

A review of the risk factors, pathophysiology, diagnosis and treatment of narcolepsy

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SUMMARY

Narcolepsy is hypothesized to be an autoimmune disease targeting the hypocretin/orexin producing neurons within the hypothalamus and is categorised into subsets based on the symptoms presented. Epidemiological studies have proposed that there is an additional requirement for an environmental trigger combined with potential genetic predisposition to trigger the onset of this spectrum of disorders. Despite epidemiological evidence which suggests the condition arises due to a breach of immunological tolerance, the absence of a defined autoantigen remains an element of debate in the aetiology of the disorder. Currently there are no definitive diagnostically useful genetic variants observed in narcolepsy sufferers which could be exploited at disease onset to support its diagnosis or clinical management

The most impactful genetic risk factor is the presence of human leukocyte antigen (HLA) allele, DQB1*06:02, which increases the risk of Narcolepsy over 20-fold. The discovery of the strong association of HLA-DQB1*06:02 and disease onset was the original trigger to shift the focus from its classification as a neurological disease to that of an immune-mediated condition. However, the presence of HLA-DQB1*06:02 alone cannot be the sole genetic risk factor to explain disease onset. Primarily, not all narcolepsy patients express DQB1*06:02 (>95%) and more importantly not all people who express DQB1*06:02 suffer from narcolepsy – only 16% of the global population who express DQB1*06:02 suffer from Narcolepsy. This has resulted in low usage of this definition test in the clinical setting due to its lack of specificity and because it is of most clinical utility if the result is negative, reducing the likelihood of a diagnosis of narcolepsy.

The diagnosis of narcolepsy is often suspected from the clinical history of the patient, supported by the results of a multiple sleep latency test (MSLT). Combined with the determination of hypocretin/orexin in the cerebral spinal fluid, these two tests are still considered the gold standard tests for diagnosis. However, there are also documented issues surrounding both tests in terms of level of sensitivity, specificity and potential complications which limit their reliability as a diagnostic resource. This review focuses on emerging evidence of genetic and environmental risk factors linked with narcolepsy onset, their link to its pathophysiology and we speculate how genetic traits could be better utilised to effectively aid a diagnosis.

Keywords: Diagnosis; Narcolepsy; Pathophysiology; Polymorphism

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INTRODUCTION

Narcolepsy is a rare, life-long disabling neurological disorder that affects 1 in 3000 individuals. It is characterised by excessive daytime sleepiness and presents as a result of a loss of hypothalamic hypocretin producing neurons [1]. A cohort of sufferers also exhibit a sudden loss of muscle tone while awake triggered by strong emotions, termed cataplexy. Patients with narcolepsy can also experience disturbed night time sleep, sleep paralysis, hypnagogic hallucinations and/or hypnopompic hallucinations [2]. The term hypnagogic describes the period when an individual falls asleep, whereas hypnopompic describes the period when a person awakens. The onset of narcolepsy is often seen during early adolescence or adulthood. The rate of concordance in monozygotic twins for NT1 onset is 20-30% [3]. Onset of the condition in a first-degree relative of a NT1 sufferer is approximately 1-2% and the relative risk for a first-degree family member of a NT1 sufferer is 10- to 40-fold higher than the general population [3].

Narcolepsy has a severe impact on the quality of life for sufferers. Excessive daytime sleepiness consists of periods of an irrepressible need to sleep during times which would normally be characterized by wakefulness, leading to sleep attacks that are promoted by sedentary activities and physical inactivity [4]. Furthermore, symptoms of narcolepsy may cause complications in relationships and family life. Narcolepsy often goes undiagnosed for many years and even following a diagnosis, it can still take time to understand and manage symptoms. Female sufferers who become pregnant may have to alter current medication as some have been shown to pose risks to the unborn child.

In 2014, the international classification of sleep disorders (ICSD3) categorised narcolepsy into two subtypes, narcolepsy type 1 (NT1) and narcolepsy type 2 (NT2) based on the presence or absence of hypocretin proteins in the patient's cerebrospinal fluid (CSF) [5]. Hypocretin (also termed orexin) is the major biomarker associated with narcolepsy [2]. Hypocretin is produced in the lateral hypothalamus, derived from the protein precursor prepro-hypocretin, which in turn is cleaved enzymatically into two peptides-hypocretin 1 and 2 [5]. Hypocretin receptors 1

and 2 (HCRTR1 & HCRTR2) are the natural ligands for hypocretin 1 and 2 respectively [6].

Patients diagnosed with NT1 have cataplexy with an absence or low levels of hypocretin 1 in their CSF, whereas NT2 patients do not have cataplexy and have normal levels of hypocretin 1 in the CSF. Patients who show low levels of hypocretin but do not exhibit the symptoms of cataplexy are also included in the NT1 category. A select group of narcolepsy patients who also suffer with cataplexy have normal levels of hypocretin, and are also categorized as NT1 by ICSD3 [7]. Previous studies have shown that the onset of NT1 is likely due to a selective loss of hypocretin producing neurons located in the hypothalamus. NT2 shares numerous clinical similarities with NT1 but currently the pathogenesis remains unknown.

The global prevalence of narcolepsy comprises a mixture of both the NT1 and NT2 subtypes. Research has shown that males have a higher risk of developing narcolepsy than females [8], but existing epidemiological studies have included too few numbers for sex differences to be fully conclusive.

