



Fc Biology and Genetic Diversity in Multiple Sclerosis: A Multi-Center Study

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OBJECTIVE

- To determine the frequency of high- and- low binding FcR and IGHG1 variants in diverse subpopulations of PwMS (people with MS) in 2 of the 6 centers participating in this study.

OVERVIEW IN BRIEF

- Cryopreserved PBMCs from a pilot data set of 349 PwMS and 38 controls were used for DNA extraction followed by qPCR and Sanger sequencing to identify single nucleotide polymorphisms in genes of interest.
- Genetic changes were analyzed in accordance with race and ethnicity to determine the prevalence of FcR and IGHG1 variants in diverse subpopulations of PwMS.
- The frequency of high and low binding CD16a, CD32a and FcRn alleles are similar in PwMS vs controls, but when the MS group is parsed by race, discordance is observed.
- Among the IGHG1 variants, G1m17 is reduced and G1m3-1 is increased in White MS cases compared to White controls and compared to Black/AA MS cases and controls.

CONCLUSIONS

- Preliminary data suggest that variation in prevalence of both the IgG1 binding receptors (CD16a, CD32a and FcRn) and the IgG1 constant region alleles in PwMS, particularly in the context of race, may impact IgG1 binding and recycling patterns and, consequently, therapeutic responses to monoclonal IgG1 antibodies.

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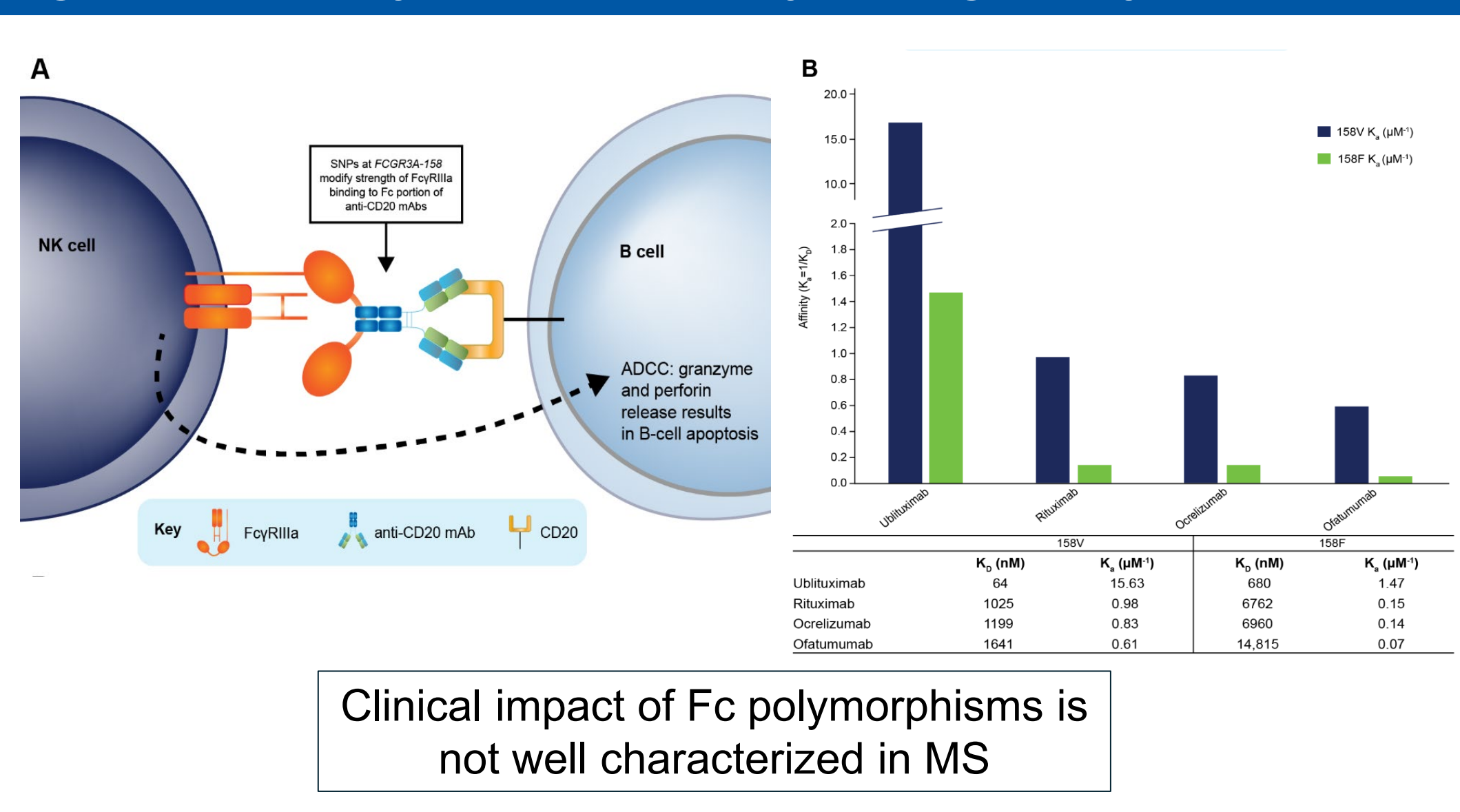
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BACKGROUND

