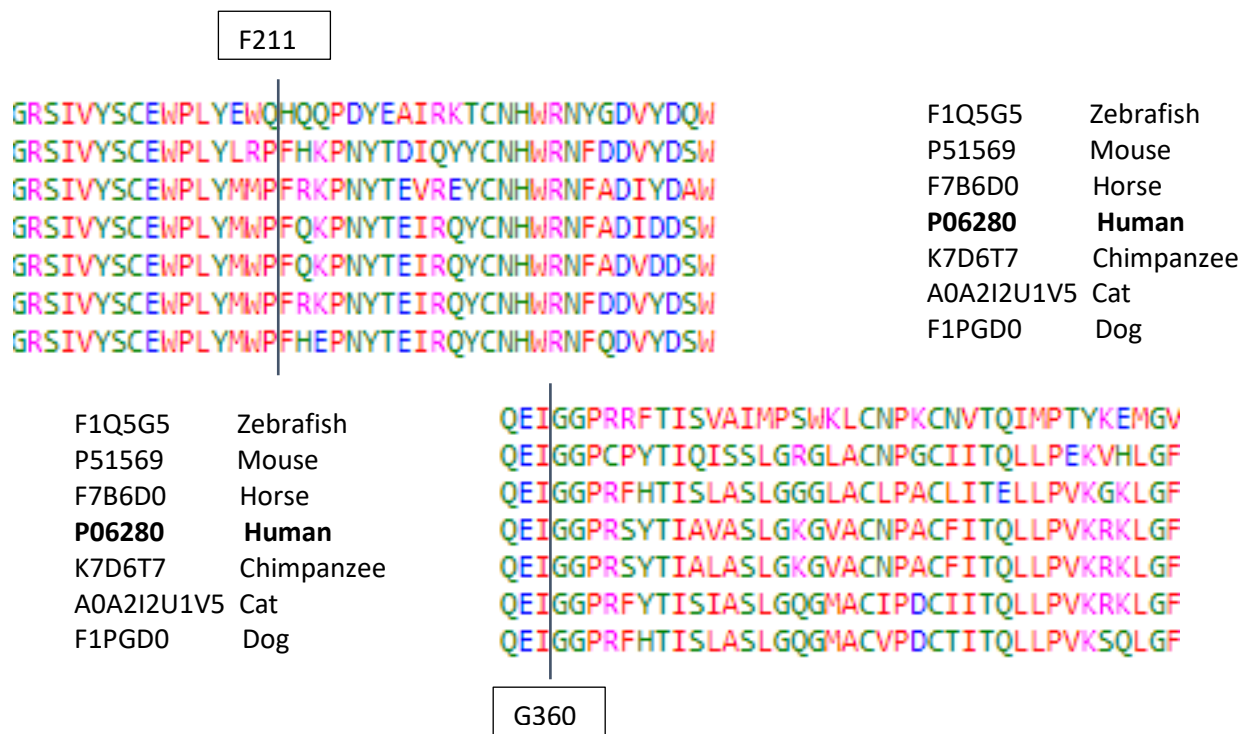
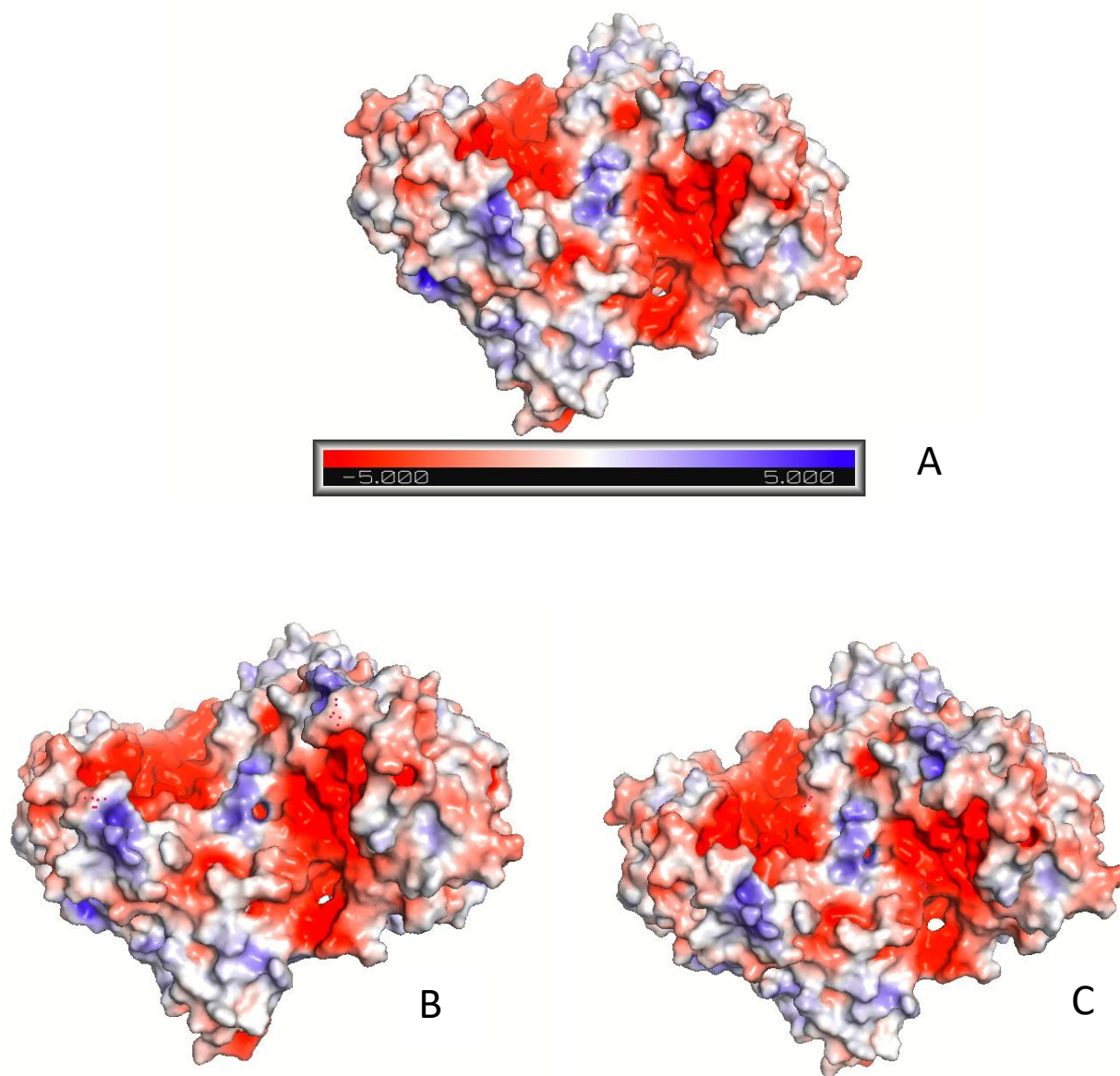