INHERITED RISK FACTORS

Human leucocyte antigens

The human leucocyte antigens (HLA) are cell-surface proteins responsible for regulation of the immune system. They are highly polymorphic including many allelic and phenotypic variants encoded by classical and non-classical loci. The classical loci are further divided into two classes, namely I and II. HLA class I loci include HLA-A, HLA-B and HLA-C, whereas class II include HLA-DRB1, -HLA-DQB1 and HLA-DPB1. The main function of HLA is to present foreign peptides to the immune system stimulating a targeted immune response. A further role of HLA is to maintain self-tolerance preventing the immune system attacking self-antigen. The HLA genes are encoded within the major histocompatibility complex (MHC) on the short arm of chromosome six – 6p21.3. The major genetic association with narcolepsy is HLA-DQB1*06:02 which is present in more than 95% of confirmed narcolepsy cases [9,10]. HLA-DQB1*06:02 is a high frequency allele, with as many as 27-32% individuals in English and European populations carrying this HLA type, and therefore a large percentage of the population could potentially be affected. HLA-DQB1*06:02 is in strong linkage disequilibrium (LD) with HLA-DQA1*01:02 and together these two genes encode separate chains which combine to form a complete HLA antigen. HLA-DQA1*01:02 is also seen in LD with other HLA-DQB1*06 alleles (including DQB1*06:03, 06:04 and 06:09) with no increase in frequency of narcolepsy onset. HLA-DQB1 is a HLA class II molecule, the primary function of which is to present antigen to CD4⁺ T cells, indicating that CD4⁺ T cells could play a central role in the pathogenesis of narcolepsy.

HLA allele frequencies vary between populations.

This also supports the association of HLA-DQB1*06:02 with the onset of narcolepsy, as populations in which there are fewer individuals who have this HLA type (for example, Greece and India) also see lower incidence of narcolepsy. Other HLA class I genes have been shown to be associated with the onset of narcolepsy including HLA-A*11:01, HLA-B*35:01 and HLA-C*04:01 [11]. In several populations, HLA-B*35:01 and C*04:01 are often inherited together, and these HLA Class I genes may also be inherited in combination with HLA-DQB1*06:02 as an extended haplotype. The role of HLA class I is to present antigens to CD8⁺ T cells suggesting a potential cytotoxic element to the pathogenesis of narcolepsy involving activated cytotoxic T cells. The associations of both HLA class I and II alleles may suggest that a full HLA haplotype of alleles inherited in combination may be linked with an increased risk of narcolepsy onset. However, DQB1*06:02 is a common allele in the general population, the presence of which alone is insufficient for narcolepsy onset. The λ value for HLA of narcoleptic patients was calculated to be between 2 and 4 which is below the relative risk in first degree family members [9]. This implies that additional genetic factors are involved in the genetic predisposition to narcolepsy onset.

T cell receptors

Polymorphisms detected in the genes encoding the T cell receptor alpha (TRA) on chromosome 14q11.2 have also been suggested to predispose patients to narcolepsy [12]. The T cell receptor (TcR) binds foreign or self-peptides presented by HLA allowing the stimulation and regulation of immune responses. In 2009, a study of 1,614 confirmed narcolepsy cases compared against 2,148 controls from mixed European, United States and Canadian ancestry showed that three single nucleotide polymorphisms (SNPs) within the TRA gene—rs12587781 ($p = 1.90 \times 10^{-13}$), rs12636646 ($p = 4.86 \times 10^{-12}$) & rs1154155 ($p = 1.90 \times 10^{-13}$) are associated with NT1 [12]. This genome wide association study (GWAS) demonstrated an increase in relative risk of narcolepsy onset if any or a combination of these three SNPs are present. All three TRA SNPs are located within the encoding region of the J segment which is central to the process of VDJ recombination. During thymic education, T cells undergo somatic recombination to increase the diversity of the TcR. Recombination of both TRA and T cell receptor beta (TRB) chains results in the generation of T cell clones with a unique complete TcR. TRA recombination occurs between the 5' end located within one of the 46 functional variable (V) regions and the 3' end located in one of the 49 functional joining (J) segments. Added to this, variation is also introduced by amino-acid junctional diversity generated by N and P inclusions in the V-J border region. Diversity in the TRB is able to produce an even more expansive repertoire of proteins as it is a result of the recombination of 48V, 2D and 13J segments [13]. Identification of these SNPs via GWAS suggests that the mutated forms of these markers may be involved in non-random VJ choices during the process of recombination, however this remains unproven

[12]. These findings coupled with the HLA-DQB1*06:02 association reinforce an indicative autoimmune basis of narcolepsy pathophysiology.

In 2009, an ImmunoChip consortium was formed to create a SNP array to target fine-mapping of loci associated with various autoimmune conditions [14]. The ImmunoChip was designed to allow deep replication from large-scale meta-analyses in nine autoimmune conditions. Using this platform, the consortium conducted a collaborative GWAS to identify genetic risk factors associated with narcolepsy in addition to HLA-DQB1*06:02 [14]. The group investigated a total of 111,240 SNP markers with a minor allele frequency of $\geq 1\%$ which were located outside the HLA region on chromosome 6. 1,886 confirmed narcolepsy cases were analysed for these markers and compared against 10,421 healthy controls of mixed European, United States and Canadian ancestry. The results showed that the strongest association with narcolepsy was a SNP located on chromosome 14 in the TRA region (rs1154155). Further fine-mapping using the ImmunoChip showed that this SNP was located close to the J10 segment of the locus confirming the results published earlier that year [12]. There are, however, weaknesses in the ImmunoChip approach. Primarily, the chip is designed for use in white European populations and is therefore less informative for other ethnicities. Another limitation of this study lies with the use of the ImmunoChip in that it does not cover the whole genome and utilises data from earlier GWAS studies for the selection of markers included in the assay [15].

