Hurler (severe), Hurler-Scheie (intermediate), and Scheie (mild), polymorphisms in the α-L-iduronidase gene are thought to modify expression and affect enzyme function.[22] In Sanfilippo syndrome type A, despite the high frequency of the R456H polymorphism (41.3% in our study), there is no evidence yet that it modifies the sulphamidase enzyme. However, expression of this polymorphism in isolation and in combination with known pathogenic mutations is necessary to investigate the possibility of such an effect.

Six mutations identified in this study, S66W, R74C, R245H, 1091delC, 1156ins6, and V486F, were found in more than one unrelated family. The 6 bp insertion has not been reported previously and appears to be unique to the British Sanfilippo A population. The novel V486F mutation was found in homozygous form in a Greek and a Czech patient and although these patients were unrelated, haplotype analysis for three common polymorphisms (R456H, IVS5+17, and IVS2-26) showed that the mutant alleles were identical, suggestive of a common ancestor. The remaining four mutations, R74C, R245H, S66W, and 1091delC, are known to be prevalent in Polish, Dutch, Italian, and Spanish populations, respectively.[8 11–13] In our study, although the majority of patients with these four mutations were British, the haplotype of the mutant alleles corresponds to that associated with the mutations and suggests that they are all ancient mutations. The most common mutation in the 15 British patients was R245H with a frequency of 20% (6/30 alleles). Two patients heterozygous and homozygous for the 1091delC mutation originated from Spain and Malta, respectively, confirming the prevalence of the mutation in this population. Altogether, the six mutations accounted for 56.5% of the mutant alleles in this study and this information in combination with knowledge of the ethnic background of patients will be important for future mutational analysis on newly diagnosed Sanfilippo A patients in the UK. However, 17 of the mutations found in this study were unique to a particular family, further highlighting the extensive heterogeneity of Sanfilippo syndrome type A at the genetic level.

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Genotype-phenotype relationship of Niemann-Pick disease type C: a possible correlation between clinical onset and levels of NPC1 protein in isolated skin fibroblasts

EDITOR—Niemann-Pick disease type C (NP-C, MIM 257220) is a fatal autosomal recessive disorder characterised by progressive neurological deterioration and hepatosplenomegaly. NP-C patients can be classified into four major groups according to the onset of neurological symptoms, that is, early infantile, late infantile, juvenile, and adult forms, and the earlier the clinical onset the more quickly progressive are the symptoms and the shorter is the life span.[1–4] Complementation analysis using cultured skin fibroblasts indicated the presence of at least two subgroups of NP-C, NPC1 (the major subgroup that comprises >90% of NP-C patients) and NPC2 (the minor subgroup).[2–4] In 1997, the NPC1 gene (*NPC1*) (accession No AF002020) that is responsible for the NPC1 subgroup was identified by positional cloning.[5 6] The number of *NPC1* mutations known to date is not far off 100,[7–11] taking into account the accumulated data from seven groups presented in a recent international workshop (International Workshop, The Niemann-Pick C Lesion and the Role of Intracellular Lipid Sorting in Human Disease, Bethesda, USA, October 1999).

Because the genomic structure of *NPC1* was unknown, initial mutation screening was performed on RT-PCR

