Aminoacids (μmol/L)	Fenofibrate (n=29)		Simvastatin (n=28)	
	Before	After	Before	After
Arginine	64·9 (15·1)	68·4 (13·1)	61·9 (13·1)	67·0 (13)*
Citrulline	20·8 (6·5)	24·0 (6·6)	23·3 (7·3)	22·1 (7·7)
Ornithine	48·7 (9·0)	51·3 (16·5)	47·1 (10·7)	49·3 (14·2)
Lysine	146·6 (25·5)	142·7 (35·2)	135·9 (26·5)	144·7 (25·5)
Histidine	68·4 (13·2)	70·8 (11·3)	69·0 (12·0)	68·8 (12·6)
Tyrosine	55·5 (11·3)	58·2 (15·2)	56·3 (12·9)	57·8 (12·6)
Phenylalanine	47·8 (5·2)	48·4 (6·8)	48·1 (7·9)	48·7 (7·8)
Leucine	109·9 (15·7)	101·9 (22·1)	111·9 (23·2)	117·3 (25·5)
Isoleucine	56·0 (10·0)	54·0 (12·4)	57·2 (14·5)	60·3 (16·1)
Proline	181·3 (64·3)	184·3 (46·9)	167·2 (62·3)	179·3 (60·2)
Hydroxyproline	7·4 (4·8)	8·2 (3·5)	10·2 (10·4)	8·6 (3·0)
Glycine	137·0 (31·9)	146·2 (29·7)	127·2 (21·6)	131·7 (26·4)
Serine	73·3 (16·4)	77·8 (12·1)	71·0 (13·8)	71·3 (14·8)
Alanine	271·6 (76·8)	250·0 (69·4)	265·2 (65·5)	283·8 (65·1)
Valine	170·2 (23·7)	153·7 (38·8)	174·8 (35·4)	179·5 (33·9)
Asp+Asn	34·6 (6·6)	39·4 (6·3)	34·7 (3·8)	36·3 (4·5)
Glu+Gln	463·6 (64·8)	476·5 (47·8)	445·8 (57·9)	463·8 (38·6)
Methionine	19·5 (3·9)	23·5 (4·6)†	18·7 (3·6)	20·9 (3·7)*
Cysteine	3·8 (2·8)	6·1 (4·8)‡	4·3 (3·3)	4·5 (3·5)
Homocysteine	11·4 (3·5)	16·6 (5·2)†	12·2 (3·9)	12·3 (5·4)

Asp+Asn=sum of aspartate and asparagine;
Glu+Gln=sum of glutamate and glutamine.
*p<0·05.
†p0·0005.
‡p<0·005.

Effects of lipid-lowering drugs on plasma aminoacids concentrations

mass index, blood pressure, smoking, blood lipids, kidney and liver function, associated drug treatment, and severity of CHD. No patient was withdrawn because of adverse effects. Nutrient consumption was stable in both groups and none of the tolerance variables was substantially altered. Both drugs significantly decreased blood total cholesterol (fenofibrate by 18%, simvastatin by 26%, both groups 7·2 [SD 0·7] mmol/L before treatment, p<0·05) and LDL cholesterol (fenofibrate by 22% and simvastatin by 37%, 5·0 [0·7] and 5·1 [0·6] mmol/L, respectively, before treatment), fenofibrate decreased concentrations of triglyceride by 43% (2·1 [0·9] mmol/L before treatment), whereas simvastatin had no effect. Neither drug modified HDL and lipoprotein-a concentrations. Most aminoacids remained stable (table). Arginine increased slightly in the simvastatin group (p<0·05). In patients receiving fenofibrate, there were significant changes in methionine (20% increase), cysteine (60% increase), and homocysteine (46% increase). Simvastatin slightly increased only methionine (11%). Cysteine and homocysteine differed significantly between groups (p<0·01).

