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Novel genes associated with hypocretin-producing neurons identified by human gene expression profiling

Marieke Vringer [a,b], Ahmed Mahfouz [c,d], Maartje G. Huijbers [a,c], Gert Jan Lammers [a,b], Jari Berkhout [c,e], Frits Koning [f], Rolf Fronczek [a,b], Mink Schinkelshoek [a,b,*]

[a] Leiden University Medical Center, Department of Neurology, Leiden, the Netherlands
[b] Stichting Epilepsie Instellingen Nederland (SEIN), Sleep-Wake Centre, Heemstede, the Netherlands
[c] Department of Human Genetics, Leiden University Medical Center, Leiden, the Netherlands
[d] Pattern Recognition and Bioinformatics, Delft University of Technology, Delft, the Netherlands
[e] Department of Endocrinology, Leiden University Medical Center, Leiden, the Netherlands
[f] Department of Immunology, Leiden University Medical Center, Leiden, the Netherlands

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ABSTRACT

Narcolepsy type 1 is a sleep-wake disorder characterized by hypocretin deficiency. It has been considered an autoimmune disorder for decades due to the strong associating with the *HLA-DQB1*06:02* allele and possible relations to the H1N1 pandemic in 2009. However, the pathophysiological mechanisms underlying the loss of hypocretin neurons is not understood. We hypothesize that a hypocretin neuron-specific antigen, other than hypocretin itself but sharing an expression pattern, may be the target of the autoimmune response leading to the development in individuals with narcolepsy type 1. In this study, we employed an *in silico* method to identify novel candidate antigens for an autoimmune response leading to the destruction of hypocretin cells. A combination of multiple publicly available datasets, based on human brain tissue from healthy individuals, was used to map the expression profile of hypocretin. Genes were categorized based on their expression pattern and its association with hypocretin expression. 15 candidate genes were identified as potentially relevant targets in the development of NT1, with varying degrees of confidence regarding the likelihood of their involvement. Six candidate genes also showed higher expression within hypocretin cells compared to other cells in the hypothalamus of which *NPVF* seems most promising. This study provides important new directions and potential targets for investigating and understanding the pathophysiology of narcolepsy type 1.

1. Introduction

Narcolepsy type 1 (NT1) is a sleep-wake disorder characterized by excessive daytime sleepiness, cataplexy, hypnagogic hallucinations, sleep paralysis, fragmented night sleep and decreased vigilance. The prevalence of NT1 is estimated to be 1 in 2000 individuals, although this figure might be underestimated because NT1 is frequently not recognized by caregivers that assess patients' symptoms (Bassetti et al., 2019; Ohayon et al., 2002). NT1 has been suspected to be an auto-immune disorder since the 1980s when an HLA-association with the *HLA-DR2* allele was discovered (Juji et al., 1984). In the 90s, an unprecedentedly strong association with the *HLA-DQB1*06:02* allele was found (Rogers et al., 1997). It took until 1998 for a new discovery when two groups simultaneously discovered hypocretin (HCRT, also known as orexin) (de Lecea et al., 1998; Sakurai et al., 1998). The specific loss of HCRT production by HCRT neurons in people with NT1 further supports the hypothesis of an auto-immune cause in the NT1 development (Peyron et al., 2000; Thannickal et al., 2000). Although the autoimmune hypothesis assumes that HCRT neurons are lost due to an immune response, other hypotheses for HCRT deficiency could still be considered. For example the hypothesis that HCRT neurons still exist, but are unable to produce HCRT due to epigenetic silencing of the *HCRT* gene (Seifinejad et al., 2023).

HCRT neurons are exclusively and specifically located within the hypothalamus (Diaz et al., 2023; Peyron et al., 1998; Sakurai et al., 1998) and project to many extrahypothalamic brain regions, except the cerebellum (Peyron et al., 1998). Two different HCRT peptides were identified: HCRT1 and HCRT2, originating from the same prepro-

* Corresponding author: Leiden University Medical Center, Department of Neurology, 2300RC Leiden, the Netherlands.
E-mail address: M.S.Schinkelshoek@lumc.nl (M. Schinkelshoek).

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hypocretin precursor protein. HCRT1 fit two receptors, HCRT-R1 and HCRT-R2, whereas HCRT2 binds only HCRT-R2. Evidence for a relationship between NT1 and HCRT deficiency has been derived from animal models and patients with NT1. Neural HCRT projections suggest a role in the sleep-wake regulation (Hagan et al., 1999; Peyron et al., 1998). Animal models show that loss of HCRT production or HCRT-R2 results in a narcolepsy phenotype (Chemelli et al., 1999; Lin et al., 1999). Low or undetectable HCRT1 concentrations in human cerebrospinal fluid are closely related to NT1 symptoms (Hansen et al., 2017; Nishino et al., 2000). Furthermore, post-mortem brain tissue from patient with NT1 show reduced amounts of HCRT-producing neurons (Shan et al., 2022; Thannickal et al., 2000) and neurons producing corticotrophin-releasing hormone (CRH) (Shan et al., 2022). HCRT has wake-promoting effects and can, among other things, influence appetite. Besides sleep related symptoms, NT1 has been associated with increased body weight, which does correlate with HCRT levels in CSF (Kok et al., 2003; Santiago et al., 2018).

More indications of an auto-immune mediated cause for NT1 comes from observations in some countries that reported an increased incidence of NT1 during and after the H1N1 pandemic and vaccination campaign in 2009–2010 (Han et al., 2011; Sarkanen et al., 2018). The hypothesis that NT1 might be caused by cross-reactive immune cells targeting both the H1N1 virus and HCRT neurons became a central focus in research on the pathophysiology of NT1. The most probable antigen was HCRT. However, more than two decades later, no definite proof of the existence of cross-reactivity (through antibodies or T cells) has been found (Luo et al., 2018; Schinkelshoek et al., 2019). Autoreactive CD4+ and CD8+ T cells to HCRT peptides have been described by several groups (Jiang et al., 2019; Latorre et al., 2018; Pedersen et al., 2019; Ramberger et al., 2017). Nevertheless, a consensus on the precise role for HLA-DQB1*06:02 is lacking (Latorre et al., 2018). Furthermore, the loss of HCRT may be a secondary effect of an immune reaction against a different antigen. Importantly, lower concentrations of these autoreactive immune cells were also found in healthy individuals (Kornum, 2020; Pedersen et al., 2019). Evidence for a HCRT-directed autoimmune response thus remains elusive.