Other immunoregulatory genes

A polymorphism within the P2Y purinoceptor 11 (P2RY11) gene on chromosome 19 has also been reproducibly associated with an increased risk of narcolepsy onset [16-18]. In 2010, a GWAS investigation conducted by a worldwide collaboration in 7122 patients showed that a SNP located downstream of P2RY11 (rs2305795) was associated with the condition [17]. P2RY11 is a member of a large family of more than 20 purinergic receptors. The purinergic signalling pathway plays a fundamental role in immune regulation, proliferation, apoptosis and chemotaxis in lymphocytes and monocytes [19,20]. P2RY11 is a low affinity receptor and detects high concentrations of extracellular adenosine triphosphate (ATP) [17]. ATP is traditionally localised intracellularly, however, during inflammation, ATP levels rise within the extracellular space and produce a cascade of concentration-dependent effects on the immune system [21]. In humans, P2RY11 expression is marked in brain tissue and leucocytes, in particular CD8⁺ T cells and natural killer cells [22]. It has been suggested that immune cell death in the presence of high ATP is controlled by a balance of multiple purinergic receptors including P2RY11 [17]. Narcoleptic patients who carried the mutated version of P2RY11 had a less efficient P2RY11 function because the presence of the minor allele (A) of rs2305795 results in decreased P2RY11 expression and therefore decreased sensitivity of

CD8⁺ T cells to an ATP-induced cell death. The association between the minor allele of P2RY11 and onset was only seen in individuals of European ancestry despite Asian and African American populations also being studied. Furthermore, P2RY11 expression is predominantly seen in CD8⁺ T cells which account for approximately ~20% of peripheral blood mononuclear cells (PBMCs). Within whole blood, P2RY11 is expressed in granulocytes, which account for 65% of all leucocytes and may reduce the ability to identify differences in the expression of P2RY11 in the CD8⁺ T cells. Hence, attempts to confirm a direct role in narcolepsy onset for this mutation in isolation may prove inconsequential.

Holm and co-workers showed that a nonsynonymous mutation in exon 21 of the DNA methyltransferase 1 (DNMT1) gene, also encoded on chromosome 19, causes a rare hereditary form of narcolepsy-associated deafness and cerebellar ataxia [23]. The P2RY11 and DNMT1 genes are in linkage so it was suggested that both mutations could be related at the pathophysiological level. This was proven not to be the case, but rs3826784 located in the intronic region of the eukaryotic initiation factor (EIF3G) gene was the most significant within the region investigated. Eukaryotic initiation factors initiate eukaryotic translation by forming a complex with the 40S ribosomal subunit and recruiting 43S to the preinitiation complex [24]. This complex recognises and binds the 5' cap structure of mRNA and regulation of the AUG initiation codon [25]. The polymorphic EIF3G gene is in high LD with P2RY11 but not with DNMT1. DNMT1 also plays a role in the differentiation of CD4⁺ T cells into regulatory T cells (Tregs) [26]. A mutation causing a lack of expression of DNMT1 could consequently lead to an absence of Treg cells and may result in a loss of immunological tolerance. The discovery of the EIF3G mutation is of particular interest as findings have implicated a role of the H1N1 pandemic virus of 2009 as a trigger for narcolepsy development [16]. The disruption of the EIF pathways seen in H1N1 influenza cases could indirectly alter the immune response to the infection and increase narcolepsy risk and the severity of symptoms in individuals who are homozygous for the mutated variant of rs3826784 (G:G).

Cathepsins are enzymes primarily located within the lysosomal/endosomal compartment of professional antigen presenting cells (monocytes, dendritic cells, B cells) [27]. They play an important role in the exogenous pathway of antigen processing which concludes with the loading of peptides onto the HLA class II protein [27]. HLA class II is then expressed on the surface of a professional antigen presenting cell initiating cellular interactions leading to elimination of the pathogen. Deficiencies in selected cathepsins have been shown to impair immune cell development resulting in defective immune effector cell function [27]. Cathepsin H (CTSH) is one of eleven cathepsins and is unique with instrumental importance in both exopeptidase and endopeptidase activities. It is expressed at high levels in immune cells which are also HLA class II positive, most notably B cells, monocytes and

dendritic cells. A global collaborative study of immune gene markers led by Faraco and co-workers in 1886 patients with narcolepsy identified two SNPs (rs34593439 and rs3825932) located in the intronic region of the gene encoding Cathepsin H located on chromosome 15 [14]. The SNP rs3825932 has a C substituted for T introducing the negatively charged amino acid alanine that could affect trafficking or the cleavage of peptides for presentation. It is speculated that changes in CTSB may alter the HLA class II peptide repertoire that is presented to CD4+ T cells. This altered repertoire of presented peptides may promote the recognition of a self-antigen, increasing the risk of developing narcolepsy.

SNPs within the Tumour Necrosis Factor ligand superfamily member 4 (TNFSF4) have also been linked with narcolepsy [14]. Located on chromosome 1, rs7553711, identified using the ImmunoChip assay, was more strongly associated with the onset of narcolepsy in Faraco's study than any other SNP [14]. Point mutations of the TNFSF4 gene are also strongly linked with other autoimmune conditions including systemic lupus erythematosus (SLE) and systemic sclerosis [28,29]. Polymorphisms upstream of the TNFSF4 gene confer susceptibility to SLE, whereas the polymorphisms identified in narcoleptic patients are located further downstream of rs7553711. The association of narcolepsy with SNPs in the TNFSF4 gene is consistent with a primary role of antigen presentation to T cells in the pathogenesis of narcolepsy. TNFSF4 is primarily expressed

on the surface of HLA class II positive professional antigen presenting cells and acts as a costimulatory ligand during the formation of an immune synapse. Changes in the structure of TNFSF4 may therefore affect the configuration of this synapse and the subsequent immune response to infection, increasing the risk of an autoimmune condition developing, including narcolepsy.

