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### In vivo characterization of CYP2D6\*12, \*29 and \*84 using dextromethorphan as a probe drug: a case report.

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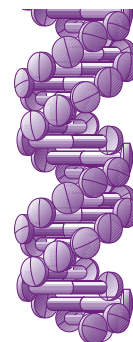
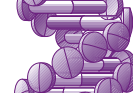
Gaedigk, Andrea; Twist, Greyson P.; Farrow, Emily G.; Lowry, Jennifer; Soden, Sarah E.; and Miller, Neil A., "In vivo characterization of CYP2D6\*12, \*29 and \*84 using dextromethorphan as a probe drug: a case report." (2017). *Manuscripts, Articles, Book Chapters and Other Papers*. 1106.  
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## Case Report

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# *In vivo* characterization of *CYP2D6*\*12, \*29 and \*84 using dextromethorphan as a probe drug: a case report

*CYP2D6*\*84 was first described in a Black South African subject, however, its function remains unknown. Astrolabe, a probabilistic scoring tool developed in our laboratory to call genotypes from whole genome sequence, identified *CYP2D6*\*84 in a trio. The father presented with intermediate metabolism when challenged with the *CYP2D6* probe drug dextromethorphan (DM/dextrorphan [DX] = 0.0839). Since his second allele, *CYP2D6*\*12, is nonfunctional, the observed activity is derived by *CYP2D6*\*84. This finding suggests that the allele's hallmark P267H causes decreased activity toward DM and that this allele should receive a value of 0.5 for Activity Score calculations. The mother's DM/DX of 0.0543 was consistent with the decreased activity classification of *CYP2D6*\*29. The child, a critically ill neonate, was not phenotyped, but predicted to be a normal metabolizer.

First draft submitted: 8 December 2016; Accepted for publication: 13 January 2017; Published online: 14 March 2017

**Keywords:** *CYP2D6* • *CYP2D6*\*12 • *CYP2D6*\*84 • dextromethorphan • phenotyping

Although *CYP2D6* is among the best characterized drug-metabolizing enzymes, there is no information regarding function for many of the allelic variants described by the Human Cytochrome P450 (*CYP*) Allele Nomenclature Database [1]. Indeed, of the 109 defined allelic variants, function for 47 variants remains unknown or has only been investigated *in vitro* per the recent update of the Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for *CYP2D6* and *CYP2C19* genotypes and dosing of tricyclic antidepressants [2]. This information void can be attributed to a number of factors including the use of de-identified subjects in the detection of novel variants through sequencing studies making subjects unavailable for follow-up phenotyping, and the relative low frequency of those variants making it difficult to not only identify individuals carrying the variant of interest but also to identify subjects with an informative diplotype. As next-generation

sequencing (NGS) platforms are increasingly employed for gene analysis in both the research and clinical settings, the detection of rare variants, including many of the 47 alleles currently classified as unknown function by CPIC, will become routine. Thus, the absence of information regarding the activity of these alleles will limit the translation of patient diplotypes into predicted phenotypes [2–5].

The *CYP2D6*\*84 allele was first described in 2013 by Dodgen *et al.* in a Black South African subject by sequence analysis [6], but no additional information regarding this variant has emerged in the literature since its discovery. We have previously identified *CYP2D6*\*84 in one subject of a trio (subject Children's Mercy Hospital [CMH] 631 [7]) using a bioinformatic tool, Astrolabe (initially named 'Constellation' [7,8]) that facilitates *CYP2D6* genotype calling from whole genome sequence data. Astrolabe has now identified this allele in two additional sub-

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jects of another trio, a father and a critically ill neonate. However, due to the unknown functional status of *CYP2D6*\*84, we were unable to predict the phenotype of the individuals carrying this allele with *a priori* data.

This case report describes confirmatory Sanger resequencing of the alleles found in the second trio including *CYP2D6*\*84. Both parents have genotypes consisting of known nonfunctional alleles that allowed us to determine the activity of *CYP2D6*\*84 and confirm activity of *CYP2D6*\*29 in these individuals with the *CYP2D6* probe drug dextromethorphan (DM). The urinary metabolic ratio of DM over its metabolite dextrorphan (DX) served as a measure of *CYP2D6* activity and thereby determines allele function and phenotype status [9].