- 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, FCGR3A (CD16a) is the most studied, with 2 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 *FCGR2A* (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 PwMS or stratified according to different demographics within this population, and the potential impact on response to therapy has not been quantified.

Figure 1. FcγR Polymorphisms Modify Binding Affinity



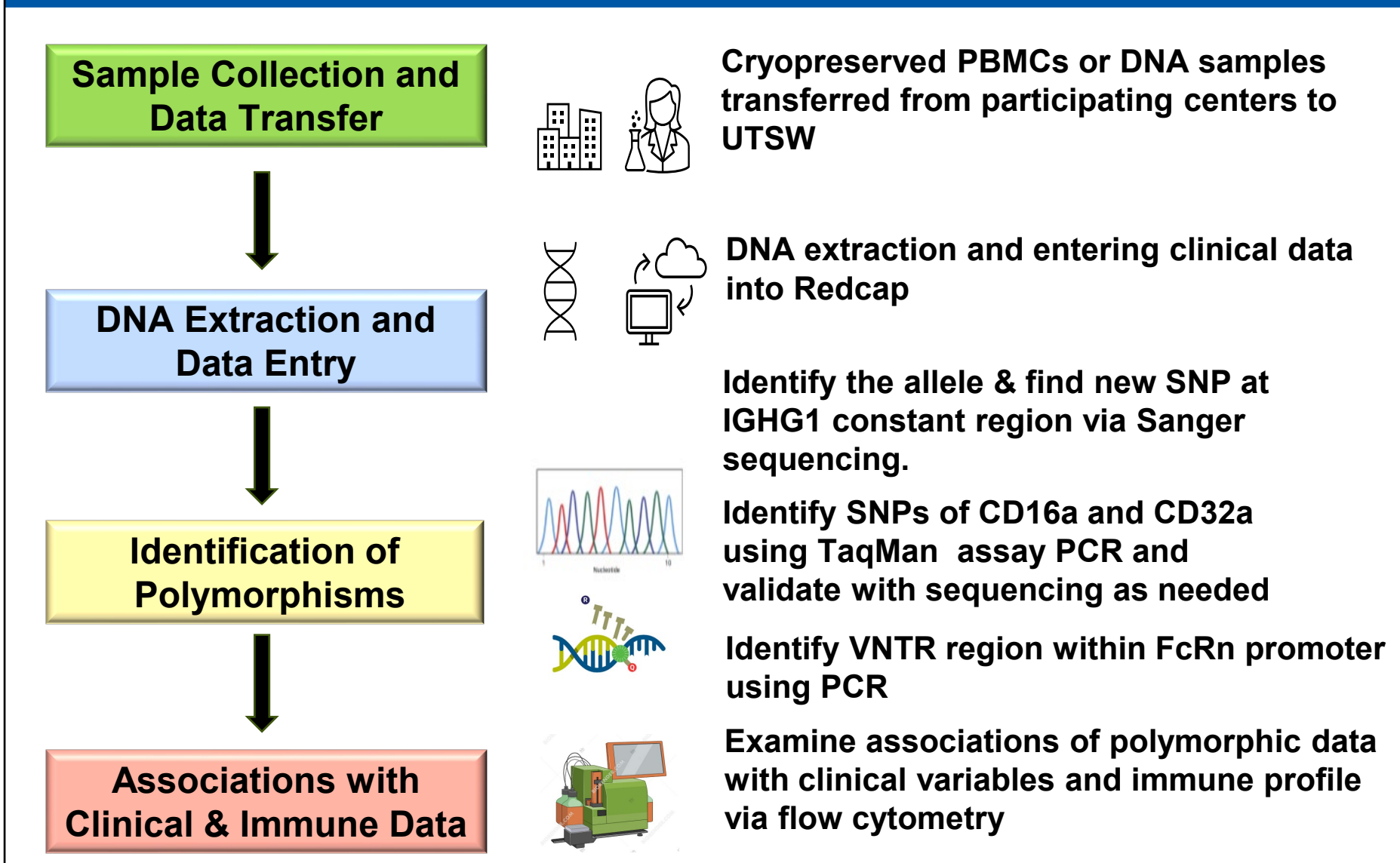
- Genetic polymorphisms on FcγRs impact binding to Fc regions on target antibodies (**Figure 1**).
- (A) *FCGR3A* polymorphisms on NK cells modulate the strength of interaction with the Fc region and determine the strength of anti-CD20 IgG1 binding. The *FCGR3A* 158F polymorphism has weaker IgG binding than the *FCGR3A* 158V polymorphism.
- (B) Ublituximab, due to its glycoengineering, has enhanced affinity for all variants of the *FCGR3A* receptor, with the highest binding and relative affinity for *FCGR3A* 158V and *FCGR3A* 158F variant receptors compared with other non-glycoengineered anti-CD20 mAbs.