Table 1 Primers for PCR amplification of the NPC1 gene exons

	Name	Sequence	Localisation*	PCR products (bp)
ex1	1FW	5'-CTG AAA CAG CCC GGG GAA GTA G-3'	−87	232
	57RV	5'-GCC TGA GCC GTC GCT GGG CC-3'	+88	
ex2	58FW	5'-ACC ATT GAG ACC CTG GTA AC-3'	−47	207
	180RV	5'-CAT TTT GTG TTC CCA GTG CC-3'	+37	
ex3	181FW	5'-GAC CTT ACT CTA ACT GTT GCC-3'	−53	230
	287RV	5'-CAC AAG TAT CTA CAG CCC AG-3'	+43	
ex4	288FW	5'-CTT GCT GGC CCT ATT ATG TGT G-3'	−56	292
	463RV	5'-CAA TTT GCT CTG CTG TCC TG-3'	+60	
ex5	464FW	5'-CCT CGT GAA TTA CAG CAA GC-3'	−57	337
	631RV	5'-GCA ATT CTC TTG CCT CAG TC-3'	+112	
ex6a	632FW	5'-ATT CCA TAG GAC GAA GCA GC-3'	−173	350
	808RV	5'-CAT ACA TGG CGT CCA AGC CAA G-3'	nt 808	
ex6b	724FW	5'-GAC TGC TCT ATT GTC TGT GGC-3'	nt 724	286
	881RV	5'-CCA TGC AAT GGT ATT CAT GGA GG-3'	+128	
ex7	882FW	5'-GAA GGC AGT AAT TAG GGA GG-3'	−130	284
	955RV	5'-TGC AAC CCA CTG AGG AAA CG-3'	+80	
ex8a	956FW	5'-GTT CCG ACT TTC AGG AAC GGC-3'	−66	159
	1049RV	5'-GGG TTT CGG ACG CAG AAA GAC-3'	nt 1049	
ex8b	986FW	5'-CAG CAT TTG AGG GCT GCT TGA G-3'	nt 986	312
	1297RV	5'-GTC CAA AGG GTA CAT CAG CTC C-3'	nt 1297	
ex8c	1235FW	5'-CCC CTC TCA CTG ACA AAC AC-3'	nt 1235	186
	1326RV	5'-AGC CCC AAA TCC CCA TCT AGC-3'	+94	
ex9a	1327FW	5'-ATT CTC TCC CTC ATC TTA GG-3'	−137	309
	1390RV	5'-CTT TCT TGT GGT CCA GCA CG-3'	nt 1390	
ex9b	1498FW	5'-CTT CAA GAC ATC TGC TTG GC-3'	nt 1498	294
	1553RV	5'-GTA AAC TTC ACA GGG CAA GG-3'	+130	
ex10	1554FW	5'-AGG GCC CAT GTT GTC CTT AG-3'	−119	285
	1654RV	5'-TGA TGC TAA TGA CAA AAC CGA G-3'	+65	
ex11	1655FW	5'-GAG ATA CAG TCC ATA GCT CC-3'	−115	288
	1787RV	5'-AAG TGC TTG CCG CAA GTG TC-3'	+70	
ex12	1758FW	5'-AAG TTT CTT ACT TAG CTG TCA G-3'	−62	315
	1947RV	5'-GAC GTT ACA CTG TGC ACT GC-3'	+63	
ex13	1948FW	5'-AAG TGG GAC AGA CAA CCC TG-3'	−56	303
	2130RV	5'-GGA GCC ATT CAC AGT CCC TG-3'	+64	
ex14	2131FW	5'-CAA GGC AGC AAG AAA TGG CG-3'	−70	261
	2245RV	5'-CAT GTT CAG GTA GCC AGC TC-3'	+76	
ex15	2246FW	5'-GAA CAT AAG ACC TGC AGA GAG C-3'	−69	263
	2373RV	5'-CCG CTA GCT GCT TCC TCT AG-3'	+66	
ex16	2374FW	5'-CTA GAG GAA GCA GCT AGC GG-3'	−51	279
	2514RV	5'-TCC TTC CCA GGC TGT CTG GC-3'	+87	
ex17	2515FW	5'-TGT ACT CCC TAT TAG CCT GTC-3'	−137	316
	2604RV	5'-CTT GCT TGA AAC ACC TAC GTG C-3'	+90	
ex18	2605FW	5'-CTT ATT CTC CGT GAT CCT CGC-3'	−80	355
	2795RV	5'-CAG TGA GAC ATT TCA GGC CTG-3'	+84	
ex19	2796FW	5'-AGA CTT CCT CCC TGT GGA GC-3'	−49	257
	2911RV	5'-GGT ATA AAC TGA GGC ACG ATG C-3'	+92	
ex20	2912FW	5'-GTA ATG CCC CTC ACT GTC AG-3'	−94	306
	3041RV	5'-GTC TTA GCC CAG TCC TCT CC-3'	+82	
ex21	3042FW	5'-AAT GTA CAG CTG GGT CTG ACC-3'	−124	378
	3245RV	5'-CAG TGT AGG CCC TTT GCT GG-3'	+50	
ex22	3246FW	5'-TGT TCG GGA GTG AGA GCG AGC-3'	−50	357
	3477RV	5'-ATG GAA TCT AAG ACA GCC AAT CC-3'	+75	
ex23	3478FW	5'-AGC ACC CAT CCT CAG AAC GG-3'	−81	265
	3591RV	5'-CTC TTC AGT CAC TGA GGA GG-3'	+69	
ex24	3592FW	5'-CAA TTA CAG GTT GGT AAA AGT GG-3'	−50	255
	3754RV	5'-ATG TCC TTC CAT TGT GCC ACC-3'	+43	
ex25	3755FW	5'-TGA GCC ACT ATG CCC AGC CAA C-3'	−81	188
	3870RV	5'-GAC ACA GTT CAG TCA GGA TG-3'	nt 3870	