## Methods

This study was approved by the Institutional Review Board of Children's Mercy Kansas City. Informed written consent was obtained from the parents of the trios.

Whole genome sequence was performed as previously described [7,10]. Briefly, a library was constructed with the TruSeq DNA PCR-Free Library Prep Kit (Illumina, San Diego, CA, USA) according to the manufacturer's protocol and sequenced on an Illumina HiSeq 2500 instrument using paired end 100-bp reads to a read depth of at least 30×. Samples were aligned to the GRCh37.p13 reference genome with *bwa-mem* v.0.7.12 and variants called with the Genome Analysis ToolKit v.3.2-2 producing variants relative to the *CYP2D6*\*2 reference [10]. Subsequently, *CYP2D6* diplotypes were called using a version of Astrolabe that has been improved since it was first published [7]. Astrolabe is available through the Children's Mercy website [11]. Variants across the entire *CYP2D6* gene locus were manually inspected to validate Astrolabe calls.

Allele-specific long-range PCR was performed as previously described [12]. Specifically, we amplified the *CYP2D6*\*84 (child), \*4, \*29 (mother) and \*12 (father) alleles and Sanger-sequenced the resulting XL-PCR fragments using BigDye Terminator chemistry and a 3730 DNA analyzer (Thermo Fisher Scientific, MA, USA).

For phenotyping, subjects self-administered 30 mg of DM (Robitussin® cough syrup, NC, USA) after voiding their bladder. Urine was collected for 4 h. DM and DX were measured by NMS Labs (PA, USA). The urinary metabolic ratio of DM/DX served as a measure of *CYP2D6* activity. Classification into poor, intermediate, normal (formerly known as extensive [13]) and ultra-rapid metabolizer phenotype groups has

previously been described. Specifically, intermediate metabolizers are defined as  $0.03 \leq DM/DX < 0.3$  [14].

The following databases were utilized to obtain information regarding allele frequencies: the CMH Variant Warehouse at the Center for Pediatric Genomic Medicine at Children's Mercy Kansas City [15], the SPHINX database, a resource of the eMERGE Network [16], the Exome Aggregation Consortium (ExAC) database [17] and the NHLBI Exome Sequencing Project Exome Variant Server (EVS) [18].

## Results & discussion

### Allelic variation

Astrolabe called the following genotypes for the trio: *CYP2D6*\*4xN/\*29 (mother), *CYP2D6*\*12/\*84 (father) and \*29/\*84 (child). Astrolabe calls were confirmed by Sanger sequencing of allele-specific *CYP2D6*\*4, \*12, \*29 and \*84 XL-PCR fragments. *CYP2D6*\*4 matched 100% with the \*4D sequence we have described previously (case 21, in Gaedigk *et al.* [19], data not shown). In contrast, as summarized in [Supplementary Figure 1](#), *CYP2D6*\*12, \*29 and \*84 deviated from the definitions listed by the P450 nomenclature database.

The *CYP2D6*\*84 allele matched 100% with the one we identified in our first trio (subject CMH 631 described by Twist *et al.* [7]), but lacks two SNPs, one in intron 7 and one in the upstream region compared with the variant described by Dodgen *et al.* [6] on which the nomenclature is based for this allele. Interestingly, the *CYP2D6*\*84 recently described by Chaudhry *et al.* [20], which matches *CYP2D6*\*84*var*, was found in a subject recruited from the same geographical region as the individual in which this variant was first discovered [6]. The *CYP2D6*\*12 allele was first described in 1996 and at that time defined by variations in exon regions only, in other words, it is unknown whether there are SNPs in introns and/or the 3'- and 5'-UTRs. Although these SNPs do not contribute to the function of this particular allele, having precise haplotype definitions facilitates automated diplotype calling using bioinformatic tools such as Astrolabe [7]. As shown in [Supplementary Figure 1](#), the *CYP2D6*\*12 in our trio features the 'key' SNP defining this allele (124G>A, G42R, rs5030862), but also carries three additional exonic SNPs (1716G>A, E155K, rs28371710; 2575C>A, rs28371718 and 3254T>C, rs28371726). However, the latter are not novel *per se*, as these SNPs are part of the *CYP2D6*\*45 and \*46 haplotype definitions. A search of the CMH Variant Warehouse for the *CYP2D6*\*12 key SNP (rs5030862) identified a second subject besides the father of the trio investigated here. Furthermore, this subject also has the three additional SNPs (1716G>A, 2575C>A and 3254T>C) suggesting that this *CYP2D6*\*12 allele is

