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RESEARCH LETTER

Thiopurine Use During Pregnancy Has Deleterious Effects on Offspring in *Nudt15*^{R138C} Knock-In Mice

Thiopurines are key immunosuppressants for the treatment of inflammatory bowel disease. Thiopurine use during pregnancy has not been prohibited, but its safety is still debated.[1,2] Additionally, it has been revealed that several genetic polymorphisms are associated with thiopurine toxicities[3]; however, the effects of parental or offspring genotype on the safety of thiopurine use during pregnancy have not been investigated. *NUDT15* (nucleoside diphosphate-linked moiety X-type motif 15) is responsible for the inactivation of thiopurines by converting thioguanosine-5'-triphosphate to thioguanosine-5'-monophosphate, and single-nucleotide polymorphisms of *NUDT15* are strongly associated with cytopenia during thiopurine use.[4,5] In particular, the c.415C>T single-nucleotide polymorphism, which induces p.Arg139Cys (R139C) and the loss of normal enzymatic activity, is clinically important in East Asians because it penetrates more than 10% of them and frequently causes severe cytopenia.[4] We recently established knock-in mice harboring a p.Arg138Cys mutation (*Nudt15*^{R138C}), which corresponds to human NUDT15 R139C, and demonstrated that thiopurine administration causes hematopoietic stem cell (HSC) toxicity in *Nudt15*$^{+/R138C}$ or *Nudt15*$^{R138C/R138C}$ mice (see Supplementary Methods).[6] In this study using our mouse model, we investigated whether thiopurine use during pregnancy differentially affects offspring, based on their NUDT15 genotype.

Our previous report demonstrated that the long-term (>2 months) survivable dose of mercaptopurine (MP) is 1.0 mg/kg for *Nudt15*$^{+/+}$, 0.5 mg/kg for *Nudt15*$^{+/R138C}$, and 0.2 mg/kg for *Nudt15*$^{R138C/R138C}$ adult mice.[6] Thus, we administered the same doses of MP to *Nudt15*$^{+/R138C}$ or *Nudt15*$^{+/+}$ pregnant mice, respectively, and then characterized the *Nudt15* genotypes of the neonatal mice. *Nudt15*$^{+/R138C}$ female mice that were mated with *Nudt15*$^{+/R138C}$ male mice generated neonatal mice in a Mendelian fashion under 0 mg/kg and 0.1 mg/kg MP treatment (see Supplementary Methods). However, few to zero *Nudt15*$^{R138C/R138C}$ neonatal mice were generated under 0.2 mg/kg or 0.5 mg/kg MP treatment, respectively (Figure 1*A*). Similarly, *Nudt15*$^{+/+}$ female mice that were mated with *Nudt15*$^{+/R138C}$ male mice failed to generate *Nudt15*$^{+/R138C}$ neonatal mice under 1.0 mg/kg MP treatment (Figure 1*B*). These data indicate that the therapeutic MP dose for pregnant mice could be deleterious to offspring harboring more *Nudt15*^{R138C} allele than the female parent.

Next, to investigate fetal abnormalities caused by MP treatment during pregnancy, we analyzed the *Nudt15* genotypes of fetal mice from *Nudt15*$^{+/R138C}$ pregnant mice that were administered 0.2 mg/kg or 0.5 mg/kg MP after mating with *Nudt15*$^{+/R138C}$ male mice. On embryonic day 14.5, the number of *Nudt15*$^{R138C/R138C}$ fetal mice was reduced under 0.2 mg/kg MP treatment and eliminated under 0.5 mg/kg MP treatment, indicating that thiopurine use during pregnancy can lead to embryonic lethality in *Nudt15*$^{R138C/R138C}$ offspring (Supplementary Figure 1). *Nudt15*$^{R138C/R138C}$ fetal mice that survived 0.2 mg/kg MP treatment during pregnancy tended to be paler (Figure 2*A*) and were significantly smaller in size than *Nudt15*$^{+/+}$ fetal mice (Figure 2*B*). We previously reported that hematopoietic tissue is promptly injured by MP treatment in *Nudt15*$^{R138C/R138C}$ adult mice, and HSCs are damaged by MP in a *Nudt15*^{R138C} allele number-dependent manner.[6] Therefore, we determined the number of HSCs in each fetal liver, the center of hematopoiesis in fetal mice. To do this, fetal HSCs that are

