A Novel *FCGR3B* Polymorphism and a New Human Neutrophil Antigen 1 Allele?

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Background

Human neutrophil antigen (HNA) 1 is encoded by the *FCGR3B** gene (CD16b) which has six known single nucleotide polymorphisms that code for five allelic variants which are attributed to four clinically relevant antigens as reflected in the current HNA nomenclature. During laboratory development of HNA-1 genotyping one blood donor sample was identified where a *FCGR3B** allele could not be assigned due to the nucleotide sequence.

Results

The novel nucleotide sequence observed in the donor sample (NM_000570.4 c.108>G, c.114>C, c.194>G, c.223>C, c.244>G, c.316>A) resulted in a novel amino acid sequence not described in the current nomenclature. Serological analysis of this sample showed it to be HNA-1 (CD16b) positive. An additional missense polymorphism was observed NM_000570.4 (FCGR3B): c.197T>G p.Leu66Arg. Results were reproduced in testing of a subsequent donation sample. Molecular dynamics calculations suggested no large conformational change due to the novel arginine substitution, as the three-carbon aliphatic stretch was undergoing similar van der Waals interactions with phenylalanine61 to leucine66.

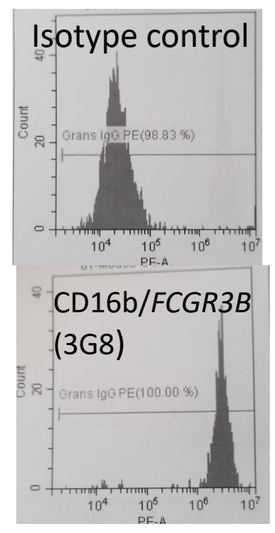
Methods

Phenotyping was performed by flow cytometry using both human monoclonal (in-house GI11) and murine monoclonal (3G8) antibodies to determine CD16b expression. Sequencing was performed by both an existing in -house Sanger sequencing-based approach and an augmented novel in-house sequence-based typing (SBT) approach.

Additionally, sequencing was performed by long read next generation sequencing (NGS) that is currently in laboratory development. Molecular dynamics calculations were performed using all-atom explicit solvent models to collect 150 nanosecond trajectories for HNA-1, -1b and the novel variant.

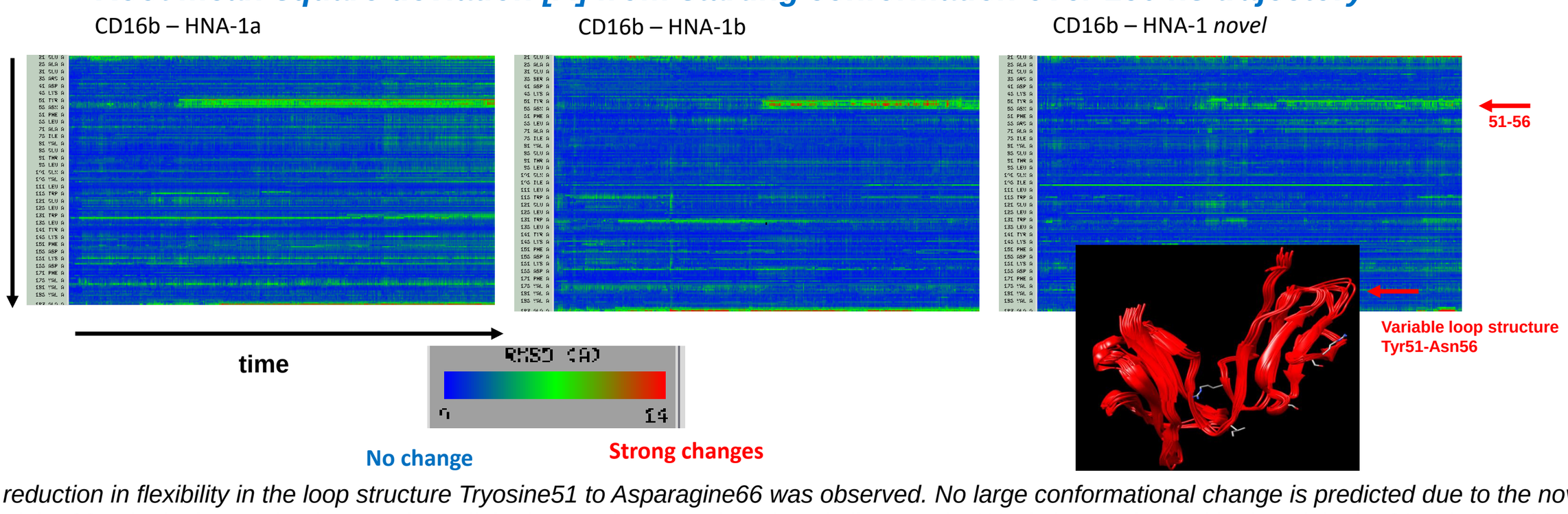
Current FCGR3B polymorphism for allele assignment