Database	Subjects (n)	MAF (%) all subjects	MAF (%) African ancestry	MAF (%) European ancestry
CMH variant warehouse	3432	0.044	NA	NA
SPHINX database	9015	0.00555	0.0072	0
ExAC	21,521	0.02567	0.2759	0
EVS	6478	0.01003	0.2964	0

SPHINX data as per update of July 2015; CMH variant warehouse and ExAC accessed 22 November 2016. The *CYP2D6*\*84-defining SNP rs148769737 (Chr22:42524219 T/A) was not observed in the ExAC database among south or east Asians subjects but was discovered in Latino and Europeans (non-Finnish) subjects at frequencies of 0.0000978 and 0.00003349, respectively.  
 CMH: Children's Mercy Hospital; EVS: Exome variant server; ExAC: Exome Aggregation Consortium; MAF: Minor allele frequency; NA: Not available.

identical to the subvariant we have Sanger-sequenced for this study. Considering the presence of these additional SNPs of which one is nonsynonymous, the *CYP2D6*\*12 identified in this study in two subjects is more accurately described as a subvariant.

*CYP2D6*\*29 is another allele for which the nomenclature database only lists variations in exons. The mother's *CYP2D6*\*29 allele carried all exonic SNPs listed by the database, but also had numerous SNPs across introns and untranslated regions (Supplementary Figure 1). This *CYP2D6*\*29 sequence matched those obtained from two Coriell samples, NA17131 and NA19109. However, this variant differed from a *CYP2D6*\*29 sequence we obtained from a subject who participated in a previous study, but was not published in that report [21].

The P450 Nomenclature database does not assign star (\*) allele designations for subvariants. Because there is currently no system in place that designates suballelic variants, the novel *CYP2D6*\*12, \*29 and \*84 variants identified in this study are labeled with 'var'.

### Allele frequencies

*CYP2D6*\*84 is a rare variant that appears to be present predominantly in subjects with Black African ancestry (Table 1). The frequency of the key SNP identify-

ing *CYP2D6*\*84 (2574C>A, P267H, rs148769737) was 0.044% in the CMH Variant Warehouse (three heterozygous subjects among 3432). One of those subjects (CMH 631 described previously [7]) was genotyped as *CYP2D6*\*17/\*84. Although the ethnicity of this subject is unknown, we speculate that this person is of African ancestry because of the presence of *CYP2D6*\*17, which is almost exclusively found in African populations or populations with African admixture (see *CYP2D6* allele frequency table available at the PharmGBK website [22,23]). The other two subjects are the father and child described in this report. Our Variant Warehouse frequency, albeit not derived from unrelated subjects only, is in line with those reported by the SPHINX and ExAC databases (Table 1). Taken together, these data suggest that *CYP2D6*\*84 is predominantly present in African populations and subjects with African ancestry. The *CYP2D6*\*12 allele also appears to be rare and, as shown in Table 2, has been found predominantly in subjects with African ancestry. However, two subjects with *CYP2D6*\*84 were found in two studies conducted in South African subjects (n = 100 [6] and n = 31 [20], respectively), which suggest an allele frequency of 0.76%, which is considerably higher compared with the frequencies reported by a number of databases (Table 1). Whether this allele

Database	Subjects (n)	MAF (%) all subjects	MAF (%) African ancestry	MAF (%) European ancestry
CMH variant warehouse	3432	0.029	NA	NA
SPHINX database	9015	0.0222074	0.148258	0
ExAC	51,296	0.003899	0.03575	0.001748
EVS	6491	0.0231	0.0455	0.0116

SPHINX data as per update of July 2015; CMH variant warehouse and ExAC accessed 22 November 2016. The *CYP2D6*\*12 defining SNP rs5030862 (Chr22: 42526670) was not observed in the ExAC database among south Asian, east Asians or Latino subjects, but was discovered in Europeans (non-Finnish) at a frequency of 0.0001748.  
 CMH: Children's Mercy Hospital; EVS: Exome variant server; ExAC: Exome Aggregation Consortium; MAF: Minor allele frequency; NA: Not available.

is indeed more common in this particular population warrants further investigation.