METHODS

- This is a multicenter study aiming to characterize polymorphisms of Fc-receptors and IgG isotypes.
- Cryopreserved peripheral blood mononuclear cells previously obtained from PwMS (N=1000) and stored at select biorepositories at 6 academic centers will be utilized for assessment of FcR genotypes and IGHG1 alleles.
- The pilot data set presented herein includes 387 samples (349 PwMS and 38 healthy controls) collected at UTSW and JHU.
- 59.3% of samples were from Caucasians and 40.7% were from Blacks or African Americans.
- 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 for future analyses.

METHODS, CONT

Figure 2. Genomic Analysis and Clinical Data Integration Workflow



Study Design

- The study schema and experimental design are outlined in Figure 2. Assessment of FcR and IGHG1 polymorphisms was conducted using DNA extraction followed by laboratory-based TaqMan qPCR and Sanger sequencing tests. Correlation with clinical variables and immunophenotypes will follow.

Table 1. Clinical Variables to be Examined for Associations with Genetic Polymorphisms

- Age
- Sex
- Race/Ethnicity
- BMI
- Smoking history
- Relapsing vs progressive diagnosis
- Disease duration
- Therapy at time of sample acquisition
- Response to current or prior mAb therapy
- History of incomplete depletion or early B-cell repopulation

- For the current data set, genetic changes were analyzed in accordance with race and ethnicity. Additional clinical variables will be collected and analyzed for associations with specific polymorphisms in future analyses. These variables include age, sex, 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 1).

RESULTS

Table 2. Demographics

	UTSW CONTROL	UTSW CASE	JHU CASE	ALL CASE
N	38	99	250	349
Sex:				
Female	27 (71.1%)	82 (82.8%)	174 (69.6%)	256 (73.4%)
Male	11 (28.9%)	17 (17.2%)	76 (30.4%)	93 (26.6%)
F:M ratio	2.45	4.82	2.29	2.75
Race:				
Black/African American	17 (44.7%)	42 (42.4%)	100 (40.0%)	142 (40.7%)
Caucasian	21 (55.3%)	57 (57.6%)	150 (60.0%)	207 (59.3%)
Age at Draw:				
Average	40.70	37.29	43.79	41.94
Standard deviation	10.21	11.39	12.87	12.79
Range	21- 59	18- 58	19-75	18-75

- The known demographics for participants included in this pilot analysis were sex, race/ethnicity, and age at time of blood draw. 73.4% of samples were from females vs. 26.6% males, 40.7% were from Blacks or African Americans vs. 59.3% from Caucasians, and the average age was 41.94 years (Table 2).