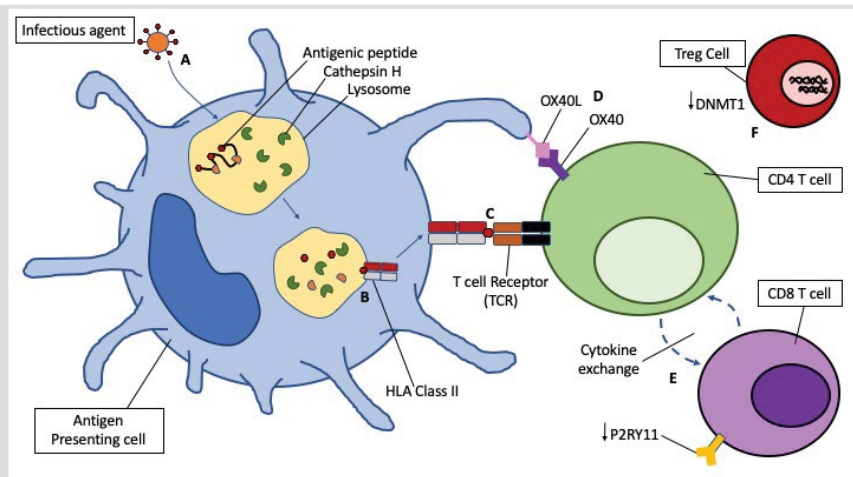
The genetic factors mentioned all encode molecules which have an essential role in the immune system (Fig. 1). The altered immunogenetic factors create an immune environment which, in the presence of an environmental agent (e.g., an infection) combine to cause selective damage to the hypocretin producing neurons. However, despite growing evidence from genetic studies in global populations of patients with narcolepsy, the specific genetic and environmental triggers of narcolepsy onset remain undetermined.

ENVIRONMENTAL RISK FACTORS

Vaccines

The most widely documented potential environmental trigger of narcolepsy is the activated monovalent vaccine used between 2009 and 2010 to protect against the H1N1 influenza virus. The H1N1 virus first emerged in Mexico and California in April 2009 and quickly spread globally and was declared a pandemic by the World Health

Fig. 1. Immunological and genetic factors leading to narcolepsy onset – A proposed mechanism. (A) Infectious agent (e.g., H1N1 or *S. pyogenes*) is phagocytosed by a professional APC, taken into a lysosome and deconstructed, a process facilitated by cathepsin H, (B) Antigenic peptides are loaded onto HLA class II antigen (potentially expressing DQB1*06:02) and are then expressed on the surface of the APC, (C) The APC forms an immune synapse with a CD4+ T cell. HLA class II ligates with a TCR, composed of TCRA and TCRB portions, (D) Co-stimulation occurs and OX40 ligates with OX40L (amongst other interactions seen during the formation of an immune synapse) allowing the T cell to become active, (E) Activated CD4+ T cells secrete various pro-inflammatory cytokines to activate CD8+ cytotoxic T cells, promoting a Th1 phenotype. The less pronounced nature of a mutated P2RY11 gene and lack of subsequent protein allow the loss of self-tolerance, potentially driven by CD8+ T cells and (F) DNMT1 plays an important role in differentiation of CD4+ T cells into T regulatory cells. Potential absence/mutation of functioning gene, leading to the absence of Treg cells could result in autoimmunity.



Organisation in June 2009 [30]. A MF-59-adjuvanted vaccine (Focteria) produced by Novartis and A (H1N1) pdm09 termed Pandemrix produced by GlaxoSmithKline were distributed to several European countries. Alternate inactivated monovalent unadjuvanted vaccines were administered in the United States of America, and the AS03-adjuvanted Arepanrix was dispersed to Canada. Arepanrix and Pandemrix had similar pH1N1 antigens and used the same AS03 adjuvant, however the manufacturing processes for the two vaccines differed. More than 1300 people who received the Pandemrix vaccine developed narcolepsy [31]. GSK acknowledged the link and some patients were awarded compensation. However, the mechanism of action that led to narcolepsy onset following vaccine administration remains unclear. Shortly after the administration of Pandemrix (August 2010) the increased incidence of NT1 was reported firstly in Finland [32], then Sweden, followed by Norway, France, Ireland and the United Kingdom [33-36]. The incidence reported was seen mainly in children and young adolescents in certain populations the relative risk for vaccinated versus non-vaccinated was significantly higher [37].

A multi-country assessment of the incidence of narcolepsy and the adjuvanted pandemic H1N1 vaccines was published in 2018 [38]. Based on a case-control analysis, it has been demonstrated that other than an increased narcolepsy incidence rate after the vaccination campaigns in Sweden, no other associations between AS03 or MF59 adjuvanted pH1N1 vaccines was seen [40]. Several limitations within this retrospective study include weak statistical power due to low vaccine coverage in some of the participating countries, diagnostic misclassification due to differing procedures, and factors that may have included selection bias.

The incidence of NT1 in China sharply increased 4 months after the 2009 H1N1 pandemic with rates returning to normal immediately afterwards [16]. This led to an observational study suggesting that genetic associations with onset of a condition can change in response to a new environmental trigger. The study strengthened the association with SNPs located in the TRA and P2RY11-DNMT1 complex and identified three new polymorphisms associated with onset (rs9648789, rs10995245 & rs2252931). A limitation with observational studies like this one by Han and co-workers is that bias cannot be completely excluded and no clear relationship to the vaccine and narcolepsy onset is acknowledged. Theoretically this may imply that the H1N1 virus alone could be the triggering environmental factor in an Asian population. This theory has been suggested by others who demonstrated that the influenza virus is capable of damaging hypocretin neurons in immunodepleted mice resulting in a narcolepsy- resembling phenotype [39].

