SHARED GENETIC PATHWAYS BETWEEN MULTIPLE SCLEROSIS AND ISCHEMIC STROKE: A REVIEW OF THE LITERATURE

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Abstract

Multiple sclerosis (MS) and stroke are both neurological diseases that affect the central nervous system (CNS) and lead to long-term motor and sensory deficits and cognitive impairment. Both diseases detrimentally affect the quality of life of patients and their families. Clinical studies on patients with MS have revealed an increased incidence of any type of stroke, including ischemic stroke (IS), hemorrhagic stroke and transient ischemic attack (TIA) compared to the general population. Both MS and stroke are heterogeneous diseases that have a genetic component. As ischemic is the most frequently encountered type of stroke, the majority of available evidence on MS patients relates to IS. The increased incidence of IS in MS patients points out the need for exploration of the underlying genetic component link of both diseases. The identification of shared risk genes between the two diseases is of great importance to develop therapies that will be more effective than the currently available treatments or will be targeted at MS patients at high risk for stroke. Here, we describe the main genetic findings from genome-wide association studies that provide evidence in favour of the genetic link between MS and IS.

Key words: multiple sclerosis, stroke, ischemic stroke, genome-wide association studies

INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory progressive autoimmune disease that is characterized by neuronal demyelination and leads to neurodegeneration [1]. The incidence of MS is higher in young adults, especially women. MS is attributed to genetic, immune and environmental influences under the control of epigenetic mechanisms [2-4]. Clinical evidence suggests that the incidence and prevalence of MS are higher in the patients' families compared to the general population [5]. Thus, the lifetime risk of MS in first-degree relatives of MS index cases is estimated at 3% and is 10- to 30-fold greater than the corresponding age-adjusted risk in the general population (0.1%-0.3%) [6-8]. This in turn points to the importance of genetic susceptibility for MS onset [5]. Despite the potential genetic heterogeneity, the Class II human leukocyte antigen *HLA-DRB1*15:01* allele in the HLA gene locus on chromosome 6p21 is strongly associated with a risk for MS and potentially MS severity [5, 9-11]. The pathogenesis of MS is complicated by interactions between Class II risk alleles, including *HLA-DRB1*15:01*, and environmental stimuli [12, 13].

MS is not a Mendelian disease. Based on the theory of common disease common variant (CDCV), which underlies genome-wide association studies (GWAS), common diseases in a population are attributed to several small common genetic variations that present with a high allelic frequency in the population. It can, therefore, be postulated that the inheritance of MS is associated with one locus that exerts a moderate effect (*HLA-DRB1*15:01*) and many loci with small (modest) effects) [14, 15]. The analysis of 47,351 MS cases and 68,284 healthy controls in the largest GWAS in MS up to date revealed 233 genome-wide loci that were related to MS susceptibility [8, 16]. Of these, 200 loci were located in the non-major histocompatibility complex (non-MHC) genome and had small contribution, accounting for approximately 20% of MS genetics [8, 16]. Most of the MS-associated gene variants resided either in intronic or intragenic regions, namely in enhancers or promoters of nearby genes, and affected the regulation of immune-system related genes and immune mechanisms [8, 16]. The same conclusion is applicable to findings from GWAS studies in other inflammatory autoimmune diseasesas well that are not limited to CNS [17].

The pathophysiology of autoimmune diseases, including MS, is to some extent common to stroke [18]. The inflammatory response (neuroinflamma-

