The Hospital for Sick Children

Technology Assessment at Sick Kids (TASK)

TECHNICAL REPORT

THIOPURINE DOSING USING THIOPURINE METHYLTRANSFERASE STATUS: A SYSTEMATIC REVIEW OF CLINICAL GUIDANCE

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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ABBREVIATIONS

6-MP	6-mercaptopurine
6-TG	6-thioguanine
AAD	American Academy of Dermatology
AASLD	American Association for the Study of Liver Diseases
AGA	American Gastroenterological Association
AGREE-II	Appraisal of Guidelines for Research and Evaluation II
AHRQ	Agency for Healthcare Research and Quality
AIH	autoimmune hepatitis
ALL	acute lymphoblastic leukemia
APAG	Asian Pacific Association of Gastroenterology
ASD	American Society of Dermatologists
AZA	azathioprine
BAD	British Association of Dermatologists
BHPR	British Health Professionals in Rheumatology
BSG	British Society of Gastroenterology
BSPAR	The British Society for Paediatric and Adolescent Rheumatology
BSPGHN	British Society of Paediatric Gastroenterology Hepatology and Nutrition
BSR	British Society for Rheumatology
CCHMC	Cincinnati Children's Hospital Medical Center
COG	Children’s Oncology Group
CPG	Clinical practice guideline
CPIC	Clinical Pharmacogenetics Implementation Consortium
ECCO	European Crohn's and Colitis Organization
EMA	European Medicines Agency
ESPGHAN	The European Society for Paediatric Gastroenterology, Hepatology and Nutrition
FDA	Food and Drug Administration
IBD	Inflammatory bowel disease

NACB	National Academy for Clinical Biochemistry (NACB)
NCCN	National Comprehensive Cancer Network
NICE	National Institute for Excellence
PMDA	Pharmaceutical Medicines and Devices Agency
TPMT	Thiopurine *S*-methyltransferase
WGO	World Gastroenterology Organization

EXECUTIVE SUMMARY

Introduction

Advances in the understanding of the relationship between genetics and drug metabolism in the field of pharmacogenetics have allowed for drug treatments to become increasingly tailored to individual patients. A considerable number of medications now include information about the contribution of genetic variation in modulating drug metabolism and/or response in product monographs. A common application of personalized medicine is testing for thiopurine *S*-methyltransferase (TPMT) status prior to treatment with thiopurine drugs, which are used to treat a number of auto-immune conditions and paediatric cancer.

Clinical guidance on the use of pharmacogenetics is required to assist healthcare professionals with decisions regarding which test to order and how test results should be interpreted in order to improve patient care. Systematic reviews of available evidence can be used to identify gaps in the literature which in turn can help inform judgments about the value of a test, as well as set research agendas. The objective of this study was to conduct a systematic review of clinical guidance documents that recommend TPMT testing prior to the administration of thiopurine drugs. The specific aims were to 1) review the breadth of guidance documents and their sources, and 2) critically appraise the quality of the guidance documents by evaluating the quality of evidence used to support the preferential use of one method (genotyping versus phenotyping) over another and used to guide dose adjustments based on TPMT status.

Methods

Guidance documents including guidelines, clinical protocols and care pathways from all medical and laboratory disciplines were eligible if they included a recommendation statement to test for TPMT status. Databases including MEDLINE, EMBASE and CINAHL along with government agency websites and online repositories of clinical guidelines were searched for eligible articles. Data extracted from eligible documents included document characteristics, recommendation statements for TPMT testing, and dosing recommendations based on TPMT status (genotype or phenotypes). Guidance documents were compared within common therapeutic areas. A quality appraisal was carried out by three independent appraisers using the AGREE-II instrument. Scores for each document were recorded for quality domains related to Scope and purpose, Stakeholder involvement, Rigor of development, Clarity of presentation, Applicability and Editorial independence. Guidance documents were ranked according to quality.

Results

A total of 20 guidance documents were included, spanning a wide range of topics including the treatment of inflammatory bowel disease (IBD) (including Crohn's disease and ulcerative colitis) (n=8), inflammatory skin disorders (n=3), autoimmune hepatitis (n=3), rheumatic disease (n=2), ALL (n=2) and general pharmacogenetic testing (n=2). Six of the included guidance documents were focused on the treatment of paediatric patients with thiopurine drugs. Results from the quality appraisal showed great variation in the quality of the included guidance documents across all AGREE domains. Five of the included guidance documents made recommendations for genotype testing and four made recommendations for phenotype testing. The remaining guidance documents included general statements about the need for TPMT status determination, without specifying the test method (genotype or phenotype). A total of 13 guidance documents included dosing recommendations based on TPMT status, with the most common recommendation being to avoid treatment in patients with extremely low or absent TPMT activity (homozygous mutant) and to reduce thiopurine doses in patients with intermediate TPMT activity (heterozygous). Five of the included guidance documents recommended adjustments of a typical dose for each TPMT genotype or phenotype. Guidance documents that included dosing recommendations were of the highest quality in terms of total AGREE-II score and the rigor of development domain.

