Evolution of Dihydropyrimidine Dehydrogenase Diagnostic Testing in a Single Center during an 8-Year Period of Time

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A R T I C L E   I N F O

Article history:
Received 23 November 2017
Revised 16 October 2018
Accepted 25 October 2018

Key words:
Dehydrogenase
Dihydropyrimidine genetic variant
Fluoropyrimidine
Pharmacogenetics

A B S T R A C T

Objective: Fluoropyrimidine treatment can be optimized based on dihydropyrimidine dehydrogenase (DPD) activity. DPD dysfunction leads to increased exposure to active metabolites, which can result in severe or even fatal toxicity.

Methods: We provide an overview of 8 years of DPD diagnostic testing (n = 1194).

Results: Within the study period, our diagnostic test evolved from a single-enzyme measurement using first a radiochemical and then a nonradiochemical assay by ultra HPLC-MS in peripheral blood mononuclear cells with uracil, to a combined enzymatic and genetic test (ie, polymerase chain reaction) followed by Sanger sequence analysis of 4 variants of the DPYD gene (ie, DPYD*2A, DPYD*13, c.2846A>T, and 11295923C>G; allele frequencies 0.36%, 0.03%, 0.29%, and 1.35%, respectively). Patients who have 1 of the 4 variants tested (n = 814) have lower enzyme activity than the overall patient group. The majority of patients with the DPYD*2A variant (83%) consistently showed decreased enzyme activity. Only 24 (25.3%) of 95 patients (tested for 4 variants) with low enzyme activity carried a variant. Complete DPYD sequencing in a subgroup with low enzyme activity and without DPYD*2A variant (n = 47) revealed 10 genetic variants, of which 4 have not been described previously. We did not observe a strong link between DPYD genotype and enzyme activity.

Conclusions: Previous studies have shown that DPD status should be determined before treatment with fluoropyrimidine agents to prevent unnecessary side effects with possible fatal consequences. Our study in combination with literature shows that there is a discrepancy between the DPD enzyme activity and the presence of clinically relevant single nucleotide polymorphisms. At this moment, a combination of a genetic and enzyme test is preferable for diagnostic testing. (Curr Ther Res Clin Exp. 2018; 79:XXX-XXX).

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Introduction

The main chemotherapeutic agents used in many types of cancer, such as colorectal, gastrointestinal, and breast cancer, are the fluoropyrimidines 5-fluorouracil (5-FU), capecitabine, and tegafur. Treatment with these agents is not well tolerated in a subgroup of patients. In 20% to 40%, moderate to severe (fatal) toxicity occurs, including nausea and vomiting, diarrhea, mucositis/stomatitis, myelosuppression, and hand-foot syndrome. The enzyme responsible for degradation of fluoropyrimidines is dihydropyrimidine dehydrogenase (DPD), the first and rate-limiting enzyme of the pyrimidine degradation pathway. DPD dysfunction leads to an increased exposure to active metabolites, which can result in severe or even fatal toxicity. From the literature, the estimated percentage of individuals who are DPD deficient is 3% to 5% in the Caucasian populations. This deficiency can in most cases be related to genetic variants in the dihydropyrimidine dehydrogenase gene (DPYD). The DPYD gene on chromosome 1p22 has 23 exons and more than 100 variants have been reported in DPYD, of which only few have been studied in relation to decreased DPD enzyme activity and/or toxicity. Of the variants studied, only 3 variants have been reported that were consistently associated with toxicity and decreased DPD enzyme activity in patients treated with...
Figure 1. Evolution of dihydropyrimidine dehydrogenase (DPD) diagnostic testing over a period of time lasting 8 years.

A total of 1194 patients were diagnostically tested (enzyme and DNA level) for the presence of complete or partial DPD deficiency in 8 years. Based on the type of screening, these were divided into 3 groups (Figure 1). Patient group 1 consisted of 256 patients, who were phenotypically (DPD enzyme activity) analyzed between 2009 and 2013. Patients with decreased enzyme activity were subsequently genotyped for the DPD*2A variant. Patient group 2 consisted of 132 patients who were simultaneously phenotypically analyzed and genotyped for the DPD*2A variant. Patient group 3 consisted of 814 patients who were simultaneously phenotypically analyzed and genotyped for DPD*2A, DPD*13, rs67376798, and rs75017182. The procedures were in accordance with the ethical standards of the Helsinki Declaration of 1975 (as revised in 1983). The study was performed alongside standard diagnostic procedures, and thus waived from institutional review board approval.