Several other hypotheses on the pathophysiology of NT1 exist, among which one that suggests that a different antigen specific for HCRT neurons may be the target of the autoimmune response leading to the development of NT1. Several studies describe genes, other than the hypocretin (*HCRT*) gene, that relate to NT1 (Degn et al., 2017; Faraco et al., 2013; Han et al., 2013; Holm et al., 2015; Miyagawa et al., 2008; Toyoda et al., 2015). However, this data is mostly derived from genome-wide association studies and information on the spatial expression data of these genes is mostly lacking. Therefore, it is unclear whether these genes are specific to HCRT neurons and potential targets in an auto-immune response leading to NT1.

In this study we used an *in silico* approach to find novel candidate target antigens involved in the development of human NT1. Our approach is exclusively based on human data since expression patterns can slightly vary between different species. We combine multiple publicly available online resources to determine the expression profile of HCRT-producing neurons in healthy individuals. Subsequently, we aimed to identify genes of interest that might be candidate antigens for the auto-immune response leading to the destruction of the HCRT-producing neurons, based on associations with the *HCRT* expression profile. Potential co-expression is an important element but the expression of alternative antigens also needs to be specific to the regions were *HCRT* is expressed.

2. Methods

2.1. Human hypocretin expression data

To identify candidate antigens that are specific to HCRT neurons, it is essential to map the *HCRT* expression profile, based on human samples. Spatially-mapped gene expression data from post-mortem samples from six healthy adult human brain donors (5 male *vs* 1 female; mean age 42 years, range 24–57) was obtained from the Allen Human Brain Atlas (AHBA; https://human.brain-map.org/) (Hawrylycz et al., 2012). A total of 3702 RNA samples were extracted from different brain areas and measured using Agilent microarrays that contained 16,000 custom probes in addition to the 4 × 44 K Agilent Whole Human Genome probes. For each probe, the sum of its Pearson correlations to all other probes was calculated in each brain separately. Connectivity of a probe was defined as the average of these six calculations. When genes were targeted by two probes or more, the one with the highest connectivity was selected. Expression data of the 20,097 genes was normalized to a z-score per brain (Hawrylycz et al., 2015; Hawrylycz et al., 2012; Keo et al., 2020). More methodological details about the initial data processing can be found in the '*Microarray Survey*' and '*Microarray Data Normalization*' technical white papers provided by the Allen Institute, which are available on their website (https://community.brain-map.org/t/documentation-human-brain-atlas/2879). The expression of the *HCRT* gene was measured using two probes, of which CUST_14616_PI416261804 (probe 1) has the highest connectivity, and is most consistent with the specific *HCRT* expression pattern, as shown in earlier research (Diaz et al., 2023; Peyron et al., 1998; Sakurai et al., 1998). This probe is located at the 5′ untranslated region of the *HCRT* gene (Fig. 1, see Fig. S1 for more details) with a sequence length of 60 bp and GC-content of 51,67%. The second probe is A_23P129989 and is located at the 3′ untranslated region. This probe has as a sequence length of 60 bp and GC-content of 71.67%. The *Z*-score values of *HCRT* gene expression were mapped to images of an anatomical atlas that was acquired from the AHBA (Hawrylycz et al., 2012).

HCRT gene expression was also provided by the Genotype Tissue Expression (GTEx) project and its portal (https://www.gtexportal.org). The portal contains gene expression information on the post-mortem tissue of 946 healthy human individuals over 54 tissues (GTEx version 10) (Consortium, 2013, 2020). More information on the methods of processing, expression quantification and number of donors per tissue are described in detail in the GTEx portal.

2.2. Gene co-expression analysis

To identify candidate genes that are specifically expressed in HCRT neurons, we focused on genes co-expressing with *HCRT*. Our approach is based on the assumption that genes with expression patterns that are similar over a set of samples are more likely to be expressed in the same cell types (Mahfouz et al., 2016; Stuart et al., 2003). We aim to identify candidate genes co-expressing with *HCRT* by using the spatially-mapped *HCRT* expression profile, as described in section 2.1, to explore their potential role in an autoimmune response against HCRT neurons leading to NT1.

BrainScope portal (www.brainscope.nl) was used as starting point for the selection of candidate genes. The BrainScope portal clusters all genes within the AHBA based on the expression level and location of a certain gene (Huisman et al., 2017). The genes are mapped and evaluated for similarity with *HCRT* gene within the human brain. The associations with *HCRT* have been computed by calculating the spatial Pearson's correlation between each gene in the AHBA and the *HCRT* gene (Huisman et al., 2017). The 142 genes (the limit in BrainScope portal) with the highest association with the *HCRT* gene, as identified in the BrainScope portal, were selected for further analysis.

Subsequently, the identified 142 genes were scored based on their expression patterns and specificity to *HCRT* expression regions. Specificity within the brain was evaluated in the BrainScope portal. The GTEx portal was used to evaluate expression levels and specificity within various brain tissues and specificity to the brain tissue. The scoring rules are shown in Table 1. The GTEx score and BrainScope score are summed and genes with a score ≥ 5 were identified as candidate genes to be co-expressing with *HCRT* if the GTEx and BrainScope scores are both

