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Short communication

Novel variant of *CYP2D6*6* is undetected by a commonly used genotyping procedure

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Abstract:
We report the identification of a novel and defective variant of the gene encoding cytochrome P450 2D6 (CYP2D6). This novel variant is a subtype of *CYP2D6*6* that was undetected by a commercially available 5' exonuclease-based assay. Because the novel variant was found in only one of 609 individuals, it represents a rare subtype of *CYP2D6*6* that may be restricted to a single family or a subpopulation. A procedure for the identification of the novel *CYP2D6*6* variant using restriction enzyme treatment of amplified fragments was developed.

Key words:
CYP2D6 genotyping, novel variant, 5' exonuclease assay

Introduction

CYP2D6 metabolizes a large number of commonly prescribed drugs [7]. Variations in the gene encoding this enzyme have been associated with individual differences in drug metabolism rates [7]. Many defective alleles of *CYP2D6* exist [1]. One of these alleles, *CYP2D6*6*, is characterized by a single nucleotide deletion, 1707delT, in exon 3 resulting in the formation of a stop codon near the deletion site (CAG·TGG·GTG>CAG·GGG·TGA; codons with 1707delT underscored). This allele has a frequency of approximately 1% in European Caucasian populations [1].

Four allelic subtypes of *CYP2D6*6* have been reported, namely, *CYP2D6*6A* [8], *CYP2D6*6B* [3, 4], *CYP2D6*6C* [6] and *CYP2D6*6D* [6]. These subtypes share 1707delT but differ at the nucleotide positions 1976, 3288 and 4180. As part of a pharmacogenetic screening program to detect defective *CYP2D6* alleles, we identified a novel variant of *CYP2D6*6*. In the current study, we report the identification of this novel variant.

Materials and Methods

DNA samples from 609 European Caucasians were examined. These DNA samples had been isolated from peripheral blood using the JETQUICK Blood & Cell Culture DNA Spin Kit (Genomed GmbH, Lőhne, Germany). Identification of *CYP2D6*6* was based on restriction fragment length polymorphism (RFLP) analysis of a fragment that was amplified by PCR using primers with the following sequences: (forward) 5'-TCCCAGCTGGAATCCGGTGTCG-3' and (reverse)

5'-GGAGCTCGCCCTGCAGAGACTCCT-3' [5]. This procedure was not able to distinguish between *CYP2D6*6* and *CYP2D6*28* because the nucleotide changes in these two alleles, i.e., 1707delT and 1704C>G, respectively, abolish the recognition site of the restriction enzyme *BtsI* that was used for the treatment of amplified fragments. All samples that lack this recognition site were subjected to a 5' exonuclease-based analysis for the identification of *CYP2D6*6* using commercially available reagents (assay ID: C_32407243_20; Applied Biosystems, Foster City, CA, USA). The 5' exonuclease-based genotyping was performed according to the guidelines of the manufacturer. Samples were selected for sequencing based on the results from this analysis. Fragments were amplified using the same primers as described above, and both strands were sequenced by the Sanger method following spin centrifugation. The amplification primers also served as sequencing primers. Sequencing was performed by Eurofins MWG Operon in Ebersberg, Germany, using capillary electrophoresis to determine fragment size.

Results and Discussion

On the basis of the results from the PCR-RFLP analysis, four individuals were suspected of being heterozygous for *CYP2D*6* or *CYP2D6*28* because the amplified fragments from these individuals were incompletely digested by the restriction enzyme *BtsI*. In contrast, the amplified products from all other individuals were completely digested. Using the 5' exonuclease-based procedure the allele *CYP2D6*6* was not identified in any of the four individuals. Sequencing revealed that three of these individuals were heterozygous with C/G at position 1704, where G at this position is characteristic of *CYP2D6*28*. The fourth sample was heterozygous for 1707delT even though *CYP2D6*6* was not detected by the 5' exonuclease-based analysis of this sample. This sample was also heterozygous with A/G at position 1749 and CT/AG at positions 1754–1755. These results suggest a novel allelic subtype of *CYP2D6*6*, i.e., a subtype that is distinct from *CYP2D6*6A*, *CYP2D6*6B*, *CYP2D6*6C* and *CYP2D6*6D* (Tab. 1). Similar to *CYP2D6*6B* and *CYP2D6*6C*, this novel variant has an A at position 1976, indicating that it is more closely related to these two allelic subtypes than with *CYP2D6*6A* and *CYP2D6*6D*. A partial sequence of the novel variant of *CYP2D6*6* spanning from intron 2 to intron 4 has been deposited in GenBank® [2] under the accession number EF442770.1. We were not able to sequence the remaining portion of the novel variant because the sample in question is no longer at our disposal. Therefore, other nucleotide changes may occur in this novel variant in addition to those reported here.

Tab. 1. Sequence variations of *CYP2D6*6* subgroups

Allele	Nucleotide changes[1]
**6A*	**1707delT**
**6B*	**1707delT**, 1976G>A
**6C*	**1707delT**, 1976G>A, 4180G>C
**6D*	**1707delT**, 3288G>A
Novel variant (this study)	**1707delT**, 1749A>G, 1754-1755CT>AG, 1976G>A

[1]Nucleotides are numbered with reference to M33388 corrected for sequencing errors. The nucleotide variation in bold is shared by all *CYP2D6*6* alleles and is responsible for the phenotype (http://www.cypalleles.ki.se)

One explanation as to why the novel *CYP2D6*6* variant was undetected by the commercially available 5' exonuclease-based assay may be that the downstream primer of this assay targets a region encompassing the polymorphic nucleotides at positions 1749 and 1754–1755, which are the nucleotides that distinguish the novel variant from known *CYP2D6*6* alleles. However, because the primer and probe sequences are proprietary information of the manufacturer of the assay reagents, we were unable to verify this hypothesis.

The occurrence of the novel variant of *CYP2D6*6* may be restricted to a single individual, or it may be present in one or more populations at low frequencies. Therefore, this variant may exhibit limited effects on the rate of false negatives of the commercially available 5' exonuclease-based assay for the detection of *CYP2D6*6*. Nevertheless, the presence of rare *CYP2D6* variants, which are undetected by commonly used procedures, may explain sporadic occurrences of unexpected pharmacokinetic responses.

We developed a procedure to identify the novel allele by exploiting the formation of a *Bsr*F1 restriction enzyme recognition site (RCCGGY) resulting from the 1755T>G substitution. Using this restriction enzyme instead of the restriction enzyme *BtsI* in the

PCR-RFLP procedure described above we demonstrated that the novel variant manifested as two fragments of 294 bp and 457 bp, respectively. In contrast, amplified fragments of all other alleles including the known variants of *CYP2D6*6* and *CYP2D*28* were undigested by the restriction enzyme *Bsr*F1. The present report emphasizes the large allelic repertoire of *CYP2D6* and the challenges that are associated with the determination of these alleles.

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