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Review

A systematic review of aspects of *NUDT15* pharmacogenomic variants and thiopurine-induced myelosuppression

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Abstract

Objectives: Evidence for *NUDT15* pharmacogenomic variants and thiopurine-induced myelosuppression (TIM), consists predominantly of association data in Asian, mixed variant homozygote/heterozygote populations. We therefore sought evidence on; (i) *NUDT15* genotype-guided thiopurine dosing. (ii) Association data for TIM in *NUDT15* variant heterozygotes with inflammatory bowel disease. (iii) Association data for *NUDT15* variants with TIM in Europeans. (iv) Health economic data for *NUDT15* genotyping in inflammatory bowel disease.

Methods: A systematic review was conducted, consisting of database searches, screening against pre-defined inclusion/exclusion criteria, and assessment of risk of bias using study-specific appraisal tools.

Key findings: Titles/abstracts of 493 articles were screened, with 29 studies included. (i) Significant reductions in TIM with genotype-guided thiopurine dosing were reported by both trials and a cohort study. (ii) TIM rates were significantly higher in *NUDT15*3* heterozygotes vs. wild type. Data were conflicting for rarer variants. (iii) Four of five studies reported an association with TIM for at least one or a combination of *NUDT15* variants in Europeans (OR 9.5–38.2), but data were conflicting. (iv) Both health economic analyses found *TPMT/NUDT15* genotyping cost-effective in Asian populations, but not when a European population was considered.

Conclusion: Limited data showed an association with TIM in *NUDT15* variant heterozygotes and Europeans and the potential for genotype-guided dosing to reduce TIM. Studies were generally small, heterogenous, and of variable quality. The low prevalence of rarer *NUDT15* variants/variants in Europeans likely contributed to contradictory findings. Further research on the clinical utility of genotyping in diverse populations will help inform future economic analyses.

Keywords: NUDT15; Nudix hydrolase 15; pharmacogenomics; pharmacogenetics; genomics; thiopurines; myelosuppression; adverse drug reactions; patient safety; health economics; systematic review

Introduction

Pharmacogenomics describes the variability in response to therapeutic medicines that can be attributed to changes in an individual's genomic make up [1]. Pharmacogenomic variants are common, with approximately 98% of the population carrying at least one variant [2]. The pharmacokinetic profile of a medicine may be altered via genomic variants affecting absorption, distribution, metabolism or elimination [3]. Therapeutic on-target and off-target sites may also be affected by genomic variation, changing the pharmacodynamics of medicine [3]. Both aspects of pharmacogenomic variation may increase the risk of treatment inefficacy or toxicity amongst other clinical factors such as co-morbidities and drug–drug interactions. Pharmacogenomic testing aims to guide prescribing, to reduce the risk of adverse drug reactions and therapeutic ineffectiveness, moving prescribing from a population-based approach to a more personalized decision.

The thiopurines, azathioprine, and 6-mercaptopurine, are indicated in the treatment of inflammatory bowel disease (IBD), other autoimmune disorders, and acute lymphoblastic leukaemia (ALL). They are associated with significant adverse effects, with thiopurine-induced myelosuppression (TIM) reported in approximately 7% of European IBD patients [4] and in up to 40% of Asian ancestry patients [5].

The active thiopurine metabolite, 6-thioguanine nucleotide (6TGN) undergoes extensive metabolism by enzymes including thiopurine-S-methyltransferase (TPMT) and nudix hydrolase 15 (NUDT15) to inactive metabolites. Pharmacogenomic variants, within the *TPMT/NUDT15* genes, may result in poorly or non-functioning enzymes and subsequent 6TGN accumulation and toxicity, including severe TIM [6]. This may require hospitalization due to life-threatening infection, or prove fatal [4]. While *TPMT* variants are more common in European ancestries (allelic frequency, AF, 0.047), *NUDT15* variants predominate in Asian, particularly East Asian populations (AF 0.12) and are rare in those of European ancestry (AF 0.0067) [7].

The multiple naming conventions [7–9] for the most common *NUDT15* variants are summarized in Supplementary Appendix 1 (Table S1). Star allele nomenclature will be used, apart from instances where *NUDT15*6* cannot be

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distinguished in reports from *NUDT15*2* due to linkage disequilibrium. *NUDT15*3*, the most common variant, results in nearly complete loss of NUDT15 activity in vitro [10], with patients homozygous for *NUDT15*3* tolerating only 8% of normal doses [11]. Less common *NUDT15* variants, *NUDT15*4-*8* are supported by less robust data and are classified as resulting in 'uncertain function' [7].

The association of *NUDT15* variants and TIM is well documented in previous systematic reviews and meta-analyses [12–15], based on data predominantly from Asian populations of mixed variant heterozygote/homozygotes. Marked variance has been reported in thiopurine doses tolerated by variant heterozygotes [10, 11]. As the majority of variant carriers are heterozygotes [7], this is a key area for further study. It is also vital to establish whether robust association data exists for *NUDT15* variants and TIM in European populations.

Despite strong data supporting the association of *NUDT15* variants with TIM, evidence of the clinical utility of *NUDT15* genotype-guided treatment is required to truly assess its potential to improve patient safety. Economic analysis is also necessary, to establish the cost-effectiveness of *NUDT15* genotyping in various thiopurine treatment pathways, to inform plans for routine adoption.

Objectives

A systematic review was undertaken with regard to the following objectives;

1. Review of evidence for the effect of *NUDT15* genotype-guided thiopurine dosing in reducing myelosuppression (all indications).
2. Review of association data for TIM in *NUDT15* variant heterozygotes with IBD.
3. Review of association data for *NUDT15* variants with TIM in patients of European ancestry (all indications).
4. Review of health economic data for *NUDT15* genotyping in IBD patients.

All thiopurine indications were included for objectives 1 and 3, as a scoping review indicated relatively few studies were

Table 1. Population, intervention, comparator, outcome and study design.

	Objective 1	Objective 2	Objective 3	Objective 4
Population(s) *types/characteristics of participants; ages of participants; health conditions of participants; etc.*	Patients prescribed thiopurines All ages All indications All ancestries	IBD indications only All ancestries	All indications European ancestry only	IBD indications only All ancestries
Intervention(s)[*] *Types/characteristics of interventions; do articles which only have your target intervention as a part of the study "count"?; etc.*	*NUDT15* genotyping and either *NUDT15* genotype-guided thiopurine dosing or alternative therapy based on genotype	NA—association studies		*NUDT15* genotyping and either *NUDT15* genotype-guided thiopurine dosing or alternative therapy based on genotype
Comparison(s)	Standard thiopurine dosing with no *NUDT15* genotyping	Thiopurine-induced myelosuppression in heterozygotes for *NUDT15* variants vs wildtype	Thiopurine-induced myelosuppression in patients of European ancestry with *NUDT15* variants vs wildtype	Standard thiopurine dosing with no *NUDT15* genotyping
Outcome(s) *Which specific outcome measures "count"? Which ones don't? What about qualitative evidence?*	Thiopurine induced myelosuppression; specifically neutropenia, leucopenia	Association of *NUDT15* variants with thiopurine induced myelosuppression in *NUDT15* heterozygotes. Rate/severity of myelosuppression (specifically leucopenia, neutropenia)	Association of *NUDT15* variants with thiopurine induced myelosuppression in patients of European ancestry. Rate/severity of myelosuppression (specifically leucopenia, neutropenia)	Health economic parameters; Quality of life Cost/life year Cost per alternative measure of effectiveness QALY, ICER Costs/resource use Costs analysis Qualitative description of costs/benefits
Study designs *Which study types "count"? Which ones don't?*	**Published studies, with full text available** Prospective randomised controlled trials Case-control studies Cohort studies			Published studies with full text available
Date or language criteria	English			
General exclusions	Unpublished data, qualitative data (Objectives 1–3), abstract only available, non-English, non-human studies, reviews, commentaries/editorials, letters, conference abstracts, case reports, systematic reviews/meta-analysis			

[*]Accepted that some studies may consider *TPMT* and *NUDT15*.

available. Question 2 considered solely IBD populations as the scoping review demonstrated that studies in ALL cohorts commonly used alterative outcome measures such as maximum tolerated thiopurine dose rather than TIM. The focus remained on IBD indications for health economic data, as the clinical and economic impact of myelosuppression in other conditions such as ALL may differ markedly from IBD. TIM was defined as either thiopurine-induced leucopenia (TIL) or thiopurine-induced neutropenia.

