseases that influence gene expression in thymus.
10 *Hum Mol Genet* **25**, 3117-3124 (2016).
- 11 61. Gaikwad, S. *et al.* Signal regulatory protein-beta1: a microglial modulator of phagocytosis in Alzheimer's
12 disease. *Am J Pathol* **175**, 2528-39 (2009).
- 13 62. Kasela, S. *et al.* Pathogenic implications for autoimmune mechanisms derived by comparative eQTL analysis
14 of CD4+ versus CD8+ T cells. *PLoS Genet* **13**, e1006643 (2017).
- 15 63. Nasr, N. *et al.* Inhibition of two temporal phases of HIV-1 transfer from primary Langerhans cells to T cells:
16 the role of langerin. *J Immunol* **193**, 2554-64 (2014).
- 17 64. Feinberg, H. *et al.* Common polymorphisms in human langerin change specificity for glycan ligands. *J Biol*
18 *Chem* **288**, 36762-71 (2013).
- 19 65. GeurtsvanKessel, C.H. *et al.* Clearance of influenza virus from the lung depends on migratory
20 langerin+CD11b- but not plasmacytoid dendritic cells. *J Exp Med* **205**, 1621-34 (2008).
- 21 66. Ward, E.M., Stambach, N.S., Drickamer, K. & Taylor, M.E. Polymorphisms in human langerin affect stability
22 and sugar binding activity. *J Biol Chem* **281**, 15450-6 (2006).
- 23 67. Ng, W.C. *et al.* The C-type Lectin Langerin Functions as a Receptor for Attachment and Infectious Entry of
24 Influenza A Virus. *J Virol* **90**, 206-21 (2015).
- 25 68. Kim-Hellmuth, S. *et al.* Genetic regulatory effects modified by immune activation contribute to autoimmune
26 disease associations. *Nat Commun* **8**, 266 (2017).
- 27 69. Thomas, J.M. *et al.* Differential responses of plasmacytoid dendritic cells to influenza virus and distinct viral
28 pathogens. *J Virol* **88**, 10758-66 (2014).
- 29 70. Lecendreux, M. *et al.* Impact of cytokine in type 1 narcolepsy: Role of pandemic H1N1 vaccination ? *J*
30 *Autoimmun* **60**, 20-31 (2015).
- 31 71. Finucane, H.K. *et al.* Partitioning heritability by functional annotation using genome-wide association
32 summary statistics. *Nat Genet* **47**, 1228-35 (2015).
- 33 72. Hartmann, F.J. *et al.* High-dimensional single-cell analysis reveals the immune signature of narcolepsy. *J Exp*
34 *Med* **213**, 2621-2633 (2016).
- 35 73. Sharon, E. *et al.* Genetic variation in MHC proteins is associated with T cell receptor expression biases. *Nat*
36 *Genet* **48**, 995-1002 (2016).
- 37 74. Roman, T.S. *et al.* Multiple Hepatic Regulatory Variants at the GALNT2 GWAS Locus Associated with High-
38 Density Lipoprotein Cholesterol. *Am J Hum Genet* **97**, 801-15 (2015).
- 39 75. Consortium, G.T. Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene
40 regulation in humans. *Science* **348**, 648-60 (2015).
- 41 76. Zheng, X. *et al.* HIBAG--HLA genotype imputation with attribute bagging. *Pharmacogenomics J* **14**, 192-200
42 (2014).
- 43 77. Dilthey, A. *et al.* Multi-population classical HLA type imputation. *PLoS Comput Biol* **9**, e1002877 (2013).
- 44 78. Hor, H. *et al.* Genome-wide association study identifies new HLA class II haplotypes strongly protective
45 against narcolepsy. *Nat Genet* **42**, 786-9 (2010).
- 46 79. Ollila, H.M., Fernandez-Vina, M. & Mignot, E. HLA-DQ allele competition in narcolepsy: a comment on Tafti *et*
47 *al.* DQB1 locus alone explains most of the risk and protection in narcolepsy with cataplexy in Europe.
48 *Sleep* **38**, 147-51 (2015).
- 49 80. Lenz, T.L. *et al.* Widespread non-additive and interaction effects within HLA loci modulate the risk of
50 autoimmune diseases. *Nat Genet* **47**, 1085-90 (2015).
- 51 81. Tafti, M. *et al.* Narcolepsy-Associated HLA Class I Alleles Implicate Cell-Mediated Cytotoxicity. *Sleep* **39**, 581-7
52 (2016).
- 53 82. Haritunians, T. *et al.* Variants in ZNF365 isoform D are associated with Crohn's disease. *Gut* **60**, 1060-7
54 (2011).
- 55 83. Miyadera, H. & Tokunaga, K. Associations of human leukocyte antigens with autoimmune diseases:
56 challenges in identifying the mechanism. *J Hum Genet* **60**, 697-702 (2015).
- 57 84. Dantoft, W. *et al.* Genomic Programming of Human Neonatal Dendritic Cells in Congenital Systemic and In
58 Vitro Cytomegalovirus Infection Reveal Plastic and Robust Immune Pathway Biology Responses. *Front*
59 *Immunol* **8**, 1146 (2017).
