Genetic Transmission of Cytochrome P450 2D6 (*CYP2D6*) Ultrarapid Metabolism: Implications for Breastfeeding Women taking Codeine

Parvaz Madadi[1,2], Catherine Ciszkowski[3,4], Andrea Gaedigk[5], J. Steven Leeder[5], Ronni Teitelbaum[6], David Chitayat[6] and Gideon Koren[*,1,2,3,4]

[1]*Clinical Pharmacology and Toxicology, and the* [2]*Motherisk Program, Hospital for Sick Children, Toronto, Ontario, Canada*

[3]*Ivey Chair in Molecular Toxicology, and the* [4]*Department of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Ontario, Canada*

[5]*Children's Mercy Hospital and Clinics, Section of Clinical Pharmacology and Experimental Therapeutics, Kansas City, Missouri, USA*

[6]*Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto, Ontario, Canada*

Abstract: The pro-drug codeine is commonly prescribed for postpartum pain relief in North America. The safety of codeine during breastfeeding is related in part to the extent of the active morphine metabolite catalyzed from codeine *via* the cytochrome P450 2D6 (*CYP2D6*) enzyme. In mothers who have greater than two functional copies of the *CYP2D6* gene (*CYP2D6* ultrarapid metabolism phenotype; UM) a substantially higher proportion of morphine is produced. Label changes on codeine-containing medications will highlight the risks associated with this genotype for breastfeeding mothers, but are not supported by translation strategies on how to incorporate this pharmacogenetic knowledge into clinical practice. To address the immediate issue of *CYP2D6* UM inheritance in family members of a breastfed infant who succumbed to fatal opioid intoxication and whose codeine-prescribed mother was a *CYP2D6* UM, we constructed a pedigree. While the pedigree approach is helpful to aid diagnosis, identify other at risk family members, and simplify pharmacogenetic analysis, its clinical usefulness is dependant on an institutional framework which is not available in most centers at this time.

Keywords: *CYP2D6*, duplication, codeine, breastmilk, ultrarapid metabolism, pedigree.

INTRODUCTION

Cytochrome P450 2D6 (*CYP2D6*) is an enzyme involved in the oxidative metabolism of about 25% of medications on the market [1] and several endogenous substrates [2, 3]. Genotype to phenotype correlations are complicated by the highly polymorphic nature of *CYP2D6* (M33388), with over 70 alleles identified [4] and by the presence of two highly homologous pseudogenes localized upstream of the *CYP2D6* gene within the locus on chromosome 22q13 [5, 6]. The *CYP2D6* ultrarapid metabolizer (UM) phenotype, which is of global importance [7], is predicted by duplications of functional *CYP2D6* genes. The frequency of *CYP2D6* gene duplication events varies depending on ethnicity (i.e. 2 – 6% of African Americans, 29% of Ethiopians, 10% of Saudi Arabians, 1% of Swedish, 7% of Spanish and Portuguese) [8-12] and are believed to result from unequal crossover events during homologous recombination [13]. In addition, unequal segregation and extrachromosomal replication of acentric DNA may explain rare multiduplication events of up to 13 *CYP2D6* copies in tandem [13].

In the case of the pro-drug codeine, *CYP2D6* UMs display on average 50% higher plasma concentrations of the pharmacologically active metabolite morphine than extensive metabolizers with only two functional gene copies, i.e. two fully functional alleles [14]. The gene-dose effect is such that increasing functional gene copies result in increased enzymatic activity and morphine production [14]. For codeine, this can cause serious toxicity even at therapeutic doses [15-17], for other drugs, the consequence may be therapeutic failure [18].

Following a fatal case of a breastfed infant whose mother was an ultrarapid metabolizer [17, 19], both the United States Food and Drug Administration [20] and Health Canada [21] have issued public health advisories highlighting a risk for opioid toxicity in breastfed infants whose codeine-prescribed mothers are *CYP2D6* UMs. With the advent of updated warning labels on codeine-containing medication for nursing mothers with the *CYP2D6* UM phenotype [21], genetic counselors and other health care professionals may increasingly encounter demands for *CYP2D6* genetic tests prior to codeine administration. In this report we describe the first analysis of *CYP2D6* genotype in the extended family of the breastfed infant who died from opioid toxicity [17, 19].