An international study conducted in 2015 set out to investigate the effect of Pandemrix and determine potential links to the onset of narcolepsy [40]. They concluded that differences in the viral proteins used to construct the vaccine may explain the association. The discovery of antibodies

directed against a protein fragment contained within the influenza virus used in the synthesis of the Pandemrix vaccine supported this theory. The fragment, which is part of the influenza nucleoprotein, cross reacted with the hypocretin receptor 2 (HCRTR2), which has shown to be associated with narcolepsy [41]. Levels of HCRTR2 antibodies were much higher in the sera of Finnish patients who had been vaccinated with Pandemrix compared to sera from normal Italian individuals who had been administered Focteria to combat the H1N1 infection. Antibodies to the HCRTR2 cross-reacted with influenza nucleoprotein peptides in 17 of the 20 samples tested from people who had since been diagnosed with narcolepsy following the Pandemrix vaccine in 2009. Further studies revealed that the influenza antigen content in the Pandemrix vaccine had significantly higher levels of the influenza nucleoprotein compared to Focteria, which had 72% less nucleoprotein than Pandemrix. However, the production of antibodies and subsequent destruction of the HCRTR2 was not suggested as a cause of the condition. One hypothesis is that the antibodies produced could occupy the HCRTR2 sites within the brain, preventing the binding of hypocretin to its natural ligand [42]. It was also reported that five of the twenty cases investigated had antibodies directed toward the nucleoprotein but did not develop narcolepsy, posing questions to the clinical relevance of these findings. Also, despite discovering an antibody against HCRTR2 post vaccine, it does not explain the selective loss of neurons seen in narcolepsy.

An investigation into the differences between Pandemrix and Arepanrix found a higher amount of structurally altered viral nucleoprotein in Pandemrix than Arepanrix resulting in an increased response to the nucleoprotein in narcoleptic children [42]. A fundamental flaw of this study was that the viral component of each vaccine was manufactured in different countries so a direct comparison of the proteomic differences cannot be performed. The purification methods employed by the manufacturers differed which may explain the variation in the structure. An additional study showed significant associations between post-Pandemrix narcolepsy cases and HLA class II genes as well as a SNP marker (rs12587781) in the gene coding for the TRA protein [37]. The genetic associations observed indicated a strong gene-environment interaction in which either antigenic constituents or the adjuvant within the vaccine used may be involved. A comprehensive meta-analysis published in 2018 showed a 5- to 14-fold increase in the incidence of narcolepsy in children and adolescents and a 3- to 7-fold increase in adults in the countries where Pandemrix was administered [43]. Based on the cases reported it was calculated that the vaccine attributable risk of narcolepsy was significant in children and adolescents (1 per 18,400). The presence of HLA-DQB1*06:02 would increase this risk to 1 case per 4500 doses administered.

Ultimately, the exact mechanism of vaccine triggered narcolepsy remains unknown. The adjuvant AS03 suggested by some research groups is an attractive model but other vaccines containing the AS03 adjuvant are not associated

with increased risk of narcolepsy [43]. The discovery of antibodies directed toward the hypocretin receptor 2 in a group of Finnish patients who were administered the Pandemrix vaccine implicates a molecular mimicry hypothesis. These antibodies may react with the influenza nucleoprotein but their role in the pathophysiology of narcolepsy remains controversial. Another caveat to the potential impact of the Pandemrix vaccine on the increased risk of narcolepsy is that despite the clear relationship between the two, narcolepsy is still considered a rare disease and the benefits of the vaccine outweighs the associated risks of narcolepsy. Of the approximately 40 million vaccinated since the outbreak, 30 million received Pandemrix [44] and of these, only 1,333 confirmed cases of narcolepsy were reported [31] generating an incidence rate of 0.0044%.

Streptococcal infections

Streptococcal infections are usually benign and self-limiting, but potentially invasive diseases with associated, well documented post-streptococcal disorders [45]. Post-streptococcal disease has been linked to neurological autoimmune conditions including Sydenham chorea [46] and encephalitis lethargica [47]. Taking this into consideration it has been suggested that the onset of streptococcal infections may initiate an autoimmune response against hypocretin producing neurons leading to the development of narcolepsy. It has been postulated that the streptococcal infections may lead to the destruction of neurons via molecular mimicry or via superantigen interactions with the HLA-TCR complex [45]. Another suggested hypothesis is that the streptococcal infections increase the permeability of the blood brain barrier, exposing the hypocretin-secreting neurons to the immune system, allowing more specific factors to trigger narcolepsy [45]. Streptococcal infections have been shown to increase the production of IL-17, a cytokine mainly produced by Th17 cells [48], which in turn has been shown to increase the permeability of the blood brain barrier [49].

A population-based case-control study detected anti-streptococcal antibodies in 65% of patients within one year of narcolepsy onset which were still elevated 1-3 years later [45]. For comparison, in uncomplicated streptococcal infections the antibody titres are reported to increase after 2 weeks, peak at 2-4 months and then decrease [50]. The persistence of these antibodies in narcoleptic patients may be due to an inability to clear the infection, possibly as a consequence of their genetic background which is able to promote and sustain a narcolepsy inducing immune reaction. This may also further qualify the role of the strongly associated HLA-DQB1*06:02. It has been shown that the presence of this HLA allele shows a degree of protection against septic shock following streptococcal infections, suggesting a superior immune response to this bacterium [51]. These findings also suggest structural similarities between *Streptococcus pyogenes* and hypocretin [5].