*The location of primers refers to intronic position from exon, and those shown by nt refer to cDNA sequences (AF002020), 1st ATG as nt 1.

products or partial genomic amplicons. In our previous study using RT-PCR products, we identified 14 different mutations in 19 alleles from 11 patients, and failed to detect mutations in the remaining three alleles.[8] Mutation screening using RT-PCR products has several drawbacks compared with screening using genomic amplicons. For example, mutations that reduce the mRNA stability may escape the screening.[12 13] To refine the screening method, we screened a CITB human BAC library (Research Genetics, Huntsville, AL) and isolated a clone 386K10 that contained all the 25 exons of *NPC1* and a 2 kb fragment of 5'UTR. Our analysis using 386K10 confirmed the exon/intron boundary sequences reported by Morris *et al*[14] and complements their data by showing the lengths of introns 1 (20 kb) and 6 (3 kb). Thus, *NPC1* spans over 70 kb in the genome.

Sets of primers to amplify each of the 25 exons of *NPC1* were designed according to the corresponding intron sequences (table 1). To include cis acting elements that participate in pre-mRNA splicing, the 3' nucleotide of nearly all the primers was placed >20 bp away from the splice junctions. For SSCP, exons 6, 8, and 9 were divided into two to three fragments by primers based on each exon sequence and named exon 6a and 6b and so on (table 1). The clinical features of the 15 Japanese and two white NPC1 subjects are summarised in table 2. All the patients were diagnosed by cholesterol accumulation in their skin fibroblasts.[15] Informed consent for gene research was obtained from all the families. Two NPC1 cell lines (GM03123 and GM110) were obtained from the Human Genetic Mutant Cell Depository, Coriell Institute for Medical Research (Camden, NJ). Fibroblasts from one healthy volunteer and three NPC2 patients were used as controls.

By SSCP analysis of genomic amplicons, we surveyed the 34 alleles from the 17 patients (including the 11 subjects in our previous study[8]), confirmed the 14 mutations that had been identified by RT-PCR SSCP, and identified one recurrent and seven novel mutations (table 3). None of the recurrent or the seven new mutations were found in over 100 normal samples, and they were thus considered to be disease causing. Mutation S954L identified in 431-1 is a recurrent mutation that has been reported by Greer *et al*[7] and also by Bauer *et al* (International Workshop, The Niemann-Pick C Lesion and the Role of Intracellular Lipid Sorting in Human Disease, Bethesda,

Table 2 Clinical features of NP-C patients

Clinical type	*Cell strain*	*Ethnic group origin*	*Sex*	*Onset of neurological signs*	*Neurological signs*	*Hepato- spleno-megaly*	*Outcome*
Late infantile	OHS	JPN	M	1.5 y	Epilepsy, psychomotor delay	HSM	1.5 y (alive)
	KUR	JPN	F	2.5 y	Epilepsy, deterioration	HSM	?
	INO	JPN	M	2.5 y	Epilepsy, deterioration	HSM	4.5 y (dead)
	TAN	JPN	M	2.5 y	Epilepsy, ataxia, deterioration	Mild SM	?
	UCH	JPN	F	2.5 y	Ataxia, deterioration	HSM	5 y (dead)
	AMA	JPN	F	3 y	Epilepsy, psychomotor delay	HSM	4 y (alive)
	YON	JPN	F	2.5 y	Epilepsy, ataxia, deterioration	HSM	12 y (alive)
	SHI	JPN	F	2.5 y	Epilepsy, deterioration	SM	4 y (alive)
	MUR	JPN	M	5 y	Epilepsy, deterioration	HSM	15 y (dead)
	YAN	JPN	F	5 y	Epilepsy, ataxia, deterioration	No	19 y (alive)
	SAK	JPN	?	?	?	?	?
	431-1	JPN	?	?	?	?	?
	GM03123	White	F	?	?	?	9 y (alive)
	GM110	White	M	5 y	Epilepsy, deterioration	?	10 y (alive)
Juvenile	SAS	JPN	F	13 y	Epilepsy, ataxia, deterioration	Mild SM	17 y (alive)
	END	JPN	M	15 y	Dystonia, ataxia, VSO	Mild SM	17 y (alive)
Adult	KAI	JPN	M	25 y	Dementia, ataxia, dystonia, epilepsy, VSO	SM	42 y (dead)