tion) that underlies acute and chronic brain diseases, including IS, MS and Parkinson's disease has been the object of investigation and is considered a potential common underlying link both early on and during disease progression [18]. Based on favorable preclinical studies, the immunomodulatory drugs natalizumab and fingolimod that are used for MS have been investigated in clinical trials for IS [19-21]. According to WHO, stroke was the second leading cause of death globally in 2019, accounting for 11% of the deaths reported worldwide, and one of the leading causes of disability [22]. There are three types of stroke: ischemic stroke (IS), intracerebral hemorrhage and transient ischemic attack [23, 24]. IS accounts for the majority (70-85%) of stroke cases [23]. The risk for stroke increases with age; its incidence is, therefore, generally higher in middle-aged and elderly people [24]. The pathophysiological mechanism of IS involves endothelial dysfunction and atherosclerotic plaque formation [24]. The etiology of stroke is heterogenous [25]. Risk factors that predispose to stroke can be both modifiable and non-modifiable and include smoking, obesity, diabetes, hypertension and hypercholesterolemia [24]. Genetics, also, contribute to the risk for stroke [25, 26]. Early GWAS studies across racial groups revealed significant correlations between stroke and *ABO* blood system locus, cardioembolic stroke and variants near *PITX2* and *ZFHX3* as well as between large-vessel stroke and *HDAC9* (histone deacetylase 9) variants and the 9p21 locus, suggesting that there might be a genetic origin in the risk for stroke, regardless of geographic and racial differences [27-31]. Subsequent GWAS have identified 35 genetic loci that conferred a risk for stroke overall or predisposed to various stroke subtypes [32, 33]. Furthermore, temporal GWAS using leukocyte counts during the first 24 hours after IS have identified that the 14q24.3 locus was associated with both leucocyte counts and IS outcomes [34]. Currently, the primary FDA-approved drug for IS is intravenous alteplase. However, thrombolysis has a limited therapeutic window and many patients with IS are not eligible because of the strict criteria for alteplase administration and the unpredictable outcomes of recanalization [20]. More emphasis should, therefore, be paid to the development of neuroprotective treatments that target other mechanisms that are implicated in IS, such as inflammation and oxidative stress [35]. It is, therefore, possible that treatments that are effectively used in inflammatory diseases of the CNS, coupled with the genetic information that emerges from GWAS studies could be exploited for the treatment of the inflammatory processes that are involved in stroke.

Clinical studies have identified an increased risk and prevalence of cerebrovascular comorbidities in patients with MS after the clinical onset of the disease compared with non-MS controls [36]. The objective of the current literature review is to describe the main findings of meta-analyses of GWAS that interconnect MS and IS. IS was used rather than stroke overall was because it is the commonest type of stroke.

ISCHEMIC STROKE IN PATIENTS WITH MULTIPLE SCLEROSIS

The meta-analysis of observational studies of various racial populations with various follow-up intervals by Hong et al. (2019) reported that both the risk and occurrence of stroke were increased in MS patients compared to the general population. IS in particular was statistically significantly more common in the MS compared to non-MS population. Additionally, the 5-year incidence of IS was 8.12/1000 person-years in people with MS and 1.48/1000 person-years in the general (non-MS) population. The incidence rate ratio of any type of stroke, including IS, hemorrhagic stroke and transient ischemic attack ranged from 2.53% to 2.85% and the incidence of IS ranged from 1.22% to 3.49% compared to non-MS individuals. Other than the common pathophysiology, common risk factors, such as obesity, and the decreased mobility that MS confers, particularly in patients with progressive forms of the disease, could account for the increased incidence of stroke in MS patients [36]. However, there is currently limited evidence on the potentially common underlying genetic component, which is increasingly investigated. Until recently, *SLC44A2* was the only common risk gene both for MS and IS. *SLC44A2* encodes solute carrier family 44, member 2 that is implicated in interleukin-enhancing binding factor 3 transcription [20, 33, 37]. The identification of shared risk genes between the two diseases has, therefore, prompted further exploration through GWAS.

GWAS IN MS AND STROKE

Li et al. (2019) performed a gene- and pathway-based meta-analysis of large-scale GWAS datasets of European/Caucasian descent to determine potential shared gene expression patterns between MS and IS. For this purpose, the large scale MS GWAS dataset from the International Multiple Sclerosis Genetics Consortium (IMSGC) derived from the Wellcome Trust Case Consortium 2 (WTCCC2) project that comprised 9,772 MS cases and 17,376 controls and the IS dataset derived from the 1000G GWAS summary results of the METASTROKE collaboration comprising 10,307 IS cases and 19,326 controls was used [33, 38]. Following identification of the significant genes for each disease ($p_{value} < 0.05$), pathway-based analysis in the following four biological pathway databases KEGG, PANTHER, REACTOME and WikiPathways as