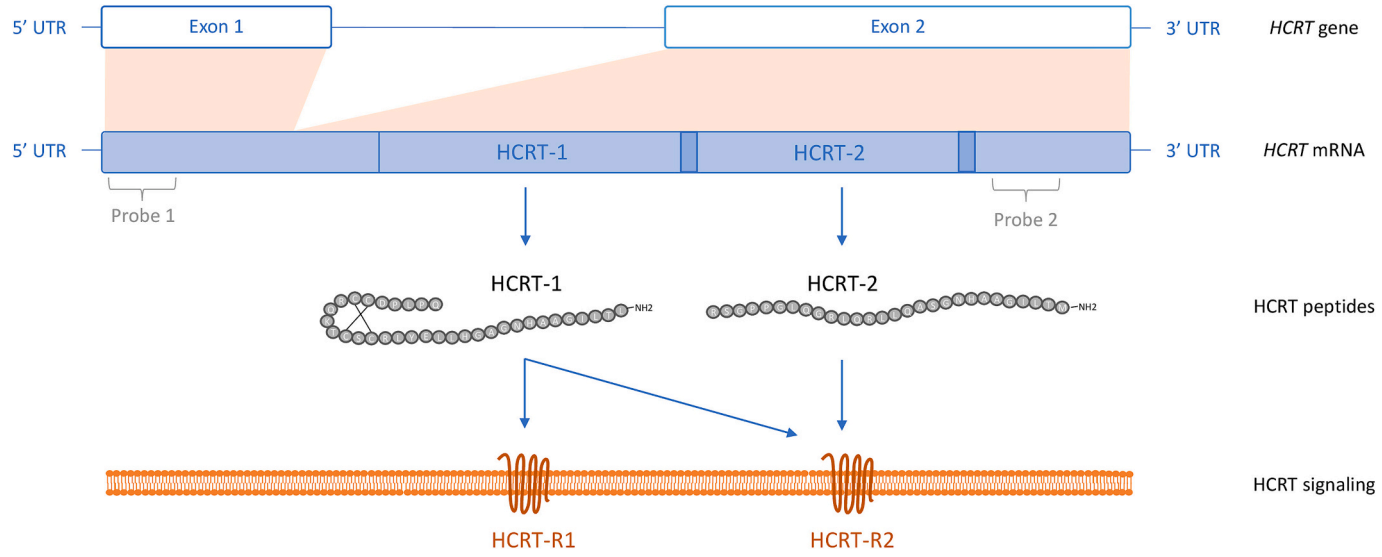

Fig. 1. Schematic visualization of probe location.

Table 1
Scoring rules for Genotype-Tissue Expression (GTEx) portal and BrainScope portal.

GTEx portal		BrainScope portal	
Scoring rule	GTEx score	Scoring rule	BrainScope score
If *only* present in hypothalamic tissue (all other tissues <2.0 TPM).	5	If *only* present in *HCRT* regions, not in other regions.	3
If highest expression level in hypothalamic region.	4	If more present in *HCRT* regions than any other region.	2
If present in hypothalamic tissue and there are only 1–3 other tissues with higher expression level.	3	If present in *HCRT* regions, but equal or less than in other regions.	1
If present in hypothalamic tissue and there are only 4–5 other tissues with higher expression level.	2	If (almost) not present in *HCRT* regions.	0
If present in hypothalamic tissue and there are 6+ other tissues with higher expression level.	1		
If not present in hypothalamic region (TPM <0.3) OR if more present in (almost) all tissues outside brain than brain tissues.	0		

HCRT = hypocretin, TPM = transcripts per million, 'HCRT regions' refers to the 'posterior hypothalamic area' and the 'lateral hypothalamic area, mammillary region' in the BrainScope portal.

minimal 1. This means that genes with GTEx scores of 4 and 5 are selected when their BrainScope score is 1 or higher, and genes with GTEx scores of 2 and 3 when their BrainScope score is 3 or 2 or higher, respectively.

2.2.1. Evaluation of candidate gene expression within the hypothalamus using single-nuclei RNA-sequencing data

To verify the association between the selected candidate genes and *HCRT*, the genes were evaluated for their expression within HCRT neurons within the publicly available human HYPOMAP dataset (Tadross et al., 2025). For this dataset, sequencing was performed on single nuclei that were isolated from post-mortem brains from eleven donors (4 female, 7 male), and includes 433,369 hypothalamic cells.

The dataset was retrieved from CELLxGENE and analyzed using Seurat v5 (Hao et al., 2024) and R v4.3.3 (Team, 2024). Genes enriched in HCRT positive neurons were calculated using the FindMarkers function, with default argument except logfc.threshold = 0. The cluster "C4-331 Mid-2 GLU-1 LHX9 HCRT" was compared against all other clusters in the "C4_named" annotation level. The Bonferroni correction method was used to correct for multiple testing, and the adjusted *p*-value is significant with $\alpha \leq 0.01$. A difference in expression level is considered biologically relevant when the log2(FC) > 0.5 or < -0.5.

2.3. Literature search for genes associated with HCRT neurons or NT1

Multiple genes have been described by earlier literature to be associated with HCRT cells or NT1, but often information on their expression data was missing. To determine if we should consider these genes as involved in the loss of HCRT cells in NT1, we evaluated their expression profiles by comparing them to the *HCRT* expression. A literature search was performed to find associating genes from earlier literature. A PubMed search on genes associated with HCRT-producing neurons was built using guidelines on performing an exhaustive literature review as published before (McKeever et al., 2015). The following PubMed search was performed: '(hypocretin neurons OR hypocretin producing neurons OR orexin neurons OR orexin producing neurons) AND (gene expression OR genes) Filter: Humans, English' (retrieved May 2024). The 'Humans' filter was used to ensure that our results are exclusively based on human data. Citation chaining was used to find additional references cited by results of the PubMed search to add missing publications to the search results.

In addition, a list of genes associated with NT1 was retrieved from DisGeNet v7.0, a database that integrates human gene-disease associations from different expert curated sources and text-mining of literature (Pinero et al., 2015). The DisGeNet database was used to identify genes that are associated with NT1. All genes in the database are assigned a 'gene disease association score' (gda-score). This score is calculated based on the number of publications or sources in various databases that support the association. A score ≥ 0.2 is considered as relevant in this manuscript.

The genes identified as associated with HCRT neurons or NT1 were evaluated for their expression similarity with the *HCRT* gene using the expression data from BrainScope portal. All genes resulting from the selection in PubMed search and DisGeNet were assessed for their correlation with *HCRT*. A gene was considered relevant if it overlaps with

the 142 genes that show the highest correlation to the *HCRT* gene in the BrainScope portal.

3. Results

3.1. Hypocretin expression in the brain and other tissues

Expression patterns of *HCRT* in the healthy human body were derived from the GTEx portal that assessed RNA expression in 13 brain areas and 41 other tissues (Fig. 2C). As expected, *HCRT* was expressed almost exclusively in the hypothalamus with a median count of 34.91 transcripts per million (TPM) ($n = 257$) compared to 0.50 TPM ($n = 285$) in the nucleus accumbens, the tissue with the second highest hypocretin expression (Fig. 2C). As expected, there is no significant expression of *HCRT* outside the brain (Fig. 2C). The AHBA data provides a higher resolution on *HCRT* expression in different human brain regions. The relative expression levels of *HCRT* varied per nucleus and per donor but were highest in the posterior hypothalamic area, various regions in the lateral hypothalamus dorsomedial hypothalamic nucleus and the perifornical nucleus (Fig. 2A and B).

