Research letters

Frequency of C3435T polymorphism of *MDR1* gene in African people

Elke Schaeffeler, Michel Eichelbaum, Ulrich Brinkmann, Anja Penger, Steven Asante-Poku, Ulrich M Zanger, Matthias Schwab

The variability of P-glycoprotein expression between individuals is linked to a C3435T polymorphism of the human *MDR1* gene. Concentration of P-glycoprotein in intestinal epithelial cells and in a subset of lymphoid cells is substantially lower in people with the T/T genotype than those with the C/C genotype. We compared allele frequencies of the C3435T polymorphism in random samples of west African, African American, white, and Japanese people. We recorded a significantly higher frequency of the C/C genotype in West Africans and African Americans (142 of 172 [83%] and 25 of 41 [61%], respectively), than in white people (139 of 537 [26%]) (p<0·0001). These findings could affect use of drugs that are P-glycoprotein substrates (such as HIV-1 protease inhibitors and ciclosporin) in African populations.

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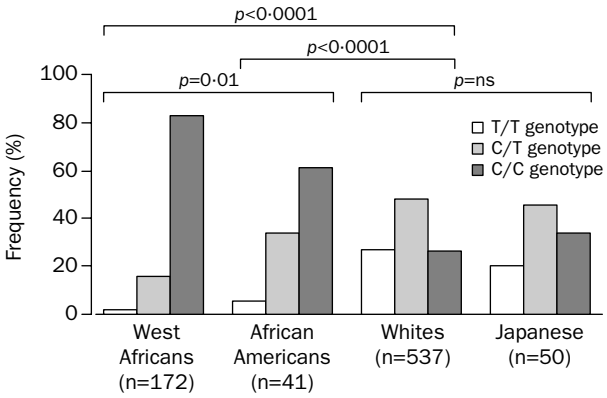
The *MDR1* gene product P-glycoprotein was first identified in cancer cells as a protein responsible for resistance to many drugs.[1] P-glycoprotein is also expressed in normal tissues where it functions as a defence mechanism against potential toxic substances ingested with the diet. In intestinal cells, renal proximal tubule cells, and endothelial cells of brain capillaries, P-glycoprotein is an energy-dependent efflux pump that transports hydrophobic xenobiotics (eg, anthracyclines) and peptides from the inside of cells to the outside.[1] *MDR1* is polymorphic and 15 mutations have been identified in the gene. The C3435T polymorphism in exon 26 correlates with expression of P-glycoprotein in the intestine, and people who are homozygous for the T allele have on average substantially lower intestinal P-glycoprotein expression than those homozygous for the C allele.[1] Since concentrations of P-glycoprotein in the intestine determine the extent of drug absorption, genotype-related differences in bioavailability of drugs are seen.

For many genetic polymorphisms, substantial differences in allele frequencies have been seen between racial groups. We compared allele frequencies of the C3435T polymorphism in 172 West Africans (Ghanians) and 41 African Americans with those of 537 white and 50 Japanese people using χ^2 analysis. All individuals in these ethnic groups were randomly selected, healthy individuals who were unrelated. Ghanians were recruited from hospital staff and medical students at Ghana Medical School, Accra, Ghana and belonged mainly to the Ga tribe. All other individuals were volunteers who participated either in phase I clinical trials or in various drug metabolism studies. Individuals were assessed by medical and physical examination. The study was approved by ethics committees and all volunteers gave written informed consent.

142 of 172 (83%) Ghanians and 25 of 41 (61%) African Americans were homozygous for the C allele, whereas only 139 of 537 (26%) white people and 7 of 50 (34%) Japanese, showed this genotype (p<0·0001, figure). The frequency of the C allele (C/T or C/C genotype) was 90% (311 of 344 alleles) in Ghanians compared with 50% (534 of 1074 alleles) in white people (odds ratio 9·5 [95% CI 6·5–13·9]; p<0·0001).



Genotype frequencies for the C3435T *MDR1* polymorphism in West Africans (Ghanians), American Africans, white, and Japanese people

NS=not significant. Genotyping was carried out by PCR restriction fragment length polymorphism analysis and denaturing high-performance liquid chromotography analysis as previously described.[5]

Bacterial and viral gastroenteritis is endemic in tropical countries and is associated with substantial infant morbidity and mortality. We propose that the much higher frequency of the C/C genotype in West Africans compared with white and Japanese populations results from a selective advantage offered by this genotype against gastrointestinal-tract infections. Overdominance of a genotype as a consequence of natural selection by infectious diseases has already been shown for the glucose-6-phosphate dehydrogenase (*G6PD*) gene, for which the predominant polymorphism, *G6PD* A-, is associated with resistance to *Plasmodium falciparum* malaria in hemizygous men and in heterozygous women in sub-Saharan Africa.[2]

P-glycoprotein plays a part in defence aganist viral infections, especially in the cellular uptake of virus particles and virus production. Overexpression of P-glycoprotein reduces susceptibility of human $CD4^+$ cells to infection with HIV-1, probably by affecting viral fusion and downstream events.[1] The proposed protective nature of raised P-glycoprotein expression resembles that of another ABC transporter gene *CFTR*. In *CFTR*, which is closely related to the *MDR1* gene, maintenance of a high frequency of a certain mutation in the *CFTR* gene (ΔF508 allele) has been explained by protection against *Salmonella typhi* and resistance to cholera toxin.[3] Our findings might be of clinical importance for treatment with P-glycoprotein substrates such as the new HIV-1 protease inhibitors (eg, nelfinavir, ritonavir, saquinavir) and the immunosuppressant ciclosporin A.[1] The consequences of higher intestinal P-glycoprotein expression due to increased frequency of the C/C genotype in Africans could be that bioavailability and plasma A in concentrations of these drugs are lower in Africans than in white people. This assumption is supported by results of studies which show

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that maximum concentrations of ciclosporin A in plasma were substantially lower in African Americans than in white people.[4] Moreover, a significantly higher P-glycoprotein-mediated rhodamine efflux from lymphoid cells has been shown in individuals with the C/C genotype.[5] Thus, the C/C genotype could be a factor restricting access of HIV-1 protease inhibitors to their major cellular target, the $CD4^+$ T-lymphocytes, which are known to express P-glycoprotein. Further studies are needed to clarify the mechanism by which the C3435T polymorphism leads to decreased P-glycoprotein expression and to define its role as a susceptibility factor for infectious diseases and drug treatment with P-glycoprotein substrates.