Conclusions

Clinical guidance on the use of pharmacogenetics is required to assist healthcare professionals with decisions regarding which test to order and how test results should be interpreted in order to improve patient care. Variations in recommendations for TPMT testing reflect the need for clarity in the clinical validity and utility of various TMPT test methods. The variability amongst these guidance documents also illustrates a lack of consistency and rigor in the methods used to develop recommendation statements. The development of high quality guidance for pharmacogenetic testing requires interdisciplinary collaboration between experts in the fields of genetics, pharmacology and the clinical disciplines responsible for administering the test-treatment combinations and careful adherence to methods for evidence-based guideline development. Systematic reviews of available evidence can be used to identify gaps in the literature which in turn can help inform judgments about the value of a test, as well as set research agendas.

1 INTRODUCTION

One of the goals of personalized medicine is to avoid life-threatening adverse events by modulating drug dosages based on the genetic profile of individual patients.[1] This is often accomplished through the *apriori* use of enzymatic assays or genetic tests which can be used to identify deficiencies in drug metabolism and subsequently, drug response.[2] Advances in the field of pharmacogenetics have made it increasingly possible for physicians to order genetic tests prior to prescribing treatments for their patients. International regulatory bodies including the United States (US) Food and Drug Administration (FDA),[3] the European Medicines Agency (EMA),[4] Japan's Pharmaceutical Medicines and Devices Agency (PMDA),[5] and Health Canada[6] have approved statements about genetic biomarkers related to drug metabolism and/or response in drug labels. To date, 122 FDA drug labels contain pharmacogenetic information on 38 unique genetic variants. Moreover, the EMA requires mandatory genetic testing for 12 medications.[7]

Translation of pharmacogenetics into clinical practice has been described as "slow" or "lagging"[8, 9] and as such, evidence regarding the clinical utility of pharmacogenetic interventions in clinical medicine is scarce.[1, 10] For physicians, the adoption of pharmacogenetic testing is impeded by a lack of education and/or awareness, uncertainty surrounding which tests to order, and skepticism that test results will translate into improved clinical outcomes.[8, 11] Clinical guidance is needed to assist physicians in the appropriate use of genetic testing to guide drug therapies.[7, 12] This requires the development of rigorous evidence-based statements, protocols, or care maps that are based on systematic reviews of evidence, assessments of clinical utility, and genotype-specific treatment recommendations.[7]

Several specialized groups have been mandated the tasks of creating pharmacogenetic clinical practice guidelines (CPGs)[13-15] but progress has been relatively slow as a result of the complexity and interdisciplinary requirements of developing high quality guidance as well as the lack of strong evidence to support the clinical utility of tests in medical practice. Clinical practice guidelines in the field of pharmacogenetics need to account for non-genetic differences between patients and how clinical factors such as age and disease may modulate drug outcomes. Specific guidelines are especially needed in the field of paediatrics given the profound developmental changes which occur throughout childhood and adolescence. These changes

are known to affect the pharmacokinetics and pharmacodynamics of a wide range of medications and may therefore render children more susceptible to drug toxicity as compared to adults in some cases. The susceptibility of children to adverse drug reactions, in combination with the fact that drug formulations are often designed for adults, adds to the challenge of achieving an optimal dose, particularly when the treatment has a narrow therapeutic index. Very few studies aimed at evaluating the clinical utility of pharmacogenetic tests are carried out in children and as a result, paediatric-specific data is often not available to guide clinical decisions.

A common application of personalized medicine in paediatrics is testing for deficiency in thiopurine *S*-methyltransferase (TPMT), the enzyme that metabolizes thiopurines.[16] Thiopurines consist of a class of immunosuppressive and chemotherapeutic drugs that are widely used to treat chronic inflammatory conditions including inflammatory bowel disease (IBD), autoimmune hepatitis (AIH), idiopathic arthritis, and a number of dermatologic conditions. Thiopurines are also used as a maintenance therapy in acute lymphoblastic leukemia (ALL) and to prevent post-transplant organ transplant rejection.[17, 18] Thiopurine based-drugs currently used in clinical practice include azathioprine (AZA), 6-mercaptopurine (6-MP) and 6-thioguanine (6-TG).

Approximately 89% of Caucasians have 'normal' (i.e. fully functional) TPMT activity, 11% have genetic variants that result in reduced activity, and 0.3% have genetic variants resulting in undetectable enzyme activity.[19, 20] Patients with reduced or undetectable TPMT activity treated with standard doses of thiopurines are at risk of serious life-threatening adverse events including myelosuppression, anemia, bleeding, leukopenia, and severe infection.[21] These adverse drug events can result in lengthy hospital admissions and substantial morbidity and reduced quality of life for patients already coping with a serious illness.[22, 23] It is therefore important to identify the presence of TPMT deficiencies in patients prescribed thiopurine drugs.