3.2. Identification of novel genes potentially co-expressing with hypocretin

To identify new candidate targets that may be responsible for NT1, we first selected genes that are associated with *HCRT* expression level and location. The selected genes were scored based on their specificity to the hypothalamic region (section 2.2). Using this strategy, we prioritized a list of 15 genes as candidate genes for co-expression with HCRT-

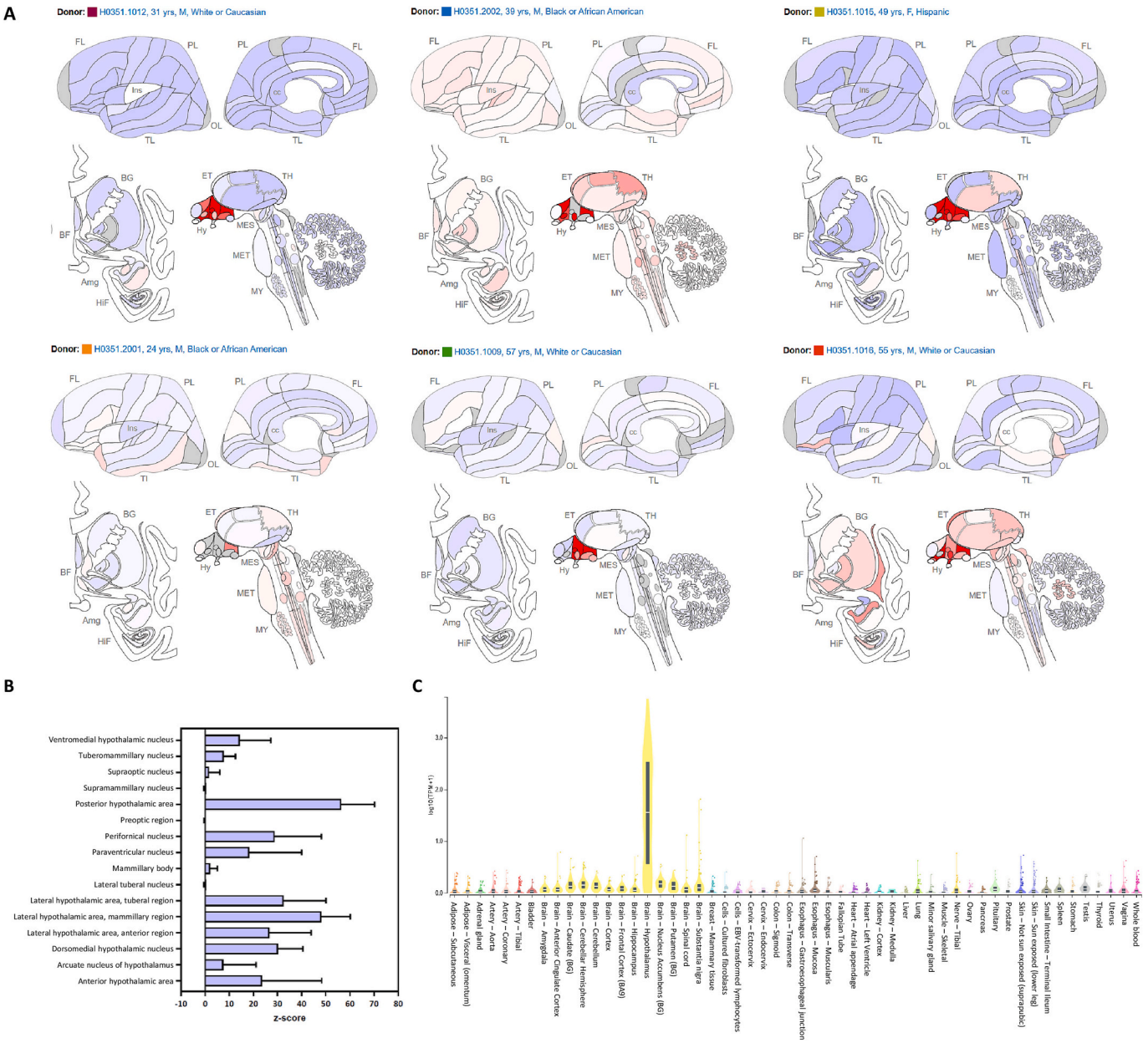

Fig. 2. Hypocretin expression. (A) Data retrieved from the Allen Human Brain Atlas (AHBA) showing the relative *HCRT* expression within the brain for all six donors donor. (B) The average *HCRT* expression level over all six donors in the AHBA. (C) Data retrieved from Genotype-Tissue Expression (GTEx) portal showing *HCRT* expression per tissue. Probe CUST_14616_PI416261804 was used in the AHBA. HCRT = hypocretin, FL = frontal lobe, INS = insula, CC = cingulate cortex, OL = occipital lobe, PL = parietal lobe, TL = temporal lobe, Amg = amygdala, BG = basal ganglia, BF = Basal Forebrain, HiF = hippocampal formation, ET = epithalamus, Hy = hypothalamus, TH = thalamus, MES = mesencephalon, MET = metencephalon, MY = myelencephalon, BA9 = Brodmann area 9, TPM = transcripts per million.

Table 2
List of candidate genes for co-expression with HCRT-producing neurons in whole human brain.

Summed score	Gene	Description
7	*(HCRT)*	(Hypocretin)
	NPVF	Neuropeptide VF precursor
6	*AVP*	Arginine Vasopressin
	CALCR	Calcitonin Receptor
	GHRH	Growth Hormone Releasing Hormone
	HDC	Histidine Decarboxylase
5	*MGC4294 / LINC01711*	Long Intergenic Non-Protein Coding RNA 1711
	NTS	Neurotensin
	OTP	Orthopedia Homeobox
	ST8SIA2	ST8 Alpha-N-Acetyl-Neuraminide Alpha-2,8-Sialyltransferase 2
	KISS1R	KISS1 Receptor
	OXT	Oxytocin / Neurophysin I Prepropeptide
	NPB	Neuropeptide B
	ZNF114	Zinc Finger Protein 114
	C22orf42	Chromosome 22 Open Reading Frame 42
	TLE6	TLE Family Member 6, Subcortical Maternal Complex Member

producing neurons (Table 2). Our strategy was validated by the fact that *HCRT* itself was in the highest scoring category with a GTEx score of 5 and a BrainScope score of 2 (summed score of 7). Next to this, *NPVF* was the highest scoring genes (summed score of 7). We furthermore identified 4 genes with a total score of 6, and 10 genes with a summed score of 5. Since those 15 genes had a summed score ≥ 5, we recognized them as most relevant candidate antigens. Five genes could not be scored because they did not exist in GTEx portal. These genes were *AC099759.1* (BrainScope score 1), *LOC100286952* (BrainScope score 1), *LOC100294396* (BrainScope score 1), *LOC283867* (BrainScope score 1) and *LOC442381* (BrainScope score 2). In Table S1, we show the scoring of all 142 genes that have the highest association with *HCRT* in the BrainScope portal.

