**56th annual European Human Genetics Conference 2023**
**(online poster)**
**Glasgow, Scotland, UK, June 10 to 13th**
**(Hybrid Conference)**

# Molecular characterization of a new *CYP21A2* allele and classification of its pathogenicity

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**Background**: The *CYP21A2* gene, coding for 21-Hydroxylase (21-OH), is located on 6p21.3 within the major histocompatibility complex, and integrated in a cluster of genes (*RP1, C4A, C4B, TNXB*) and pseudogenes (*RP2, CYP21A1P, TNXA*). This genomic region is variable in size and gene copy number. Due to the high homology between genes and their pseudogenes, recombination is common, deletions, insertions and duplications are frequent. The great diversity of this cluster and rare alleles contributes to additional difficulties on molecular analysis and pathogenicity classification.
**Methods**: The *CYP21A* cluster was characterized using genomic DNA obtained from four healthy brothers (parents not available). Two long-PCR products, specific for each *CYP21A2* copy of a trimodular allele (with two *CYP21A2* copies), and for a normal/bimodular allele present in this family, were characterized by Sanger cycling sequencing and MLPA (MRC-Holland, P050-C1 kit).
**Results**: The molecular studies revealed that one sister, who asked for genetic counselling, has a very rare trimodular allele, with two *CYP21A2* genes. One of these genes has a deletion covering exons 4 to 7 and an insertion of exons 4 to 7 of the pseudogene (*CYP21A1P*) which has the pathogenic variants c.518T>A, c.710T>A, c.713T>A, c.719T>A, c.844G>T and c.923dupT, all in phase. This alteration can be described as: *CYP21A2*ex4_7delins*CYP21A1*Pex4_7.
**Conclusion**: The developed molecular approach, which was specifically designed for this family and included segregation analysis of all brothers, allowed the characterization of a new *CYP21A2* trimodular allele that, even containing six pathogenic variants, is non-pathogenic as it also has (in phase) a normal *CYP21A2* copy.