In the absence of TPMT testing, patients begin treatment with standard doses of thiopurines and are monitored for neutropenia by means of white blood cell counts. In these patients, up- or down-titration is often required to achieve an optimal therapeutic dose, but with delayed benefit for patients with fully functional TPMT activity, and risk of toxicity for patients with reduced or deficient TPMT activity.[24] When TPMT status is known, patients achieve an optimal therapeutic dose faster and avoid the risk of toxicity.[25] There are two approaches to testing for TPMT status. The most common is a phenotype test that measures the level of TPMT enzyme activity. Unfortunately, results of the enzymatic assay can be confounded by concomitant medications or

blood transfusions.[26-33] Genotype tests that detect the presence of variant genes responsible for expressing the TPMT enzyme are more versatile, but most commercially available tests capture only a proportion of known genetic variants[34, 35] It remains uncertain whether the enzymatic assay (phenotype) or genotype test is the most appropriate strategy for clinical practice. Nonetheless, TPMT testing is an application of personalized medicine that been cited as having significant clinical uptake.[36]

1.1 Objective

The objective of this study was to conduct a systematic review of clinical guidance documents that recommend TPMT testing prior to the administration of thiopurine drugs. The specific aims were to 1) review the breadth of guidance documents and their sources, and 2) critically appraise the quality of the guidance documents by evaluating the quality of evidence used to support the preferential use of one method (genotyping versus phenotyping) over another and used to guide dose adjustments based on TPMT status.

2 METHODS

2.1 Literature search

The electronic databases, Medline, Embase, and the Cumulative Index to Nursing and Allied Health Literature (CINAHL) were searched between 1980 and September 2012 using search strategies provided in Appendix 1. Medical subject headings (MeSH) included 'Practice Guideline', 'Guideline', 'Clinical Protocols', 'Critical Pathways', 'Decision Support Systems, Clinical', '6-mercaptopurine', 'azathioprine'', and 'thioguanine.' Keywords included but were not limited to 'TPMT', 'thiopurine methyltransferase', 'recommendation', 'clinical consensus', and 'consensus statement'. Grey literature searches included the National Guideline Clearinghouse, Guidelines International Network, and a number of other international guideline databases, national guideline agency websites, government agency websites, and medical organization websites (see Appendix 2). Reference lists of identified articles were also hand-searched for eligible guidelines.

2.2 Selection of guidance documents

2.2.1 Inclusion criteria

Inclusion of guidance documents was based on the following predefined eligibility criteria:

Type of documents

Clinical practice guidelines, clinical protocols, and care pathways were eligible if they included recommendation(s) for TPMT testing. Guidelines for any clinical condition, specialty, or discipline, as well as those spanning multiple conditions, specialties, or disciplines were eligible.

Target population

Guidance documents that included recommendations for the treatment of human subjects of any age (adults, children, mixed populations) with a thiopurine drug were eligible.

Type of tests

Documents that included recommendations for TPMT testing regardless of the testing strategy (genotype or phenotype test), and regardless of the laboratory assay or test method were eligible.

Recommendation focus

Guidance documents that made a statement or statements regarding testing for TPMT status were eligible. Recommendations of interest focused on the method of testing and any dose modifications as a result of TPMT status.

2.2.2 Exclusion criteria

Guidance documents that discussed TPMT activity but failed to make a recommendation for or against testing based on the information provided were excluded. Laboratory protocols and non-English articles were also excluded.

2.2.3 Article review

Results from the literature search were exported into a single Endnote library and duplicate documents were removed. Titles and abstracts were screened by a single reviewer (HB) to determine eligibility. The same reviewer then examined the full text of all remaining studies, applying the inclusion and exclusion criteria.

2.3 Data extraction

A data extraction spreadsheet was used to systematically collect relevant data from each guidance document. Data included basic characteristics such as publication year, authors,

target audience, target population or condition, organization or group the guideline was produced for or endorsed by, and whether or not systematic methods were used to produce the guideline. Details of TPMT testing recommendations were also extracted and included recommendation statements for test type and dosing, evidence grades or quality assigned to TPMT recommendations, and the sections of the guideline where TPMT recommendations were found.

TPMT recommendations were categorized based on whether or not they provided recommendations for genotype testing or phenotype testing. Recommendations that included vague statements to “test”, “measure”, “check” or “assess” TPMT were categorized as those without specification of a test type. Guidelines that recommended genotype or phenotype testing were also categorized as those without specification of a test type. Recommendations that explicitly referred to genotyping or gene polymorphisms, including those that referred to testing only a specific patient population (e.g. patients that had undergone a recent blood transfusion) or genotyping prior to another adjunct test were categorized as genotyping recommendations. Recommendations that referred to TPMT levels, and/or thiopurine metabolites were categorized as phenotype testing recommendations.