The simultaneous increase in all three sulphur aminoacids after fenofibrate is difficult to interpret. Whether increased methionine and cysteine concentrations has clinical implications is unclear. Baseline cysteine concentrations seemed quite low in the two groups and may be unreliable. However, plasma was obtained and stored in similar ways in patients taking fenofibrate or simvastatin, which suggests modification of cysteine metabolism by fenofibrate. Further studies are needed to investigate at which step of the pathway the alteration occurs and whether it can be corrected without decrease of the hypolipidaemic effect. B6 supplementation has been shown to normalise altered sulphur aminoacid status induced by nicotinic acid.[4]

Several studies suggest that increased concentrations of homocysteine are associated with an increased risk of CHD and several toxic effects of homocysteine on the vascular endothelium and coagulation may predispose to CHD.[2] A reduction in the risk of CHD by lowering homocysteine concentration has not been shown, and a causal relation between high homocysteine concentrations and CHD remain uncertain. Whether the increase in homocysteine outweighs the benefit of fenofibrate on lipids, fibrinogen, and uric acid needs to be assessed. A report has shown that bezafibrate, another fibric acid derivative, did not decrease CHD after a 5-year follow-up, which might be a first indication.[5] Finally, from the available data and in contrast to the other classes of lipid-lowering drugs, simvastatin seems to have no effect on homocysteine, which may partly explain its efficacy in CHD. If this effect is true, more data on the entire class of drug are important. The effect of simvastatin on arginine, the precursor of nitric oxide (a major mediator in cardiovascular physiology) also warrants further investigation.

1 Wierzbicki AS, Lumb PJ, Semra YK, Crook MA. Effect of atorvastatin on plasma fibrinogen. *Lancet* 1998; **351:** 569–70.
2 Townend J, O'Sullivan J, Wilde JT. Hyperhomocysteinaemia and vascular disease. *Blood Rev* 1998; **12:** 23–34.
3 de Lorgeril M, Renaud S, Mamelle N, et al. Mediterranean alpha-linolenic acid rich diet in secondary prevention of coronary heart disease. *Lancet* 1994; **343:** 1454–59.
4 Basu TK, Mann S. Vitamin B6 normalizes the altered sulfur amino acid status of rats fed diets containing pharmacological levels of niacin without reducing niacin's hypolipidemic effects. *J Nutr* 1997; **127:** 117–21.
5 Hotline on the Bezafibrate Infarction Prevention Trial. XXth Congress of the European Society of Cardiology. August 21–26, 1998. Vienna, Austria.

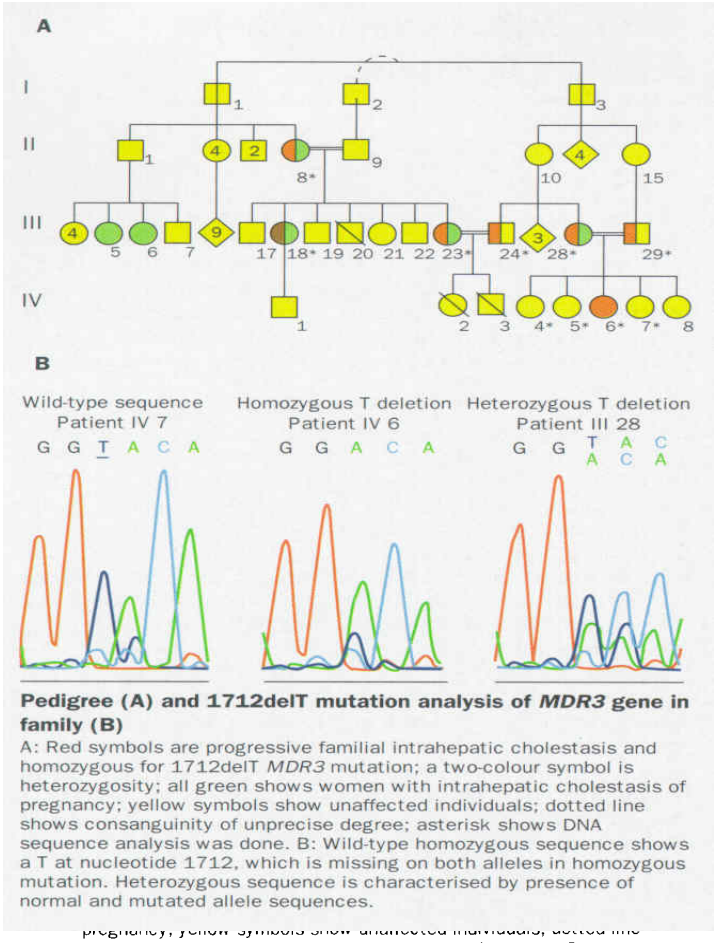
Explorations Fonctionnelles Cardiorespiratoires et Métaboliques, niveau 6, CHU Nord, 42055 Saint-Etienne, France (M de Lorgeril); **Département de Cardiologie, CHU de Rennes, Rennes and Fédération de Biochimie, Hôpital Edouard Herriot, Lyon**