JPN: Japanese, HSM: hepatosplenomegaly, SM: splenomegaly, VSO: vertical supranuclear ophthalmoplegia.

Table 3 Mutations of NPC1 gene in Niemann-Pick C families

Clinical type	*Cell strain*		*Genomic mutation*	*cDNA change*	*Amino acid change*	*Genotype*
Late infantile	OHS	Exon 19	G2867A	Missense transition	C956Y	Cmpd hetero?
	KUR	Exon 9	G1553A	Missense transition & splicing error	R518Q	Homo
	INO	Exon 9	G1553A	Missense transition & splicing error	R518Q	Homo
	TAN	Exon 9	G1553A	Missense transition & splicing error	R518Q	Cmpd hetero
		Exon 24	*C3614G	Missense transversion	T1205R	
	UCH	Exon 9	A1529C	Missense transversion	H510P	Homo
	AMA	Exon 4	350(or351)AG(orGA) del	2 bp del	aa 119 frameshift-aa126/stop	Cmpd hetero
		Exon 5	T529G	Missense transversion	C177G	
	YON	Exon 20	*T2987G	Missense transversion	M996R	Cmpd hetero
		Exon 24	*3615[-3618]A del	1 bp del	aa 1205 frameshift-aa1241/stop	
	SHI	Exon 13	*T2108C	Missense transition	F703S	Cmpd hetero
		Exon 16	*C2438G	Missense transversion	S813X	
	MUR	Exon 5	C629A	Nonsense transversion	S210X	Cmpd hetero
		Exon 9	T1417C	Missense transition	S473P	
	YAN	Exon 24	*G3707A	Missense transition	G1236E	Cmpd hetero?
	SAK	Exon 19	G2867A	Missense transition	C956Y	Cmpd hetero
		Exon 24	*3615[-3618]A del	1 bp del	aa 1205 frameshift-aa1241/stop	
	431-1	Exon 24	*3615[-3618]A del	1 bp del	aa 1205 frameshift-aa1241/stop	Cmpd hetero
		Exon 19	†C2861T	Missense transition	S954L	
	GM03123	Exon 6	C709T	Missense transition	P237S	Cmpd hetero
		Exon 21	T3182C	Missense transition	I1061T	
	GM110	Exon 21	T3182C	Missense transition	I1061T	Cmpd hetero
		Exon 14	*2215(or2217) TCCTTT(orCTTTTC) del	6 bp del	2 aa deletion (740F,741S)(in frame)	
Juvenile	SAS	Exon1	44(or45) TG(orGT) del	2 bp del	aa 16 frameshift-aa56/stop	Cmpd hetero?
	END	Exon 22	A3263G	Missense transition	Y1088C	Cmpd hetero
		Exon 24	G3639C	Missense transversion	L1213F	
Adult	KAI	Exon 18	G2665A	Missense transition	V889M	Cmpd hetero
		Intron 20	IVS20 -2A del	Splicing error (54 bp del)	18 aa deletion (1015-1032)(in frame)	

*: new mutation, †: recurrent mutation, Cmpd hetero: compound heterozygous, Homo: homozygous, (): not confirmed, del: deletion.

USA, October 1999). Of the seven novel mutations, five were found in new subjects whereas the remaining two were found in one allele of TAN (C3614G) and of SAK (3615 (−3618) A del), respectively. It is not known why these two mutations escaped RT-PCR SSCP. Allelic mutations were not detected in three patients (OHS, SAS, and YAN) (table 3). In summary, SSCP analyses of genomic amplicons showed 21 disease causing mutations in 31 out of 34 alleles from 17 patients. Additionally, six different variants were identified (table 4).