2.4 Quality appraisal

The quality of all included guidance documents was assessed by three independent appraisers (HB, WC, RT) using the Appraisal of Guidelines for Research and Evaluation II (AGREE-II) Instrument.[37] AGREE-II was used to assess the methodological rigor and transparency of each included document across six independent domains (23 items): scope and purpose (3 items), stakeholder involvement (3 items), rigor of development (8 items), clarity of presentation (3 items), applicability (4 items), and editorial independence (2 items) (see Appendix 3). Websites of guideline developers were examined for additional information when necessary. Independent scoring of each item was carried out using a 7-point scale (anchored at 1-strongly disagree and 7-strongly agree). Higher rated items result in higher domain scores. Quality scores were entered into a scoring spreadsheet which was used to assess agreement across independent appraisals.[1] Scores assigned by each appraiser for individual guidance documents were required to be within 2 points of agreement. When disagreement occurred, face-to-face discussions were carried out until consensus within 2 points was reached. Domains scores

were calculated by summing the scores for each item and each reviewer within a domain and scaling the total as a percentage of the maximum possible score for that domain (assuming all 7's). This allowed standardization of domain scores from 0 (lowest score) to 100 (best score). Domains scores were used to rank the quality of each guideline for each domain. An overall score for each guideline was determined from the mean of the domain scores. Standard deviations were calculated for each domain in order to quantify the variance between the three appraisers.

Guidance document characteristics and quality scores were grouped according to the following clinical categories and results were reported by category throughout the report: inflammatory bowel disease, inflammatory skin disease, autoimmune hepatitis, rheumatic diseases, acute lymphoblastic leukemia, and general pharmacogenetic testing.

3 RESULTS

A total of 370 guidance documents were identified and reviewed for eligibility, 158 of which were excluded because they were not guidance documents, and 104 and 88 because they did not include a TPMT recommendation statement or were written in a language other than English. A total of 20 guidance documents were included, spanning a wide range of patient populations: IBD (including Crohn's disease and ulcerative colitis) (n=8), inflammatory skin disorders (n=3), autoimmune hepatitis (n=3), rheumatic disease (n=2), ALL (n=2) and general pharmacogenetic testing (n=2). Six of the included guidance documents were focused on the treatment of paediatric patients with thiopurine drugs. Table 1 provides an overview of the characteristics of all included clinical guidance documents.

3.1 Quality of recommendations

Results from the quality appraisal showed great variation in the quality of the included guidance documents across all AGREE domains (see Table 2). The mean total score for all documents was 47.14 (SD =18.94), with scores ranging from 10.42 to 78.59. The three highest quality documents were the IBD guideline produced by that National Institute for Health and Clinical Excellence (NICE),[38] the paediatric IBD guideline produced by Cincinnati Children's Hospital (CCHMC)[39] and the rheumatology guideline produced by the British Health Professionals in

Rheumatology (BHPR).[40] Overall, the included guidance documents scored the highest in terms of objective and scope (domain 1) and lowest in terms of applicability (domain 5).

For objective and scope (domain 1) the highest quality guidance documents were the IBD guidelines produced by NICE[38] and Cincinnati Children's[39] and the rheumatology guideline produced by the BHPR.[40] (see Figure 1). The mean score across documents for domain 1 was 57.8 (SD 22.2). This domain was the highest scoring domain.

For stakeholder involvement (domain 2) the highest quality guidance documents were also the IBD guidelines produced by NICE[38] and Cincinnati Children's[39] as well as the 2011 guidelines produced by the British Association of Dermatologists (BAD)[41] (see Figure 2). The mean score for domain 2 was 42.9 (SD 22.8), representing the second lowest scoring domain. Low scores were a result of very few documents providing sufficient information on members of the guideline development group and the fact that the views and preferences of patients were not sought in the development process.

