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 (\(w_{\text{wm}}\)) 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 (\(w_{\text{wm}}\) DNA-TG/\(w_{\text{wm}}\) Ery-TGN ratio) 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 (\(P = 8.4 \times 10^{-6}\) and \(1.3 \times 10^{-3}\), respectively). The association was mostly driven by differences in \(w_{\text{wm}}\) Ery-TGN, but in regression analyses adjusted for \(w_{\text{wm}}\) Ery-TGN (\(P < 0.0001\)), rs72846714-A genotype was also associated with a higher \(w_{\text{wm}}\) DNA-TG (\(P = 0.029\)). Targeted sequencing of NT5C2 did not identify any missense variants associated with rs72846714 or \(w_{\text{wm}}\) Ery-TGN/\(w_{\text{wm}}\) 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.