The 22 mutations included 15 missense mutations, two nonsense mutations, two in frame deletions, and three deletions that cause a frameshift and a premature stop codon. In accordance with our identification of T3182C (I1061T substitution) as a frequent mutant allele in patients of western European descent,[13] this mutation was found in the genome of two white cell lines. None of the Japanese patients possessed this mutant allele, clearly highlighting an ethnic difference in the mutation frequency. Instead of T3182C, G1553A appears to be a relatively frequent mutation in Japanese patients, found in five alleles in three patients. This mutation is unique for two reasons; one is that it is predicted to cause both an amino acid substitution (R518Q) and an alternative exon skipping[8] and the other is that the skin fibroblasts from patients homozygous for this mutation (KUR and INO) retained considerable levels of NPC1 protein (see below).

Table 4 New polymorphisms of NPC1 gene

	Nucleotide location	*Influence on amino acids*
Exon 1	A-22C	Silent
Exon 8a	G1014T	Silent
Intron 12	IVS12+8~+10GGG del	Silent
Exon 18	T2618C	Missense transition (V873A)
Exon 18	C2775T	Silent
Exon 21	C3159T	Silent

With regard to the structure-function relationship of *NPC1*, mutagenesis studies have shown several functionally important domains of NPC1 protein including an NPC domain and a sterol sensing domain (SSD).[16 17] In addition, Greer *et al*[9] suggested the functional importance of the cysteine rich extracellular loop between TM9 and TM10 based on the segregation of point mutations in this region. The 14 missense mutations and the one in frame deletion found in the present survey are widely distributed on *NPC1* cDNA and appeared to be classified into five groups according to their location (fig 1A). Each group of mutations gives some insight into the structure-function relationship of *NPC1*. First, two mutations (F703S and del 740-741) in

