Germline variants in NT5C2 alter thiopurine metabolism and are associated with acquired relapse mutations in childhood acute lymphoblastic leukemia

The antileukaemic drug 6-mercaptopurine is converted into thioguanine nucleotides (TGN) and incorporated into DNA (DNA-TG), the active end metabolite. In a series of genome-wide association studies, we analysed time-weighted means ($\text{wm}$) of erythrocyte concentrations of TGN (Ery-TGN) and DNA-TG in 1009 patients undergoing maintenance therapy for acute lymphoblastic leukaemia (ALL). In discovery analyses (454 patients), the propensity for DNA-TG incorporation ($\text{wm}_{\text{DNA-TG}/\text{wm}_{\text{Ery-TGN}}}$) was significantly associated with three intronic SNPs in NT5C2 (top hit: rs72846714; $P = 2.09 \times 10^{-10}$, minor allele frequency 15%). In validation analyses (555 patients), this association remained significant during both early and late maintenance therapy ($P = 8.4 \times 10^{-8}$ and $1.3 \times 10^{-3}$, respectively). The association was mostly driven by differences in $\text{wm}_{\text{Ery-TGN}}$, but in regression analyses adjusted for $\text{wm}_{\text{Ery-TGN}}$ ($P < 0.0001$), rs72846714-A genotype was also associated with a higher $\text{wm}_{\text{DNA-TG}}$ ($P = 0.029$). Targeted sequencing of NT5C2 did not identify any missense variants associated with rs72846714 or $\text{wm}_{\text{Ery-TGN}}/\text{wm}_{\text{DNA-TG}}$. rs72846714 was not associated with relapse risk, but in a separate cohort of 180 children with relapsed ALL, rs72846714-A genotype was associated with increased occurrence of relapse-specific NT5C2 gain-of-function mutations that reduce cytosol TGN levels ($P = 0.03$). These observations highlight the impact of both germline and acquired mutations in drug metabolism and disease trajectory.