well as GO datasets was performed [28]. The subsequent analysis revealed that MS and IS shared 9 significant ($p_{value} < 0.05$) pathways in KEGG [including the natural killer (NK) cell-mediated cytotoxicity pathway], 2 in PANTHER (the Cadherin and the Wnt signaling pathway), 14 in REACTOME [including the cell-cell communication pathway and the interferon-gamma (IFN-γ) signaling pathway), 1 in WikiPathways [the thymic stromal lymphopoietin (TSLP) signaling pathway] and 194 in GO annotations. In KEGG, the pathways could be broadly divided into six groups: immune system, environmental information processing, drug resistance and endocrine, nervous system, cancers and infectious diseases. In GO annotations the shared significant pathways concerned biological processes (85 pathways), cellular components (78 pathways) and molecular function (31 pathways). Out of all these significant shared pathways, 4 key pathways correlated with both the immune and the nervous system. These were the NK cell-mediated, the Toll-like receptor signaling (TLR), the Th1 and Th2 cell differentiation and the neurotrophin signaling pathways. The cytolytic function of NK cells is important for immune homeostasis and the regulation of immune cells of both innate and adaptive immunity. Thus, the contribution of NK cells to various autoimmune diseases, including MS, has been increasingly investigated [39-42]. The dysfunction of NK cells strongly correlates with the pathophysiological mechanisms of MS and the response of several patients to selected MS treatments [42]. One of the 3 NK subtypes, the weakly cytotoxic $CD56^{bright}$ NK cells, can acquire cytotoxic qualities and regulate immune responses via cytokine production upon stimulation [43]. Certain immunotherapies that are administered in MS, such as daclizumab and IFN-β, selectively expanded $CD56^{bright}$ NK cells that in turn correlated with decreased disease flares in MS patients [44-46]. Compared to untreated patients, NK cells from daclizumab-treated patient samples showed increased cytotoxicity toward $CD4^+$ autologous activated T cells [47]. NK cells are also important in the pathophysiology of IS [48]. The release of fractalkine by neurons in acute IS can attract lymphocytes, including NK cells in the ischemic area, and NK cells, in turn, augment neuronal death and accelerate brain infarction via the secretion of cytokines and glutamate [48]. A meta-analysis of 12 GWAS of all types of stroke revealed that the NK cell signaling pathway is the only pathway that is significantly shared by all types of stroke, including IS subtypes [28]. The TLR protein family includes pattern-recognition receptors (PRRs) that recognize microbe-specific pathogen-associated molecular patterns (PAMPs) and self-derived damaged cell-derived danger-associated molecular patterns (DAMPs) [49]. The disruption of TLR signaling is implicated in autoimmunity and inflammatory diseases, as PRRs produce immune system mediators that activate innate immune responses [49, 50]. The Wnt and the cell surface TLR2 signaling pathway are implicated in impaired remyelination in the animal model of MS (experimental autoimmune encephalomyelitis, EAE), with enhanced expression of TLR2 on oligodendrocytes in MS lesions only [50, 51]. However, the enhanced expression of TLR2 was not observed on oligodendrocytes in normal areas [50, 51]. The cell surface TLR4 can, also, promote inflammation in EAE, whereas the inflammatory response following IS was reduced in TLR4-deficient mice [52, 53]. TLR2 and TLR4 DAMP-mediated activation enhance the production of pro-inflammatory cytokines in various chronic inflammatory conditions, including autoimmune diseases [49, 53]. Furthermore, a clinical trial on IS demonstrated that the intracellular TLR7 and TLR8 correlated with poor outcomes at 3 months and infarct volume [54]. The microbiome also contributes to the regulation of innate immunity via the provision of microbial products in the systemic circulation to induce TLR2 tolerance; however, TLR2 tolerance induction is disrupted in MS patients, thus, the contribution of the microbiome in MS onset warrants further investigation [55]. Regarding the $CD4^+$ T cell differentiation into T-helper 1 and 2 (Th1 and Th2) cells in response to stimuli, through which Th1 cells are stimulated by IL-12 (interleukin-12) to produce IFN-γ and IL-2 and Th2 cells are activated by IL-4 and IL-2 to produce a range of cytokines, a shift from Th1 to Th2 cytokine production was associated with increased susceptibility to bacterial infections and conferred differential effects in infarct size in preclinical models of IS [56-58]. The susceptibility was in turn attributed to stroke-induced immunosuppression. Furthermore, the $CD4^+$ Th17 cells are also involved in the pathophysiology of autoimmune diseases via the production of IL-17[57]. Blocking IL-17 or treatment with IFN-γ in clinical trials on MS patients conferred reduction in brain MRI lesion activity or MS symptom exacerbation respectively [59, 60]. Therefore, clinical evidence supported the preclinical evidence on the effect of Th cell subsets (Th1, Th2 and Th17) in the course of MS [59, 60]. The last key shared pathway, the neurotrophin signaling pathway, is crucial for the differentiation and survival of neurons. Mammalian neurotrophins include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3) and neurotrophin 4 (NT-4) [61]. The neurotrophins activate the tropomyosin-related kinase (Trk) family of tyrosine kinase receptors (Trk) and p75 neurotrophin receptors (p75NTRs) [61]. The latter are involved in matrix remodelling and limit scar formation and are upregulated after tissue injury, such as after stroke [62]. Additionally, an increase of glial p75NTR expression in MS plaques, but not controls has been observed [63]. BDNF/Trk B family signaling