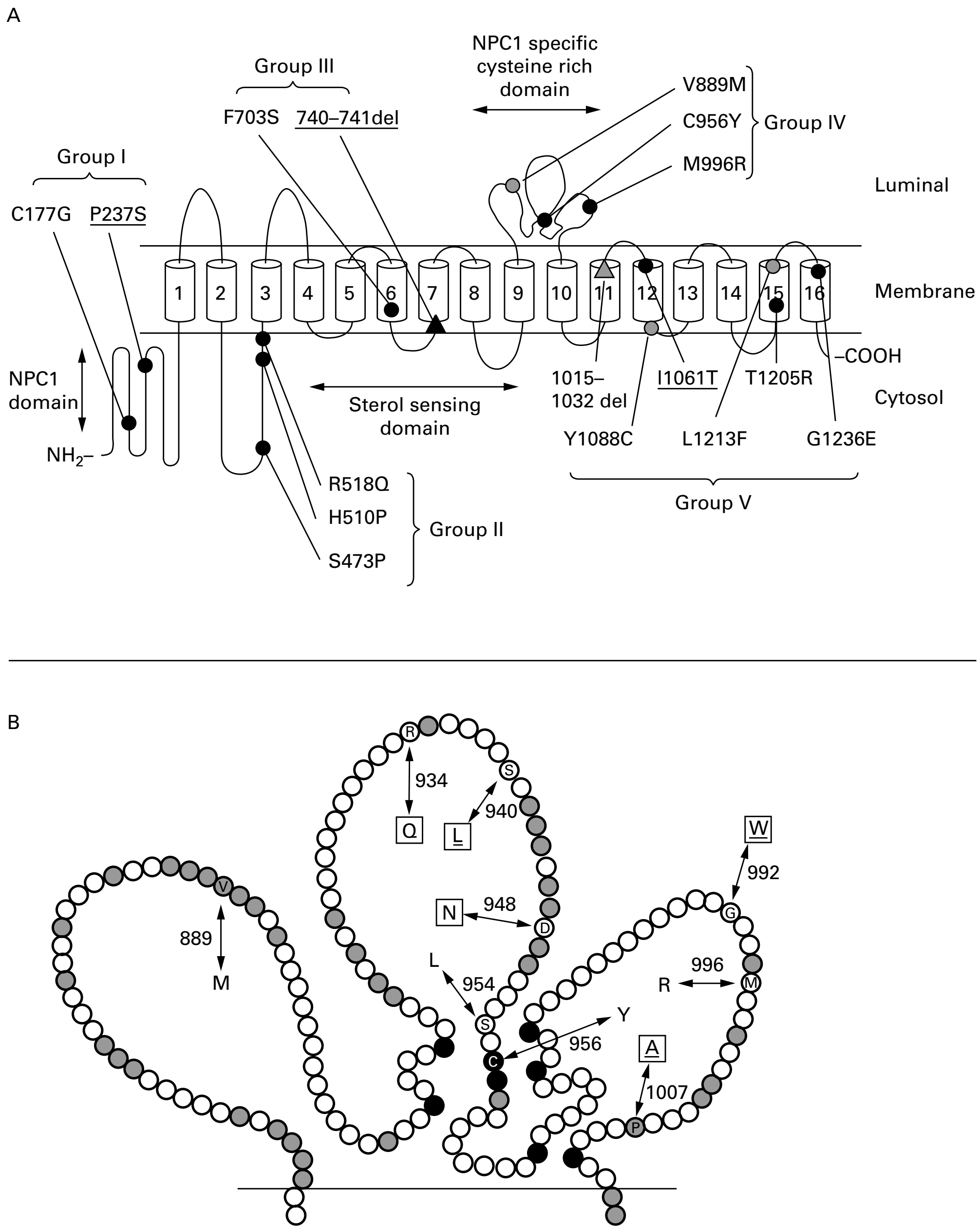

Figure 1 Distribution of mutations in NPC1. (A) Missense mutations and in frame deletions identified in this study are depicted. Circles and triangles indicate missense mutations and in frame deletions, respectively. Black circles and triangles are the mutations found in late infantile form patients and grey ones are from juvenile and adult form patients. Underlined are mutations found in white cell lines. (B) Mutations in NPC1 specific cysteine rich domain. Black circles are cysteines that may form disulphide bonds and grey ones are conserved amino acids. Squares are mutations reported by Greer et al.[7 9] Underlined are mutations found in patients with moderate or mild phenotypes. The model for the organisation of NPC1 is according to Greer et al.[9] Still tentative, it may have to be slightly altered in the future.[19]

group II are located in the sterol sensing domain. Second, four mutations in group VI are located in the cysteine rich extracellular loop. Interestingly, C956Y is the mutation of the cysteine residue itself that is supposed to be involved in the secondary structure formation and the other two mutations (V889M and M996R) were located in the conserved motif sequences in this loop (fig 1B). Thus, the mutations in groups III and IV appear to reinforce the functional significance of SSD and the cysteine rich domain, respectively. By analogy, one may infer the presence of functionally important domains that correspond to groups I, II, and V mutations and this should be the subject of a future study. No wild type mutations were found in the NPC domain, although the functional importance of this domain is obvious from mutagenesis studies.[17]

To investigate the impact of mutations on expression of the translation product, we quantified the levels of NPC1 protein in membrane preparations from cultured fibroblasts by anti-NPC1 immunoblotting[18] (fig 2). The anti-NPC1 detected two bands on the blot of the control membrane preparations, a major band at ~170 kDa and a minor band at ~190 kDa. These two bands have been shown to represent the same protein with differential glycosylation.[16]

In NPC1 cell lines, there appeared to be a distinct difference in the NPC1 protein levels between the late infantile and juvenile/adult forms. In the late infantile forms, there was a clear reduction of the NPC1 protein level regardless of the type of mutation, and five fibroblast lines (MUR, OHS, SHI, GM3123, and GM110) expressed undetectable levels of NPC1 protein. An exception was