Methods

Ethics approval was granted by the University of Exeter (reference 043/22/11/07). The review was registered on the PROSPERO database (reference CRD42023406846) and is reported in line with the Preferred Reporting Items for Systematic Review (PRISMA) guidelines [16].

Search strategy and selection criteria

Articles were identified via searches of Ovid Medline, Embase, and the Cochrane library using defined keywords (thiopurin*, thioguanine, nudix*, mercaptopurine, azathioprine, nucleoside disphosphate*, 6-mercaptopurine, tioguanine, NUDT*, 6-MP, 6MP, MTH2, MutT*). Additional references were sought via forwards and backwards citation chasing. A Population, Intervention, Comparator, Outcome (PICO) framework for each objective was utilized (Table 1). Databases were searched from the date of inception up to 14 and 15 March 2023. No database filters were employed. The search strategy is detailed in Supplementary Appendix 1, Fig. S1.

Screening and data extraction

Search results were exported to Endnote and duplicates were removed. Titles and abstracts were screened against inclusion and exclusion criteria for objectives 1–4 (Table 1) and the remaining studies were retrieved in full text for further screening against inclusion and exclusion criteria. All studies screened on title and abstract and a random sample of 25% of the studies screened in full text, were independently screened by a second reviewer, and any disagreements were resolved via discussion. Relevant study data were extracted to an Excel spreadsheet by one reviewer, for outcomes specified in the PICO framework (Table 1). Other data fields collected are specified in Supplementary Appendix 1, Table S2. Lead authors were emailed to clarify points or to request further data if necessary. Where data on patient ancestry was not provided, the country in which the study was conducted was recorded. Where follow-up duration was not specified, to inform the use of the Newcastle Ottawa scale for cohort and case-control studies, the length of follow-up was approximated from the latest outcome reported. For the comparison of thiopurine dosing, the 6-mercaptopurine dose was converted to equivalent azathioprine dose by multiplication by a factor of 2.08 [17].

Outcome measures and analysis

Meta-analysis was not undertaken due to heterogeneity across the included studies. No restriction was placed on the use of particular outcome measures due to the low number of studies identified via initial scoping reviews. Studies were also included that did not include a specific outcome measure but reported rates of TIM for wildtype and variant groups. Where a multi-variate analysis (MVA) was also undertaken, the MVA odds ratio (OR) was quoted in the results. Studies investigating the association of the same *NUDT15* variants, with the same outcome measures were directly compared, and an overarching narrative review of study design, results, and data quality was conducted. Results for objectives 1–3 were tabulated. For illustration, a forest plot was utilized to display the unadjusted risk difference (and normal approximated 95% CIs) in TIM events, between *NUDT15* variant heterozygotes and wildtypes, for all cohort studies in Asian populations included in Objective 2.

Review of statistical analysis

Statistical significance was defined as a P value < .05 for case control or cohort studies and the Bonferroni testing threshold of $P < 5 \times 10^{-8}$ for exome-wide association studies (EWAS) or genome-wide association studies (GWAS) [18].

Quality assessment

No quality threshold was employed for inclusion due to the small number of studies. The quality of each study was assessed by one reviewer using the following study-specific critical appraisal tools: Rob-2 tool for randomized controlled trials [19], Newcastle-Ottawa scale for cohort and case-control studies [20] and QHES for health economic evaluations [21]. Areas of uncertainty were raised with a second reviewer to independently review.

For the Newcastle-Ottawa scale, thiopurine dose was determined as the most important factor to control for in non-European ancestry patients and *TPMT* genotype in Europeans. The following recognized parameters were utilized to convert the Newcastle-Ottawa scores for cohort and case-control studies to the Agency for Health Research Quality (AHRQ) standards (good, fair, poor) [22]:

Good quality: 3 or 4 stars in selection domain AND 1 or 2 stars in comparability domain AND 2 or 3 stars in outcome/exposure domain

Fair quality: 2 stars in selection domain AND 1 or 2 stars in comparability domain AND 2 or 3 stars in outcome/exposure domain

Poor quality: 0 or 1 star in selection domain OR 0 stars in comparability domain OR 0 or 1 stars in outcome/exposure domain

Results

Following the search strategy detailed in Fig. 1, a total of 29 studies were included in the review, with some meeting criteria for more than one objective. Two studies [23, 24] may include some of the same patients, but unfortunately, verification could not be obtained from the lead author via email so both studies were included. Further details were obtained via email from the lead authors for two studies to clarify information included in the data extraction process. Details of studies excluded on full-text screening are provided in Supplementary Table S3 (Appendix 1).

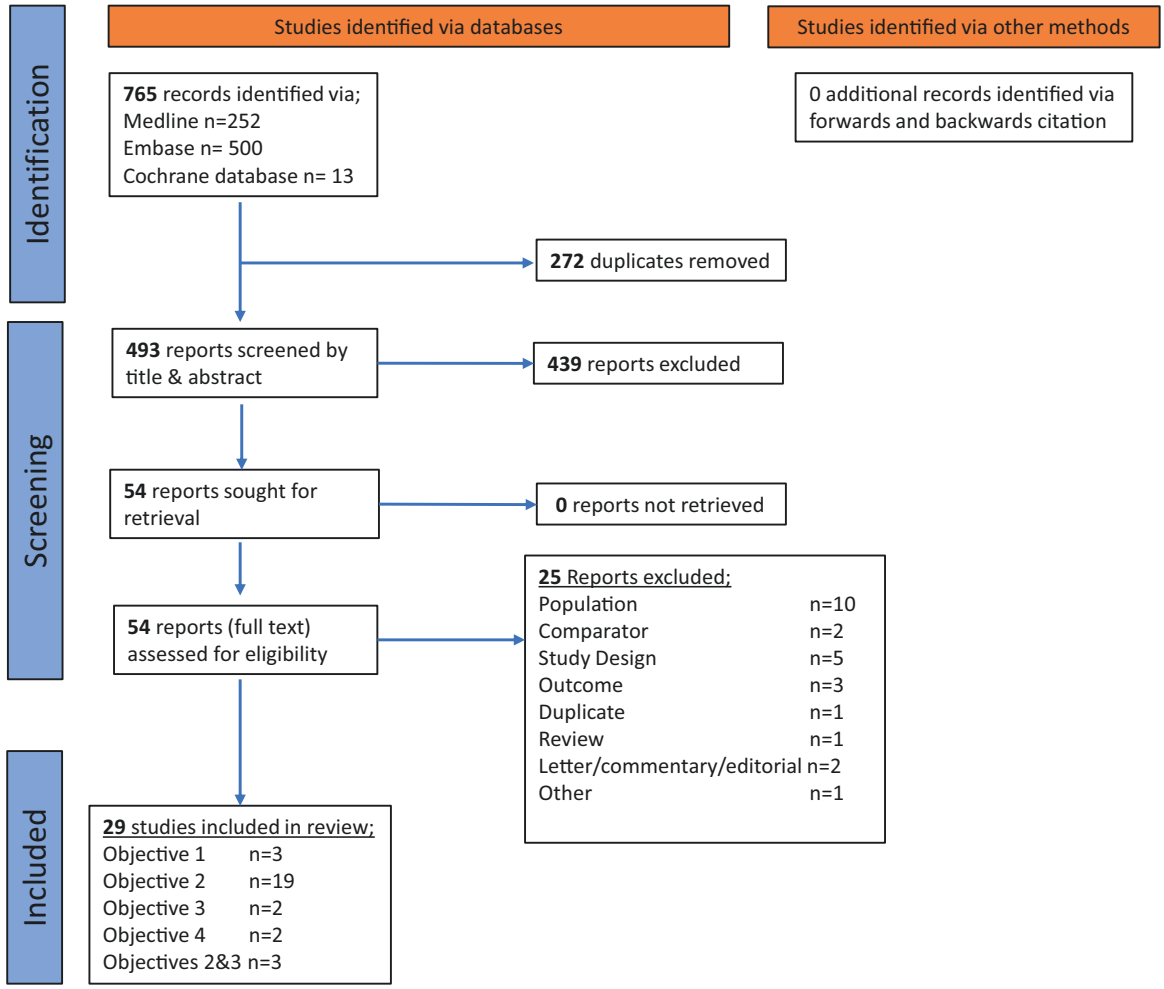

Figure 1. PRISMA diagram [16] summarizing results of the search strategy.