METHODS

Genomic DNA was isolated with informed consent from blood and tissue (infant) using the QIAamp DNA Blood and Tissue Mini Kits (Qiagen, Valencia, CA, respectively).

*Address correspondence to this author at the Motherisk Program, Division of Clinical Pharmacology and Toxicology, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada; Tel: 416-813-5781; Fax: 416-813-7562; E-mail: gkoren@sickkids.ca

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CYP2D6 genotyping was conducted for the functional *CYP2D6 *2* allele, null alleles **3, *4, *5, *6, *7, *8, *11, *40*, reduced functional alleles **9, *10, *17, *29, *41*, and the presence of **1xN, *2xN, *4xN* gene duplications in the deceased infant and his parents. *CYP2D6* copy number variation was quantified. Limited analysis was performed for the grandparents and aunts of the deceased baby (**2, *3, *4, *5, *6, *7, *9, *10, *41, *1XN, *2XN*). Subsequent siblings of the deceased infant were genotyped only for the **2* and **2XN* alleles. Allele designations were as defined by the CYP450 Allele Nomenclature Committee [4].

RESULTS AND DISCUSSION

The results illustrate an autosomal transmission of the functional *CYP2D6*2Ax2*, a single allele carrying two copies of the fully functional *CYP2D6*2A* variant, from the maternal grandmother to the mother, one of the aunts, and a subsequent sibling of the deceased infant (Fig. **1**). The *CYP2D6*2* allele has demonstrated a high susceptibility to multiduplication [8-10, 22] and comprises 72% of all duplications among Caucasians [23, 24]. All other family members were genotyped as extensive metabolizers, with two functional *CYP2D6* alleles. The deceased infant, however, may have been physiologically incapable of metabolizing codeine and morphine which were invariably present in breast milk. *CYP2D6* genotype to phenotype concordance has been observed from 2 weeks of postnatal age onwards [25, 26], but the overall clearance rate in newborns may be compromised until renal function and phase II drug metabolizing enzymes involved in morphine elimination are fully matured [27]. Thus, at 12 days of age the infant's cause of death was attributed to prolonged exposure to excessive morphine metabolite transmitted through the breast milk.

The *CYP2D6* UM phenotype is not necessarily inherited in an autosomal dominant fashion. This is because gene amplification per se does not always translate into the UM phenotype. For one, the nature of the second allele has to be considered. But one also has to bear in mind that not all gene duplications are equal. For example, *CYP2D6*4xN* is a non-functional allele and *CYP2D6*10xN* and *CYP2D6*41xN* constitute duplications of genes encoding enzymes of reduced function. The latter confer higher activity compared to their 'single gene' counterparts, but are likely not reaching the same level of ultrarapid activity conveyed by *CYP2D6*1xN* and **2xN* (duplications of fully functional genes). Noteworthy, in Caucasians, the majority of gene duplications are fully functional (*CYP2D6*1xN* and **2xN)*, while almost half of all duplication events in African American populations are composed of non-functional genes (*CYP2D6*4xN*) [23]. Testing strategies which include determination of copy number variation are necessary to elucidate the relative increase in enzymatic activity. For *CYP2D6*, copy numbers between 2 and 13 alleles have been reported [8-10, 22].

CYP2D6 inheritance depends on the combination of maternal and paternal alleles in accordance with Mendel's second law of independent assortment. This is exemplified by differential genotypes in the three sisters of the maternal line and also in their offsprings. Thus, siblings must be individually genotyped to determine the potential for risk of sedation and toxicity when taking codeine and/or breastfeeding their children. As *CYP2D6* is subject to inhibition by a wide range of chemical substrates, clinical counseling must also include potential drug-drug interactions that can result in phenocopying of reduced or null genotypes. Evidently, in addition to maternal genotype, factors such as maternal codeine dose, duration, neonatal milk intake, and neonatal metabolic clearance capacity may also contribute to adverse drug reactions in breastfed infants of codeine-prescribed mothers [28, 29].

