healthy subjects *(26)*. Because the adverse effects of homocysteine are most likely related to its prooxidant properties *(6)*, a direct involvement of the amino acid in this phenomenon may be therefore hypothesized.

In conclusion, PD patients undergoing regular treatment with L-DOPA have higher plasma homocysteine concentrations than healthy subjects. The increase seems to be related to the methylated catabolism of the drug, although other factors, such as enzymatic defects in the remethylation pathway of homocysteine, are likely to play a substantial role. Increased risk of cerebro- and cardiovascular diseases has been reported in PD patient populations, although the issue is highly disputed *(27, 28)*. Whether the increase in plasma homocysteine occurring in PD patients plays a role in the progression of the disease remains to be established.

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The *CYP3A43 Allele: Is It Really Rare?** *Ron H.N. van Schaik,[1*] Saskia N. de Wildt,[2] Rebecca Brosens,[2] Marianne van Fessem,[1] John N. van den Anker,[2] and Jan Lindemans[1]* (Departments of [1] Clinical Chemistry and [2] Pediatrics, University Hospital Rotterdam, PO Box 2040, 3000 CA Rotterdam, The Netherlands; * author for correspondence: fax 31-10-4367894, e-mail vanschaik@ckcl.azr.nl)

Enzymes of the cytochrome P450 system are involved in the metabolism of a broad range of foreign compounds, such as drugs, environmental pollutants, and carcinogens *(1)*. The most abundant enzyme in the human liver is cytochrome P450 3A4 (CYP3A4) *(2)*. This enzyme is involved in the metabolism of >50% of all drugs used in humans *(3, 4)*, and the interindividual differences in the pharmacokinetics of these drugs are thought to be related to variations in CYP3A4 activity *(4–6)*. These variations may be caused by age and disease-related differences, by drugs inducing or repressing transcription/translation, or by genetic polymorphisms. Although the *CYP3A4* gene was initially thought not to be polymorphic, recent reports have described three genetic variants of this gene: *CYP3A4*1B*, *CYP3A4*2*, and *CYP3A4*3 (7, 8)*. The allelic frequency for the *CYP3A4*1B* allele, which contains an A(−290)G substitution in the promoter region of *CYP3A4*, ranges from 0.0% in Chinese and Japanese Americans to >54% in African Americans *(8, 9)*. American and European Caucasians were reported to have an allelic frequency of ~4–5% *(8–11)*. The *CYP3A4*2* allele, which encodes a Ser222Pro change, has an allelic frequency of 2.7% in the white (Finnish) population *(8)*. Because variant alleles that are found in >1% of the population are defined as genetic polymorphisms *(12)*, both the *CYP3A4*1B* and the *CYP3A4*2* allele are consid-

ered to be genetic polymorphisms of *CYP3A4*. In addition, a variant allele found in the DNA of a single Chinese subject contained a T1437C substitution *(8)*. Because this allele, encoding a Met445Thr change, was not found in any other of the 91 subjects investigated in that study, it was referred to as a rare allele.

In this study, we developed a PCR-restriction fragment length polymorphism (RFLP) procedure for the detection of the *CYP3A4*3* allele. We used this assay to determine the allelic frequency of *CYP3A4*3.* EDTA-whole blood was obtained from 499 healthy Dutch Caucasian volunteers after informed consent. The study was approved by the Medical Ethical Committee of the University Hospital Rotterdam. We isolated genomic DNA from 300 μL of blood, using the GenomicPrep Blood DNA Isolation reagent set (Amersham Pharmacia Biotech). DNA yields were estimated by measuring the absorbance at 260 nm (A_{260}). A total of ~50 ng of genomic DNA was used in a PCR volume of 50 μL. The PCR mixture contained 1× buffer [10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, and 0.01 g/L gelatin (Perkin-Elmer)], 0.2 mM each dNTP (Roche), 1.25 U of AmpliTaq Gold (Perkin-Elmer), and 40 pmol of each of forward primer (5′-TGG ACC CAG AAA CTG CAT ATG C-3′; nucleotides 23255–23276; GenBank sequence AF209389) and reverse primer (5′-GAT CAC AGA TGG GCC TAA TTG-3′; nucleotides 23483–23503; GenBank sequence AF209389). The nucleotides underlined are mismatches with the *CYP3A4* sequence, creating a *Nsi*I restriction site in the wild-type *CYP3A4* PCR product. When the *CYP3A4*3* allele is amplified, this *Nsi*I site is disrupted. PCR conditions were as follows: 7 min at 94 °C; 35 cycles of 1 min at 94 °C, 1 min at 55 °C, and 1 min at 72 °C; and finally 7 min at 72 °C. The 249-bp PCR products of five DNA samples were sequenced, confirming that indeed only the *CYP3A4* gene was amplified.