To further delineate which candidate antigens may be associated with NT1 due to a strong co-expression correlation with *HCRT* we evaluated the expression of the 15 candidate genes in *HCRT* positive neurons using the single-nuclei RNA-sequencing (snRNA-seq) HYPOMAP dataset generated from hypothalamic brain tissue from eleven unique donors (Tadross et al., 2025). We found that *HCRT* positive neurons express significantly higher levels of *NPVF, AVP, MGC4294/LINC01711, OXT, NPB* and *C22orf42* than other *HCRT* negative cells within the HYPOMAP dataset. The remaining nine candidate genes show no biologically relevant difference in expression level when comparing *HCRT* positive cells to *HCRT* negative cells (Table 3 and Fig. 3). While the differential expression analysis identifies many additional genes with significant enrichment in *HCRT* positive cells (Fig. 3 upper right dots), those are not *per se* relevant since this analysis does not consider their expression levels outside the hypothalamus. In conclusion, six out of 15 candidate target antigens are highly specific for expression in *HCRT* positive cells and share a brain expression pattern highly similar to *HCRT*. Both *AVP* and *NPVF* are, like the GTEx and BrainScope scoring, two of the strongest associations and form interesting candidates.

3.3. Associated genes described in literature do not co-express with hypocretin

Next to our *in silico* analysis of transcriptomic databases, we performed a literature search for genes associating with HCRT-producing neurons or NT1, and evaluated their expression similarity with the *HCRT* gene using the expression data from the BrainScope portal. The literature search consisted of evaluating results from a PubMed search and the gene-disease association scores in the DisGeNet database. The PubMed search resulted in 94 articles, of which 9 articles were original studies that linked expression of specific genes to HCRT neurons (Degn et al., 2017; Dommer et al., 2019; Holm et al., 2015; Honda et al., 2009; Khajavi et al., 2023; Sasson et al., 2006; Shimada et al., 2020; Tanaka et al., 2010; Wiemerslage et al., 2017). An additional 9 articles were added by the citation chaining method (Blouin et al., 2005; Crocker et al., 2005; Faraco et al., 2013; Han et al., 2013; Hohjoh et al., 2000; Juji et al., 1984; Miyagawa et al., 2008; Seifinejad et al., 2023; Toyoda et al., 2015). As a result of the PubMed search, the 18 articles indicated a total of 37 genes as being associated with HCRT neurons or NT1 (Table S2). Data from the DisGeNet database was used to find genes that have previously been described in the literature to be associating with NT1. DisGeNet indicated 17 genes, other than *HCRT*, that associate with NT1 with a gda-score ≥ 0.2 (Table S2). Nine of these genes overlap with the outcomes of the PubMed search (Fig. 4 and Table S2).

The genes described in literature have been evaluated for their expression pattern in BrainScope portal and GTEx to evaluate similarity with the *HCRT* gene. None of the genes identified from the PubMed search or DisGeNet selection overlap with the 15 candidate genes resulting from section 3.2 or all the 142 genes that were indicated by BrainScope portal to have the most similar expression pattern with *HCRT* (Table S2 and Fig. S2). Nevertheless, visual inspection of all genes resulting from the literature search in the BrainScope data reveals that the genes *PENK* (only identified by DisGeNet), *PDYN* and *IL10RB* (only identified by the PubMed search) show an expression pattern that is relatively close to the 142 selection (Fig. S2). However, these genes

Table 3
Expression level of candidate genes in *HCRT* positive and *HCRT* negative cells.

Gene	log2(FC): (*HCRT* positive *vs* negative)	Expressed in % of *HCRT* positive cells	Expressed in % of *HCRT* negative cells	Adjusted P-value
NPVF	5.72	1.6	0.1	1.18×10^{-28} *
AVP	2.04	49.6	9.2	6.47×10^{-189}*
CALCR	−2.80	3.3	8.8	1
GHRH	−0.49	1.6	0.6	1
HDC	0.04	17.9	3.8	4.23×10^{-47} *
MGC4294 / LINC01711	3.75	1.2	0.1	9.19×10^{-10} *
NTS	−1.37	4.7	3.1	1
OTP	−0.24	1.6	2.4	1
ST8SIA2	−2.35	1.2	4.2	1
KISS1R	0.92	3.5	2.8	1
OXT	1.89	25.9	4.1	3.56×10^{-110} *
NPB	2.80	14.8	3.0	8.34×10^{-43} *
ZNF114	−1.49	0.5	1.2	1
C22orf42	1.22	6.8	2.6	5.31×10^{-4} *
TLE6	1.06	5.4	3.7	1

The 15 candidate genes within the single-nuclei HYPOMAP are listed and their expression levels in HCRT positive and HCRT negative cells are compared. *adjusted p-value is significant with $\alpha \leq 0.01$. A difference in expression level is considered biologically relevant when the log2(FC) > 0.5 or < -0.5.