Table 1: Characteristics of guidelines that include recommendations for TPMT testing

Identifier, year	Organization	Guidance type	Focus	Target audience	Target condition/field
Inflammatory bowel disease					
ECCO[42]	European Crohn's and Colitis Organization*	CPG	Uni-disciplinary	Gastroenterologists (paediatric)	Paediatric ulcerative colitis
NICE, 2012[38]	National Institute for Health and Clinical Excellence	CPG	Uni-disciplinary	Gastroenterologists	Crohn's disease
APAG, 2010[43]	Asian Pacific Association of Gastroenterology	Consensus statement	Uni-disciplinary	Gastroenterologists	Inflammatory bowel disease
BSG, 2010[44]	British Society of Gastroenterology	CPG	Uni-disciplinary	Gastroenterologists	Inflammatory bowel disease
WGO, 2010[45]	World Gastroenterology Organization	CPG	Uni-disciplinary	Gastroenterologists	Inflammatory bowel disease
BSPGHN, 2008[46]	British Society of Paediatric Gastroenterology Hepatology and Nutrition	CPG	Uni-disciplinary	Gastroenterologists (paediatric)	Paediatric inflammatory bowel disease
CCHMC, 2007[39]	Cincinnati Children's Hospital Medical Center	CPG + algorithm	Uni-disciplinary	Gastroenterologists (paediatric)	Paediatric inflammatory bowel disease
AGA, 2006[47]	American Gastroenterological Association	Medical Position Statement	Uni-disciplinary	Gastroenterologists	Inflammatory bowel disease
Inflammatory skin disorders					
BAD, 2011[41]	British Association of Dermatologists	CPG	Uni-disciplinary	Dermatologists	Inflammatory dermatoses
BAD, 2004[48]	British Association of Dermatologists	CPG	Uni-disciplinary	Dermatologists	Inflammatory dermatoses
AAD, 2009[49]	American Academy of Dermatology	CPG	Uni-disciplinary	Dermatologists	Psoriasis

Autoimmune hepatitis					
BSG, 2011[50]	British Society of Gastroenterology	CPG	Uni-disciplinary	Gastroenterologists	Autoimmune hepatitis
AASLD, 2010[51]	American Association for the Study of Liver Diseases	CPG	Uni-disciplinary	Gastroenterologists	Autoimmune hepatitis
AASLD, 2003[52]	American Association for the Study of Liver Diseases	CPG	Uni-disciplinary	Gastroenterologists	Autoimmune hepatitis
Rheumatic diseases					
BSPAR, 2011[53]	The British Society for Paediatric and Adolescent Rheumatology	Medical Position Statement	Uni-disciplinary	Gastroenterologists	Paediatric rheumatology
BHPR, 2008[40]	British Health Professionals in Rheumatology	CPG	Multi-disciplinary	Healthcare professionals, health service managers, patients, national societies	Rheumatic and dermatological conditions
Acute lymphoblastic leukemia					
NCCN, 2012[54]	National Comprehensive Cancer Network	CPG	Uni-disciplinary	Oncologists (paediatric)	Acute lymphoblastic leukemia
COG, 2008[55]	Children's Oncology Group	Clinical Protocol	Uni-disciplinary	Oncologists (paediatric)	Acute lymphoblastic leukemia
General pharmacogenetic testing					
CPIC, 2011[56]	Clinical Pharmacogenetics Implementation Consortium	CPG	Multi-disciplinary	Clinicians	TPMT genotyping and dosing
NACB, 2010[57]	The National Academy of Clinical Biochemistry	CPG	Multi-disciplinary	Medical practitioners (physicians, nurses, pharmacists, clinical researchers)	Pharmacogenetic testing

Note: The ECCO guideline[42] was also endorsed by the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition. The British Health Professionals in Rheumatology guideline[40] was also endorsed by the British Society for Rheumatology

