Immune Response Profiling of Serum Identifies Autoantibodies Specific to Moyamoya Patients

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Introduction

Moyamoya Disease (MMD) is characterized by the progressive occlusion of the internal carotid arteries (ICA), as well as the proximal anterior and middle cerebral arteries (ACA and MCA respectively), together with the development of fragile, collateral vessels. Early diagnosis is often made angiographically, following clinical presentation of hemorrhage or other severe neurological deficits. The etiology of MMD is currently unknown, although several MMD-associated loci and individual gene mutations have been identified. Existing research suggests that MMD may have an autoimmune-related component, given that MMD is also associated with several other disorders, including neurofibromatosis and Graves's disease. In addition, other groups have reported elevated levels of several autoantibodies, such as anti-cardiolipin, anti-thyroid and anti–a-fodrin.

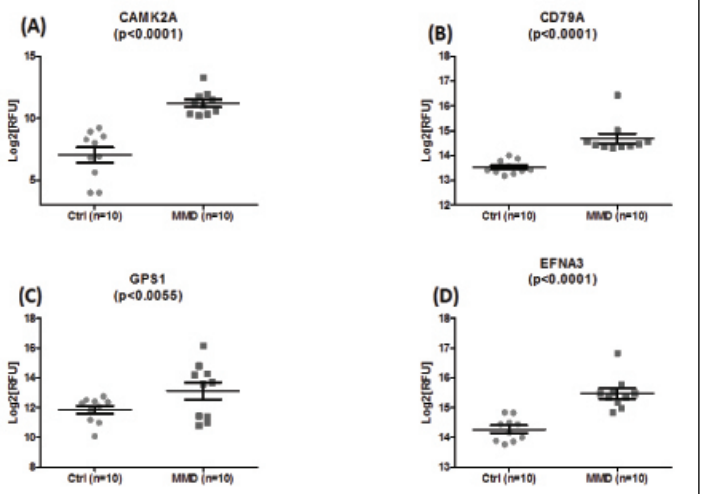
Methods

To examine the potential role of autoimmunity in MMD, ProtoArray® Human Protein Microarrays (v4.0 Invitrogen, Carlsbad, CA) were used to profile autoantibody expression in the serum of angiographically diagnosed MMD patients (n=5, matched pre- and 6 mo post-surgery; mean age 27.6±10.7 yr), compared to healthy control samples (n=10; mean age 29.3± 13.1 yr). Following array analysis, we identified MMD-specific proteins through an integration of antibodyomic, genetic, and genomic data using a bioinformatics method called Functional Interpolating SNPs (fitSNPs). This subset of autoantibodies were further validated by custom-designed ELISA in a separate group of MMD patients (n=59) and cerebrovascular control patients (n=25) which included carotid occlusion (n=9), carotid stenosis (n=7), and arteriovenous malformation (n=9).

Results

Using high density autoantibody arrays, we identified over 186 circulating autoantibodies with increased expression in the sera collected from MMD patients compared to healthy controls (Figure 1). We identified 6 autoantibodies by further filtering this dataset through an integration of antibodyomic, genetic, and genomic data using fitSNPs.

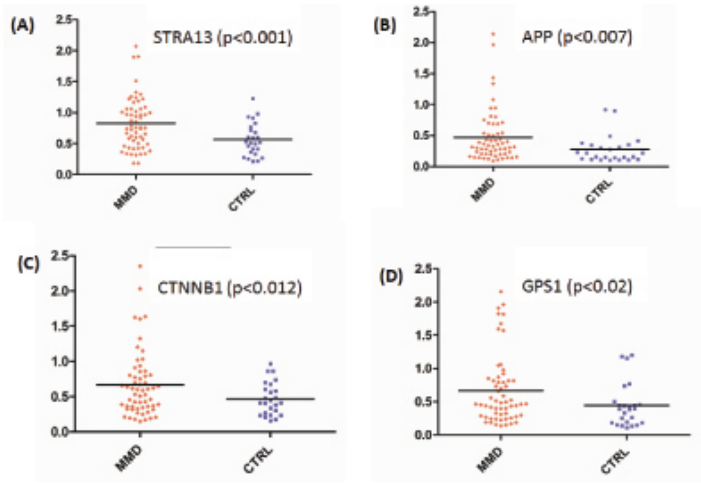
Figure 1. Autoantibody Profiling Reveals Increased Expression in Moyamoya Patients



A subset of autoantibodies were increased in MMD compared to healthy controls, including: (A) calcium/calmodulin-dependent protein kinase II alpha (CAMK2A); (B) B-cell antigen receptor complex-associated protein alpha-chain (CD79A); (C) G protein pathway suppressor 1 (GPS1); (D) ephrin-A3 (EFNA3).RFU=relative fluorescent units.

Custom-designed antibody ELISA assays were successfully generated for 4 of these MMD-specific autoantibodies. Using these ELISAs, we validated levels of autoantibodies against APP, GPS1, STRA13 and CTNNB1 in MMD patients compared to cerebrovascular disease controls (CVD) (Figure 2). A significant association with T cell receptor signaling, axon guidance, and ErbB signaling was observed.

Figure 2. Validation of Moyamoya-specific Autoantibody Expression by Custom-designed ELISAs



All four antigens were found to be significantly increased in MMD (n=59) compared to CVD control (n=25): (A) STRA13 (p<0.001), (B) APP (p<0.007), (C) CTNNB1 (p<0.012), and GPS1 (p<0.02). OD=optical density at 405 nm.

Conclusions

Autoantibodies may be produced as a result of exposure to protein antigens during tissue injury or remodeling, expression of elevated or mutated forms of a protein, or molecular mimicry. Future studies will be required to determine the biological significance of the elevated expression of these autoantibodies. These findings may provide a basis for novel diagnostic and therapeutic approaches and will advance our understanding of MMD basic disease biology.

Learning Objectives

By the conclusion of this session, participants should be able to describe and discuss the potential role of the autoimmune system in the context of Moyamoya Disease.