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Crigler-Najjar syndrome type II resulting from three different mutations in the bilirubin uridine 5'-diphosphate-glucuronosyltransferase (*UGT1A1*) gene

EDITOR—Crigler-Najjar syndromes (CN, MIM 218800) are inborn errors of metabolism characterised by unconjugated hyperbilirubinaemia resulting from the defective activity of the hepatic enzyme bilirubin uridine 5'-diphosphate-glucuronosyltransferase (B-UGT).

CN syndrome has been classified into two types according to the degree of hyperbilirubinaemia and to the response to phenobarbital administration. The more severe CN type I is characterised by severe chronic non-haemolytic unconjugated hyperbilirubinaemia with high levels of serum bilirubin owing to the absence of bilirubin UGT activity. In the milder CN type II, bilirubin UGT activity is only decreased and a consistently significant reduction is obtained with phenobarbital treatment, which does not occur in CN I.

Like other members of the UGT isozyme family, the two human liver bilirubin UGT isozymes, UGT1A1 and UGT1D, are encoded by the *UGT1* gene complex through a mechanism of alternative splicing. Each gene has a unique promoter and a unique exon 1, while exons 2-5 are common to both genes.[1] Most of the enzymatic activity results from the expression of the *UGT1A1* gene.[2]

At the molecular level, CN I results from a number of different defects; nonsense (or frameshift) and missense mutations are represented in almost the same amounts both in homozygosity and in the compound heterozygous state.[3 4] The milder phenotype in CN II patients seems to be mainly the result of homozygosity for missense mutations[5] and more rarely of the genetic compound for nonsense (or frameshift) and missense mutations or an interaction between missense mutations and a homozygous TA insertion in the TATAA promoter element, $A(TA)_7$ TAA, instead of the normal $A(TA)_6$ TAA.[6] The presence of the TA insertion in the TATAA promoter element of the *UGT1A1* gene reduces the expression of bilirubin-UDP-glucuronosyltransferase.[7] Homozygosity for the TA insertion has proved to be associated with Gilbert's syndrome.[7]

Here, we report a case of CN II, which appears to be the result of the interaction of two different mutations and homozygosity for the promoter polymorphism $(TA)_7$.

Blood samples were collected, after informed consent, from a 13 year old male CN type II patient, from both his parents, his older brother, and from 100 unrelated normal subjects as controls. The patient was born after a 40 week gestation to clinically normal, non-consanguineous parents. His weight at birth was 3450 g. Jaundice requiring phototherapy appeared during the neonatal period. At 8 days of age, the total, direct, and indirect bilirubin levels were 204, 20, and 184 µmol/l, respectively. During infancy and childhood, the indirect bilirubin levels ranged between 170 and 284 µmol/l. The highest values were related to episodes of stress and intercurrent acute illness.

Serum bilirubin levels (STB) were lowered to 30 µmol/l (80% less than the steady state level) by administration of phenobarbital (10 mg/kg/day) for 40 days. The proband showed normal somatic and developmental milestones. He had no complaints except for jaundice. The bilirubin levels of the other family members were in the normal range and are shown in table 1.

Table 1 Clinical and molecular data

	Father	*Mother*	*Proband*	*Brother*
TSB (µmol/l)	27,2	18,7	308,1	20
Mutation	AG del	V224G	AG del/V224G	AG del
TATAA box	TA_6/TA_7	TA_6/A_7	TA_7/TA_7	TA_6/TA_7

By sequence analysis of both strands of the *UGT1A1* gene, including the promoter region from nucleotide −227 and all the exons,[8] the patient was found to be a genetic compound for two novel mutations, a T→G transition at codon 224 (V224G) and a 2 bp deletion (−AG) at codons 238-239-240. Both mutations reside in the specific exon 1 of *UGT1A1*. This finding is consistent with the notion that *UGT1A1* codes for the only relevant enzymatic isoform in bilirubin glucuronidation.

The AG deletion is easily detectable by polyacrylamide gel electrophoresis of a PCR product (fig 1A). For the molecular screening of the V224G mutation we set up an allele specific PCR using primers shown in fig 1B.

The proband inherited the GTG→GGG transition from his mother and the deletion (−AG) at codons 239/240/241 from his father (table 1). Furthermore, he was found to be homozygous for the sequence variation $(TA)_7$ in the promoter region. This means that the mutated *UGT1A1* alleles in both parents are in cis to the $(TA)_7$ variation.

His healthy brother proved to be heterozygous for both the AG deletion and the $(TA)_7$ variation.

Analysis of the *UGT1D* sequence showed a neutral polymorphism at codon 157 (TGC→TGT).