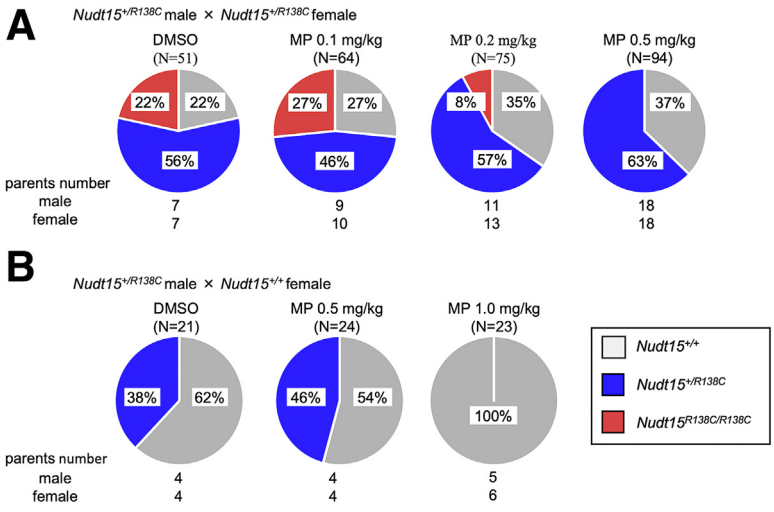

Figure 1. Thiopurine use during pregnancy induces harmful effects on offspring. The frequency of *Nudt15* genotype in neonatal mice generated by *Nudt15*$^{+/R138C}$ female mice (*A*) or *Nudt15*$^{+/+}$ female mice (*B*) that were mated with *Nudt15*$^{+/R138C}$ male mice and treated with the indicated MP dose during pregnancy. Dimethyl sulfoxide was administered instead of MP as a control. The numbers of analyzed neonatal mice and parental mice are presented for each MP dose. DMSO, dimethyl sulfoxide.