Heterozygous non-sense mutation of the *MDR3* gene in familial intrahepatic cholestasis of pregnancy

Emmanuel Jacquemin, Danièle Cresteil, Sylvie Manouvrier, Odile Boute, Michelle Hadchouel

The pathogenesis of intrahepatic cholestasis of pregnancy is obscure, although a role of female sex hormones or metabolites, and exogenous and genetic factors impairing bile secretion, has been suggested.[1,2] We have reported previously that homozygous non-sense mutations in the human multidrug resistance 3 (*MDR3*) gene lead to a subtype of progressive familial intrahepatic cholestasis (PFIC), with high serum γ-glutamyltransferase activity.[3] MDR3 P-glycoprotein is a canalicular phospholipid translocator involved in the biliary secretion of phospholipids.[4] In our previous study,[3] we mentioned that the heterozygous mother of an affected child experienced recurrent episodes of intrahepatic cholestasis of pregnancy. This finding suggests the possible role of a *MDR3* gene defect in the pathogenesis of intrahepatic cholestasis of pregnancy, which is hepatocellular in origin as PFIC.

We now report the coexistence of PFIC and intrahepatic cholestasis of pregnancy associated to a *MDR3* gene defect in a large consanguinous family (figure). The proband (patient IV 6) had PFIC and received a liver graft at age 6 years. RT-PCR was done on liver RNA and sequence analysis of the RT-PCR products covering the complete coding sequence of the *MDR3* cDNA showed a homozygous single-nucleotide deletion (1712delT) starting at codon 571. This deletion results in a frameshift and introduces a stop codon 15 codons downstream, leading to an inactive truncated protein.[3,5] The homozygosity of patient IV 6 for the mutation and the heterozygosity of the parents (III 28, III 29) was confirmed by sequencing and restriction analysis of amplified genomic DNA, since the



Pedigree (A) and 1712delT mutation analysis of *MDR3* gene in family (B)

A: Red symbols are progressive familial intrahepatic cholestasis and homozygous for 1712delT *MDR3* mutation; a two-colour symbol is heterozygosity; all green shows women with intrahepatic cholestasis of pregnancy; yellow symbols show unaffected individuals; dotted line shows consanguinity of unprecise degree; asterisk shows DNA sequence analysis was done. B: Wild-type homozygous sequence shows a T at nucleotide 1712, which is missing on both alleles in homozygous mutation. Heterozygous sequence is characterised by presence of normal and mutated allele sequences.

pregnancy; yellow symbols show unaffected individuals; dotted line shows consanguinity of unprecise degree; asterisk shows DNA sequence analysis was done. B: Wild-type homozygous sequence shows a T at nucleotide 1712, which is missing on both alleles in homozygous mutation. Heterozygous sequence is characterised by presence of normal and mutated allele sequences.

mutation deletes a *Rsa*I restriction site. Within this family, six women had had at least one typical episode of intrahepatic cholestasis of pregnancy, characterised by pruritus, abnormal serum liver tests with a cholestatic pattern in the third trimester (eg in one women alkaline phosphatase 179 IU/L, alanine aminotransaminase 252 IU/L, γ-glutamyl transferase 41 UI/L and bile acids 119 μmol/L), resulting in fetal deaths in three women and spontaneous and progressive disappearance of cholestasis after delivery. Restriction and sequencing analyses of amplified genomic DNA was available in four women, and showed that they were heterozygous for the 1712delT mutation.

We have provided evidence for a genetic basis of intrahepatic cholestasis of pregnancy. The heterozygous state for a *MDR3* gene defect probably represents a genetic predisposition in this family, since the disease is not constant for every pregnancy in an affected women. The coexistence of non-genetic factors, such as female sex hormones and metabolites, can modify heterozygous *MDR3* expressivity directly by decreasing normal allele expression, or indirectly by impairing the function of transport systems involved in bile secretion.[1,2] Such events could favour the transient decompensation of the heterozygous state for a *MDR3* gene defect during pregnancy, which could lead to intrahepatic cholestasis of pregnancy. As for PFIC, the disease could be caused by bile in which the toxic effect of detergent bile salts is not inactivated by phospholipids.[3,4] This preliminary report may justify to search for a *MDR3* gene mutation in intrahepatic cholestastis of pregnancy, especially when serum γ-glutamyltransferase activity is raised.