Objective 1: Review of evidence for the effect of *NUDT15* genotype-guided thiopurine dosing in reducing myelosuppression (all indications)

Two randomized trials [25, 26], plus a comparison of a genotype-guided treatment to a historical control [27] met the inclusion criteria (Table 2). Chang *et al*. considered variants in three genes, reporting 15/72 patients heterozygous for *NUDT15* variants, eight for *FTO* variants, and one for *TPMT* [25]. No significant association with myelosuppression was demonstrated for the *FTO* variant across intervention and control groups [25]. The remaining studies investigated *NUDT15*3* genotyping only [26, 27]. All used a 'no genotyping' comparator.

Both randomized trials were unblinded, conducted in small numbers of IBD/Crohn's Disease (CD) patients, and utilized similar strategies for genotype-guided treatment. Statistically significant reductions were reported in the genotype-guided arms compared to the control arm: Chao relative risk (RR) of 0.73 (95%CI 0.53, 1.00) for thiopurine-induced leucopenia (TIL) [26]; Chang hazard ratio (HR) of 0.37 (95% CI 0.18, 0.77) for TIM [25]. Results from Chang *et al*. [25] could not be separated by individual variants.

Wang *et al*. [27] considered patients with autoimmune disorders in the genotyping cohort and reported a statistically significant lower rate of TIL compared to historical controls: 0.4% vs. 7.6%. In contrast to the randomized trials [25, 26], variant heterozygotes were switched to alternative treatment rather than having their thiopurine dose reduced. Thiopurine dosing in wildtype and control patients could not be ascertained from the study report and the control rate of myelosuppression was substantially lower than that reported in both trials.

Some concerns regarding the risk of bias were highlighted in the quality assessment of the study conducted by Chang *et al*. [25], but the study conducted by Chao *et al*. [26] was deemed to be at low risk of bias (Supplementary Appendix 1 Table S4). The study undertaken by Wang *et al*. [27], was assigned a quality score of 5/9 according to the Newcastle-Ottawa scale, translating to a poor quality rating against AHRQ standards, mainly due to patient selection and comparability of cohorts (Supplementary Appendix 1 Table S5).

Objective 2: Review of association data for TIM in IBD patients heterozygous for *NUDT15* variants

Twenty-two studies were included [23, 24, 28–47], three of which also met the criteria for objective 3. Studies considered predominantly Asian patients and were mainly of retrospective cohort design, with some case-control and genome-wide (GWAS) or exome-wide association studies (EWAS). Fig. 2 summarizes the number of TIM events in wildtype

Table 2. Characteristics of interventional *NUDT15* genotyping studies.

First author/ Year/ Location	Study type and patient numbers	Thiopurine indication	Genotyping	Thiopurine dosing (azathioprine equivalent)	Outcome	Results (TIM/TIL rate)	Outcome measure (95% CI)
Chang 2020 [25] Korea	Prospective, randomized controlled, multi-centre, unblinded *N* = 92 control *N* = 72 genotyping	IBD	*TPMT* *FTO* *NUDT15*3*	**WT and control;** 50 mg initial to max 2–2.5 mg/kg/day **Heterozygotes;** 50 mg/day **Homozygotes;** alternative treatment	**TIM; one of;** WCC < 3 × 10^9/L Hb <10 g/dL Platelets <100 × 10^9/L	16.7% intervention vs 35.9% control *P* = .005	**MVA HR (reduction in TIM);** 0.37 (0.18, 0.77) *P* = .008
Chao 2021 [26] Chinese-Han	Prospective, randomized, controlled, multi-centre, unblinded *N* = 219 genotyping *N* = 204 control	Crohn's Disease	*NUDT15*3*	**WT and control;** 2 mg/kg/day **Heterozygotes;** 50% dose **Homozygotes;** alternative treatment	**TIL;** WCC<3.5 × 10^9/L	23.7% intervention vs 32.4% control *P* = .049	**RR leucopenia;** 0.73 (0.53, 1.00)
Wang 2022 [27] Taiwan/China	Prospective intervention cohort vs historical control Non-randomized Non-blinded *N* = 1056 genotyping *N* = 3244 control	Auto-immune disorders	*NUDT15*3*	**WT;** Not stated **Historical controls;** Not stated **Heterozygote and homozygote variant carriers;** alternative treatment	**TIL;** WCC<3 × 10^9/L	0.4% intervention vs. 7.6% control (historical) *P* = 9.3 × 10^{-20}	Not stated

WT = wildtype, TIM = thiopurine-induced myelosuppression, TIL = thiopurine-induced leucopenia, MVA = multi-variate analysis, HR = hazard ratio, WCC = white cell count, Hb =haemoglobin.

and *NUDT15* variant heterozygote groups, plus the unadjusted risk difference for TIM events, for all cohort studies of Asian patients. Table 3 summarizes details of all 22 studies included in Objective 2, including outcome measures, where provided, from univariate and multivariate analysis, specific to *NUDT15* variant heterozygotes. Five cohort studies considered solely variant heterozygotes vs wildtype patients [28–32] (Fig. 2 and Table 3), eight further studies reported statistical analysis for variant heterozygote subsets [35, 36, 38, 42–44, 46, 47] (Table 3) and in total, eight studies reported at least one outcome measure specific to *NUDT15* variant heterozygotes (hazard ratio *n* = 1) [36], (odds ratio, *n* = 7) [30, 31, 35, 38, 44, 46, 47] (Table 3).

Azathioprine was prescribed across all studies, with 16 also including patients prescribed 6-mercaptopurine for IBD. Most studies investigated the relationship between the *NUDT15*3* variant and thiopurine-induced leucopenia (TIL), while some used a composite outcome of leucopenia/neutropenia, or reported neutropenia separately (Table 3). Heterogenous thresholds were utilized in the definition of leucopenia or neutropenia, follow-up durations were variable and 95% CI were wide. Study quality scores, assessed via the Newcastle-Ottawa Scale, ranged from 5 to 9/9 and conversion to AHRQ ratings, resulted in 10 studies categorised as good quality, 3 of fair quality and 9 of poor quality (Table 3, further details in Supplementary Appendix 1, Table S5 and S6). Potential confounding factors which could affect thiopurine metabolism or alter the risk of TIM, such as *TPMT* genotype, drug interactions with allopurinol, azathioprine equivalent dose or concomitant corticosteroids, were variably reported (Supplementary Appendix 1, Table S7).

All five cohort studies of solely variant heterozygote populations vs. wildtype, reported statistically significant increased rates of TIM in the *NUDT15* variant heterozygote group [28–32].