Phenotypic DPD analysis

In the presence of reduced nicotinamide adenine dinucleotide phosphate, DPD converts thymine and uracil into 5,6-dihydrothymine and 5,6-dihydrouracil, respectively. Until December 2014, DPD activity was measured according to the method of van Kuilenburg et al.7 All subsequent samples were measured using a nonradiochemical method with uracil as substrate instead of 14C-labelled thymine. Peripheral blood mononuclear cells were isolated from 10 mL EDTA-anticoagulated blood within 24 hours after blood draw. Pellets were suspended in 200 μL milli-Q Merck, (Darmstadt, Germany) and cells were lysed by sonication for 2 minutes (microtip MS2 (Hielscher GmbH, Teltow, Germany), cycle 0.5, amplitude 80%). Cell debris was removed by centrifugation at 11,500 g for 20 minutes at 4 °C. The supernatant was used for the enzymatic analysis. Protein concentration was determined spectrometrically using the Pierce™ BCA Protein Assay Kit: Thermo (Fisher Scientific, Waltham Massachusetts). The assay mixture contained 35 mM potassium dihydrogen phosphate pH 7.4, 2.5 mM magnesium chloride, 1 mM dithiothreitol (DTT), 250 μM NADPH, 25 μM uracil, 5 μM internal standard, and 50 μg total protein in a final volume of 100 μL. The reaction tubes were incubated at 37°C for 60 minutes using a dry heating block. The reaction was terminated by the addition of 25 μL ice-cold 10% perchloric acid. The samples were placed on crushed ice for 10 minutes to obtain complete precipitation. After centrifugation at 11,000 g for 5 minutes at 4°C, subsequently 10 μL supernatant and 500 μL mobile phase ammonium formate buffer was transferred into a Costar spin-X (Corning, New York) centrifuge tube filter, 0.2 μm nylon membrane, and centrifuged at 11,000 g for 1 minutes at 4°C. The flow-through was transferred into a 96-well collection plate. Components were separated using a Waters Acquity ultra-high performance liquid chromatography (Waters, Etten-Leur, the Netherlands) and analyzed on the Waters Xevo TQ-S tandem mass spectrometer. DPD activity was expressed as the amount of 5,6-dihydrouracil formed per milligram total protein per hour. All samples were run in duplicate. Data acquisition and chromatographic analysis was performed by using MassLynx software (Waters, Milford, Massachusetts). The threshold for decreased DPD enzyme activity was determined by testing the enzyme activity of controls and set at a cut-off of 30% of the lower end of the spectrum. For more analytical information regarding the measurement of DPD enzyme activity we refer to the supplementary information.

Materials and Methods

Patients

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Genetic DPDY analysis

Total DNA was extracted from blood using the Blood L Kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) and the Hamilton Microlab STAR Line (Hamilton, Bonaduz, Switzerland) according to the protocol of the manufacturer. Specific primers with an additional M13-tag were used to amplify the protein coding exons and immediate flanking intronic regions of the fragments containing the DPDY c.1905+1G->A (rs3918290), c.1679T->G (rs55886062), and c.2846A->T (Asp949Val).1,3 Allele frequencies for these variants are, respectively, 0.58%, 0.03%, and 0.29%. More recently, a meta-analysis indicated that also the 1129-5923C->G (rs75017182) variant (with an allele frequency of 1.35%) is a clinically relevant predictor for fluoropyrimidine toxicity.3

Several methods have been described to directly or indirectly determine whether a patient is DPD deficient.9 Each of these methods has advantages and disadvantages and currently, no assay is stated to be the most optimal in terms of predicting toxicity, sensitivity and specificity, and cost-effectiveness. In this article, we provide an overview of 8 years of DPD diagnostic testing, in which our method changed from only DPD enzyme activity measurement to a combination of DPD enzyme activity assessment in peripheral blood mononuclear cells and DPDY genotyping for 4 variants (ie, DPDY*2A, DPDY*13, rs67376798, and rs75017182).

