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

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 (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 (wmDNA-TG/wmEry-TGN ratio) was significantly associated with three intronic SNPs in NT5C2 (top hit: rs72846714; P = 2.09 × 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 × 10⁻⁶ and 1.3 × 10⁻³, respectively). The association was mostly driven by differences in wmEry-TGN, but in regression analyses adjusted for wmEry-TGN (P < 0.0001), rs72846714-A genotype was also associated with a higher wmDNA-TG (P = 0.029). Targeted sequencing of NT5C2 did not identify any missense variants associated with rs72846714 or wmEry-TGN/wmDNA-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.