RESULTS, CONT

Table 3. Frequencies of High and Low Affinity FCGR2A (CD32a) in PwMS vs Controls Stratified by Race/Ethnicity

		HIGH affinity (%) (A/A; 131H)	LOW affinity (%) (G/G; 131R)	Het (%) A/G
Reference	n	24.00	24.00	52.00
ALL				
Controls	38	20.00	27.50	52.50
MS Cases	349	25.30	28.40	44.30
BLACK/AA				
Controls	17	17.60	35.30	47.10
MS Cases	142	19.90	32.20	45.20
CAUCASIAN				
Controls	21	23.80	23.80	52.40
MS Cases	207	29.00	25.60	45.40

- The frequencies of high- and- low binding FCGR2A (CD32a) are similar in PwMS and healthy controls. However, when the MS group is parsed by race, 29% of Caucasian MS participants expressed the CD32a high-affinity 131H polymorphism compared to 19.9% of Black/AA MS participants. In correlation, 25.6% of Caucasian MS participants and 32.2% of Black/AA MS participants expressed the low-affinity 131R polymorphism, respectively, demonstrating a trend favoring expression of the low-affinity binding receptor in the Black/AA group (Table 3).

Table 4. Frequencies of High- and- Low Affinity FCGR3A (CD16a) in PwMS vs Controls Stratified by Race/Ethnicity

		HIGH affinity (%) (T/T; 158V)	LOW affinity (%) (G/G; 158F)	Het (%) T/G; 158VF
Reference	n	7.00	38.00	55.00
ALL				
Controls	38	10.30	43.60	46.20
MS Cases	349	12.50	42.50	45.00
BLACK/AA				
Controls	17	12.50	56.30	31.30
MS Cases	142	11.30	40.10	48.60
CAUCASIAN				
Controls	21	9.50	28.60	61.90
MS Cases	207	14.00	44.40	41.50

- The frequencies of high- and- low binding FCGR3A (CD16a) are similar in PwMS and healthy controls. When the MS group is parsed by race, 14.0% of Caucasian MS participants expressed the CD16a high-affinity polymorphism 158V compared to 11.3% of Black/AA MS participants. In correlation, 44.4% of Caucasian MS participants and 40.1% of Black/AA MS participants expressed the low-affinity 158F polymorphism, respectively, demonstrating that the homozygous frequency distribution of CD16a was not significantly different by race (Table 4).

Table 5. Frequencies of High- and- Low Affinity FcRn in PwMS vs Controls Stratified by Race/Ethnicity

		HIGH affinity (%) (VNTR3/3)	LOW affinity (%) (VNTR2/3)
ALL			
Controls	38	52.50	22.50
MS Cases	349	64.60	21.70
BLACK/AA			
Controls	17	23.50	29.40
MS Cases	142	36.90	31.90
CAUCASIAN			
Controls	21	80.00	15.00
MS Cases	207	83.10	15.00