Prediction of variants of unknown significance

For in silico prediction we used similar methods as described in Offer et al11 but in more detail and using other additional fea-
Statistical Analysis

Enzyme activities were measured with 2 different methods: a radiochemical enzyme activity method (n = 380) and a nonradiochemical method using ultra-HPLC–MS (n = 814). The methods gave slightly different values, the cut-off point for decreased enzyme activity for the radiochemical enzyme activity method is 7.57 nmol/mg protein/h and for the mass spectrometry method 8.69 nmol/mg protein/h. Differences between groups were assessed using Pearson χ² test or ANOVA. Pearson correlation and χ² tests were performed to identify relationships between variables. Analyses were performed using SPSS version 22.0.0.1 (IBM-SPSS Inc, Armonk, New York).

Results

General characteristics of the patient population

In a period of 8 years, 1311 DPD diagnostics were processed in our center. For 117 samples, only DNA analysis was performed. Therefore, only 1194 patients were included in the comparisons shown below. Data on the development of toxicities were not available. An increase in the number of tests requests is observed in the last years (Supplementary Table S2). Within this period, the diagnostic approach evolved from a focus on enzyme activity measurement to a combined analysis of DPD enzyme activity and DPYD genetic testing of 4 genetic variants (Figure 1). The mean age of the patients analyzed for DPD in the diagnostic setting was 64.9 years and 50.1% were men (Table 1).

Enzyme activities

Enzyme activity was measured with 2 different methods. The radiochemical method was replaced by a nonradiochemical method in December 2014. Accuracy, precision, and reproducibility of this method was ascertained (ISO 15189 accreditation). In group 3, only the nonradiochemical method was used. The reference value for low enzyme activity appeared to be slightly different for the radiochemical and nonradiochemical method (7.57 vs 8.69 nmol/mg protein/h, respectively). Therefore, we analyzed both groups separately. The percentage of patients with decreased enzyme activity is lower in the patients analyzed in group 3. This is in line with the transition of only screening after evidence of toxicity in a patient to pretreatment screening of all patients. In total, DPD enzyme activity was measured in 1194 patients (380 patients measured with the radiochemical method [groups 1 and 2] and 814 patients measured with the nonradiochemical method [group 3]). We observed a small but significant correlation between age and DPD enzyme activity in groups 1 and 2 (Pearson correlation = 0.127; P = 0.043 and Pearson correlation = 0.218; P = 0.014, respectively). In group 3, no significant correlation was observed (P = 0.423). Sex did not influence enzyme activity.

Enzyme activity distribution is shown for group 3, the largest group tested for 4 variants (measured by the nonradiochemical method), in Figure 2 with 1 patient (0.1%) displaying 0 activity, 94 (11.5%) with intermediate activity (between 0 and 8.69 nmol/mg protein/h), and 719 (88.3%) with normal activity (>8.69 nmol/mg protein/h).

Genetic DPYD analyses

Standard DPYD analysis of the 4 clinically relevant variants (group 3) showed that only 18% of patients with a genetic variant had decreased enzyme activity (i.e., below the threshold) (Figure 2). Their mean (SD) enzyme activity was significantly lower (P < 0.001) than the patients without a genetic variant (11.0 [5.0] and 15.7 [5.6], respectively). The correlation between the enzyme activity and the presence of the *2A variant was most clear, with 83.3% of patients showing decreased enzyme activity (Table 2). In group 3, we observed 95 patients with decreased enzyme activity and of these only 24 (25.3%) carried at least 1 of the 4 tested variants.

In a subgroup of patients (n = 47) derived from patient groups 1 and 2, the entire coding region of the DPYD gene was analyzed. The subgroup consisted of patients with a decreased enzyme activity without the DPYD*2A variant (n = 46) who were routinely screened at that time and 1 patient of whom only DNA was available. A genetic variant was identified in 11 (23.4%) patients. In 3 patients, DPYD*13 was identified. One of these patients showed an absence of enzyme activity (0 activity). In 4 patients, rs75017182 was identified and in 1 patient rs67376798 was identified. All patients were heterozygous for the detected variants. In 3 other patients, a variant of unknown significance was identified: c.601A>C p.(Ser201Arg) (rs72549308), c.2279C>T p.(Thr760Ile), and c.2843T>C p.(Ile948Thr).