The cellular arm of the immune response has also been shown to play a role in narcolepsy onset post-streptococcal infection. Ambati and co-workers published a study in 2015

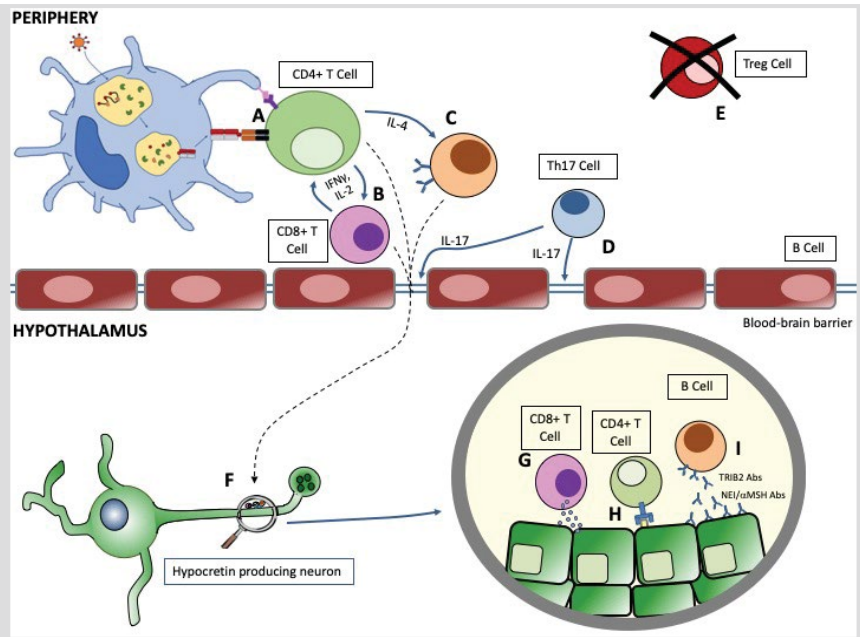
demonstrating evidence of an increase in the amount of IFN γ in the peripheral circulation of confirmed narcolepsy cases in response to stimulation with streptodornase B (a streptococcal protein) and streptococcus M proteins [52]. This suggests a pro-inflammatory Th1 phenotype is involved in streptococcal induced narcolepsy cases. In contradiction, no inflammatory processes, including lymphocytic infiltration have been observed in the hypothalamus during post-mortem brain analysis of long-term disease sufferers putting this hypothesis into question [53,54].

PATHOPHYSIOLOGY

The underlying pathological mechanism of NT1 is the loss of hypocretin signalling. The hypocretin system is made up of ~70,000 neurons producing hypocretin located in the hypothalamus. All narcoleptic patients are born with these neurons and the hypocretin deficiency develops later in life. The exact mechanism that leads to the destruction of the hypocretin neurons in narcoleptic patients is still unknown. The only potential proposed hypothesis for the development of narcolepsy is due to a loss of immunological tolerance—the autoimmune hypothesis. An autoimmune basis for narcolepsy has long been suspected and data from genetic, epidemiologic and immunologic studies strongly suggest this to be the case. The development of an autoimmune condition is multifactorial and includes both underlying genetic predispositions combined with an environmental trigger(s) (Fig. 2.). In narcolepsy, both have been demonstrated with strong genetic associations with HLA-DQB1*06:02 and other immune based polymorphisms, as well as suggested environmental triggers including streptococcal infections, H1N1 infections and H1N1 vaccination.

In order to prove that a disease or condition is the result of a loss of immunological tolerance, the presence of autoreactive immune cells or autoantibodies must be demonstrated and an autoantigen identified. Despite many years of research, it remains unclear how the genetic differences and environmental triggers actually contribute to the onset of this condition. As a result, very few studies in narcolepsy have produced confounding evidence to establish the autoimmune pathogenesis. In 2010, two independent studies showed the presence of antibodies against tribbles homologue 2 (TRIB2)—a protein produced by many cells including hypocretin neurons in narcoleptic patients [55]. These autoantibodies were originally described in uveitis and were found to have higher titres in a small group of narcoleptic patients when compared to healthy controls [56]. This was the first study to report the impact of the humoral immune response in narcolepsy. The impact of TRIB2 autoantibodies is debatable and other studies have shown that the levels of these antibodies are not increased in confirmed narcolepsy cases [57]. Also, as TRIB2 is expressed on other cell populations both in the CNS and periphery, these antibodies are unlikely to have a direct role in the onset of narcolepsy. TRIB2 antibodies have also been

Fig. 2. The Autoimmune Hypothesis. (A) Professional antigen presenting cell interacts with a CD4+ T helper cell, resulting in activation (B) Activated CD4+ T cells orchestrate an effective immune response by secreting cytokines. IFN γ and -IL-2 are released to promote a proinflammatory, cytotoxic response (Th1), (C) IL-4 can also be secreted by an activated CD4+ T cell that promotes a Th2 phenotype. Activated B cells secrete antigen specific antibody, (D) Activated local Th17 cells secrete IL-17 which is able to increase the permeability of the blood brain barrier, allowing immune cells to penetrate into the hypothalamus, (F and E) Suppression of regulatory T cells, possibly to pre-existing genetic mutations allows an autoimmune response to occur, (G) Activated cytotoxic T cells are able to injure hypocretin producing neurons through the granule exocytosis pathway, (H) CD4+ T cells are able to directly bind to potential autoantigens on the surface of the neuron, promoting a localised immune response and (I) B cells secrete TRIB2 and NEI/ α MSH inflicting further damage to the neuron.



detected in individuals who do not suffer from narcolepsy adding further question to the relationship between TRIB2 antibodies and disease onset. It has been postulated that TRIB2 antibodies may be produced as a downstream effect of neuron loss. Passive transfer of antibodies from narcoleptic patients to murine models leads to sleep behavioral disturbances or neurological changes [58] but the specific mechanism by which these antibodies are able to induce changes in the brain remains unknown.

Autoantibodies directed towards the C-terminal epitope of neuropeptide glutamic acid-isoleucine/ α -melanocyte-stimulating hormone (NEI/ α MSH) have also been identified in narcoleptic patients following vaccination with Pandemrix [59]. Investigation of narcolepsy patients' sera or cerebral spinal fluid using immunohistochemistry found a significant number to have NEI/ α MSH antibodies which were passively transferred into rats and their electroencephalography (EEG) and behaviour analysed. Results showed an increase in sleep fragmentation when purified IgG antibodies were transferred from narcoleptic patients that was not observed for the control group. Apart from sleep fragmentation, no other symptoms of narcolepsy were seen [59]. Despite the presence of potential autoantibodies in narcoleptic cases, none of the corresponding antigens (TRIB2, NEI/ α MSH, anti HCRT receptor 2) are exclusively expressed in hypocretin producing neurons. This, plus the observation that these antibodies have been detected in some healthy controls, creates debate as to their role in the pathogenesis of narcolepsy.