CPG = clinical practice guideline

Table 2: **Results of AGREE-II quality appraisal**

Identifier, year	D1 - Scope and Purpose		D2 - Stakeholder Involvement		D3 - Rigor of Development		D4 - Clarity of Presentation		D5 - Applicability		D6 - Editorial Independence		Overall Score	Overall Rank
	Score	Rank	Score	Rank	Score	Rank	Score	Rank	Score	Rank	Score	Rank		
Inflammatory bowel disease														
ECCO, 2012	75.9	5	35.2	11	50.7	9	63.0	8	16.7	12	56.0	9	49.6	8
NICE, 2012	94.4	1	79.6	2	86.8	1	64.8	7	70.8	2	75.0	6	78.6	1
APAG, 2010	53.7	10	55.6	7	43.8	11	53.7	11	11.1	14	55.6	10	45.6	11
BSG, 2010	68.5	7	68.5	3	46.5	10	37.0	16	20.8	7	38.9	13	46.7	10
WGO, 2010	35.2	17	11.1	18	5.6	20	48.1	14	18.1	11	0.0	19	19.7	19
BSPGHN, 2008	68.5	7	46.3	8	42.4	12	66.7	6	25.0	6	75.0	6	54.0	7
CCHMC, 2007	87.0	3	81.5	1	79.2	3	87.0	1	50.0	3	69.4	8	75.7	2
AGA, 2006	42.6	16	59.3	6	31.9	15	55.6	10	20.8	7	33.3	15	40.6	13
Inflammatory skin disorders														
BAD, 2011	53.7	10	68.5	3	83.3	2	85.2	2	34.7	4	80.6	5	67.7	4
BAD, 2004	31.5	18	24.1	15	61.8	4	59.3	9	30.6	5	88.9	4	49.3	9
AAD, 2009	31.5	18	20.4	16	56.9	6	53.7	11	9.7	16	97.2	1	44.9	12
Autoimmune hepatitis														
BSG, 2011	51.9	12	27.8	14	34.0	13	72.2	4	11.1	14	13.9	16	35.1	15
AASLD, 2010	48.1	13	38.9	9	32.6	14	50.0	13	8.3	17	38.9	13	36.1	14
AASLD, 2003	48.1	13	35.2	11	30.6	16	33.3	17	8.3	17	2.8	17	26.4	18
Rheumatic diseases														
BSPAR, 2011	9.3	20	9.3	19	9.7	19	31.5	18	0.0	19	2.8	17	10.4	20
BHPR, 2008	88.9	2	68.5	3	56.9	6	42.6	15	77.8	1	91.7	3	71.1	3
Acute lymphoblastic leukemia														
NCCN, 2012	48.1	13	29.6	13	19.4	18	16.7	20	19.4	10	47.2	11	30.1	17
COG, 2008	81.5	4	NA	NA	52.8	8	68.5	5	NA	NA	NA	NA	67.6	5
General pharmacogenetic testing														
CPIC, 2011	72.2	6	37.0	10	59.0	5	79.6	3	20.8	7	97.2	1	61.0	6
NACB, 2010	64.8	9	18.5	17	27.1	17	31.5	18	12.5	13	41.7	12	32.7	16
Mean (SD)	**57.8 (22.2)**		**42.9 (22.9)**		**45.6 (22.6)**		**55.0 (19.1)**		**24.6 (20.8)**		**52.9 (32.5)**		**47.1 (18.9)**	

Figure 1: AGREE-II results for domain 1 (objective and scope)

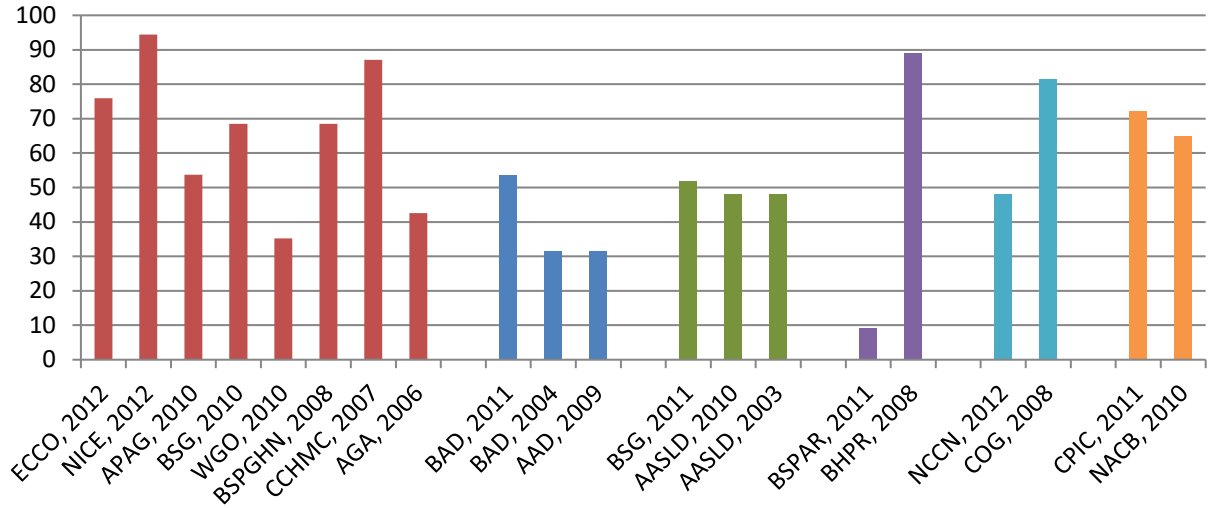
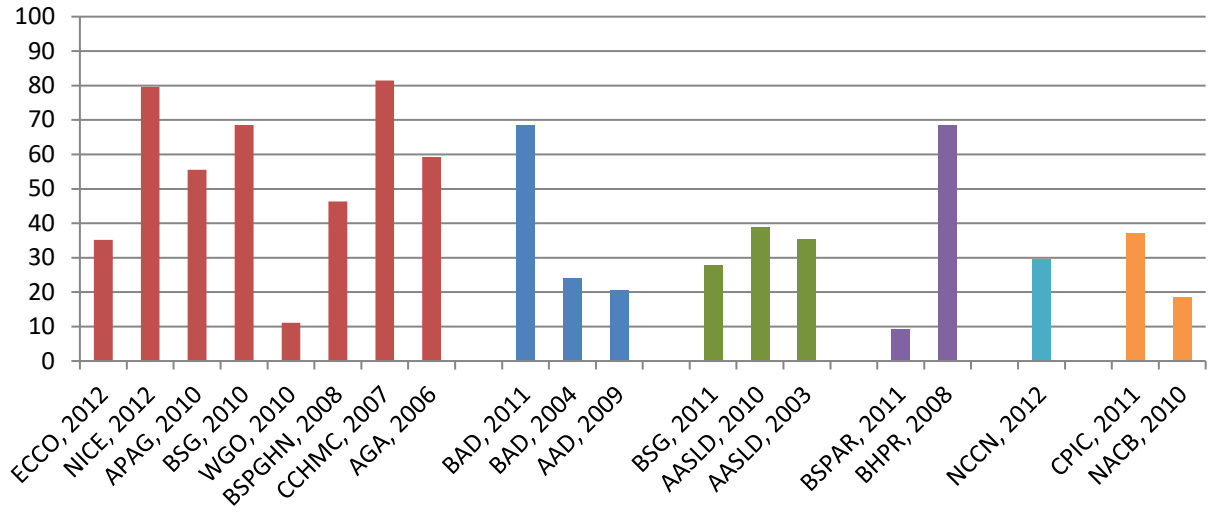