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Dr Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany (E Schaeffeler PhD, Prof M Eichelbaum MD, U M Zanger PhD, M Schwab MD)**; Divison of Clinical Pharmacology, Eberhard-Karls-University, Tübingen, Germany** (Prof M Eichelbaum MD)**; Epidauros Biotechnology, Pharmacogenetics Laboratory, Bernried, Germany** (U Brinkmann PhD, A Penger PhD)**; and Department of Biochemistry, University of Ghana Medical School, Accra, Ghana** (S Asante-Poku PhD)

Correspondence to: Dr Matthias Schwab, Dr Margarete Fischer-Bosch Institute of Clinical Pharmacology, Auerbachstrasse 112, 70376 Stuttgart, Germany
(e-mail: matthias.schwab@ikp-stuttgart.de)

Prediction of severe disseminated adenovirus infection by serum PCR

Marcela Echavarria, Michael Forman, Maarten J D van Tol, Jaak M Vossen, Patricia Charache, Aloys C M Kroes

Adenoviruses are increasingly recognised as viral pathogens that can cause fatal infections in immunocompromised patients, particularly recipients of haematopoietic stem-cell grafts. Adenovirus infections are not easily diagnosed and the development of a severe infection cannot be predicted by standard culture techniques. In a pilot study, we investigated the value of adenovirus DNA detection in serum as a marker of disseminated disease in 14 patients with defined patterns of adenovirus infections. The results show that the appearance of adenoviral DNA in serum preceded the development of a severe or fatal adenovirus infection. Because proper management is dependent on early diagnosis and differentiation from other conditions, this test may be a valuable tool in the management of adenovirus infection.

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Adenoviruses are a common cause of self-limiting infections of the respiratory or gastrointestinal tract. Adenoviruses are frequently found in the serum of immunocompromised patients, and these viruses may persist in a latent form after infection. In these cases, the reactivated virus may be shed asymptomatically or may cause disease—eg, pharyngitis, conjunctivitis, pneumonia, haemorrhagic cystitis, colitis, hepatitis, or encephalitis.[1] These infections may cause fatal disease—particularly in young patients—by widespread dissemination, which can be difficult to recognise. An increasing incidence of adenovirus infections has been observed in recipients of stem-cell transplantation, with mortality rates as high as 25%.[2,3] Early detection of dissemination would permit accurate diagnosis and the prompt initiation of appropriate clinical management. Localised disease cannot be distinguished from disseminated disease by viral cultures.

The presumed pathogenesis of viral reactivation originating from the respiratory or gastrointestinal tract would imply that the appearance of adenovirus in serum is associated with an early stage of viral dissemination. In this pilot study, and with a PCR method described in 1998,[4] we investigated the value of adenovirus DNA detection in serum as a marker of disseminated disease in stem-cell-transplantation recipients.

The incidence and outcome of adenovirus infection were retrospectively studied in 328 consecutive paediatric recipients of an allogeneic stem-cell grafts transplanted during 1985–99 at the Leiden University Medical Center, Leiden, Netherlands. Urine, throat, and stool samples were cultured at least once every 2 weeks during the initial 6 months after transplantation and subsequently as clinically indicated. Adenovirus was recovered from 38 (12%) of recipients. 17 (45%) of these patients had symptoms of adenovirus disease, and the infection was fatal in seven (18%) of them. No clustering of distinct serotypes or a typically seasonal distribution of the first occurrence of positive cultures was evident within this population. 14 patients were selected for investigation of the presence of adenovirus DNA in blood and divided into four clinical categories: A=control cases (with no evidence of adenovirus infection and with negative culture results); B=symptom-free patients, with adenovirus culture-positive samples; C=patients with localised adenovirus-disease—ie, enteritis or haemorrhagic cystitis; and D=patients with a clinical diagnosis of fatal disseminated adenovirus-disease, supported by cultures or necropsy findings. Clinical data and viral serotypes are given in the table.

48 serum samples from these 14 patients were tested in a blinded manner at Johns Hopkins Medical Institutions, Baltimore, USA, for the presence of adenovirus DNA by PCR, using methods described previously for urine,[4] and subsequently optimised for serum (sensitivity of adenovirus detection: 0·1 plaque-forming units/mL). A positive result was defined as the presence of a 139 base-pair band in the agarose gel with corresponding signal after Southern blot hybridisation and autoradiography.

PCR results of the 14 patients are summarised in the table. All sera from control patients, those with symptom-free shedding, and those with localised disease (categories A, B, and C) were negative for adenovirus by PCR. Of four patients with disseminated adenovirus disease (category D), three had strongly positive PCR results on multiple samples. One of these patients had a positive adenovirus-PCR even before transplantation. These three patients had hepatitis and multiorgan failure with or without enteritis. The fourth patient, with pneumonia, had only one weakly positive PCR result on day 57 after transplantation. This patient had continuously positive adenovirus-culture stool samples, and died on day 97. On necropsy this patient was found to have adenovirus-positive cultures from the liver, colon, and lung.

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