The Design of a Study to Evaluate Fc Biology and Genetic Diversity in Multiple Sclerosis

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OBJECTIVE

- To present the design of an epidemiological study characterizing Fc biology and genetic diversity in a large population of PwMS.

OVERVIEW IN BRIEF

- The experimental design of a multi-center study aiming to characterize polymorphisms of Fc-receptors and IgG isotypes in PwMS is described.
- Cryopreserved PBMCs will be used for DNA extraction followed by qPCR and Sanger sequencing to identify single nucleotide polymorphisms in genes of interest.
- Genetic changes will be analyzed in accordance with clinical variables to determine the prevalence of FcR and IGHG1 variants in diverse subpopulations of PwMS.

CONCLUSION

- Determining the prevalence of Fc genetic variants with potential therapeutic implications could be important for individualizing treatments for PwMS.

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BACKGROUND

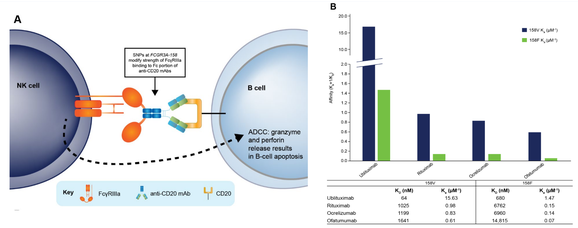
- Fc Receptors (FcRs) are expressed on various immune and endothelial cells. The Fc–FcR interaction is critical for both the function of endogenous antibodies and therapeutic monoclonal antibodies (mAbs), such as those used for the treatment of multiple sclerosis (MS).[1-3]
- Several reported genetic polymorphisms can affect the expression level, binding affinity, or function of FcRs and have been associated with response to therapy in people with malignancy or autoimmune disease.[1-4]
- Within the family of Fcγ receptors, FcγRIIIa (CD16a) is the most studied, with two major polymorphisms identified at amino acid residue 158 (F, phenylalanine and V, valine).[5-6]
- The 158F polymorphism represents the more difficult to bind variant and is expressed in approximately 40% of the healthy population.[6]
- Additional FcR polymorphisms exist, including those in CD32a, which has 2 known polymorphisms at position 131 (R, arginine and H, histidine) that have been shown to impact binding to immunoglobulin and subsequent effector function, and FcRn, which plays an important role in extending serum IgG lifespan however these are not well characterized in the context of autoimmunity.[1,7]
- Functional consequences of human immunoglobulin constant heavy G chain (IGHG) polymorphisms in the Fc region of antibodies are largely unknown, but biological importance has been suggested by multiple disease associations with this locus.[8-9]
- The frequency of FcR polymorphisms and corresponding IgG isotype alleles, have not been reported in people with MS (PwMS) or stratified according to different demographics within this population, and the potential impact on response to therapy has not been quantified.

METHODS

- This will be a multi-center study aiming to characterize polymorphisms of Fc-receptors and IgG1 isotypes.
- Cryopreserved Peripheral Blood Mononuclear Cells previously obtained from PwMS **(n=1,000)** and stored at select biorepositories at 5 academic centers will be utilized for assessment of FcR genotypes and IGHG1 alleles.
- Demographic and history of disease data will be captured in an electronic database and assessment of immune cell subsets will be conducted to correlate genotypes with immunophenotypes.

STUDY DESIGN

Figure 1. FcRyR Polymorphism Modify Binding Affinity



Clinical impact of Fc polymorphisms is not well characterized in MS

Genetic polymorphisms on FcγRs can impact binding to Fc regions on target antibodies. **(Figure 1).**
(A) FcγRIIIa polymorphisms on NK cells modulate the strength of interaction with the Fc region and determine the strength of anti-CD20 IgG1 binding. The FcγRIIIa 158F polymorphism has weaker IgG binding than the FcγRIIIa 158V polymorphism.
(B) Ublituximab, due to its glycoengineering, has enhanced affinity for all variants of the FcγRIIIa receptor, with the highest binding and relative affinity for FcγRIIIa 158V and FcγRIIIa 158F variant receptors compared with other, non-glycoengineered anti-CD20 mAbs.