Results specific to *NUDT15*3*

Across the 17 studies reporting leucopenia in *NUDT15*3* variant heterozygotes, rates ranged from 30% to 73% [42, 43]. An association with leucopenia was demonstrated for *NUDT15*3* heterozygotes across all cohort studies in Asian patients (Fig. 2). Three studies providing odds ratios (OR) specific to *NUDT15*3* heterozygotes, reported statistically significant associations with TIM (ORs ranging from 5.23 to 9.16) [30, 31, 46] (Table 3). Kakuta *et al.* [38] and Yang *et al.* [46] also reported significant OR (13.04 and 88.06, respectively) in *NUDT15*3* heterozygote patient subsets for early leucopenia. Banerjee *et al.* [36] reported a statistically significant *NUDT15*3* heterozygote HR of 11.1 for leucopenia and a further three studies demonstrated statistically significant differences in TIL rates between WT and *NUDT15*3* variant heterozygotes [28, 32, 42].

Conversely, the only study not to look at people with Asian ancestry did not find a significant association with TIM for European *NUDT15*3* heterozygotes alone, via GWAS or EWAS [47].

Results for other *NUDT15* variants and combined variant analysis

In two studies, significant association with TIM was reported for the combined analysis of several *NUDT15* variants: **3*, **6*, **9* in Walker *et al.* with an OR of 20.9 (95% CI 6.4–68.6)

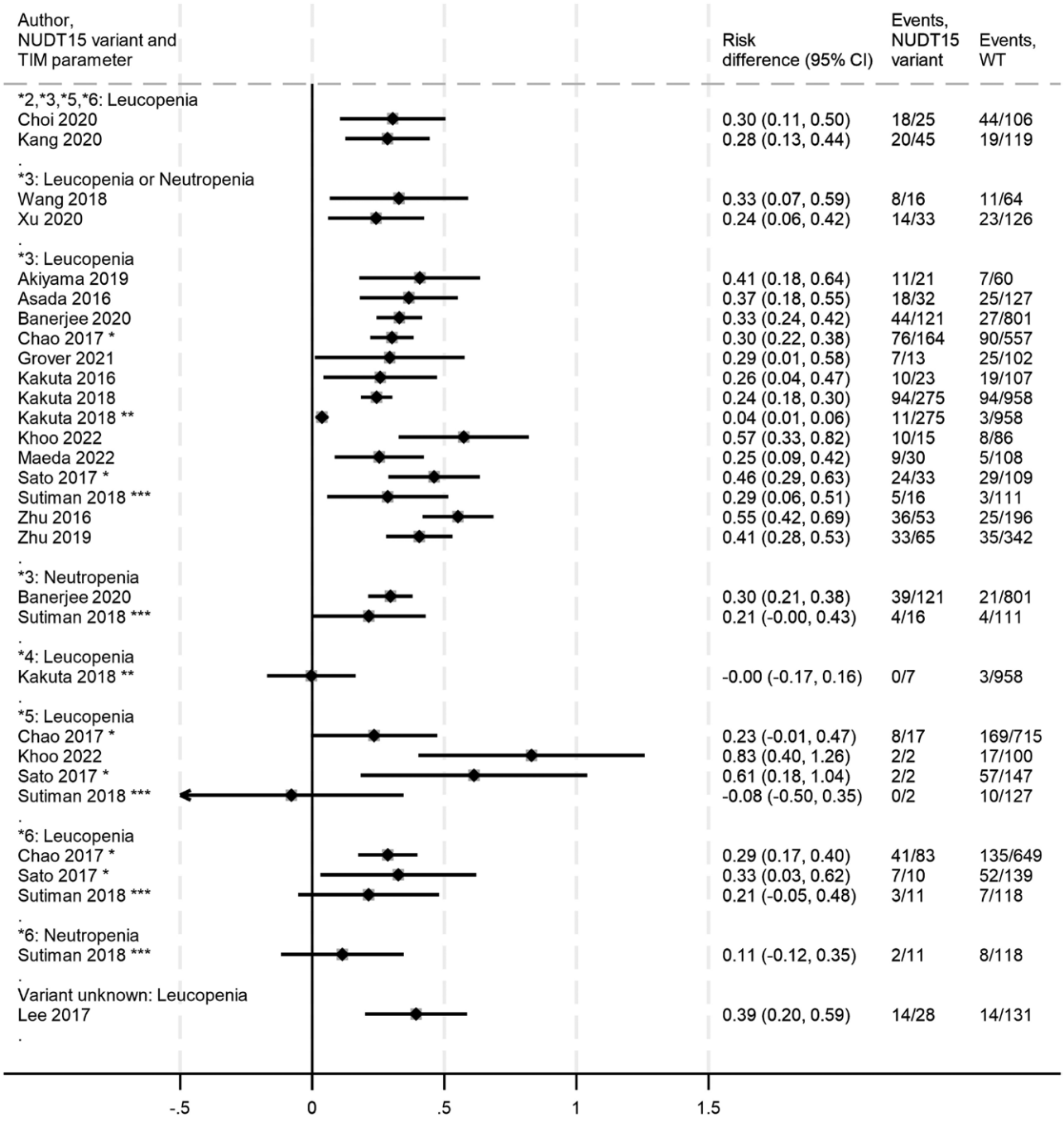

Figure 2. Forest plot of unadjusted risk difference for TIM events in wildtype vs. *NUDT15* variant heterozygotes (cohort studies, Asian populations). **Chao 2017* & Sato 2017*;** WT for *NUDT15*3* subset represents those not carrying *NUDT15*3*, WT for *NUDT15*5* subset represents those not carrying *NUDT15*5*. **Kakuta 2018**;** Subset results for acute, severe leucopoenia (WCC < $2x10^9$/L within 2 weeks). **Sutiman 2018***;** WT represents those not carrying any of the variants investigated (*NUDT15*3, *4, *5, *6*).

[47] in the EWAS section of their study and **2, *3, *5, *6* in Choi *et al.* [29].

Association data for other *NUDT15* variant heterozygotes were uncertain and conflicting. Studies contained low patient numbers, with as few as two variant heterozygotes in *NUDT15*5* subsets in two studies [43, 44] (Table 3). Statistically significant associations with TIM were reported for *NUDT15*5, *6, *9* variant heterozygotes in three studies [35, 44, 47] (OR 4.63–5.95) [35, 47], but no significant association for *NUDT15*5* [43, 44, 46], *NUDT15*4* [38], or *NUDT15*6* [43] variant heterozygotes in 4 studies (Table 3). Walker *et al.* found no association for individual *NUDT15* variant subsets in Europeans in the GWAS section of the study [47]. An association with leucopenia was demonstrated via the unadjusted risk difference for Sato *et al.* [43] for *NUDT15*5* and **6* heterozygotes (Fig. 2), but differences in leucopenia rates failed to reach statistical significance using non-parametric methods [43]. The calculated risk difference for *NUDT15*5* and *NUDT15*6* leucopenia rates, reported by Chao *et al.* [35] and Sutiman *et al.* [44], displayed 95%

Table 3. Association data for *NUDT15* variant heterozygotes and TIM (IBD indications only, all ancestries)

