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Letter to the Editors

The combination of nuclear and mitochondrial mutations as a risk factor for idiosyncratic toxicity

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Idiosyncratic toxicity is an unpredictable and often life-threatening complication of drug therapy. The underlying mechanisms are largely unknown, but the evidence favours the view that reactive intermediates generated during phase I metabolism of a parent drug are inefficiently detoxified and cleared. These metabolites accumulate intracellularly and covalently bind to, and modify host proteins or DNA. If the adduct is presented to the immune system, a hypersensitivity reaction occurs (i.e. the hapten hypothesis) [1]. One caveat, however, is the fact that a high incidence of idiosyncratic reactions is not found with all drugs known to form reactive intermediates. This dilemma has led to the formulation of the 'danger, or risk hypothesis', wherein the potential for an idiosyncratic drug reaction is enhanced by certain host risk factors such as infection, injury or cell stress [2]. The challenge is to identify these risk factors and to elucidate their role in the toxic cascade. Here we report the case of a 68-year-old woman who possesses a combination of nuclear and mitochondrial defects. We submit that this combination presents a previously unrecognized risk factor and we advocate its consideration, along with other genetic determinants, in the evaluation of drug idiosyncrasy.

The subject had suffered a lifelong seizure disorder, myopathy and myoclonus, and had been treated over the years with a wide variety of drugs, including antimicrobials, antiepileptics, anaesthetics and sedative-hypnotics. At normal doses, these drugs had caused severe and occasionally life-threatening toxicity. For example, phenytoin had produced symmetrical target lesions on her extremities, trunk and mouth, progressing to blisters and denudation within a week of exposure. Also, 2–3 days following the initiation of sulfamethoxazole/trimethoprim for a urinary tract infection, the patient had developed a malar rash upon exposure to the sun, accompanied by symmetric arthralgias. All symptoms had resolved after discontinuation of the sulfamethoxazole/trimethoprim. The patient was referred to our service for further investigation into the potential causes of her idiosyncratic drug toxicity, to recommend potentially safe medications for her to take and to identify putative substances to protect her from future adverse drug reactions.

Genotyping revealed defects at multiple gene loci, including regions that encode phase I and phase II drug-metabolizing enzymes. Analysis of three cytochrome P450 (CYP) genes that are known to confer clinically important polymorphisms confirmed the presence of two allelic variants: *CYP2C19*1/2** and *CYP2D6*1/4** (Table 1). The third gene, *CYP2C9*, was not polymorphically expressed [i.e. the subject was found to be homozygous for the wild-type *CYP2C9* allele (*CYP2C9*1/*1*) (Table 1)]. As shown (Table 1), the patient was homozygous for the commonly occurring N-acetyltransferase 2 allele, *NAT2*5B*, which confers slow acetylator status. Conversely, no polymorphisms were detected at the *NAT1* locus (i.e. the patient was homozygous for the wild-type *NAT1*4* allele). Genotyping at the glutathione S-transferase (GST) locus revealed that the patient was homozygous null for both the *GSTM1* and *GSTT1* genes (*GSTM1*0/*0*, *GSTT1*0/*0*). Reportedly, a physiological function of GSTs is to remove reactive metabolites by conjugation with glutathione (GSH) [3]. Our findings therefore reinforce the realization that the double null GST mutation, which occurs in approximately 5–10% of US Whites and African-Americans [4], is a risk factor for drug toxicity [5, 6]. However, an imbalance in phase I and phase II drug metabolism is not sufficient to explain idiosyncratic drug reactions; other risk factors must be present [7].

Table 1
Polymorphisms in phase I and phase II drug metabolizing enzymes

Enzyme	Genotype	Phenotype
cytochrome P450		
CYP2C9	*1/*1	extensive metabolizer
CYP2C19	*1/*2	poor metabolizer
CYP2D6	*1/*4	extensive metabolizer
N-acetyltransferase		
NAT1	*4/*4	normal activity
NAT2	*5B/*5B	catalytically inefficient
glutathione S-transferase		
GSTM1	*0/*0	no activity
GSTT1	*0/*0	no activity
GSTP1	*A/*A	normal activity

*Genotyping results for drug metabolizing enzymes: In cytochrome P450 nomenclature the gene name and allele are separated by an asterisk, and the number 1 is given to the wild-type version of the gene. CYP2C9*1/*1 encodes wild-type CYPC2C9. NAT1*4/*4 encodes a wild-type isoenzyme with normal catalytic activity. In GST nomenclature *0 represents a complete gene deletion, and individuals who are homozygous for the *0 allele (null genotype) lack any functional GST protein. GSTP1*A/*A encodes functional wild-type GSTP1. Cytochrome P450 polymorphisms were identified by DA Flockhart, Indiana University School of Medicine, Indianapolis, IN, N-acetyltransferase polymorphisms by PK Knoefel, University of Louisville School of Medicine, Louisville, KY, and glutathione S-transferase polymorphisms in collaboration with M-A Loriot, Hôpital Européen Georges Pompidou, Paris, France.*

Our patient's history of myopathy and myoclonus suggested that mitochondrial dysfunction might be an additional risk. Biopsy of her left quadriceps muscle showed mild atrophy, mildly elevated lipid content in some areas, and some ragged red fibres, features potentially indicative of a metabolic disorder. Sequencing of mitochondrial DNA (mtDNA) from the biopsied muscle tissue revealed a missense mutation at position 11204, resulting in a T to C transition that converts a highly conserved phenylalanine to a leucine in the ND4 subunit of respiratory complex I (C1). Mutations within the C1 subunits have been shown to inhibit cell respiration and to increase the production of reactive oxygen species (ROS) with the subsequent development of chronic oxidative stress [8]. This possibility was confirmed by measuring the oxidation/reduction status of leucocytes isolated from the patient. The fluorescent signal from the redox-sensitive dye, dichlorodihydrofluorescein, was 54 ± 8% greater in cells from the patient compared with those of controls [9]. Additional clinical evidence for the presence of oxidative stress was the finding that the subject's GSH/glutathione disulphide (GSSG) ratio was 11, a value considerably below the normal range of 200–500. (The subject's blood GSH content was 5.18 μmol gHb^{-1} and her GSSG concentration was 464 nmol gHb^{-1}.) Unfortunately, the exact cause of her abnormal GSH/GSSG ratio is unclear at present.

We propose a new paradigm for drug-induced idiosyncrasy: the superimposition of oxidative stress, caused in this case by a mitochondrial mutation, upon a deficiency in the clearance of reactive metabolites, caused by the double null GST mutations. The consequences can be appreciated by a discussion of the patient's response to sulfamethoxazole (SMX). First, her slow acetylation status coupled with normal CYP2C9 oxidation favours an enhanced production of the SMX hydroxylamine [10]. Second, the hydroxylamine is normally auto-oxidized to a highly reactive intermediate [10] and this process is exacerbated by the superimposition of oxidative stress. Lastly, the nitroso intermediate is cleared by conjugation with GSH [10], but this process occurs minimally as a result of her deficiency in GST activity and low GSH content. Thus, the combination of defects favours the generation of reactive intermediates and promotes the formation of drug adducts by mass action. Interestingly, there are striking similarities between our case and the sulphonamide idiosyncrasy seen in human immunodeficiency virus patients [10]. In essence, our hypothesis is an extension of the hapten hypothesis, and we urge investigators to consider this risk factor when identifying the putative causes of idiosyncratic drug reactions.

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