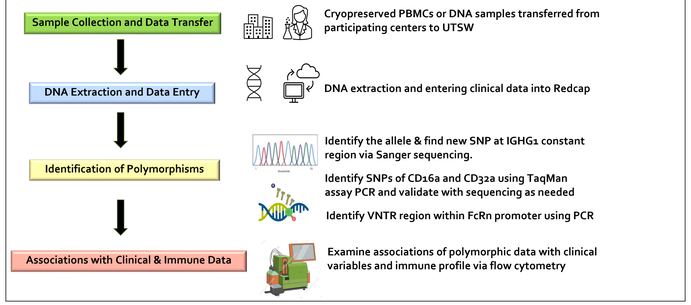
Abbreviations: ADCC antibody dependent cell-mediated cytotoxicity, *CD* cluster of differentiation, *Fc* fragment crystallizable, *FCGR* Fc gamma receptor gene, *FcγR* Fc gamma receptor, *IgG* immunoglobulin G, *Ka* association constant, *KD* equilibrium dissociation constant, *mAb* monoclonal antibody, *NK* natural killer, *SNP* single-nucleotide polymorphism.

Table 1. Study Objectives

Primary Objective: To report the frequency of polymorphisms in CD16a, CD32a, FcRn, and IgG1 isotypes within a large and diverse population of PwMS
Secondary Objective: To examine associations of polymorphism data with clinical variables and immune profiles

- The primary objective of the study is to report the frequency of polymorphisms in CD16a, CD32a, FcRn, and IgG1 isotypes within a large and diverse population of PwMS. The secondary objective is to examine associations of polymorphism data with clinical variables and immune profiles, including predefined immune cell subsets obtained by CyTOF or flow cytometry via a subgroup analysis **(Table 1).**

Figure 2. Genomic Analysis and Clinical Data Integration Workflow



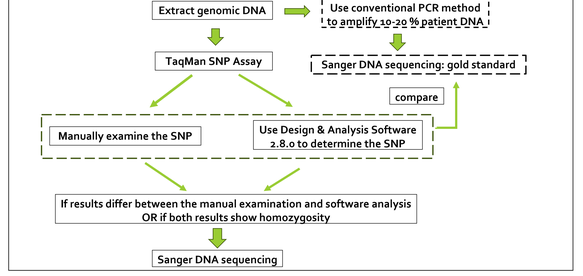
- The study schema and planned experimental design are depicted above. Assessment of FcR and IGHG1 polymorphisms will be conducted using DNA extraction followed by laboratory based Taqman qPCR and Sanger sequencing tests. Correlation with clinical variables and immunophenotypes will follow **(Figure 2).**

Table 2. Clinical Variables to be Examined for Associations with Polymorphisms

Age	Relapsing vs Progressive Diagnosis
Sex	Disease Duration
Race/Ethnicity	Therapy at time of sample acquisition
BMI	Response to current or prior mAb therapy
Smoking History	History of incomplete depletion or early B cell repopulation

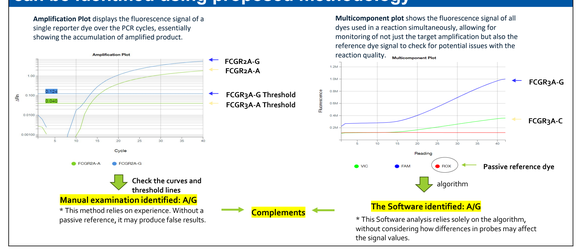
- Clinical variables available will be collected and analyzed for associations with specific polymorphisms identified in the study. These variables include age, sex, race, ethnicity, BMI, smoking history, MS diagnosis (relapsing vs progressive), disease duration, therapy at time of sample acquisition, response to current or prior mAb therapy, and history of incomplete depletion or early B cell repopulation while on prior B-cell depleting therapy, if applicable **(Table 2).**

Figure 3. Methodology for identifying FcR polymorphisms



- The experimental approach that will be used to ascertain the FcR genotypes of each individual person with MS included in the study is shown below. Methodology will vary slightly when identifying IGHG1 and FcRn genotypes, but all will involve sequencing and PCR. Feasibility experiments have been done to ensure that all assays are working correctly **(Figure 3).**

Figure 4. FCGR2A heterozygous positive control (FCGR2A-A/G) can be identified using proposed methodology



- TaqMan qPCR results for a known heterozygous FCGR2A genotype are shown below. This result confirms the ability to consistently and reliably identify the genotype of interest using a positive control sample **(Figure 4).**

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