First author & Year	Patient ancestry/ study location & no. patients	TIM threshold (x10⁹/L)	Incidence of TIM	Number (%) *NUDT15* variant heterozygotes	Odds ratio, OR (95% confidence intervals) or alternative outcome measure	Newcastle- Ottawa Score (max 9) & Quality rating
Akiyama 2019 [28]	Japanese *N* = 81 (83 wider cohort)	WCC < 3	22.2%	21/83 (25.3%) ***NUDT15*3*** (no homozygotes)		7 (poor)
Choi 2020 [29]	Korea *N* = 131	WCC < 3	47.3%	25/131 (19.1%) ***NUDT15*2,*3,*5 or *6*** (no homozygotes)		6 (good)
Grover 2021 [30]	Indian *N* = 119	WCC < 3	27.7%	13/119 (10.9%) ***NUDT15*3*** (no homozygotes)	Leucopenia OR 3.59 (1.10–11.7) *P* = .0336 Cytopenia MVA OR 5.23 (1.44–19.04) *P* = .012	8 (good)
Wang 2018 [31]	Chinese *N* = 80	WCC < 3.5 **Or** neutrophil < 1.5	23.8%	16/80 (20%) ***NUDT15*3*** (no homozygotes)	Leucopenia MVA OR 7.663 (1.89–31.02), *P* = .004	7 (good)
Xu 2020 [32]	China *N* = 159	WCC < 3.5 **Or** neutrophil < 2 **Or** decrease in blood count requiring drug cessation	23.2%	33/159 (20.8%) ***NUDT15*3*** (no homozygotes)		9 (good)
Afrin 2022 [33]	European (95%), Asian, African, *n* = 291	WCC < 2.5 **Or** neutrophils < 1	4.1%	4/291 (1.4%) ***NUDT15*3*** (plus 1 homozygote)	TIM HR for variant allele = 15.35 (3.25-72.47), $P = 5.63 \times 10^{-4}$	5 (poor)
Asada 2016 [34]	Japanese *N* =161	WCC < 3	27.9%	32/161 (19.9%) ***NUDT15*3*** (plus 2 homozygotes)	Leucopenia MVA OR for *NUDT15* variant = 5.38 (1.91–15.2), *P* = .001	8 (good)
Chao 2017 [35]	Han Chinese *N* = 732	WCC < 3.5	24.2%	164/732 (22.4%) ***NUDT15*3*** (plus 11 homozygotes)	Leucopenia OR for *NUDT15*3* variant = 4.45 (3.23–6.12), $P = 2.51 \times 10^{-22}$ Leucopenia MVA OR for *NUDT15*3* variant = 4.54 (3.02–6.8), $P = 2.69 \times 10^{-13}$	7 (good)
				17/732 (2.3%) ***NUDT15*5*** (no homozygotes)	Leucopenia OR for *NUDT15*5* heterozygote = 2.83 (1.08–7.39), *P* = .041 Leucopenia MVA OR for *NUDT15*5* heterozygote = 4.63 (1.65–13), *P* = .004	
				83/732 (11.3%) ***NUDT15*6*** ≠ (plus 1 homozygote)	Leucopenia OR for *NUDT15*6* variant = 3.52 (2.26–5.49), $P = 4.39 \times 10^{-9}$ MVA OR not significant	

Table 3. Continued

First author & Year	Patient ancestry Study location and no. patients	TIM threshold (×10⁹/L)	Incidence of TIM	Number (%) *NUDT15* variant heterozygotes	Odds ratio, OR (95% confidence intervals) or alternative outcome measure	Newcastle- Ottawa Score (max 9) & Quality rating
Banerjee 2020 [36]	Indian *N* = 935	WCC < 3 **or**	9%	121/935 (12.9%) ***NUDT15*3*** (plus 13 homozygotes)	Leucopenia OR for *NUDT15*3* variant = 19.35 (11.55–32.42), *P* < .0001 Leucopenia MVA HR *NUDT15*3* heterozygote = 11.31 (6.85–18.03) *P* < .0001	8 (good)
		neutrophils < 1.5	7.5%		Neutropenia OR for *NUDT15*3* variant = 21.41 (12.25–37.41), *P* < .0001 Neutropenia MVA HR *NUDT15*3* heterozygote= 13.04 (7.65–22.22)	
Kakuta 2016 [37]	Japanese *N* = 135	WCC <3	25.2%	23/135 (17%) ***NUDT15*3*** (plus 5 homozygotes)	**Early** leucopenia OR for *NUDT15*3* variant allele = 28.4 (9.78-82.3) $P = 4.38 \times 10^{-15}$ **Early** leucopenia MVA OR for *NUDT15*3* variant allele = 51.9 (9.46-1256), $P = 4.65 \times 10^{-4}$ No association found for late leucopenia	8 (good)
Kakuta 2018 [38]	Japanese *N* = 1291	WCC < 3	18.1%	275/1291 (21.3%) ***NUDT15*3*** (plus 49 homozygotes)	Leucopenia OR for *NUDT15*3* variant allele = 6.59 (5.19-8.36), $P = 2.2 \times 10^{-63}$	6 (poor)
		WCC < 2 within 8 weeks	3.7%		Early severe leucopenia OR for *NUDT15*3* heterozygote = 13.4 (3.7–48.4), $P = 7.43 \times 10^{-5}$	
		WCC < 2, within 8wks	3.7%	7/1291 (0.54%) ***NUDT15*4*** (no homozygotes)	No association found	
Kang 2020 [39]	Korean, *N* = 167 Paediatric IBD patients	WCC < 3	25.1%	**45/167 (26.9%) *NUDT15*2,*3,*5,*6*** (plus 3 variant homozygotes)	Leucopenia OR for *NUDT15* variant = 4.84 (2.29–10.24), *P* < .001 Leucopenia MVA OR for *NUDT15* variant = 5.78 (2.57–13.03), *P* < .001	9 (good)
Khoo 2022 [40]	Malay, Chinese Indian *N* = 102	WCC <3	18.6%	15/102 ***NUDT15*3*** (18.3%) (plus 1 homozygote) 2/102 (1.96%) ***NUDT15*5*** (no homozygotes)	Leucopenia OR for *NUDT15* variant = 41.49 (9.55–180.28), *P* = <.001	6 (poor)

First author & Year	Patient ancestry/ study location and no. patients	TIM threshold (×10⁹/L)	Incidence of TIM	Number (%) *NUDT15* variant heterozygotes	Odds ratio, OR (95% confidence intervals) or alternative outcome measure	Newcastle- Ottawa Score (max 9) and quality rating
Lee 2017 [41]	South Korea *N* = 140 (wider cohort 165)	WCC <3	27.1% leucopenia	28/165 (17%) ***NUDT15* variant** (plus 6 variant homozygotes)	Leucopenia OR for *NUDT15* variant = 3.44 (1.21–9.78)	7 (poor)
Maeda 2022 [42]	Japan *N* = 142	WCC <3	12.7%	30/142 (21.1%) ***NUDT15*3*** (plus 4 homozygotes)		6 (poor)

Table 3. Continued

First author & Year	Patient ancestry/ study location and no. patients	TIM threshold (×10⁹/L)	Incidence of TIM	Number (%) *NUDT15* variant heterozygotes	Odds ratio, OR (95% confidence intervals) or alternative outcome measure	Newcastle- Ottawa Score (max 9) and quality rating
Sato 2017 [43]	Japanese *N* = 149 (160 wider cohort)	WCC <3	36.9%	35/160 (21.9%) ***NUDT15*3*** (plus 8 homozygotes) 11/160 (6.9%) ***NUDT15*6* (pVal18_Val19ins GlyVal)**≠ (no homozygotes) 2/160 (1.2%) ***NUDT15*5*** (no homozygotes)		6 (poor)
Sutiman 2018 [44]	Chinese, Malay Indian *N* = 129	WCC < 3 Neutrophils <1.5	WCC <3; 7.75% Neutrophil <1.5;7.75%	16/129 (12.4%) ***NUDT15*3*** (plus 2 homozygotes) 11/129 (8.5%) ***NUDT15*6*** (c.36_37insGGAGTC)≠ (no homozygotes) 2/129 (1.6%) ***NUDT15*5*** (no homozygotes)	OR leucopenia for *NUDT15*3* variant = 22.9 (5.17–101.4), $P = 3.71 \times 10^{-5}$ OR neutropenia for *NUDT15*3* variant = 13.4 (3.3–54.2), $P = 2.79 \times 10^{-4}$ OR leucopenia for *NUDT15*6* variant heterozygote = 5.95 (1.29–27.5), *P* =.022 OR neutropenia for *NUDT15*6* variant heterozygote = 3.06 (0.56–16.6), *P* = 0.196 No association found with neutropenia or leucopenia	7 (poor)
Zhu 2016 [23]~	Chinese-Han *N* = 253	WCC <3.5	25.7%	53/253(20.9%) ***NUDT15*3*** (plus 4 homozygotes)	OR leucopenia for *NUDT15*3* variant= 10.8 (5.89–19.83), $P = 8.61 \times 10^{-19}$	6 (poor)
Zhu 2019 [24]~	China *N* = 411	WCC <3.5	17.5%	65/411(17.9%) ***NUDT15*3*** (plus 4 homozygotes)		6 (fair)