Discussion

This is to our knowledge the first article presenting 8 years of routine DPD testing in a clinical setting. Within this period, our prognostic approach has evolved from a focus solely on enzyme activity measurement to a combined analysis of DPD enzyme activity...
and DPD genetic testing of 4 clinically relevant genetic variants. We observed a strong interindividual variability in DPD enzyme activity within different DPD genotypes. Patients with the DPD*2A variant showed most consistently enzyme activity below the defined threshold. The genotype–phenotype correlation for the other variants tested was less clear.

In line with previous publications, we show that there is a weak correlation between DPD enzyme activity and the presence of the variants tested. With respect to this correlation, we observed a sensitivity of 0.25 (24 / [24 + 71]) and a specificity of 0.93 (667 / [667 + 52]); that is, most patients do not carry a variant as expected and all these patients have normal DPD enzyme activity (ie, high specificity). However, only 25% of patients with reduced activity carry a variant. The majority of patients with the DPD*13, rs67376798, or rs75017182 variants showed enzyme activity within the normal range. Similar results are reported for the rs67376798 variant in functional assays, in which this variant still showed ~50% activity compared with controls, whereas the DPD*2A variant showed a complete absence of activity when homozygously expressed. These observations are also in line with previous studies in which dose reductions based on the presence of a certain variant showed safe treatment with capecitabine and are reflected in existing pharmacogenetic dosing guidelines. The Dutch Pharmacogenetics Working Group recommends for DPD*2A, DPD*13, and rs75017182 variant carriers a starting dose of 50%, 50%, and 75%, respectively. Guidelines from the Clinical Pharmacogenetics Implementation Consortium (both DPWG and CPIC PGx guidelines available at www.pharmgkb.org) recommend starting with 50% in general (independent of the variant) followed by a monitored dose increase. Patients homozygous for the rs75017182 variant were found to still have a remaining DPD enzyme activity of 30%, indicating that also this variant does not result in a completely dysfunctional enzyme. These differences are likely caused by the different effects of the variant on enzyme function. For DPD*2A, exon 14 skipping due to the induced splicing defect leads to a completely nonfunctional enzyme. DPD*13 is believed to lead to destabilization of a sensitive region of the DPD protein, although the exact functional consequences are not clear. The rs67376798 variant is believed to interfere with cofactor binding or electron transport due to a structural change in the DPD protein. The rs75017182 variant leads to

Figure 2. Dihydropyrimidine dehydrogenase (DPD) genotype/phenotype correlation in the diagnostic patient population screened for 4 variants.
a premature stop codon in exon 11 due to aberrant splicing, but does not result in solely the presence of mutant transcript because wild-type mRNA can still be present, even in persons who are homozygous for this variant.13

After sequencing the entire coding region of the DPYD gene in patients with decreased enzyme activity without carrying the DPYD*1A variant (from group 1), 3 variants of unknown significance were identified. The c.601A>C p.(Ser201Arg) variant (rs72549308) was identified homozygously in a patient without DPD activity. The variant was not reported in the Exome Sequencing Project or the 1000 Genomes Project,14 whereas the Genome Aggregation Database browser (277,264 alleles from unrelated individuals) showed an allele frequency of 0.0029%. In silico prediction indicated that the variant was probably damaging and expression of the genetic variant in HEK293T/C17 cells performed by Offer et al15 showed a clear decrease in enzyme activity comparable to cell lines with a DPYD*2A variant (<12.5% activity). This explains the absence of DPD activity in the homozygous patient (the presence of a deletion/depletion was excluded; data not shown). The c.2279C>T p.(Thr760Ile) variant (ie, rs112766203) was also classified as probably damaging by us and others and only present in Genome Aggregation Database with an allele frequency of 0.061%. Offer et al15 showed a decrease in DPD enzyme activity after functional analysis comparable to the clinically relevant rs67376798 variant (~50% activity). These results indicate that the presence of the variant in homozygous state can explain the decreased DPD enzyme activity found in the patient. The c.2843T>C p.(Ile948Thr) variant was classified as probably damaging by Alamut Visual and was not present in the Exome Sequencing Project and 1000 Genomes Project and showed an allele frequency of 0.00041% in the Genome Aggregation Database. Recently, Kullenburg et al15 showed residual DPD activity of 30% after functional analysis of this variant. Based on this information, the variant identified in our patient may explain the observed decreased enzyme activity. One patient (heterozygous DPYD*13) showed 0 enzyme activity, whereas no other DPYD variants were detected by sequencing the entire coding region of the DPYD gene (including rs75017182). Moreover, no deletions or duplications were observed (data not shown) to explain this absence of activity. The patient might have a genetic variant in a regulatory region of the DPYD gene located outside the sequenced coding region or other non-genetic factors might play a role.