RESULTS, CONT

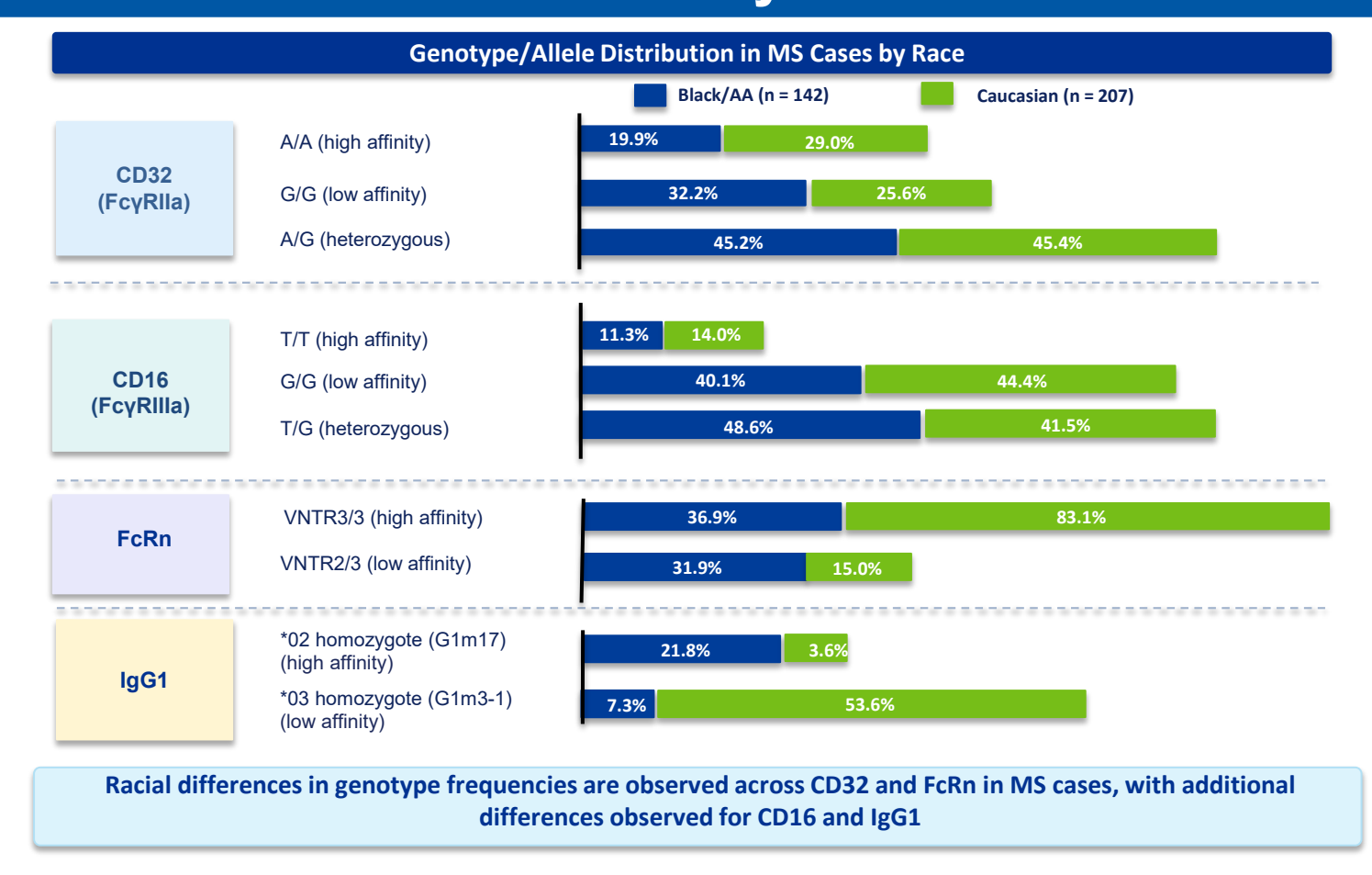
- The frequencies of high- and- low binding affinity FcRn are similar in PwMS and healthy controls. However, when the MS group is parsed by race, 83.1% of Caucasian-MS participants expressed the FcRn high-affinity polymorphism VNTR 03/03 compared to only 36.9% of Black/AA MS participants and 23.5% of Black/AA controls. In correlation, 15.0% of Caucasian MS participants and 31.9% of Black/AA-MS participants expressed the low-affinity polymorphism VNTR 02/03, respectively, suggesting a predisposition to lower expression levels of the neonatal Fc receptor which could result in less efficient recycling and faster degradation of antibodies, including therapeutic monoclonal antibodies, in the Black/AA population (Table 5).

Table 6. Frequencies of High- and- Low Affinity IgG1 Alleles in PwMS vs Controls Stratified by Race/Ethnicity

		HIGH affinity (%) *02 homozygote G1m17	LOW affinity (%) *03 homozygote G1m3-1
ALL	n		
Controls	38	16.70	16.70
MS Cases	349	10.80	34.90
BLACK/AA			
Controls	17	15.40	0.00
MS Cases	142	21.80	7.30
CAUCASIAN			
Controls	21	17.60	29.40
MS Cases	207	3.60	53.60

- The G1m17 allotype of IGHG1 binds FcRn efficiently and was expressed by 3.6% of Caucasian MS participants compared with 21.8% of Black/AA MS participants. The *03/G1m3-1 allele displays the lowest affinity for FcRn among the IgG1 alleles and is most often detected in Caucasian populations. 53.6% of Caucasian MS participants expressed *03/G1m3-1 compared to 7.3% of Black/AA MS participants, supporting the possibility of a higher IgG index and synthesis rate in the CSF of Black/AA compared to Caucasian PwMS and a more aggressive disease course. Additionally, these differences in IGHG1 allotypes could impact the development of anti-drug antibodies and rate of clearance of IgG1 therapeutic monoclonal antibodies for Black/AA PwMS vs Caucasians (Table 6).

Figure 3. Fc Receptor and IgG1 Genotype Distribution in MS Cases by Race



- Graphical summary of racial differences in genotype frequencies that were observed across CD32a, CD16a, FcRn and IgG1 alleles in MS cases (Figure 3).