In 2013, an article by De la Herran-Arita and co-workers was published suggesting that hypocretin was an autoantigen. The group demonstrated functional analysis of CD4+ T cells from narcoleptic patients that were able to recognise hypocretin peptides presented by dendritic cells homozygous for the heterodimer HLA-DQB1*06:02/DQA1*01:02. These cells could also recognise peptides

derived from H1N1 suggesting a potential link due to molecular mimicry between hypocretin peptides and similar peptides from H1N1. Unfortunately, the authors were unable to replicate the findings resulting in the retraction of the article [60].

Different studies of the autoimmune origins of narcolepsy have yielded alternative hypotheses to determine the etiology of the condition. Based on the facts that there are both genetic and environmental links with the disease there is a strong evidence base to support narcolepsy being an autoimmune condition. The majority of the evidence that contributes to this theory suggests that it is mainly driven by the humoral response. This is further supported by the absence of autoreactive CD4+ T cells in confirmed narcoleptic cases [61] and a lack of T cell infiltrations in affected hypothalamic areas seen in the majority of post-mortems [62]. In the absence of evidence, a defined cellular response cannot be completely ruled out. The lack of cellular infiltrations seen during post mortems could simply be explained if the neurological damage was historic, completed many years previously and recent studies which have shown a global increase of activated T cells in NT1 patients retain the potential for a role of the cellular immune response [63,64]. Nevertheless, crucial pieces of information are missing to fulfil the autoimmune hypothesis, including the lack of an autoantigen, a self-derived structure for the immune system to target. Further work into the autoimmune pathogenesis is required to determine the autoantigen in a condition that shows all the traditional aspects of an autoimmune condition without the essential trigger.

DIAGNOSIS

The current diagnostic classification for narcolepsy is outlined in the third edition of the International Classification of Sleep Disorders (ICSD3) published in 2014 [7]. Often patients attend sleep clinics long after symptom

onset and often following several incorrect diagnoses. The diagnosis of narcolepsy may be suspected from the clinical history of the patient but is confirmed with an overnight polysomnography, followed by a multiple sleep latency test (MSLT). The overnight sleep study helps to rule out other potential causes of daytime sleepiness and in people with narcolepsy it may reveal fragmented, light sleep and an early (within the first 15 minutes) transition into rapid eye movement (REM) sleep. During the MSLT the patient is encouraged to fall asleep every 2 hours for 20 minutes over an 8 to 10-hour period. Patients who suffer with narcolepsy will tend to fall asleep in less than 8 minutes compared to healthy individuals who take on average a minimum of 15 minutes [65]. Combined with the shorter time to fall asleep, patients with narcolepsy often have REM sleep during at least two of the daytime naps during the MSLT, whereas healthy individuals rarely have any daytime REM sleep [66]. The presence of sleep-onset REM periods (SOREMPs)-the occurrence of REM sleep within 15 minutes is key in the diagnosis of narcolepsy. The MSLT is the gold standard in the diagnosis of narcolepsy and as a result must be performed under the strictly defined conditions. Medications that suppress REM sleep should be discontinued well in advance of the MSLT and the patient should try to attain a sufficient amount of sleep the night before the test. A significant group of patients tested do not fully meet the criteria for a diagnosis of narcolepsy. This group of patients show and suffer the same symptoms as narcoleptic patients (excessive daytime sleepiness, hypnagogic hallucinations, hypnopompic hallucinations) but fail the MSLT (lack of two incidences of REM sleep). These patients are often diagnosed with idiopathic hypersomnia and the symptoms are managed with a working diagnosis of NT2.

Despite the clear and strong link between HLA-DQB1*06:02 and narcolepsy, the presence of this allele is of limited diagnostic value due to a high background population frequency, it is more beneficial as a negative predictor. Measuring hypocretin 1 levels in the CSF is a highly specific and sensitive technique for the diagnosis of narcolepsy (NT1 only). When compared to a recognised reference standard, hypocretin 1 levels of ≤ 110 pg per ml is highly specific for a diagnosis of NT1 [7]. Care must be taken when interpreting these results as low hypocretin 1 values in the CSF are also seen in patients suffering other non-sleep related conditions [67]. CSF levels must, therefore, be used to help build a diagnosis when combined with the MSLT and overnight polysomnography.

The Brighton Collaboration in 2013 published the criteria for the diagnostic accuracy of narcolepsy for patients who showed symptoms of the condition post vaccination with Pandemrix [68]. The case definition and levels of severity are broken down into three levels and covers criteria including the presence of excessive daytime sleepiness, CSF hypocretin deficiency, results of the MSLT and SOREMP. The recently developed Narcolepsy Severity Scale (NSS),

a self-administered scale that evaluates the severity of the condition is a welcomed and accurate approach to assess NT1 symptom severity, however the NSS is only useful following the confirmation of disease diagnosis [69].

TREATMENT

The management of narcoleptic patients can be achieved using both pharmacological and non-pharmacological agents. Often a combination of both are employed in order to manage the condition. Non-pharmacological agents largely consist of developing set behavioural strategies. Each strategy is individualised to each patient and consists of avoiding sedentary activities, maintaining a regular night time routine and planning daytime naps [70]. Caffeine and energy drinks are also often consumed to fight bouts of excessive daytime sleepiness but are not sustainable and have been shown to have sideeffects which are detrimental to subsequent sleep [71].