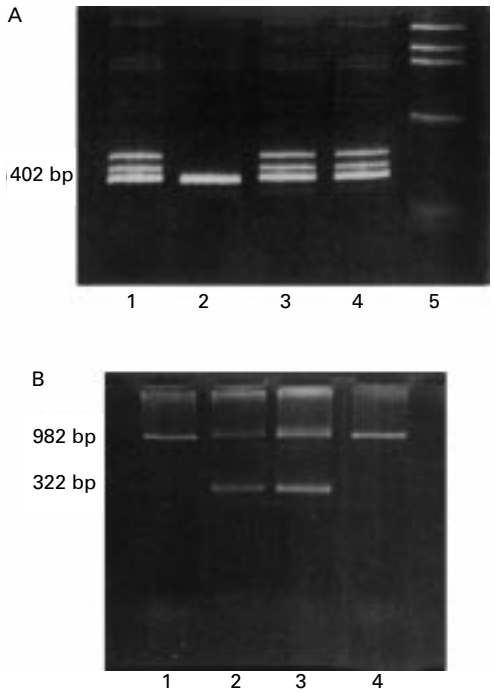

Figure 1 (A) Polyacrylamide gel electrophoresis of a 402 bp amplified DNA fragment containing the AG deletion at codons 239/240/241 of exon 1 of the UGT1A1 gene. Lanes 1, 3 4: father, proband, and brother, respectively, showing the heteroduplexes owing to heterozygosity for the AG deletion. Lane 2: mother, without the AG deletion. (B) Allele specific amplification (ARMS) to detect the V224G mutation. DNA from normal subjects (father and brother, lanes 1 and 4) does not give a 322 bp PCR product when amplified with a mutant primer complementary to the mutation (sense mutant primer: TGCCTTTCACAGAACTTTCTGTG CGAGGG; antisense primer: TCTCAGAATGCTTGCTCAG). Using the same primers, DNA from the mother (lane 2) and proband (lane 3) shows a 322 bp PCR product indicating the presence of the V224G mutation. A 982 bp PCR fragment is simultaneously amplified as a control.

One hundred normal subjects from the same area were analysed but none was found to carry the V224G mutation. The fact that we did not find normal subjects with the V224G substitution could suggest that it is a disease causing mutation. A comparative analysis of the sequence of the B-UGT protein in man, mouse, and rat indicate that the valine residue is highly conserved. Furthermore, from an analysis of the GOR secondary structure prediction,[9] it seems that Val-Gly substitution could lead to loss of the beta sheet structure. For these reasons, it can be postulated that V224G can affect the function of bilirubin-UDP-glucuronosyltransferase 1 by reducing its activity, thus causing a decrease in bilirubin glucuronidation.

The deletional event that was found in the paternal allele causes the premature appearance of a stop signal after 15-17 codons. This mutation does not exert any dominant negative effect, probably because of the very short truncated encoded protein. Moreover, as for other mutations, it is silent at the heterozygous level as shown by the normal STB of the mother.

The brother, in spite of inheriting the chromosome carrying both the AG deletion and the in cis $(TA)_7$ variation, was normal. We can hypothesise that the presence of the $(TA)_7$ polymorphism in cis to the missense mutation could reduce its negative effect.

The number of cases studied so far are very heterogeneous both at the clinical and at the molecular level making genotype-phenotype analysis very difficult. In our case, the CN II phenotype is the result of the additive effect of the interaction of a deletional event and the missense mutation V224G in association with the $(TA)_7$ promoter polymorphism.

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I705 variant in the low density lipoprotein receptor gene has no effect on plasma cholesterol levels

EDITOR—Familial hypercholesterolaemia (FH) is an autosomal dominant inherited lipoprotein disorder characterised by raised plasma low density lipoprotein (LDL) levels, xanthomas, premature coronary heart disease, and a family history of one or more of these. Homozygous FH occurs in one in a million people and they are severely affected, while heterozygotes are moderately affected and occur at a frequency of 1 in 500 in genetically heterogeneous populations. FH is caused by a mutation in the LDL receptor gene (*LDLR*) and over 700 have been reported[1] (http://www.ucl.ac.uk/fh). Among these, a missense mutation, T705I in exon 15 (FH Paris-9), was originally reported in a compound heterozygote ("homozygous" FH subject) of French-American origin,[2] but has now been observed in several heterozygotes who also carry another mutation in the coding region of the LDL receptor protein.[3 4] The presence of the I705 variant has also been reported in two normocholesterolaemic subjects in the heterozygous and homozygous form, which led to the suggestion that the T705I change is a non-FH causing variation.[5] The most recent report of this variation was in a Spanish family where the hypercholesterolaemia segregated with the I705 substitution and no other mutation was identified.[6] Possible explanations for these contradictory findings have been that the exon 15 variant is only pathogenic when another environmental or genetic factor is present, or that in some subjects the I705 variant is in linkage disequilibrium with a second, as yet unidentified causative mutation.

We have set up a clinical genetic diagnostic service for FH[7 8] and the I705 variant was identified in a subject with a clinical diagnosis of possible FH who was referred for FH genetic testing (data not shown). To investigate the pathogenicity of the amino acid substitution at codon 705, we have determined the frequency of the I705 variant in 2287 healthy UK men and examined the effect of this variant on plasma lipid levels.

An assay was designed for the T705I substitution where an *Nsi*I restriction site was introduced into the rare C allele (I) by a mismatch in the sense primer (underlined): sense primer: 5'-CAG TGG CCA CCC AGG AGA CAT GCA-3' and antisense primer: 5'-ATC TCC ACC GTG GTG AGC CCA-3'. PCR conditions were as described previously, the *Nsi*I enzyme was added directly to the PCR product, and the products (139 bp uncut = T705 and 115 bp + (non-detected) 14 bp cut) were separated on a 7.5% MADGE[9] and stained with ethidium bromide. The I705 carriers were then analysed for the 1061-8C variation in intron 7 by a natural *Ear*I restriction digest, after amplification of exon 8 (197 bp) with primers FH119 (FH website) and FH27 (FH website) and reaction conditions as previously described.[8] A restriction site was lost in the