Supported in part by the Assistance Publique-Hôpitaux de Paris (CRC number 97001) and Association Française contre les Myopathies, Paris, France.

1 Leevy CB, Koneru B, Klein KM. Recurrent familial prolonged intrahepatic cholestasis of pregnancy associated with chronic liver disease. *Gastroenterology* 1997; **113:** 966–72.
2 Meng LJ, Reyes H, Axelson M, et al. Progesterone metabolites and bile acids in serum of patients with intrahepatic cholestasis of pregnancy: effect of ursodeoxycholic acid therapy. *Hepatology* 1997; **26:** 1573–79.
3 De Vree JML, Jacquemin E, Sturm E, et al. Mutations in the *MDR3* gene cause progressive familial intrahepatic cholestasis. *Proc Natl Acad Sci USA* 1998; **95:** 282–87.
4 Smith AJ, de Vree JML, Ottenhoff R, Oude Elferink RPJ, Schinkel AH, Borst P. Hepatocyte-specific expression of the human MDR3 P-glycoprotein gene restores the biliary phosphatidycholine excretion absent in Mdr2 (-/-) mice. *Hepatology* 1998; **28:** 530–36.
5 Jacquemin E, de Vree JML, Cresteil D, et al. MDR3 deficiency; progressive familial intrahepatic cholestasis (PFIC) due to *MDR3* gene mutations. *Hepatology* 1998; **28:** 316A.

Hepatology Unit, Department of Pediatrics, and INSERM U 347, Bicêtre Hospital, Le Kremlin Bicêtre, France; and Medical Genetics, Department of Pediatrics, Jeanne de Flandre Hospital, 59037 Lille Cedex, France (E Jacquemin; e-mail emj@kb.inserm.fr)

Monitoring HIV-1 treatment in immune-cell subsets with ultrasensitive fluorescence-in-situ hybridisation

Bruce K Patterson, Mary Ann Czerniewski, John Pottage, Michelle Agnoli, Harold Kessler, Alan Landay

Studies of HIV-1 viral dynamics have shown that HIV-1 replicates in cells producing over 1 billion viral particles per day.[1] Highly active antiretroviral treatment (HAART) reduces the amount of HIV-1 RNA in plasma within several weeks. Viral reservoirs, however, exist in cells within lymph nodes and tonsils that contribute to a second order of viral decay.[2] Cellular reservoirs of HIV-1 that remain in spite of effective treatment may reconstitute the viral burden to pre-treatment levels after treatment failure or non-compliance.[3]

Blood samples were obtained from five patients at baseline, 4 weeks, 12 weeks, and 24 weeks after treatment with zidovudine (200 mg, three times a day), lamivudine (150 mg twice a day), and indinavir (800 mg three times a day). Amplicor reverse transcriptase (RTPCR) was used to determine the response of plasma viral load to HAART. In three of five patients studied (table), plasma viral load dropped to undetectable levels by week 4. In the other two patients, plasma viral load dropped to less than 400 copies by 4 weeks and undetectable levels until week 24.

To investigate whether viral production in specific cell types might explain the immunological changes and identify treatment failures, we did three-colour, simultaneous immunophenotyping and ultrasensitive in-situ hybridisation (UFISH).[4] We identified and quantified productively infected, $CD4^+$, $CD45RO^+$, gag-pol mRNA T lymphocytes in all patients. The percentage of productively infected cells initially decreased in all five patients by week 4. In 3 of the 5 patients, the percentage of productively infected cells increased while on therapy. This increase was accompanied by an increase in plasma viral load in one patient but not in the others. The explanation for this pattern in the latter group may be that a reservoir of infected cells remains during treatment that contains transcriptionally active virus without abundant release.

Much as the CD4 count is a clinical pointer rather than a predictor of disease activity, viral load is a virological pointer rather than a marker of disease activity. In-vitro analyses, show that the CD4, CD45RO T cell supports