

Comments on Seven novel mutations of the UGT1A1 gene in patients with unconjugated hyperbilirubinemia by D'Apolito et al.

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Dear Editor,

We read with great interest the letter by D'Apolito et al, *Seven novel mutations of the UGT1A1 gene in patients with unconjugated hyperbilirubinemia*.¹ The authors analysed UGT1A1 gene in 11 unrelated patients with neonatal hyperbilirubinemia. They described seven novel mutations responsible for Crigler-Najjar syndrome type I or II. However, this paper raises several questions that we wish to address.

Some of these mutations are not novel and have been previously reported. Indeed, the c.513_515delCTT deletion, identified in patients 1 and 2, has been initially reported by Ritter et al in 1993.² We also discussed this deletion in one of our previous publication.³ Wild-type UGT1A1 contains two consecutive phenylalanine in position 170 and 171. Modification of this hydrophobic region of UGT1A1 (these two phenylalanine are located in a membrane-embedded helix) may be responsible for a modification of the enzymatic activity.⁴ In this repetitive genomic sequence, it is impossible to determine exactly which nucleotides are deleted. This deletion could be referred to 508_510delTTC, 509_511delTCT, 510_512delCTT, 511_513delTTC, 512_514delTCT or 513_515delCTT because it is undistinguishable at the molecular level.³ Since the recommendations established by Antonarakis and the Nomenclature Working Group in 1998,⁵ the most 3' is arbitrarily assigned to describe small deletions in repetitive sequences. In consequence, the deletion described as p.Phe170del by Ritter et al. in 1993 is the same one as this described by D'Apolito et al.

The c.652_653insT insertion (responsible for the modification of the protein sequence p.Ser218PhefsX40), identified at the heterozygous state in patient 4 has already been described in our laboratory in 2004.⁵ At the date of this publication, we only found this insertion at the heterozygous state in association with the heterozygote A(TA)₇TAA polymorphism in the TATAbox in a Polish Crigler Najjar type I patient. Currently, the second allelic mutation was identified in this patient and surprisingly was the same as that observed by D'Apolito et al. in their patient 4. The observation of two rare heterozygote genetic variations in two different families affected by a rare genetic disease, originating from two different countries is a very exceptional genetic situation. It would be interesting to confront our observations and to collaborate on this specific point.

There is confusion in the nucleotide numbering of the first mutation described in this paper. 1 In the UGT1A1 cDNA referring sequence (Genbank accession number NM_000463), nucleotides in positions 717 and 718 are

not AG but GA. This genetic region presents a quadruple AG-repetition. As indicated previously, genetic modifications in repetitive sequence have to be arbitrarily assigned on the 3' side. 5 Thus, this deletion has to be located at position 722_723 referring to the UGT1A1 cDNA sequence. The peptide sequence is not modified until the glutamic acid in position 241 which is replaced by a glycine. The deletion introduces a stop codon at position 16 (p.Glu241GlyfsX16).

There is an error in the description of the predicted protein consequence of the last reported mutation. 1 The deletion of the cytosine in position 210 results in the substitution of the aspartic acid in position 70 (...AGA-GACGGA...aspartic acid codon in bold-type, cytosine in position 210 underlined) not in a glutamine (CAG or CAA) but in a glutamic acid (GAG).

These different points illustrate the difficulty to perform molecular studies in such a condition as Crigler Najjar disease whose allelic heterogeneity has long been known.⁶

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