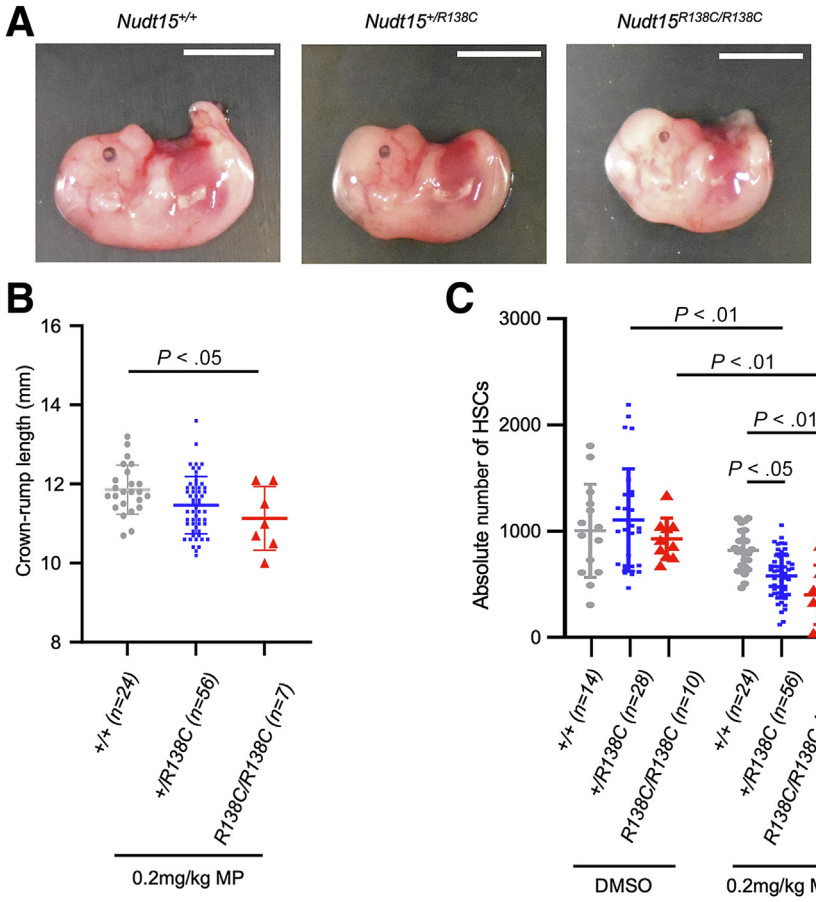

Figure 2. Thiopurine induces hematopoietic toxicity in fetal mice. Representative appearance (*A*) and the crown-rump length (*B*) of *Nudt15*$^{+/+}$, *Nudt15*$^{+/R138C}$, or *Nudt15*$^{R138C/R138C}$ fetal mice on embryonic day 14.5 generated by *Nudt15*$^{+/R138C}$ female mice that were mated with *Nudt15*$^{+/R138C}$ male mice and treated with 0.2 mg/kg MP during pregnancy. Scale bars indicate 5 mm. The numbers of parental male and female mice are 14, and 15, respectively. (*C*) The absolute number of HSCs in *Nudt15*$^{+/+}$, *Nudt15*$^{+/R138C}$, or *Nudt15*$^{R138C/R138C}$ fetal liver on embryonic day 14.5 is plotted. The means and standard deviations are indicated by *horizontal bars* and *vertical bars*, respectively. Significant *P* values with analysis of variance followed by Tukey test are described. DMSO, dimethyl sulfoxide.

phenotypically enriched in the CD48^{-}CD150^{high}Lineage^{-}c-Kit^{+}Sca-1^{+} population using multicolor staining[7] were counted on embryonic day 14.5 (see Supplementary Methods and Supplementary Figure 2). The number of fetal HSCs was not altered in any of the *Nudt15* genotypes without MP treatment (Figure 2*C*). However, it was significantly reduced by MP treatment in *Nudt15*$^{+/R138C}$ fetal mice ($P < .01$; mean number 1104 in dimethyl sulfoxide and 577 in MP) and in *Nudt15*$^{R138C/R138C}$ fetal mice ($P < .01$; mean number 927 in dimethyl sulfoxide and 402 in MP), but not in *Nudt15*$^{+/+}$ fetal mice ($P = .39$; mean number 1004 in dimethyl sulfoxide and 820 in MP). Finally, the number of HSCs in *Nudt15*$^{R138C/R138C}$ fetal livers was significantly reduced to less than 50% of that in *Nudt15*$^{+/+}$ fetal livers by MP treatment during gestation ($P = .0089$).

The current study clearly demonstrates that thiopurine use during pregnancy can cause serious damage to fetal mice, depending on the *Nudt15* genotype of the offspring. In particular, *Nudt15*$^{+/R138C}$ offspring in *Nudt15*$^{+/+}$ pregnant mice and *Nudt15*$^{R138C/R138C}$ offspring in *Nudt15*$^{+/R138C}$ pregnant mice are not safe when the pregnant mice are exposed to therapeutic MP dose. Because the placental permeability of thiopurines and metabolites is unknown in mice, our data may possibly overestimate thiopurine fetal toxicity in humans. However, it has been reported that thioguanines including thio-guanosine-5'-triphosphate, which is the active thiopurine metabolite for cytotoxicity and is directly metabolized by NUDT15, can cross human placenta.[8] In addition, cases of anemia in thiopurine-exposed infants have been reported, although there is no description of NUDT15.[9] Our data also show that fetal HSCs can be damaged by thiopurine use during pregnancy in a *Nudt15* allele number-dependent manner.

In summary, given the ethical difficulty of conducting further prospective clinical studies, thiopurine use during pregnancy should be considered with caution based on the *NUDT15* genotype. Particularly, the paternal *NUDT15 R139C* alle is recommended to be examined in the decision to use thiopurine during pregnancy because it is critical to determine the Nudt15 genotype of offspring.

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Conflicts of interest

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