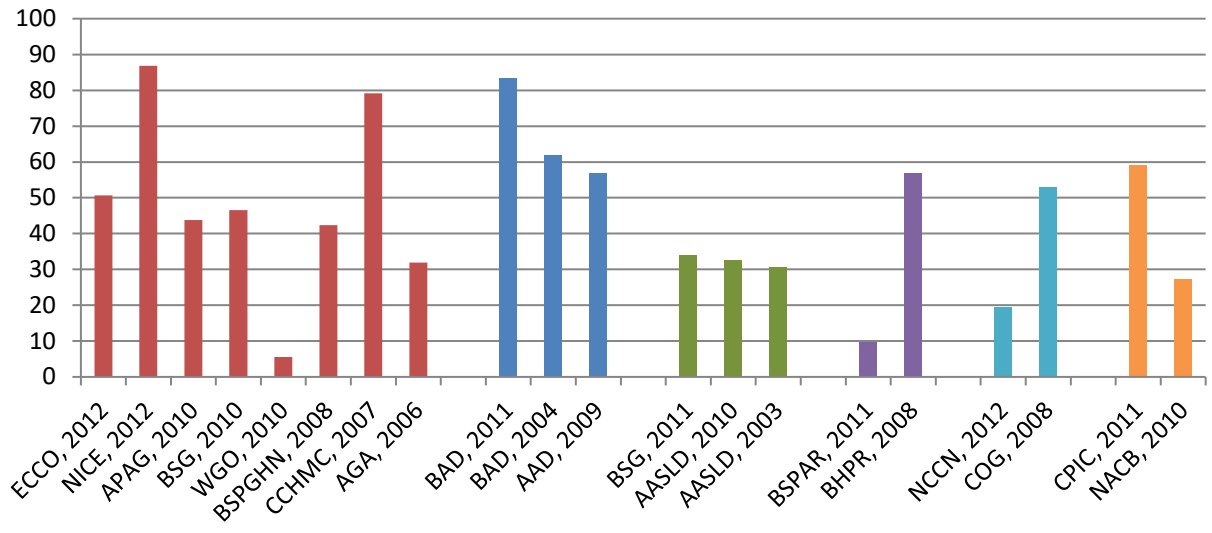
Figure 2: AGREE-II results for domain 2 (stakeholder involvement)



For rigor of development (domain 3) the highest quality guidance documents were again the IBD guidelines produced by NICE[38] and Cincinnati Children's[39] as well as the 2011 guidelines produced by the BAD[41] (see Figure 3). The mean score for domain 3 was 45.6 (SD 22.6). Assessments of items within this domain focused specifically on the quality of TPMT recommendations. In general, guidance documents assigned a low score failed to use appropriate systematic methods in their development of recommendation statements. In cases where systematic reviews were carried out, very few documents provided sufficient evidence to support recommendations or failed to link recommendations with supporting evidence. In some cases evidence used to support recommendations contradicted recommendation statements.