The PCR product (15 μL) was digested with 10 U of *Nsi*I (Roche) for 2 h at 37 °C, and analyzed on a 3% MP agarose/Tris-borate-EDTA gel with ethidium bromide staining. The fragments produced were 226 and 23 bp for the wild-type sequence (Fig. 1, lane wt/wt) and 249, 226, and 23 bp for heterozygous sequences (Fig. 1, lane wt/*3); the 23-bp fragments are not visible on the gel. Samples that produced a heterozygous signal were reanalyzed using the PCR-RFLP assay. Subsequently, heterozygosity for *CYP3A4*3* was confirmed by sequencing of the PCR product with the reverse primer on an automated ABI 310 capillary sequencer (Perkin-Elmer) using the Big Dye Terminator Cycle Sequencing reagent set (Perkin-Elmer). To check reproducibility, the *CYP3A4*3* assay was performed 10 times for a wild-type and 10 times for a heterozygous sample; the genotype was identified correctly each time.

The *CYP3A4*3* PCR-RFLP assay was applied to 499 genomic DNA samples obtained from Dutch Caucasians. In 488 cases, digestion of the 249-bp PCR product produced the 226- and 23-bp fragments, as expected for wild-type samples, whereas in 11 cases (2.2%), the heterozygous signal was produced. No homozygotes were detected. The allelic frequency of *CYP3A4*3* in these Caucasians was therefore 1.1%. These allelic and genotypic frequencies are in Hardy-Weinberg equilibrium ($P = 0.80$). In the heterozygous samples, direct sequencing showed a mixed T/C peak corresponding to position 1437, indicating that the nucleotide change was indeed T1437C in all cases.

Variant *CYP3A4* alleles in the population may contribute to interindividual variability in CYP3A4 activity, and detecting genetic polymorphisms may help to predict an individual's ability to respond to certain drugs. The *CYP3A4*3* allele, which has a T1473C change that produces a Met445Thr substitution in exon 12, was found in only 1 Chinese subject from Shanghai and could not be detected in 91 other individuals *(8)*. Because of this, *CYP3A4*3* was described as being a rare allele, which may lead researchers to assign a low priority to performing functional studies on this allele. Our data indicate that the *CYP3A4*3* allele is not limited to a single individual, but has an allelic frequency of 1.1% in Caucasians. This implies that the variant *CYP3A4*3* allele is not a rare allele, but instead represents a genetic polymorphism that can be found in a substantial part of the population. The identification of the *CYP3A4*3* variant allele as a genetic polymorphism, in addition to the *CYP3A4*1B* and **2* polymorphisms, has implications for the number of variant *CYP3A4* alleles to be expected in the population. The *CYP3A4*1B* allele potentially alters the transcription efficiency and thus the overall enzymatic activity of CYP3A4. Although initial reports suggested decreased activity in vivo *(7, 13, 14)*, increased activity in vitro *(15, 16)* and no effect *(10, 14, 17)* have also been reported. For the variant allele *CYP3A4*2*, a decreased enzymatic activity was observed for nifedipine, but not for testosterone *(8)*. For *CYP3A4*3*, the location of the amino acid that is changed in the CYP3A4 protein is near the cysteine that is involved in the active site of the enzyme *(8)*. This might induce structural differences, leading to alteration in enzymatic

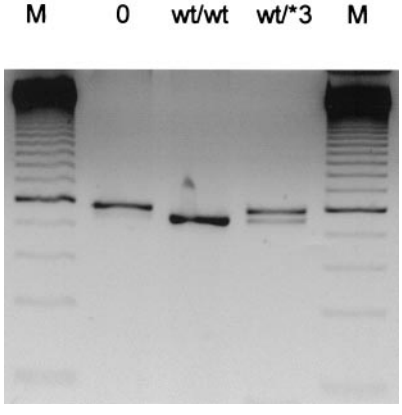

Fig. 1. PCR-RFLP procedure for the *CYP3A4*3* allele.