- 60 85. Hu, X. *et al.* Additive and interaction effects at three amino acid positions in HLA-DQ and HLA-DR molecules
61 drive type 1 diabetes risk. *Nat Genet* **47**, 898-905 (2015).

- 1 86. Tsutsumi, C. *et al.* Class II HLA genotype in fulminant type 1 diabetes: A nationwide survey with reference to
2 glutamic acid decarboxylase antibodies. *J Diabetes Investig* **3**, 62-9 (2012).
- 3 87. Ahmed, S.S. *et al.* Antibodies to influenza nucleoprotein cross-react with human hypocretin receptor 2. *Sci*
4 *Transl Med* **7**, 294ra105 (2015).
- 5 88. Cvetkovic-Lopes, V. *et al.* Elevated Tribbles homolog 2-specific antibody levels in narcolepsy patients. *J Clin*
6 *Invest* **120**, 713-9 (2010).
- 7 89. Kawashima, M. *et al.* Anti-Tribbles homolog 2 (TRIB2) autoantibodies in narcolepsy are associated with
8 recent onset of cataplexy. *Sleep* **33**, 869-74 (2010).
- 9 90. Lind, A. *et al.* A/H1N1 antibodies and TRIB2 autoantibodies in narcolepsy patients diagnosed in conjunction
10 with the Pandemrix vaccination campaign in Sweden 2009-2010. *J Autoimmun* **50**, 99-106 (2014).
- 11 91. Sadam, H. *et al.* Prostaglandin D2 Receptor DP1 Antibodies Predict Vaccine-induced and Spontaneous
12 Narcolepsy Type 1: Large-scale Study of Antibody Profiling. *EBioMedicine* (2018).
- 13 92. Bernard-Valnet, R. *et al.* CD8 T cell-mediated killing of orexinergic neurons induces a narcolepsy-like
14 phenotype in mice. *Proc Natl Acad Sci U S A* **113**, 10956-61 (2016).
- 15 93. Dauvilliers, Y. *et al.* Hypothalamic immunopathology in anti-Ma-associated diencephalitis with narcolepsy-
16 cataplexy. *JAMA Neurol* **70**, 1305-10 (2013).
- 17 94. Bomfim, I.L. *et al.* The immunogenetics of narcolepsy associated with A(H1N1)pdm09 vaccination
18 (Pandemrix) supports a potent gene-environment interaction. *Genes Immun* **18**, 75-81 (2017).
- 19 95. Ollila, H.M. *et al.* Genetics of vaccination-related narcolepsy. *bioRxiv* 169623; doi:
20 <https://doi.org/10.1101/169623> (2017).
- 21 96. Nicolazzi, E.L., Iamartino, D. & Williams, J.L. AffyPipe: an open-source pipeline for Affymetrix Axiom
22 genotyping workflow. *Bioinformatics* **30**, 3118-9 (2014).
- 23 97. Howie, B., Fuchsberger, C., Stephens, M., Marchini, J. & Abecasis, G.R. Fast and accurate genotype imputation
24 in genome-wide association studies through pre-phasing. *Nat Genet* **44**, 955-9 (2012).
- 25 98. Howie, B., Marchini, J. & Stephens, M. Genotype imputation with thousands of genomes. *G3 (Bethesda)* **1**, 457-
26 70 (2011).
- 27 99. Wellcome Trust Case Control, C. Genome-wide association study of 14,000 cases of seven common diseases
28 and 3,000 shared controls. *Nature* **447**, 661-78 (2007).
- 29 100. Liu, J.Z. *et al.* Meta-analysis and imputation refines the association of 15q25 with smoking quantity. *Nat*
30 *Genet* **42**, 436-40 (2010).
- 31 101. Lee, S.H., Wray, N.R., Goddard, M.E. & Visscher, P.M. Estimating missing heritability for disease from
32 genome-wide association studies. *Am J Hum Genet* **88**, 294-305 (2011).
- 33 102. Wang, C. *et al.* High-throughput, high-fidelity HLA genotyping with deep sequencing. *Proc Natl Acad Sci U S*
34 *A* **109**, 8676-81 (2012).
- 35 103. Chang, C.C. *et al.* Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience*
36 **4**, 7 (2015).
- 37 104. Westra, H.J. *et al.* Cell Specific eQTL Analysis without Sorting Cells. *PLoS Genet* **11**, e1005223 (2015).
- 38 105. Lee, M.N. *et al.* Common genetic variants modulate pathogen-sensing responses in human dendritic cells.
39 *Science* **343**, 1246980 (2014).
- 40 106. Ye, C.J. *et al.* Intersection of population variation and autoimmunity genetics in human T cell activation.
41 *Science* **345**, 1254665 (2014).
- 42 107. Marchini, J. & Howie, B. Genotype imputation for genome-wide association studies. *Nat Rev Genet* **11**, 499-
43 511 (2010).
- 44