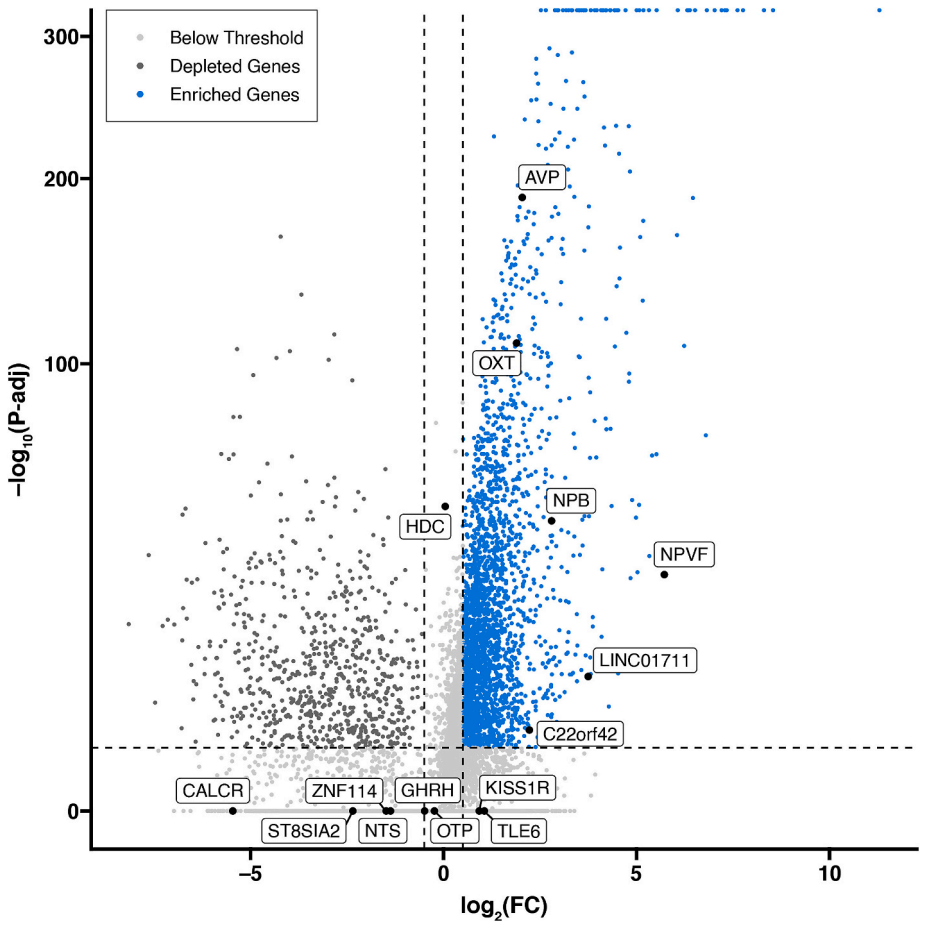

Fig. 3. Expression of candidate genes within hypocretin cells. *NPVF, AVP, MGC4294/LINC01711, OXT, NPB* and *C22orf42* are more expressed in *HCRT* positive than in *HCRT* negative hypothalamic cells within the single-nuclei HYPOMAP dataset. The adjusted *p*-value is significant with $\alpha \leq 0.01$. A difference in expression level is considered biologically relevant when the log2(FC) > 0.5 or < -0.5.

show no specific expression in the hypothalamic structures. The data from three genes (*TCRA, TPGS2/PGs2* and *TCRB*) where not available in the BrainScope portal and therefore not further investigated. The genes *CHKB* (indicated by both the PubMed search and DisGeNet database) and *PDYN* (indicated by the PubMed search, not by DisGeNet) show higher expression levels in *HCRT* positive neurons compared to *HCRT* negative cells in the hypothalamus, based on the snRNA-seq HYPOMAP dataset (Fig. 4). *CHKB* and *PDYN* do not appear in the results from the combined BrainScope-GTEx strategy, nor within the 142 genes with the highest *HCRT* expression similarity in BrainScope portal. In conclusion, the literature based search shows very limited overlap with the expression atlas data, with CHKB and PDYN forming the only overlap between datasets.

4. Discussion

Here we present a new strategy to search for potential autoimmune targets in narcolepsy combining expression pattern overlap with *HCRT* in the human brain from publicly available *in silico* databases with literature-based candidate selection. Consistent with earlier reports *HCRT* expression is mostly limited to hypothalamic tissues. We hypothesized that alternative targets for an autoimmune response leading to the development of NT1 can be identified by searching for genes with a *HCRT-like* expression profile. We employed multiple independent expression databases and identified 15 candidate genes: *NPVF, AVP, CALCR, GHRH, HDC, MGC4294/LINC01711, NTS, OTP, ST8SIA2, KISS1R, OXT, NPB, ZNF114, C22orf42* and *TLE6*, of which the first five show an expression pattern most similar to *HCRT*. A snRNA-seq dataset showed more expression of *NPVF, AVP, MGC4294/LINC01711, OXT, NPB* and *C22orf42* within *HCRT* positive cells than in other cells, which increases interest in these six candidate genes. *HDC, OTP, ZNF114 and C22orf42* are expressed intracellularly. Because NT1 has a strong HLA-association (Juji et al., 1984), intracellular antigens could be considered of special interest. Intracellular antigens can only be targeted by T-cells after presentation through the HLA system, whereas extracellular antigens are rather targets for B cells (Janeway et al., 2001). However, extracellular antigens cannot be excluded.

The presence or absence of some candidate antigens seems to contradict earlier (rodent) research. On the one hand, several genes emerge as candidates in our *in silico* approach using human data, due to their specific and localized expression near *HCRT*, even though they are described to be present in other cell types close to, but probably different from, HCRT neurons in rodent data. For example *OXT* and *AVP* are mainly expressed in the PVN and the supraoptic nucleus (Grinevich and Ludwig, 2021). On the other hand, some expected genes described to be colocalizing with HCRT, like *NARP*, *PDYN* and *QRFP* (Chou et al., 2001; Crocker et al., 2005; Seifinejad et al., 2023), do not appear in the results because they are also expressed in other brain regions and therefore lack specificity. Furthermore, some candidate antigen genes are described in earlier literature and show functional consistency or inconsistency with the NT1 phenotype, as is detailed below.

Even though not all 15 candidate genes are extensively described in the literature, there are some studies that show functional consequences of gene expression that are in line with the NT1 phenotype. Functions of *NPVF* gene products are described in several animal models. They are related to sleep duration (Lee et al., 2017; Madelaine et al., 2017),