Genotyping of 4 genetic variants can only explain the genetic background of a small part of the patients with decreased enzyme activity. Presence of other variants in regions of the DPYD gene that have not been sequenced and genetic variants in other (ie, modifier) gene regions (eg, miR27a and miR27b)5,8 that determine DPD enzyme activity might be the reason that we cannot explain all cases of a decreased enzyme activity. In addition, other (eg, environmental) factors determine part of DPD enzyme activity. In our population, we observed a weak but statistically significant correlation with age, which is in line with a previous publication.16 We could not confirm a relationship between sex and DPD enzyme activity,17–19 although this has been published before.16 Several reports indicated that DPD enzyme activity is partly determined by circadian rhythm,20 although this circadian expression seems to be abolished in patients with gastrointestinal carcinomas.21 In addition, it is not unlikely that comedication can influence the expression of DPD enzyme. Studies have indicated that oxaliplatin (an often used anticancer agent) results in a reduced DPD activity.22,23 This observation could not be confirmed by Boisdron-Celle et al,24 although they did show an effect of oxaliplatin on 5-FU plasma clearance. In addition, the time from blood draw to processing of the samples is known to influence enzyme activity. Therefore, our laboratory flow is set up in such a way that the samples are processed within 18 hours. Within this time frame it is expected that enzyme activity is stable.25,26 In practice, our turnaround time from when we receive patient blood to finishing the report is maximal 7 working days. Usually we have the results around 4 to 5 working days. The referring oncologists have agreed with our turnaround-times because this aligns well with other procedures required for the treatment of these patients and does not affect the start of treatment or outcome compared with not testing for DPD deficiency.

Table 2: DPYD genetic variants identified in the diagnostic setting.

<table>
<thead>
<tr>
<th>Group</th>
<th>Genomic test (n [%])</th>
<th>Variant (n)</th>
<th>Mean enzyme activity (min-max)</th>
<th>Patients with a genetic variant and decreased enzyme activity (n [%])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (n = 254)</td>
<td>21 (8.3)</td>
<td>DPYD*2A (n = 6)</td>
<td>4.9 (1.8–7.4)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>Group 2 (n = 126)</td>
<td>126 (100)</td>
<td>DPYD*2A (n = 2)</td>
<td>7.5 (75–75)</td>
<td>2 (100)</td>
</tr>
<tr>
<td>Group 3 (n = 814)</td>
<td>814 (100)</td>
<td>DPYD*1A/2A (n = 12)</td>
<td>5.4 (1.2–10.5)</td>
<td>10 (83.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DPYD*1A/2A (n = 3)</td>
<td>12.8 (9.6–18.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heterozygous rs67376798 (n = 12)</td>
<td>10.8 (4.9–18.7)</td>
<td>3 (25)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>heterozygous rs75017182 (n = 46)</td>
<td>12.8 (4.5–23.3)</td>
<td>8 (17.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs75017182/ rs67376798 (n = 2)</td>
<td>4.3 (3.3–5.3)</td>
<td>2 (100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>homozygous rs75017182 (n = 1)</td>
<td>8.06</td>
<td>1 (100)</td>
</tr>
<tr>
<td>Subgroup entire screen (n = 47)</td>
<td>47 (100)</td>
<td>DPYD*1A (n = 3)</td>
<td>3.8 (0.75)</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DPYD*1A (n = 4)</td>
<td>6.1 (5.0–7.0)</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs67376798 (n = 1)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.601A-C (n = 1)</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.2279C&gt;T (n = 1)</td>
<td>3.2</td>
<td></td>
</tr>
</tbody>
</table>

DPYD = dihydropyrimidine dehydrogenase.