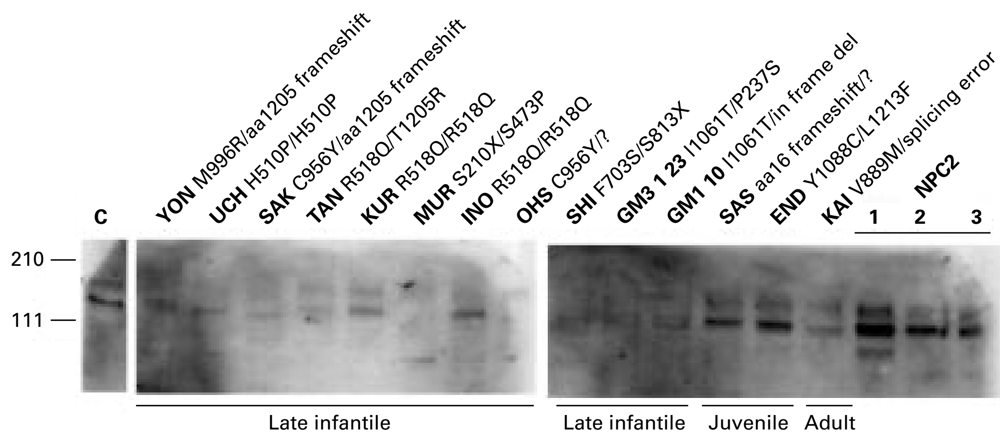

Figure 2 Western blot of membrane proteins extracted from skin fibroblasts of NP-C patients and normal controls (C). Numbers 1 to 3 indicate NPC2 patients. Molecular weight (kDa) is given on the left. A rabbit polyclonal anti-NPC1 antibody was a kind gift from Dr S C Patel and was used at 1:100.

KUR and INO, both of whom have R518Q homozygous mutations and levels of NPC1 protein in their fibroblasts were close to those of controls.

Patients with a late clinical onset were distinct in that all of their skin fibroblasts expressed considerable levels of mutant NPC1 protein (fig 2). Two of the three patients (END and KAI) with a late clinical onset were compound heterozygotes for the groups IV and V mutations, whereas at least one allelic mutation of the 14 patients with a late infantile form belonged to group I, II, or III (fig 1A). In another study, skin fibroblasts from a patient with an adult neurological onset (homozygous for a V950M mutation)[11] appeared to retain normal expression of NPC1 protein (G Millat, M T Vanier, C Tomasetto, unpublished data). These results led us to form a tentative conclusion that the relatively mild form of NPC1 is caused by mutations located on the C-terminal side of the transcript that do not interfere with expression/turnover of the translation product. Future studies with an increased number of patients will verify this conclusion.

Finally, we also found that NPC2 fibroblasts expressed normal, or rather increased levels of NPC1. Similar results were achieved in a parallel study conducted with another antibody (G Millat, M T Vanier, C Tomasetto, unpublished data). Because of the identical biochemical phenotype of NPC1 and NPC2, the NPC2 protein is assumed to be located close to NPC1 both spatially and functionally. At one extreme, there has been a hypothesis that the biochemical phenotype of NPC2 is the result of the secondary absence of NPC1.[2–4] Our findings clearly exclude this hypothesis but do not exclude that NPC2 is required for the normal function of NPC1.

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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).

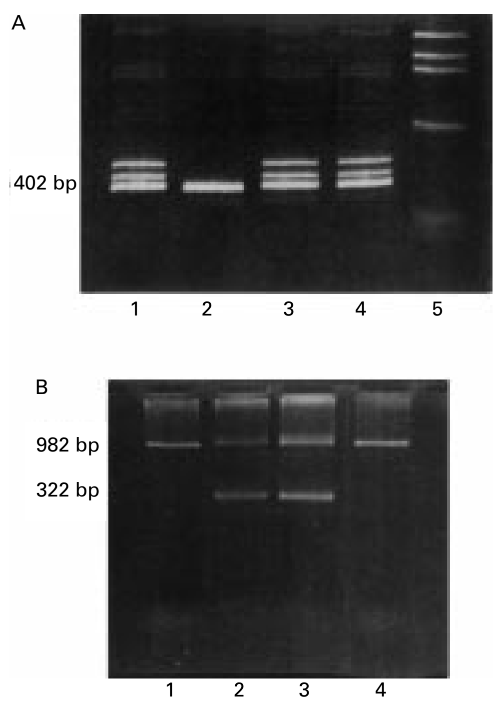

Figure 1 (A) Polyacrylamide gel electrophoresis of a 402 bp amplified DNA fragment containing the AG deletion at codons 239/240/241 of exon 1 of the UGT1A1 gene. Lanes 1, 3 4: father, proband, and brother, respectively, showing the heteroduplexes owing to heterozygosity for the AG deletion. Lane 2: mother, 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: TGCCTTTTCACAGAACTTTCTGTG 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.