**Fig 1.** α-Gal A three-dimensional structural map of the two novel amino acid substitutions together with the two active sites (spheres) in the A and B chains of the enzyme. Pymol



**Fig. 2.** Alignment of wild-type human  $\alpha$ -Gal A amino acids with other organisms sequences. The rectangles indicates the amino acids at position 211 and 360 that we found mutated in the index cases.



**Fig 3.** Electrostatic Surface potential of the mutant proteins, products of the novel missense variants. The color scale ranges from  $-5KT/e$  (red) to  $5 KT/e$  (blue). A) Wild type protein; B) p.Phe211Ser; C) p. Gly360Ala. Alterations in the potential of the electrostatic surface in the mutant proteins are observed.

| Variant           | Tool | p.Phe211Ser<br>(c.632T>C) | p.Gly360Ala<br>(c.1079G>C) |
|-------------------|------|---------------------------|----------------------------|
| SIFT              |      | Tolerated                 | Deleterious                |
| Poly-Phen2        |      | Benign                    | Probably damaging          |
| FATHMM            |      | Harmful                   | Harmful                    |
| M-CAP             |      | Harmful                   | Harmful                    |
| MetaLR            |      | Harmful                   | Harmful                    |
| MetaSVM           |      | Harmful                   | Harmful                    |
| Mutation Assessor |      | Moderate                  | Moderate                   |
| Mutation Taster   |      | Neutral                   | Harmful                    |
| PROVEAN           |      | Neutral                   | Harmful                    |
| SNP & GO          |      | Disease causing           | Disease causing            |
| SAAP              |      | No effects                | Pathogenic                 |
| PhD - SNP         |      | Benign                    | Benign                     |

**Table 1.** Predictions of the effect of novel variants on  $\alpha$ -Gal-A protein.