First author & Year	Patient ancestry/ study location and no. patients	TIM threshold (x10⁹/L)	Incidence of TIM	Number (%) *NUDT15* variant heterozygotes	Case control results	Odds ratio, OR (95% confidence intervals) or alternative outcome measure	Newcastle- Ottawa Score (max 9) and Quality rating
Bangma 2020 [45]	European (non-Finnish), *n* = 500 (wider cohort *n* = 695)	WCC <2.5 **and/or** neutrophil< 1.	4.2%	13/695 (2%) heterozygous for ***NUDT15*3,*6,* or **9*** (Plus 2 variant homozygotes)	*NUDT15* variant in; 4/29 (15%) cases vs 4/470 (1%) controls $P = 7.88 \times 10^{-5}$ variant allele	**UVA OR 24.3** (6.06–104) $P = 5.67 \times 10^{-6}$ *NUDT15* variant **MVA OR 20.2** (4.4–94.4), $P = 7.88 \times 10^{-5}$	6 (fair)

Table 3. Continued

First author & Year	Patient ancestry/ study location and no. patients	TIM threshold (x10^9/L)	Incidence of TIM	Number (%) *NUDT15* variant heterozygotes	Case control results	Odds ratio, OR (95% confidence intervals) or alternative outcome measure	Newcastle- Ottawa Score (max 9) and Quality rating
Yang 2014 [46]	Korean; *N* = 978 total Discovery; 33 cases and 307 controls Replication; 33 early TIL 280 late TIL 325 control	WCC < 3	35.4% (wider cohort)	176/978 (18%) ***NUDT15*3*** (plus 14 homozygotes)	Discovery and replication cohorts: *NUDT15*3* in 59/66 (89.4%) cases vs 43/632 (6.8%) controls	***NUDT15*3* heterozygote OR 9.16** (6.26–13.38), $P = 1.97 \times 10^{-35}$ (*NUDT15*3* heterozygote early leucopenia OR 88.06 [47.47–206.93], $P = 2.96 \times 10^{-38}$ and late leucopenia OR 6.28 [4.21–9.36], $P = 9.37 \times 10^{-21}$)	7 (good)
Walker 2019 [47]	European (non-Finnish) *N* = 311 cases, 608 controls	WCC <2.5 or neutrophil <1	Not stated		No statistically significant association found for *NUDT15* via GWAS. **EWAS analysis;**		7 (fair)
					NUDT15*3 heterozygotes 8/328 (2.4%) cases vs 0/633 controls	NA, P 1.8×10^{-4} (insignificant)^^	
					NUDT15*9 heterozygotes 19/328 (5.8%) cases vs 1/633 (0.2%) controls	**TIM OR 38.2** (5.1–286.1), $P = 1.3 \times 10^{-8}$	
					NUDT15*6 heterozygotes 5/328 (1.5%) cases vs 2/633 (0.3%) controls	**TIM OR 5.2** [1.0–26.6] $P = .04$ (insignificant)^^	
					Exploratory analysis; *NUDT15*9, NUDT15*3 or NUDT15*6* 31/328 (9.5%) cases vs 3/633(0.5%) controls	**TIM OR 20.9** (6.4–68.6) $P = 1.5 \times 10^{-12}$	

Blue= studies or data specific to NUDT15 variant heterozygotes
^^ Significant *P* value for GWAS/EWAS data stated as $P < 5 \times 10^{-8}$.
≠ In strong linkage disequilibrium with *NUDT15*3*, where both present, defined as *NUDT15*2*.
UVA = univariate analysis; **MVA** = multi-variate analysis; **HR** = hazard ratio; **WCC** = white cell count; **TIM** = thiopurine-induced myelosuppression.

confidence intervals crossing the significance threshold of 0, but each study reported a statistically significant *P*-value via non-parametric methods and adjusted analysis respectively [35, 44].

Objective 3: review of association data for *NUDT15* variants with TIM in patients of European ancestry (all indications)

Five studies comprising cohort, case-control, and GWAS/EWAS methodology met objective 3 criteria, considering association data for *NUDT15* variants and TIM in patients of European ancestry. Differing myelotoxicity parameters, thresholds, and outcome measures were utilized and a variety of patient populations were considered (IBD, autoimmune disease, paediatric ALL) (Table 4). Notably, Schaeffeler *et al*. [48] assessed TIM via a questionnaire and/or on laboratory criteria, in contrast to laboratory criteria alone, and undertook a comparison to population data via the GnomAD database. Wahlund *et al*. [49] studied the association with neutropenia in paediatric ALL patients undergoing ALL chemotherapy treatment. *TPMT* genotype was assessed by all studies, with Yang *et al*. excluding those with variants [46] and several cases of concurrent *NUDT15/TPMT* variants were reported. All contained only very low numbers of patients with *NUDT15* variants.

Four studies demonstrated a statistically significant association for at least one or a combination of *NUDT15* variants with TIM, with ORs ranging from 9.5 to 38.2 [46, 47], but data were conflicting and confidence intervals, where reported, were very wide (due to the small number of patients with *NUDT15* variants). Walker *et al*. reported no association for *NUDT15* variants and TIM via GWAS, or for EWAS data for *NUDT15*3 or NUDT15*6* [47]. This conflicted with findings from Yang *et al*. [46], which indicated a significant association for *NUDT15*3*. Walker *et al*. demonstrated significant associations with TIM for *NUDT15*9* and a combination of *NUDT15*3,*6,*9* (OR 20.9, 95% CI 6.4–68.6) [47]. Similarly, Bangma *et al*. reported OR of 20.9 (95% CI 4.4–94.4) for a combination of *NUDT15*3, *6, *9* [45]. Association for *NUDT15*6* reached the *P* value threshold of .05 in data reported by Schaeffeler *et al*. [48], indicating non-significance and Wahlund *et al*. [49] reported no association with neutropenia in paediatric ALL patients.

Objective 4: review of health economic data for *NUDT15* genotyping in inflammatory bowel disease patients

Two health economic analyses for *NUDT15* genotyping to inform azathioprine treatment in IBD were included.

Zarca *et al*. [50] used a decision tree model to compare the cost of combined *TPMT/NUDT15* genotyping to *TPMT* genotyping or next-generation sequencing (NGS), in preventing severe TIM for Caucasian and Asian ancestry patients from the perspective of French healthcare reimbursement policies. Authors presumed that no patients heterozygous for *NUDT15* variants would experience severe TIM and all variant homozygotes would experience severe TIM requiring hospitalization. The model included costs for genotyping or NGS, azathioprine treatment and monitoring, alternative treatments, and severe TIM requiring hospitalization. The study generated an ICER of 7 491 281 euro for combined genotyping vs. *TPMT* genotyping in Caucasian patients and an ICER of 619 euro in Asian patients. Sensitivity analysis demonstrated ICERs were highly sensitive to the prevalence of severe myelotoxicity, the sensitivity of combined genotyping, and the cost of myelotoxicity [50]. Authors concluded there was 99% probability (via an acceptability curve), that combined *TPMT/NUDT15* genotyping was cost-effective vs. *TPMT* genotyping in Asian ancestry patients, but not for those of European ancestry due to an exorbitant ICER [50].