† Table only includes patients for which the enzyme activity was measured.

2 All patients were heterozygous for the variants; only c.601A>C was homozygous.

1 Enzyme measurements were performed for 3 samples.
this situation. Patients either partially deficient (ie, decreased DPD enzyme activity) or carrying 1 of the 4 relevant SNPs are at risk according to the existing guidelines and may receive an adjustment of the dose accordingly. In our opinion, only focusing on the enzyme test would ignore patients with low-normal activity who could still carry another DPYD variant and thus may be at risk for developing toxicity. On the other hand, focusing only on the genotype results is not ideal because currently we only test 4 DPYD variants, which are certainly not the only variants that may cause DPD deficiency.27 Starting all patients on a reduced dose and monitoring them afterward is not a realistic option because most patients will not have a (partial or complete) DPD deficiency and will experience a period of undertreatment. Because each test has its own advantages and disadvantages, we prefer the combination of both the enzyme and genotype tests as the best predictor for the development of side effects.

A limitation of our study is that we did not systematically collect information concerning the development of side effects related to fluoropyrimidine treatment. Therefore it is unclear whether the genetic variants or the enzyme activity predicts treatment outcome best. Currently, a study is being performed in the Netherlands addressing this issue (ClinicalTrials.gov identifier: NCT02324452). The literature indicates that genotype-guided dosing of fluorouracil based on DPYD*2A results in a reduction of grade ≥ 3 toxicity of 45% (from 73% in DPYD*2A carriers with standard dose to 28% in DPYD*2A carriers with genotype-guided dosing).28 In addition, for DPD enzyme activity measurements, 56% of patients with decreased enzyme activity develop severe complications (eg, grade 4 neutropenia) versus 29% of patients with normal enzyme activity.29 Based on our data, 76 patients (in group 3) carried a genetic variant, of whom 24 showed decreased activity, whereas 95 patients showed decreased enzyme activity in total. This means that severe toxicity might have been prevented in ~18% of patients. The evidence that DPD testing is beneficial is also reflected in the increase of the number of diagnostic DPD requests. In the initial years of clinical DPD testing, it was expected that most patients who had been tested were screened after the development of side effects. During the last year of clinical testing for this study, we observed an increase in requests for DPD testing before 5-FU treatment, which includes a shift to pretreatment screening in centers that only performed testing after toxicity, but also hospitals that never requested this type of test before. It is expected that more and more patients will be tested before treatment because it also has become evident that pharmacogenetic screening is likely cost-effective28 and more extended studies are underway (eg, ClinicalTrials.gov identifiers: NCT01547923 and NCT02324452).

Conclusions

DPD status should be determined before treatment with fluoropyrimidines to prevent patients from developing possible fatal toxicity and to prevent unnecessary side effects. Our study in combination with the literature shows that there is a discrepancy between DPD enzyme activity and the presence of clinically relevant SNPs. Therefore, in our view, a combination of a genetic and enzyme test is preferable for diagnostic testing until it is clear which method most reliably predicts the observed side effects.

Conflicts of Interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

Author Contributions

All authors listed were involved in the study design and interpretation of data for the work. Aimée Paulussen, Marc Breuer, Martijn Lindhout, Demis Tserpelis, Jörgen Bierau, Anja Steyls, Bianca van den Bosch collected data. Martijn Lindhout was responsible for enzyme activity measurements, genotyping was performed by Anja Steyls and Demis Tserpelis. Data analysis was performed by Marijke Coenen and Bianca van den Bosch, with input from the other investigators. The manuscript was written by Marijke Coenen and Bianca van den Bosch, all other authors edited the manuscript and gave final approval for publication. Marijke Coenen and Bianca van den Bosch had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Acknowledgments

The authors thank Dennis Visser, Jeannine Pachen-Voges, Janine Grashorn, and Lidewij Boersma from the Department of Clinical Genetics, Maastricht University Medical Centre, Maastricht, the Netherlands, for providing technical assistance.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.curtheres.2018.10.001.

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