Analysis on a 3% agarose/Tris-borate-EDTA gel. *Lane 0*, undigested PCR product; *lane wt/wt*, *Nsi*I-digested PCR fragments of 226 and 23 bp for a wild-type DNA sample; *lane wt/*3*, *Nsi*I-digested PCR fragments of 249, 226, and 23 bp for a heterozygous DNA sample. The 23-bp fragment is not visible. *Lanes M*, base pair marker (50-bp ladder). Gel is printed as a negative.

activity. However, expression studies need to be performed to confirm this. Taking into account the allelic frequencies of the genetic polymorphisms in *CYP3A4* (10% heterozygous for *CYP3A4*1B*, 5.4% heterozygous for *CYP3A4*2*, and 2.2% heterozygous for *CYP3A4*3*), this implies that ~15% of the (Caucasian) population may carry a genetic polymorphism in this allele. Because genetic polymorphisms may exhibit strong differences in occurrence among different ethnic groups, other populations need to be investigated to determine the allelic frequency of *CYP3A4*3*.

In conclusion, we have described and validated a PCR-RFLP assay for the *CYP3A4*3* allele. The frequency of this variant allele in the Caucasian population (1.1%) indicates that it might be important in predicting CYP3A4 activity based on genotype. Future research should be directed toward elucidating the effect of this polymorphism on CYP3A4 enzymatic activity and toward establishing whether this is solely a genetic, or also a functional, polymorphism.

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Increases in Nitric Oxide Concentrations Correlate Strongly with Body Fat in Obese Humans, *Jong Weon Choi,[1*] Soo Hwan Pai,[1] Soon Ki Kim,[2] Masafumi Ito,[3] Chang Shin Park,[4] and Young Nam Cha[4]* (Departments of [1] Clinical Pathology and [2] Pediatrics, College of Medicine, Inha University Hospital, Inchon, 400-103, South Korea; [3] Department of Pathology, Nagoya University Hospital, Nagoya, 466-0065 Japan; [4] Department of Pharmacology and Toxicology, Medicinal Toxicology Center, College of Medicine, Inha University, Inchon, 400-103, South Korea; * address correspondence to this author at: Department of Clinical Pathology, Inha University Hospital, 7-206, 3-ga, Shinheung-dong, Jung-gu, Inchon, 400-103, South Korea; fax 82-32-890-2529, e-mail jwchoi@inha.ac.kr)

NO is produced in many different cells and is involved in the regulation of such physiological and pathophysiological processes as inflammation, vasodilation, and metabolism *(1)*. Depending on the cell type, NO is produced in an enzymatic reaction catalyzed by one of the three isoforms of NO synthase (NOS): neuronal NOS, endothelial NOS, and inducible NOS *(2)*. Measurement of the NO metabolites, nitrate and nitrite (NOx), is also important as a marker of NOS enzyme activity.

Obesity is a condition involving an excess accumulation of body fat, and the prevalence of obesity is rapidly increasing worldwide. Excessive weight and obesity are leading to nutrition-related disorders of clinical and public health concern. Recent studies have suggested a role for NO in the regulation of food intake in an animal experiment *(3, 4)*. Endothelial and inducible NOS have been shown to be present in adipose tissue of the rat *(5)*, suggesting that adipose tissue may be a potential source of NO production. Previous reports demonstrated that NOS activity and inducible NOS protein were also present in human subcutaneous adipose tissue *(6)* and showed that inhibition of NOS led to increased lipolysis in this tissue *(7)*. However, how NO production changes as body mass index (BMI) increases in apparently healthy subjects has not been studied extensively. Moreover, correlation studies of serum NOx concentrations, body fat mass, and blood lipid concentrations in healthy subjects are limited. Therefore, in the present study, we investigated the changes in NOx concentrations according to BMI and evaluated the relationships among NOx concentrations, total body fat, and lipid profiles in adolescents.