Zeng *et al*. [51] utilized a decision tree model informed by real-world, retrospective data from an IBD patient database at a single centre and published data for TIM, from the perspective of the Chinese healthcare setting. A comparison of combined *TPMT/NUDT15* genotyping vs. *TPMT* genotyping vs. *NUDT15* genotyping vs no genotyping was conducted. An outcome of avoidance of severe myelotoxicity was utilized, with an outcome measure of cost per quality-adjusted life year (QALY) and a willingness to pay a threshold of 30 425 US dollars per QALY. The probability of severe myelotoxicity was based upon retrospective data and phenotype analysis, with the prevalence of *TPMT/NUDT15* variants set according to single-centre retrospective data for base-case analysis and ethnic-specific published data for sensitivity analysis [51]. Utility values for IBD remission and severe TIM were based on published data from Hong Kong and UK settings respectively. In comparison to combined *NUDT15/TPMT* genotyping, no genotyping and *TPMT* genotyping were estimated to be more expensive but less effective. The authors concluded that there was a 91.7% probability of combined genotyping being cost-effective at the willingness to pay threshold vs. either genotyping alone or no genotyping. When compared to combined *NUDT15/TPMT* genotyping, *NUDT15* genotyping was estimated to be less expensive, but also less effective (and was not considered cost-effective at the given willingness to pay threshold). The ICER value was most sensitive to changes in costs of myelotoxicity, plus costs of *TPMT* genotyping.

Both studies set time horizons of 1 year (discounting therefore inapplicable) and undertook sensitivity and probabilistic sensitivity analysis, but due to the healthpayer perspective taken, neither considered indirect costs due to productivity loss. Accounting for exchange rates, markedly different costs were utilized for the treatment of myelotoxicity and TNF-alfa treatment: A cost of 7000 euro (2019) was utilized by Zarca *et al*. [50], vs. $363 (2020) by Zeng *et al*. [51], for the treatment of severe TIM and the cost of TNF-alfa treatment and monitoring was set at 4200 euro/year (2019) by Zarca *et al*. [50] vs. $20 457/year by Zeng *et al*. [51]. Study quality was assessed via the QHES instrument [21], with scores of 66/100 and 86/100 for the study by Zarca *et al*. [50] and Zeng *et al*. [51] respectively (Supplementary Appendix 1, Table S8).

Discussion

Objective 1

Data for *NUDT15* genotype-guided dosing was limited to two trials of relatively low patient numbers [25, 26] plus a cohort study [27], all in Asian populations. Both trials were prospective, multi-centre and randomized, but unblinded. Results from the larger clinical trial (n = 423), conducted by Chao *et al*. [26], reported a RR of 0.73 (0.53-1) for *NUDT15*3* genotyping. However, as the RR 95% CI reached the threshold of 1, there is a chance that genotyping may not in fact reduce the risk of TIM. The trial was assessed via the Rob-2 tool

Table 4. Studies of association of *NUDT15* variants with TIM in European populations (all indications).

First author and Year	Patient no., ancestry/ country, indication	TIM threshold ($\times 10^9$/L)	Incidence of TIM	Number (%) *NUDT15* variant carriers	TIM rate: wildtype WT vs *NUDT15* variant carriers		*P* value	Outcome measure (95% CI)	Newcastle-Ottawa Score (max 9) and Quality rating
					WT	variant			
Wahlund 2020 [49]	*N* = 101 Swedish ALL	Neutrophils <0.5	100% Median 9 episodes/ patient	4/102 (3.9%) ***NUDT15*3*** 1/4 also carried *TPMT* variant	NI	NI	**0.96**	**HR 1.01 (0.71–1.42)**	6 (fair)
Bangma 2020 [45]	*N* = 500 (from 695) European (non-Finnish) IBD	Probable case: WCC <2.5 and/or neutrophils < 1	5.8%	15/695 (2.2%) ***NUDT15*3,*6, or *9***	NA—case control study. *NUDT15* variant in; 4/29 (15%) cases 4/470 (1%) controls		7.88×10^{-5}	**UVA OR 24.3** (6.06–103) $P = 5.67 \times 10^{-6}$ **MVA OR 20.2** (4.4–94.4) $P = 7.88 \times 10^{-5}$	6 (fair)
Schaeffeler 2019 [48]	*N* = 107, IBD and auto-immune diseases *N* = 689, ALL cohort European	CTCAE criteria if lab results available & questionnaire	NA	Case control study. *NUDT15* variant in 14/107 (13%) cases. *NUDT15* & *TPMT* variant in 6% cases. 5 implicated *NUDT15* variants; ***NUDT15*9*** ***NUDT15*3*** ***NUDT15*6*** **c.3G>C, *NUDT15*19 (novel)*** **c.217delA, *NUDT15*18 (novel)***			**Comparison to GnomAD AF** $P = 4.0 \times 10^{-16}$ (and absent in ALL cohort) $P = 1.5 \times 10^{-12}$ (and absent in ALL cohort) $P = .05$ NA – novel variant NA – novel variant	3 (poor)
Yang 2014 [46]	*N* = 1188 US cohort IBD	WCC <3	NA	Case-control study; ***NUDT15*3*** AF; 2.74% cases vs 0.31% controls			$P = 4.64 \times 10^{-4}$	**OR 9.5** (CI not stated)	7 (good)
Walker 2019 [47]	See Table 3								

AF= allelic frequency;ALL = acute lymphoblastic leukaemia; IBD = inflammatory bowel disease;OR = odds ratio.
UVA = univariate analysis; MVA = multi-variate analysis; HR = hazard ratio; WCC = white cell count; TIM = thiopurine-induced myelosuppression; NI= not indicated.

as being at low risk of bias (Supplementary Appendix 1), although a full trial protocol was not published in advance of study commencement.

Although the remaining two studies reported a significant reduction in TIM, concurring with recent meta-analyses of broader pharmacogenomic testing in inflammatory bowel disease [52], results should be interpreted with caution. The randomized trial conducted by Chang *et al.* (n = 164) [25] reported a statistically significant reduction in TIM (HR 0.37, 95% CI 0.18–0.77), with combined genotyping for *NUDT15*3*, *TPMT* and *FTO* variants. Thiopurine dose escalation/adjustment was left to clinician discretion, which may have introduced bias into this unblinded trial, although this is perhaps more reflective of clinical practice. Results could not be separated by individual genes: Although subset analysis showed that *FTO* variants were not significantly associated with TIM in this study, findings from previous research into the influence of *FTO* variants on thiopurine toxicity are equivocal [43, 53]. A recent meta-analysis has considered data generated by Chang *et al.* to solely represent *NUDT15* genotyping [52], due to only a single *TPMT* variant carrier and lack of *FTO* association with TIM. However, it is unclear if the trial undertaken by Chang *et al.* [25] was adequately powered to assess the impact of solely *NUDT15* genotyping. Assessment via the Rob-2 tool raised 'some concerns' of bias, regarding data analysis in accordance with a pre-specified plan. The pre-specified outcome in the US clinical trials registry states simply 'myelosuppression', with no details of which haematological parameters and thresholds were to be used. A full trial protocol, published in advance of study commencement, was not found.

Wang *et al.* [27] reported a significant reduction in TIM with *NUDT15*3* genotyping vs a historical control. Markedly lower rates of TIM were reported for intervention and control groups. The use of alternative therapy for variant heterozygotes, rather than thiopurine dose adjustment, is likely to have substantially reduced the rate of TIM in the intervention group. However, the TIM rate (7.6%) in historical controls was much lower than that reported in both trials (32.4–35.95%) [25, 26] and remains unexplained. The use of a historical control and non-randomized setting provided lower quality results: The cohort study was assigned a quality score of 5/9 on the Newcastle Ottawa scale, translating into 'poor' quality against AHRQ standards.

Objective 2

A significant association with TIM was demonstrated for all cohort studies considering solely *NUDT15* variant heterozygotes [28–32] and for all *NUDT15*3* heterozygote patient subsets in case-control and cohort studies. Conflicting data for rarer *NUDT15* variant patient subsets is likely to have been influenced by very low patient numbers [35, 36, 38, 43, 44, 46]. Disparities for rarer variants, between unadjusted risk difference for TIM events (Fig. 2) and *P* values reported in studies, may be due to the use of parametric vs non-parametric statistical methods and unadjusted vs adjusted analysis. Although Walker et al [47] found no significant association for *NUDT15*3* alone, via GWAS or EWAS analysis, this study focused on people of European ancestry, rather than Asian ancestry. The prevalence of *NUDT15*3* in those with European ancestry is extremely low and only 8 patients with *NUDT15*3* variants were found in the EWAS analysis [47].