Allele	c.108	c.114	c.194	c.233	c.244	c.316
*01, -	G	C	A	C	G	G
*02, -	C	T	G	C	A	A
*03, -	C	T	G	A	A	A
*04, -	G	C	A	C	G	A
*05, -	C	T	G	C	G	A
Null (*FCGR3A*)	G	C	G	C	G	A



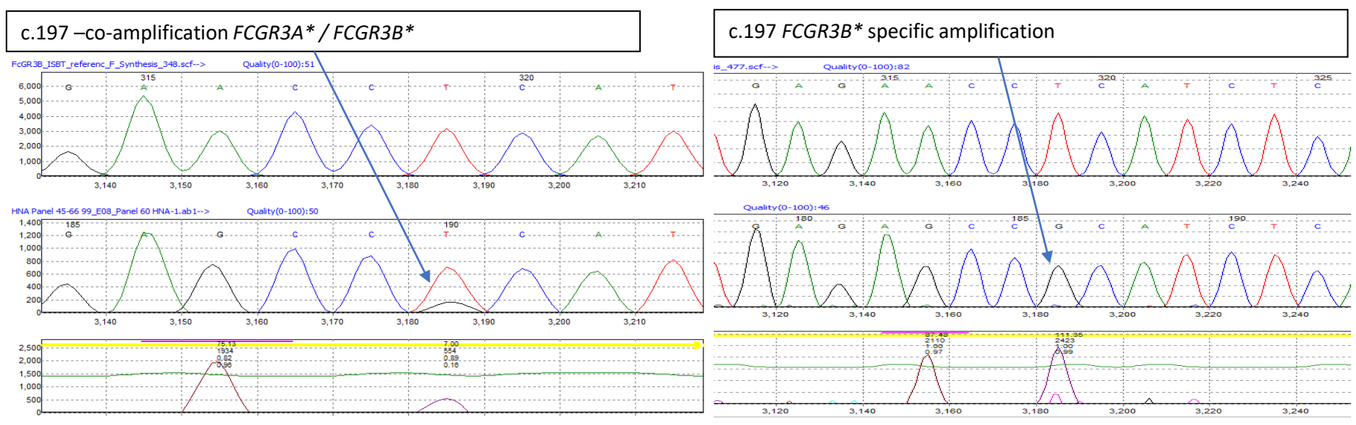
Blood donor assignment as Null but FCGR3B was phenotypically positive (right)

Root mean square deviation [Å] from starting conformation over 150 ns trajectory



A reduction in flexibility in the loop structure Tryosine51 to Asparagine66 was observed. No large conformational change is predicted due to the novel arginine66 substitution as the three-carbon aliphatic stretch was undergoing similar van der Waals interactions with phenylalanine61 to leucine66.