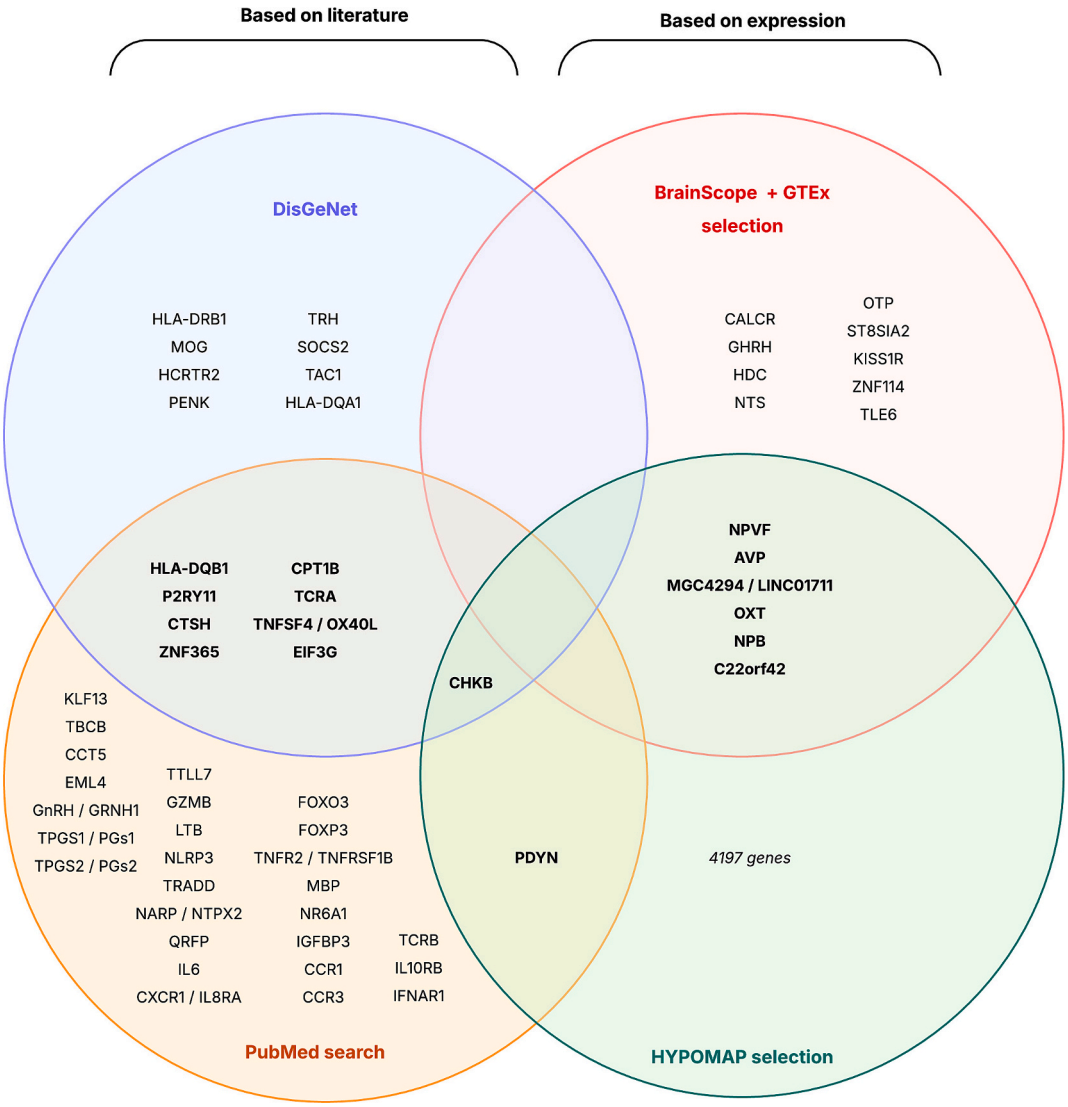

Fig. 4. Venn diagram from genes associating with hypocretin in literature and expression datasets. The genes resulting from the literature search do not overlap with the 15 candidate genes most similar in expression to hypocretin in BrainScope portal.

feeding, energy metabolism (Koller et al., 2021) and sensitivity to pain (Ubuka, 2023). Additionally, administration of *Npvf*'s products leads to increased CRH-production. A massive reduction of CRH-positive neurons within the paraventricular nucleus (PVN) have been observed in the post-mortem brain tissue of people with NT1 (Shan et al., 2022). Therefore, potential loss of *NPVF* expression in NT1 may be involved in this reduction of CRH neurons within the PVN. *In vivo* experiments on the co-expression with *HCRT* is an important indicator for the plausibility of the 15 candidate genes to be a target in an auto-immune response in NT1 as an alternative for *HCRT*. In zebrafish, *Hcrt* and *Npvf* are both expressed in the dorsomedial periventricular zone of the hypothalamus (Sagi et al., 2024). Earlier studies in zebrafish show that *Npvf* is specifically expressed next to the Hcrt-producing neurons but co-localization was not detected (Madelaine et al., 2017; Yelin-Bekerman et al., 2015). However, a recent study (Sagi et al., 2024) using snRNA-seq demonstrates that a subset of *Hcrt* positive neurons do colocalize *Npvf* and show that those *Hcrt*-positive-*Npvf*-positive cells differ from other Hcrt-producing neurons – without *Npvf* expression – regarding genetic profile, projections and activity patterns. In mice, *Hcrt* is mostly expressed in the lateral hypothalamus while *Npvf* is expressed in the dorsomedial hypothalamus. Consistently, colocalization was not observed in mice (Sagi et al., 2024) and rats (Legagneux et al., 2009). *Nts* is in zebrafish expressed in cells close to the *Hcrt* positive neurons in the lateral hypothalamus, but also expressed in 10%–25% of Hcrt-producing neurons itself (Leinninger et al., 2009; Levitas-Djerbi et al., 2015). A mouse study revealed that 82% of the *Hcrt* positive cells in the lateral hypothalamus co-express *Nts*' gene product neurotensin and showed that 84% of Hcrt positive cells express *Nts* mRNA. Also, 27% of the neurotensin producing neurons in lateral hypothalamus expressed *Hcrt* mRNA (Furutani et al., 2013). This favors the possibility that *NTS* has a role in the destruction of HCRT-producing neurons in an autoimmune attack.

Besides *NPVF,* no co-localization experiments have been performed regarding the other 14 candidate genes but relevant literature about *AVP, GHRH* and *HDC* is available. Similar to *NPVF, AVP* is also linked to CRH in the PVN since a subset of these CRH neurons co-express *AVP* (Scott and Dinan, 1998). However, the AVP-positive cells in the post-mortem tissue of people with NT1 are mostly spared (Shan et al., 2022), which makes *AVP* less plausible as candidate gene target.

Despite the existing literature describing a role for *GHRH* in sleep regulation, most literature point towards effects that are not in line with an autoimmune hypothesis with *GHRH* as target gene in NT1 development. *GHRH* shows to have sleep promoting effects (Steiger, 2003) and the secretion of growth hormone – which is released by *GHRH*'s gene product– correlates with slow-wave sleep (Overeem et al., 2003).

Furthermore, rat studies with Hcrt peptide administration show inhibition of *Ghrh* expression and release of growth hormone (Lopez et al., 2004; Seoane et al., 2004). However, antibodies against *Ghrh* in rodents reduces both non-REM and REM sleep periods during the inactive light period of the day (Obal Jr. et al., 1992) and suppressed growth hormone secretion has been observed during sleep onset in people with NT1 while having sleep onset REM periods (SOREMPs) (Higuchi et al., 1979).