CYP2D6 also mediates the metabolism of other commonly used opioid analgesics- oxycodone, hydrocodone, and tramadol- into potent, pharmacologically active

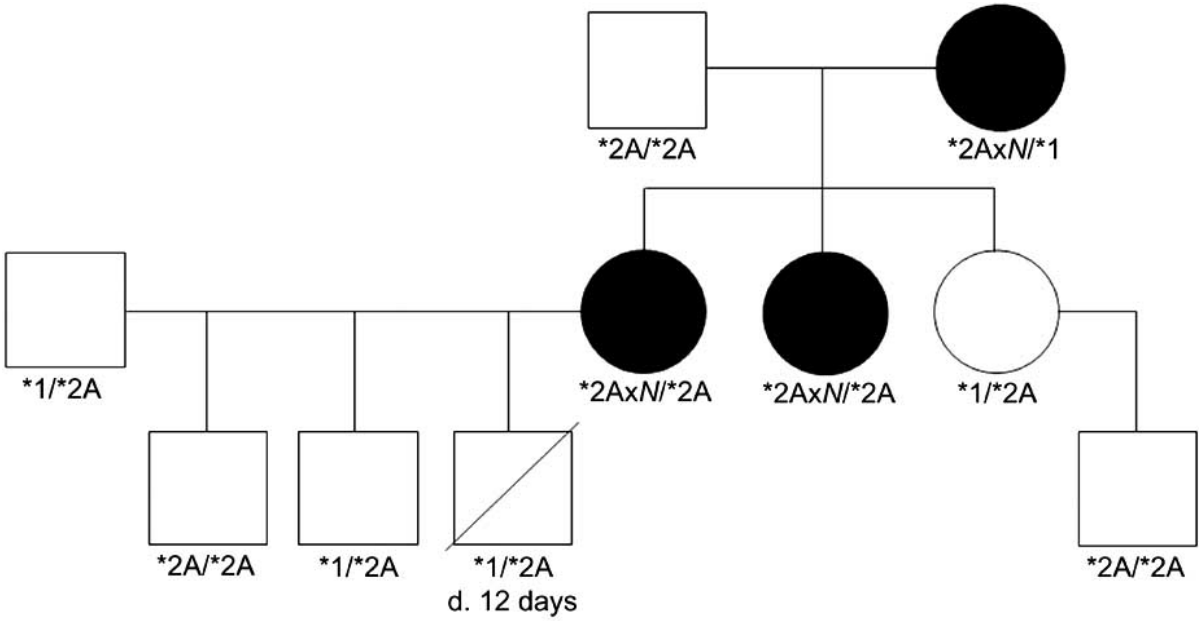

Fig. (1). Pedigree structure for cytochrome P450 2D6 (*CYP2D6*). Individuals with functional gene duplications resulting in the ultrarapid metabolizer phenotype are shown by blackened symbols. Unaffected individuals are identified by unblackened symbols, and deceased individuals are indicated by a slash (/).

metabolites. The relationship between *CYP2D6* UM and opioid toxicity has been less studied in these analgesics as compared to codeine, although an adverse drug event had been reported [30]. Until more safety data is generated, it is advised that *CYP2D6* UM breastfeeding mothers should also avoid oxycodone, hydrocodone, and tramadol. A recent systematic review reports that non-steroidal anti-inflammatory drugs (NSAIDs) may be an equipotent alternative to codeine-acetaminophen for the treatment of post-abdominal surgery pain [31].

Pedigree analysis for *CYP2D6* is a useful clinical tool to help identify at risk family members, aid diagnosis, and help establish a pattern of inheritance [32]. The interpretation of *CYP2D6* genotype into UM phenotype necessitates an understanding of the highly polymorphic nature of the *CYP2D6* gene. This pharmacogenetic knowledge, alongside patient education and maternal and neonatal monitoring, can be used as preventative clinical tools to avoid opioid-related toxicity. Thus, educational and clinical methods must be developed to enable health care providers to provide needed counselling and genetic services in order to meet the growing shortage of professional genetic personnel [33]. As strategies on translating and incorporating new pharmacogenetic knowledge into clinical practice lag considerably behind rapid scientific advances, more studies are needed to address these shortcomings.

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