Antidepressants are widely used for the treatment of NT1, particularly for the treatment of cataplexy. However, despite their popularity, antidepressants have never been proven for indication in narcolepsy in controlled clinical trials. Antidepressants act by blocking norepinephrine and serotonin reuptake resulting in a reduction in REM sleep and cataplexy [72,73]. Both serotonin and norepinephrine increase alertness, promote vigilance and focus attention. Antidepressants are well tolerated and very few suffer side effects although some experience issues including weight gain, diarrhoea and anorexia [73]. There is however a severe risk of cataplexy rebound if antidepressants are suddenly removed from a patient's treatment plan [74].

Various pharmacological agents are approved by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) that are available for NT1 sufferers. Modafinil is one of the first lines of treatment of narcolepsy and helps promote wakefulness; however, the mechanism of action remains undetermined. The drug is long lasting with a half-life of 13.8 hours and the maximum concentration in the periphery is achieved in 2-4 hours [73]. Overall, Modafinil is well tolerated in both adults and children and side effects are rare and mild. Modafinil can be combined with other agents such as sodium oxybate to improve narcolepsy management. Sodium oxybate is the sodium salt of gamma hydroxybutyrate and is seen as another front-line therapy. Sodium oxybate activates the GABA type B receptor, but the precise mechanism of sodium oxybate remains to be elucidated [75]. Sodium oxybate has been shown to effectively improve sleep quality, increase slow-wave sleep and promote wakefulness [70]. Additionally, sodium oxybate is the only medication approved by the FDA and EMA for the treatment of cataplexy. However, sodium oxybate is not approved in the UK, and NICE approval is required. Randomised control trials and meta-analysis have shown that sodium oxybate is currently the only available drug that can improve all of

the core narcolepsy symptoms, and reduce the frequency of cataplectic episodes [76]. The half-life of sodium oxybate is only 40-60 minutes but the effects of the drug persist much longer [73]. The administration of the drug is a staged process, with low amounts (4.5g/night) taken initially and then increased over a number of subsequent weeks (increase of further 1.5g/night) to achieve the desired dosage (max 9g/night). The drug is well tolerated if titrated correctly, but adverse side effects have been observed (if incorrectly titrated) including dizziness, headache, nausea, weight loss and depression. Due to the power of sedation of this drug, patients are advised to administer at the bedside.

When both modafinil and sodium oxybate are ineffective, second line therapies like methylphenidate can be administered. Methylphenidate (also known as Ritalin) is a stimulant that blocks the re-uptake of monoamines, primarily dopamine, allowing it to remain in circulation for longer. Despite its wide utilization only one evidence-based study has shown the significance of methylphenidate in the treatment of narcolepsy [70]. Again, like modafinil and sodium oxybate, the incidence of side effects is low but some users can suffer tachycardia, hypertension, anxiety and weight loss [77]. Another pharmacological agent recently approved by the EMA but not the FDA is Pitolisant. Pitolisant is a histamine H3 receptor agonist able to activate histamine neurons and has been shown to improve wakefulness in healthy animals and decrease daytime sleepiness in orexin knockout mice [78]. Like methylphenidate, Pitolisant is seen as an alternative therapy if modafinil and sodium oxybate are ineffective. The drug is generally well tolerated by both adults and children. Amphetamines are considered a third line therapy for narcolepsy. They are able to increase the concentration of dopamine and norepinephrine and have been shown to reduce excessive daytime sleepiness. However, dextroamphetamine is the only amphetamine approved for treatment of narcolepsy [73,79].

The treatment options for NT2 include modafinil, sodium oxybate and methylphenidate as the main symptom is excessive daytime sleepiness, but often less severe than NT1. The reduced severity of symptoms is often reflected in the lower dosages administered.

FUTURE DIRECTIONS

The past several years has seen great advances in understanding the pathogenesis, underlying genetics, and development of new therapeutic agents. However, many questions remain unanswered. Future work should be directed toward the clarification of the pathological process that destroys the hypocretin producing neurons. All the current data suggests that NT1 is an autoimmune condition. All the factors are present for this to be the case;

the genetic association, an environmental trigger and an increase in peripheral immune cells have been classified. Despite this, the lack of the elusive autoantigen remains a constant frustration of researchers. Discovery of an autoantigen would aid in the production of new effective therapeutic drugs that target the condition rather than treating the symptoms and may potentially result in the development of a cure.

Interestingly, despite the increasing evidence promoting an autoimmune hypothesis little success has been made with treatments that are able to manipulate the immune system. Therapies including corticoids, intravenous immunoglobulin, plasmapheresis, immunoadsorption have all been tested with variable efficacy [80-82]. These therapies target the humoral arm of the immune response potentially suggesting that antibodies are weakly associated with the development of narcolepsy. However, a single patient case study was published showing that intravenous immunoglobulin treatment administered 15 days after disease onset fully reversed the symptoms of the narcolepsy and cataplexy [83]. This highlights the importance of early diagnosis and if treated early the symptoms may be reversed and enough hypocretin neurons may be saved to prevent progression to disease. Future work should also focus on the adaptive side of the immune system to determine how significant a role it plays in development of narcolepsy and immunomodulatory agents may be synthesised as a result.

For NT2, very little is known and the condition is poorly defined. Further work in this area should focus on the pathogenesis and the causative agents. The main issue in narrowing the gap in knowledge of NT2 is the poor classification of the condition in a group of highly heterogeneous patients. Genetic profiling of patients suffering from NT2 or idiopathic hypersomnia may provide further clarification on the condition and should be transferred into the clinical setting. The severity of the condition may be linked to the number of genetic mutations an individual possesses or the strength of the initial environmental trigger or a combination of both. A clearer understanding pre-diagnosis combined with the NSS may allow for a more specific and less invasive diagnosis as well as giving the clinical team scope to tailor the treatment options.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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