and TrkB-FL/TrkB-T1 balance have been exploited as targets for stroke therapies [64, 65], wheras the expression of the precursor of BDNF (pro-BDNF) was upregulated in circulating lymphocytes and infiltrated inflammatory cells both in clinical studies at the lesion sites of the brain and spinal cord of MS patients as well as in EAE [66]. Furthermore, the ciliary neurotrophic factor (CNTF) is neuroprotective in EAE and the cortex of MS patients [67, 68].

Tian et al. (2020) performed a gene-based meta-analysis of large-scale MS GWAS and IS GWAS with the aim to identify significant transcriptional changes in overlapping genes between MS and IS [20]. The MS GWAS dataset comprised 9,772 MS cases from IMSGC and 17,376 controls from the WTCCC2 and the IS dataset from METASTROKE comprised 10,307 IS cases and 19,326 controls (all of European descent). Overall 24 shared genes were identified and 5 genes (*FOXP1*, *CAMK2G*, *CLEC2D*, *LBH* and *SLC2A4RG*) with significant expression differences in the MS and IS datasets. The expression of *FOXP1* was elevated in both MS and IS datasets. *FOXP1* is located at 3p13 and encodes the forkhead box protein P1, a member of the FOX family of transcription factors [69]. *FOXP1* is essential to both immune system function and CNS development. FOXP1 is important in the early development and maturation of B cells and in the differentiation of macrophages and T cells via negative transcriptional modulation in the differentiation of $CD4^+$ follicular Th cells [69-72]. Pathological *FOXP1* upregulation impairs germinal center B cell function and distribution, thus contributing to lymphomagenesis [73]. Furthermore, FOXP1 is required for the FOXP3-mediated IL2-dependent function and responsiveness of regulatory T cells [69]. Preclinical studies have demonstrated that *FOXP1* affects the neurogenesis of neural stem cells (NSCs) via Notch signaling and triggers embryonic NSC differentiation *in vitro* [72] or modulates the neuronal migration and morphogenesis of cortical neurons during neuronal development [74]. The importance of *FOXP1* in neuronal development is evidenced by the identification of mutations or variants that are associated with several neurological disorders, such as Huntington disease [75], autism [76], and epilepsy [76, 77]. Bot et al. (2011) demonstrated that *FOXP1* is also expressed in various cells and is related to atherosclerotic plaque stability and severity through the transforming growth factor-beta (TGF-β) pathway. In the same study, *FOXP1* overexpression correlated positively with IL-2 and IL-4 levels, which may be of relevance to immunomodulatory diseases [78]. Based on this evidence it has been proposed that atherosclerosis could be effectively prevented and treated via targeting immunomodulatory pathways [79]. A strong association between *FOXP1* expression and MS may exist in large-vessel atherosclerosis, one of the major subtypes of IS, that should be explored [20]. This is further supported by *in vivo* evidence that *FOXP1* silencing delayed EAE onset and prevented mature dendritic cell-induced T-cell maturation [80]. Another gene with upregulated expression in both data sets was *CAMK2G. CAMK2G* is located at 10q22.2 and encodes the γ isoform of the calcium (Ca^{2+})/calmodulin-dependent protein kinase II (CaMKIIγ) [81, 82]. *CAMK2G* is implicated in vascular diseases and was reported as an enhancer gene for coronary artery disease in a GWAS meta-analysis [83]. Additionally, CAMK2G/CaMKIIγ enhanced neuronal survival in an experimental model of acute ischemia/reperfusion via activating protective signalling pathways [84]. Furthermore, the expression of *CAMKIIγ* in macrophages induces atherosclerotic plaque necrosis [85]. The third of the 5 genes *CLEC2D* that is located at 12p13.31 next to the NK gene complex and encodes the lectin-like transcript 1 (LLT1) was upregulated in MS but