Direct comparison of studies was hindered by differing TIM parameters/thresholds, choice of outcome measures, and paucity of outcomes specific to variant heterozygotes. Extraction of data from heterozygote patient subsets was a suboptimal approach. Where OR specific to *NUDT15* variant heterozygotes were reported, the 95% CI was very wide, indicating considerable uncertainty regarding the outcome and the retrospective nature of most studies may affect data quality. Other potential confounding factors were variably reported (Supplementary Appendix 1). Notably, the TIM group in 3 studies received a higher mean thiopurine dose. Although this was significantly associated with TIM in multi-variate analysis (MVA), the independent association of *NUDT15* variants, was also demonstrated via MVA in all three studies. The large variance in leucopenia rates may be impacted by *TPMT* genotype [7], although this is less prevalent in Asian populations, corticosteroids which may cause leucocytosis [54] and allopurinol, which interacts to increase thiopurine levels [55] (Supplementary Appendix 1). Some analyses also reported the age and sex of participants to be of significance [28, 30, 36, 43]. Where studies considered the combined effect of several *NUDT15* variants, the inclusion of indeterminate variants, *NUDT15*4-*8* [7], may have diluted outcomes. The inclusion of GWAS/EWAS data may have increased the risk of spurious associations in the absence of further functional studies [56] and analysis of association data for *NUDT15*6* was impeded by strong linkage disequilibrium with *NUDT15*3* (considered together as *NUDT15*2*).

As mean time to onset of TIM has been variably reported [4, 5, 57] it was challenging to define 'adequate' study follow up and this varied considerably amongst studies. A 12 week follow-up threshold was utilized in the assessment of study quality, but may have introduced bias. As the Newcastle-Ottawa scale allows three parameters to be defined by assessors, a degree of subjectivity is unavoidable and variation in scoring is apparent both between previously published systematic reviews and also with this analysis [14, 52].

No published reviews or meta-analyses to date specifically consider variant heterozygotes. Our findings indicate that there is a significant association with TIM in non-European *NUDT15*3* heterozygotes. The rate of leucopenia in *NUDT15*3* heterozygotes varied substantially (30%–73% [42, 43]), reflective of variability reported in clinical practice [10, 11] and may indicate the influence of other contributory factors. Data were conflicting for rarer *NUDT15* variants, likely influenced by extremely low patient numbers. This is not in accordance with some meta-analyses of combined heterozygote/homozygotes data, which report significant associations with TIM for rarer variants [13, 14], but concurs in part with allele function ratings of 'uncertain' for *NUDT15*4-*8* assigned by the Clinical Pharmacogenetics Implementation Consortium guidelines [7] and findings from a previous systematic review [15].

Objective 3

Despite a very low prevalence of *NUDT15* variants in the European cohorts studied, significant associations with TIM were reported in at least one aspect of four of the five studies included. Substantial uncertainty in findings was highlighted by conflicting results, very wide confidence intervals and it was not always possible to separate results for patients with concurrent *TPMT* variants. Although Schaeffeler *et al.* [48] reported statistically significant differences in variant allelic

frequency between TIM cases and gnomAD population data, the study was assigned a low study quality score (3/9, mainly due to selection methods and comparability of case and controls, plus differing methods of ascertainment of outcome between groups). Furthermore, gnomAD data cannot be considered a true control, as patients were not thiopurine-exposed and therefore could not be declared as being unaffected by TIM. Wahlund *et al.* [49] found no association with neutropenia in paediatric ALL patients. In a population where neutropenic episodes are prevalent due to concurrent chemotherapy, this is unsurprising and maximum tolerated thiopurine dose may be a better outcome in ALL cohorts, as thiopurine dosing is also more regularly adjusted to WCC than in IBD. The complex findings from Walker et al [47] indicate the requirement for further research in this area in large patient populations.

Objective 4

Both health economic analyses conclude that combined *TPMT/NUDT15* genotyping was cost-effective in Asian IBD populations in comparison to *TPMT* genotyping [50] and no genotyping, *TPMT* or *NUDT15* genotyping, respectively [51]. Zarca *et al.* [50] also notably found *NUDT15* genotyping not to be cost-effective in Caucasian patients. However significant presumptions are made regarding the incidence and severity/treatment of myelosuppression [50], unsupported by data in some instances. The assumption that no heterozygotes experience severe TIM, and all homozygotes require hospitalization with TIM may underestimate and overestimate the effectiveness of *NUDT15* genotyping respectively. Given the cost of myelotoxicity treatment is a highly significant factor in sensitivity analysis [50, 51], this may undermine data quality. The use of real-life data from a single centre by Zeng *et al.* [51], rather than regional or national data, may introduce bias and limit wider applicability. Large differences in costings for myelosuppression between studies also indicate the variability of data or perhaps emphasise the perils of extrapolating health economic data from other healthcare systems.

Areas for further work

A meta-analysis could potentially provide a more robust analysis of data, but this would only consider more limited data, as several studies will be excluded due to the use of heterogenous outcome measures. Analysis of data for early leucopenia, occurring before 8 weeks, may provide more conclusive results as demonstrated by Yang *et al.* [46] and Kakuta *et al.* [38]. As large proportions of TIM cases remain unexplained by either *NUDT15* or *TPMT* genotype, analysis of further factors such as age, sex, rarer genetic variants in *FTO* and *ITPA* and the stratification of patients via biomarkers such as 6-thioguanine nucleotides may provide further insights. Inclusion of grey literature may also reduce the risk of positive publication bias.

Conclusion

This review provides a new collation of data on the association of *NUDT15* variants with TIM in variant heterozygotes and European ancestry patients.

An association with TIM was found for non-European *NUDT15*3* heterozygotes, but association data for rarer *NUDT15* variant heterozygotes was conflicting. Limited data suggests an association of *NUDT15* variants with TIM in Europeans, with four of five studies reporting a significant association with TIM for at least one or a combination of *NUDT15* variants. Analysis was impeded by heterogenous study design and was based upon predominantly retrospective data. Wide 95% CI reported for outcome measures are likely to be a result of low patient numbers and for analysis in European ancestry patients, a low prevalence of *NUDT15* variants.

Two randomized trials plus a cohort study, report statistically significant reductions in TIM with the use of genotype-guided thiopurine dosing in IBD and autoimmune indications. Larger, randomized, controlled, double-blinded trials, in populations of diverse ancestries, are required to further establish the clinical utility of *NUDT15* genotyping, building on the established association data in Asian populations [9, 11], but these may be practically challenging to conduct Although two health economic analysis reported *TPMT/NUDT15* genotyping to be cost effective in Asian IBD populations, the clinical utility of genotyping in diverse populations should be further evaluated, to inform future economic analyses.

Schaefeler *et al.* [48] demonstrated that after accounting for *TPMT* and *NUDT15* polymorphism and major drug interactions, around 50% of cases of TIM in Europeans remain unexplained. This highlights the need to investigate the role of rarer variants and emphasises that pharmacogenomics is not a panacea, but, must be considered in parallel with clinical parameters such as organ function, age and other predisposing factors for myelotoxicity.

Supplementary Material

Supplementary data are available at *RPS Pharmacy and Pharmacology Reports* online.

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Author contributions

R.P. led in formulating the research question, designing the study, carrying out the literature search, analysing the data and writing the article. As an educational supervisor, J.P. supervised the aforementioned activities, undertook an independent review of all studies screened by full text, a proportion of articles screened via title and abstract and independently reviewed aspects of the critical appraisal. JP also contributed significantly to writing the article.

Conflict of interest

RP has received an honorarium from Hartley Taylor Medical Communications. JP has no conflicts of interest to declare.

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Data availability

All data utilized in the review is available via published articles. The template data collection form is available on request.

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