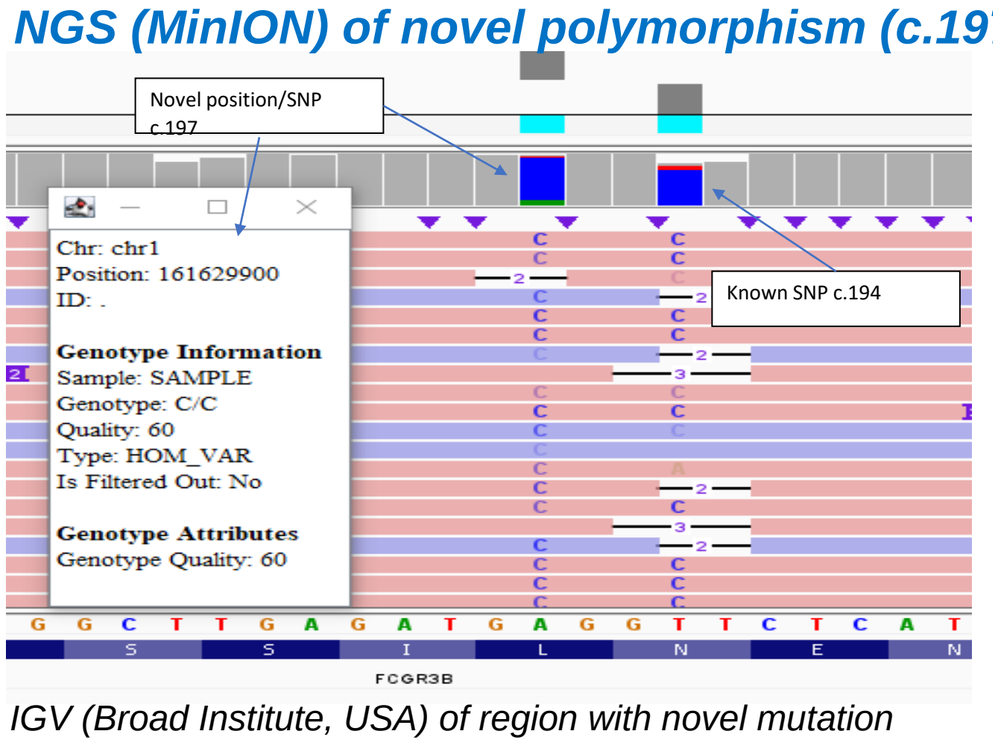
SBT of novel missense polymorphism



Mutation Surveyor® view of novel polymorphism at c.197.

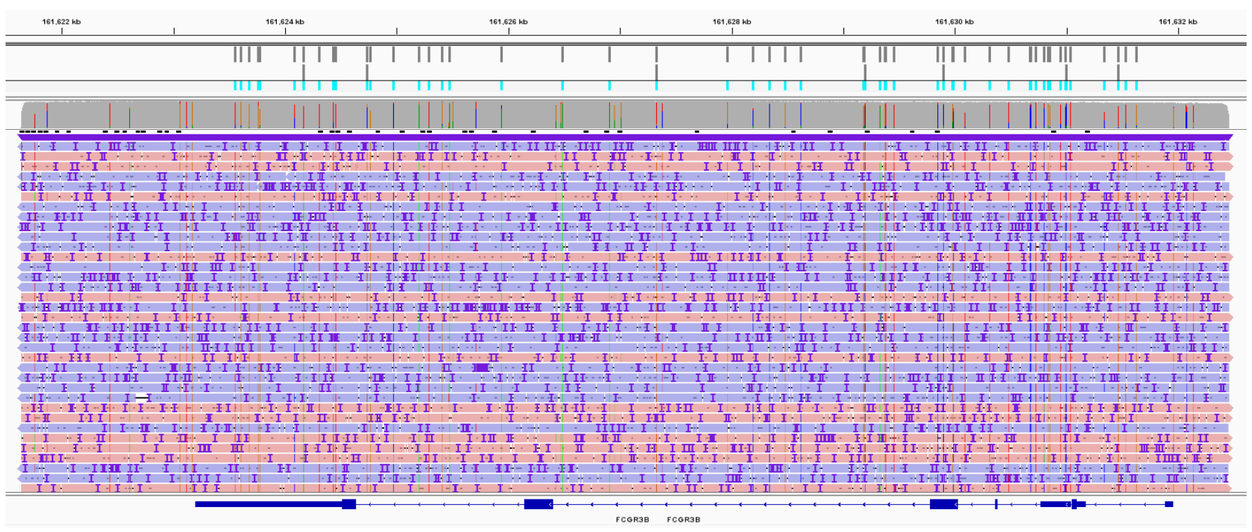
Current HNA genotyping (left) and proposed FCGR3B specific method (right).

NGS (MinION) of novel polymorphism (c.197)



IGV (Broad Institute, USA) of region with novel mutation

NGS (MinION) of whole FCGR3B gene



Integrated Genomics Viewer (IGV, Broad Institute, USA) of whole FCGR3B gene. Each read is ~10.5kb across the whole gene (pink = sense, purple = anti-sense).

Conclusion

In current laboratory testing antigen expression is inferred from exon 3 genotyping as described in the current nomenclature and this sample would have been incorrectly reported as a HNA-1 null phenotype. Confirmatory *FCGR3B* gene sequencing testing is currently being confirmed by an external reference laboratory. Comparative conformational studies are also underway to assess possible implications of post-translational modifications (glycosylation).