down-regulated in IS datasets [86, 87]. LLT1 has been reported as a negative ligand for CD161 receptor in humans [88] and suppressed CD161-mediated NK cell cytotoxicity [89] or affected B-cell activation in germinal centers [90]. Moreover, *LLT1* is expressed by TLR-activated cells of innate and adaptive immunity, such as dendritic cells or activated B cells [91]. Another shared gene with significant expression difference (downregulation in the MS and opposite alterations in IS datasets) was *SLC2A4RG* that is located at 20q13.33 and encodes the SLC1A4 regulator, a sodium-dependent neutral amino acid transporter [92]. *SLC2A4RG* is not solely expressed in neuronal cells and is implicated in early neuronal development; it may, thus, affect neurological diseases [93]. SLC2A4RG is also a TF that regulates the expression of *SLC2A4* [94, 95]. A large-scale GWAS provided evidence that *rs2256814/SLC2A4RG* is a novel gene with immune function that is related to MS susceptibility [37]. Additionally, Dhaouadi et al. (2014) reported that SLC2A4RG might enhance to a small extent the expression levels of cytokine TGF-β1 that has a protective effect in human atherosclerosis [96]. The last gene with altered expression in the datasets (downregulation in MS datasets and opposite alterations in IS) was the embryonic transcription cofactor *LBH* (limb-bud and heart) that is located at 2p23.1 and regulates cell development in various tissues [97-99]. The expression of *LBH* in neoplasms and the epithelium is in turn regulated by the Wnt signaling pathway. The latter is tightly regulated to preserve neurovascular functions and its disruption is involved in hemorrhagic stroke and traumatic brain injuries [100-102]. Interestingly, GWAS in 991 MS patients that experienced 2,231 relapses from a single institute in Europe identified a genetic variant of the Wnt signalling pathway (variant *rs11871306* of *WNT9B*) that was associated with relapse occurrence

in MS [103]. The alternations in expression patterns warrant further investigation but could be attributed to the heterogeneity of IS and its subtypes.

CONCLUSION

Science has made great progress in the past few decades. GWAS and meta-analyses have provided evidence of the genetic component of MS and stroke and revealed gene variants and genetic pathways that predispose to an increased risk for either of these neurological diseases. Determining and understanding the genetic correlations between MS and stroke can fill in the missing information on their interactions or their heterogeneity under the influence of environmental stimuli. The genetic component could also partly account for the increased risk and incidence for stroke in MS patients and complement the existing evidence on their pathophysiology link. A potential limitation in the generalization of the available data is that the majority of GWAS in any disease or trait, including MS or stroke, are performed on populations of European ancestry or self-reported as of European ancestry. Nevertheless, geographic and/or racial differences affect genetics, suggesting that genetic susceptibility could be subject to variation in diverse populations. Thus, further GWAS studies in other racial groups should be performed. It would, also, be interesting to perform temporal GWAS studies to determine if there is a genetic link that underlies IS onset and MS relapses. Conclusively, genetic research has provided us with new information and at the same time generated new questions and hypotheses that should be exploited further. This new information will guide us to the development of new targeted treatments that will be more effective or allow a more personalized treatment approach for MS patients who are more likely to suffer from stroke based on the evaluation of risk factors, environmental influence and genetic background.

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