Furthermore, *Hdc*-knock out (KO) mice show an overall normal development, but they seem less reactive and have greater body weight than WT mice. Furthermore, the *Hdc*-KO mice had a shorter sleep onset time, and their wake and slow wave sleep periods are more fragmented. *HDC* seems important for maintaining the wake state (Parmentier et al., 2002). Pathway analysis also show its involvement in the synthesis of the wake-promoting neurotransmitter histamine, which is decreased in the CSF of people with NT1 (Bassetti et al., 2010; Kanbayashi et al., 2009). However, investigation of postmortem brain tissue of individuals with NT1 show rather an enhancement of histaminergic cells (Shan et al., 2023). Since these observations partially overlap with the NT1 phenotype, *HDC* remains a relevant target gene in the autoimmune response in NT1 development. *NTS* is also related to the NT1 phenotype since its gene product neurotensin is involved in metabolism, pain modulation and stimulates CRH release (Leinninger et al., 2011; Mazzocchi et al., 1997). Blocking the Nts receptor effects in rodent models results in reduced Hcrt cell activity, reduced wake time (Furutani et al., 2013) and increased REM sleep during the active dark phase (Fitzpatrick et al., 2012).

Additionally to the identification of candidate genes, we assessed the expression pattern of genes that were suggested by earlier (genome-wide association) studies (Degn et al., 2017; Dommer et al., 2019; Faraco et al., 2013; Han et al., 2013; Hohjoh et al., 2000; Holm et al., 2015; Honda et al., 2009; Juji et al., 1984; Khajavi et al., 2023; Miyagawa et al., 2008; Sasson et al., 2006; Shimada et al., 2020; Tanaka et al., 2010; Toyoda et al., 2015; Wiemerslage et al., 2017) to potentially co-express with *HCRT*. None of the genes resulted from these studies described in the literature showed an expression pattern close to *HCRT* in either the AHBA (BrainScope portal) or GTEx portal. This suggests that their gene products are unlikely to be involved in eliciting an auto-immune response specifically targeting HCRT neurons, and they were not selected using our approach. However, some showed an expression pattern that was still relatively close to our selection in BrainScope (see Fig. S2). For example PDYN, PENK and IL10RB. PDYN and PENK encode precursor proteins for several opioid neuropeptides and are mainly expressed in the brain (Benko et al., 2021; Horikawa et al., 1983; Konig et al., 1996). IL10RB encodes a cytokine receptor and is therefore related to the immune system (Sheppard et al., 2003). See Table S3 for more details on the genes resulting from the literature search.

Combining our findings *NPVF* is the most promising candidate because it demonstrates the most similar expression pattern to *HCRT* based on BrainScope and GTEx portal data, it shows increased expression within *HCRT* cells according to a snRNA-seq dataset and earlier findings in the literature are in line with a hypothesis of deficient *NPVF* in NT1.

Our strategy also holds important limitations. For example, the AHBA contains data from only six individuals, of which five were female. Furthermore, in this exploratory study, we devised a scoring system to evaluate all genes in the same way. We have used the HYPOMAP dataset as a first validation step, but further research is necessary to determine the extent to which the current rules are biologically relevant. Moreover, among the 142 genes that most strongly correlate with the expression of the *HCRT* gene in BrainScope, there were five that did not exist in the GTEx portal*: AC099759.1, LOC100286952, LOC100294396, LOC283867* and *LOC442381*. Also, the data for three out of 42 genes resulting from the literature search and DisGeNet was not available in the BrainScope portal: *TCRA, TPGS2/PGs2* and *TCRB*. Therefore, these genes could not be further analyzed with our approach. However, expression information of the TPGS2/PGs2 gene is available in GTEx portal and does not show any specificity to the hypothalamic region in the brain. Therefore, TPGS2/PGs2 is not a likely candidate. Furthermore, TCRA and TCRB are also unlikely targets for an autoimmune reaction, as they both encode proteins that are part of the T-cell receptor protein complex.

To conclude, the *in silico* methods used in this study yielded novel genes of interest in the context of NT1. The identified candidate genes may potentially play a role in the auto-immune process of NT1. It will be important to validate our candidate targets by for example co-localization studies of *HCRT* in post-mortem brain tissue of healthy people and people with NT1 and through identification of B- or T-cells that recognize the gene products of these candidate genes in NT1 patients. Furthermore, it should be noted that our *in silico* approach is a dynamic technique since it depends on the data that is available in any of the databases used in this process. The more data will become available, the more detailed the information will be that can be retrieved by using our methodology.

5. Conclusion

Using a unique combination of *in silico* methods, we were able to map the human expression profile of *HCRT* and identify 15 candidate genes that have expression patterns associating *HCRT* expression, of which 6 also showed higher expression within hypocretin cells compared to other cells in the hypothalamus. Those genes may be alternative targets for an auto-immune response in the destruction of HCRT-producing neurons as seen in NT1. Based on our analysis, *NPVF* is the most promising candidate gene since its expression pattern is most similar to *HCRT*, the gene shows enhanced expression in HCRT-positive cells, and earlier findings show its involvement in various aspects of the NT1 phenotype. Future experiments should confirm whether the identified candidate genes are indeed targets of an autoimmune response causative of NT1.

Data statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Marieke Vringer: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Ahmed Mahfouz:** Writing – review & editing, Resources, Methodology, Conceptualization. **Maartje G. Huijbers:** Writing – review & editing, Visualization, Conceptualization. **Gert Jan Lammers:** Writing – review & editing, Conceptualization. **Jari Berkhout:** Visualization, Formal analysis, Data curation. **Frits Koning:** Writing – review & editing, Conceptualization. **Rolf Fronczek:** Writing – review & editing, Conceptualization. **Mink Schinkelshoek:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jneuroim.2026.578936.

Data availability

Data will be made available on request.

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Glossary

AHBA: Allen Human Brain Atlas
CRH: Corticotrophin releasing hormone
Gda-score: Gene disease association-score
GTEx: Genotype Tissue Expression
HCRT: Hypocretin
HLA: Human leukocyte antigen
KO: Knock-out
NT1: Narcolepsy type 1
PVN: Paraventricular nucleus
snRNA-seq: Single-nuclei RNA-sequencing
SOREMPs: Sleep onset REM periods
TPM: Transcripts per million