### Phenotype prediction from genotype data & *in vivo* phenotyping using DM

Because of the 'unknown' function status of *CYP2D6\*84*, the father's phenotype was predicted to be either a poor metabolizer (activity score = 0), intermediate metabolizer (activity score = 0.5) or normal metabolizer (activity score = 1) depending on whether *CYP2D6\*84* is a no, decreased or normal function allele. The mother's genotype received an activity score of 0.5 predicting an intermediate metabolizer phenotype status based on the decreased function categorization of *CYP2D6\*29*. The prediction was intermediate or normal metabolizer for the child (translation into phenotype according to the updated CPIC translation table [22]).

The father uniquely presented us with a rare opportunity to measure CYP2D6 activity *in vivo* that is solely derived from *CYP2D6\*84*. Only about 1 in 10,000 individuals carries a genotype consisting of a non-functional allele that is paired with *CYP2D6\*84* such as the father's, in other words, *CYP2D6\*12/\*84*. *In vivo* phenotyping determined a urinary metabolic ratio of DM/DX of 0.0839 categorizing the father as intermediate metabolizer. The allele-defining SNP in exon 5 (2574C>A) causes an amino acid change (P267H) that is predicted by SIFT to be damaging (score of 0.03) [24] and by PolyPhen to be probably damaging (score of 0.977) [25]. Decreased activity, as indicated by a urinary metabolic ratio of DM/DX of 0.0839 is consistent with the *in silico* predictions and might be explained by a difference in the charge caused by the proline to histidine change. Based on these findings, *CYP2D6\*84* should be classified as 'decreased' and receive a value of 0.5 for Activity Score calculations.

The mother's DM/DX ratio was 0.0543 classifying her as an intermediate metabolizer that was expected given her genotype (*CYP2D6\*4x2/\*29*). Decreased activity is consistent with published data describing *CYP2D6\*29* as a variant encoding protein with compromised function [21,26].

The child, a critically ill neonate was not subjected to phenotyping. Based on the parent's phenotype information, the child's diplotype (*CYP2D6\*29/\*84*) was calculated to have an Activity Score of 1 predicting normal metabolism, which provided valuable information to parents and providers caring for this complex patient.

### Conclusion

*In vivo* phenotyping of an individual with an informative *CYP2D6* diplotype allowed us to determine *CYP2D6\*84* activity for the first time. This information is invaluable to predict CYP2D6 activity in patients carrying this allelic variant.

### Future perspective

As genotype testing is increasingly performed using NGS platforms, rare alleles will reveal themselves, but will elude interpretation due to the lack of data. The development of protocols allowing follow-up phenotype studies are warranted to functionally characterize the increasing number of allelic variants and diplotype combinations discovered by NGS to accurately predict phenotype and aid thereby move individualized drug therapy forward.

### Supplementary data

To view the supplementary data that accompany this paper, please visit the journal website at: [www.futuremedicine.com/doi/full/10.2217/pgs-2016-0192](http://www.futuremedicine.com/doi/full/10.2217/pgs-2016-0192)

### Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

### Executive summary

- *CYP2D6\*84* is a rare variant predominantly observed in Africans and subjects with African ancestry. It was first discovered in a South African individual in 2013.
- Because *CYP2D6\*84* function remained unknown, phenotype could not be predicted, or only approximated, from diplotypes containing this allele.
- Astrolabe, a tool to call genotypes from next-generation sequencing data, correctly identified the *CYP2D6\*84* allele in three individuals of two trios (one trio described in this report).
- Allele calls were verified by Sanger sequencing and an individual with a *CYP2D6\*12/\*84* genotype subjected to *in vivo* phenotyping using the CYP2D6 probe drug dextromethorphan.
- The urinary metabolic ratio of dextromethorphan to its metabolite dextrorphan (DM/DX) indicated that *CYP2D6\*84* encodes decreased function. This finding is consistent with *in silico* predictions.

### Informed consent disclosure

The authors state that they have obtained verbal and written informed consent from the patient/patients for the inclusion of their medical and treatment history within this case report.

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