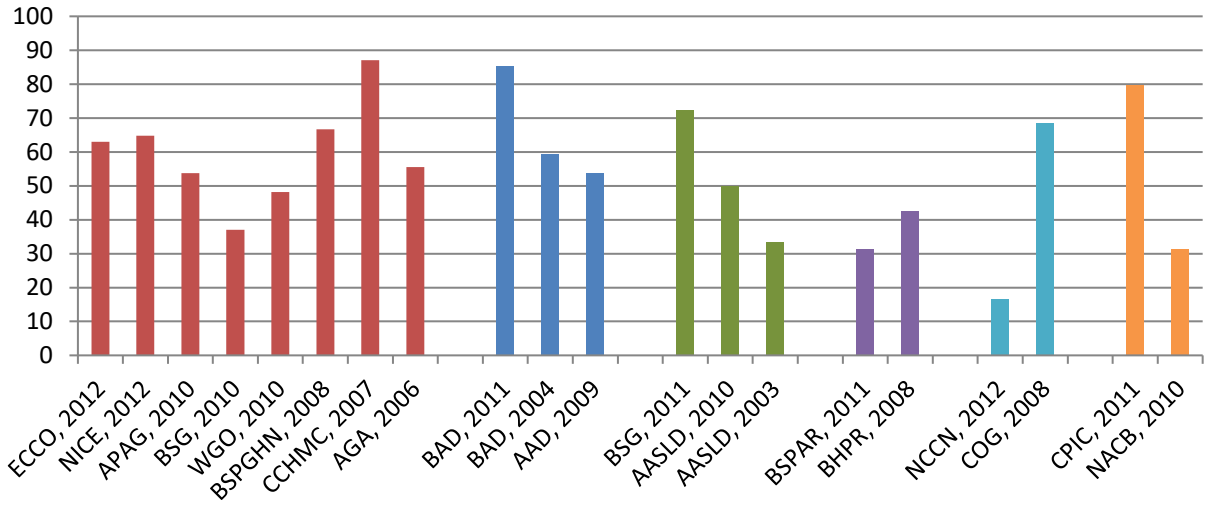
An example of this inconsistency was observed in the 2010 British Society of Gastroenterology guideline (BSG)[44], which referred to several studies illustrating that TPMT status is a poor predictor of myelosuppression and other adverse events in patients with IBD; therefore, the evidence to support TPMT testing prior to treatment with thiopurines was deemed "controversial". However, the authors then recommended "all patients be tested for TPMT levels before starting thiopurines, to avoid administration in patients with no functional TPMT in whom thiopurine administration may be fatal." Similarly the 2011 American Association for the Study of Liver Disease (AASLD) guidance document[51] stated that thiopurine toxicity was not well predicted by "genotyping or phenotyping for TPMT activity" and recommended TPMT testing as a "reasonable precaution" that should be considered in all patients, especially those with pretreatment cytopenia, those with cytopenia that developing during therapy, or those patients that require higher than conventional doses of AZA.

Figure 3: AGREE-II results for domain 3 (rigor of development)



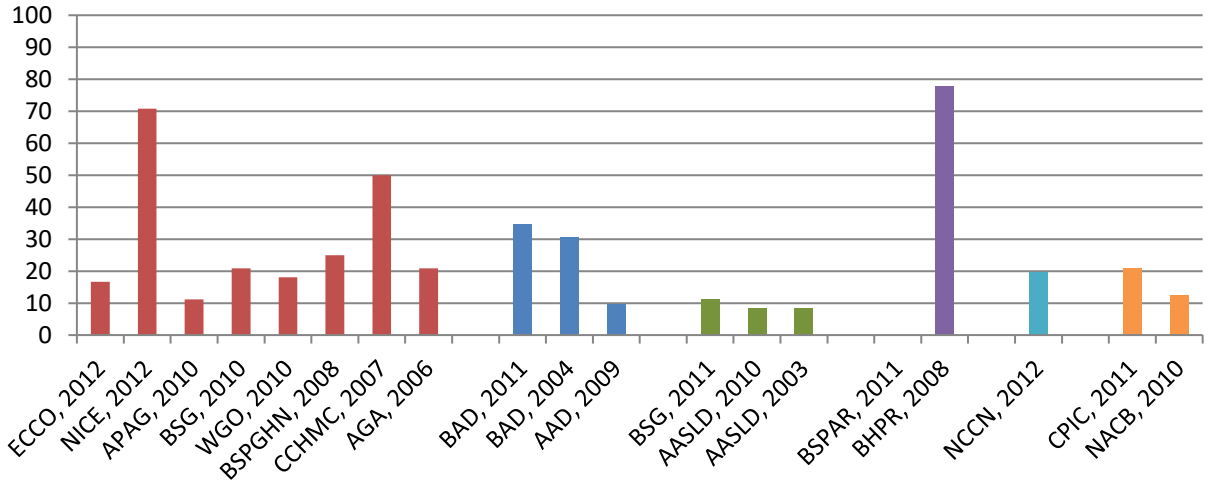
For clarity of presentation (domain 4) the highest quality guidance documents were produced by Cincinnati Children's,[39] the BAD[41] and the Clinical Pharmacogenetics Implementation Consortium (CPIC)[56] (see Figure 4). The mean score for domain 4 was 55.0 (SD 19.1), representing the second highest scoring domain.

Figure 4: AGREE-II results for domain 4 (clarity of presentation)



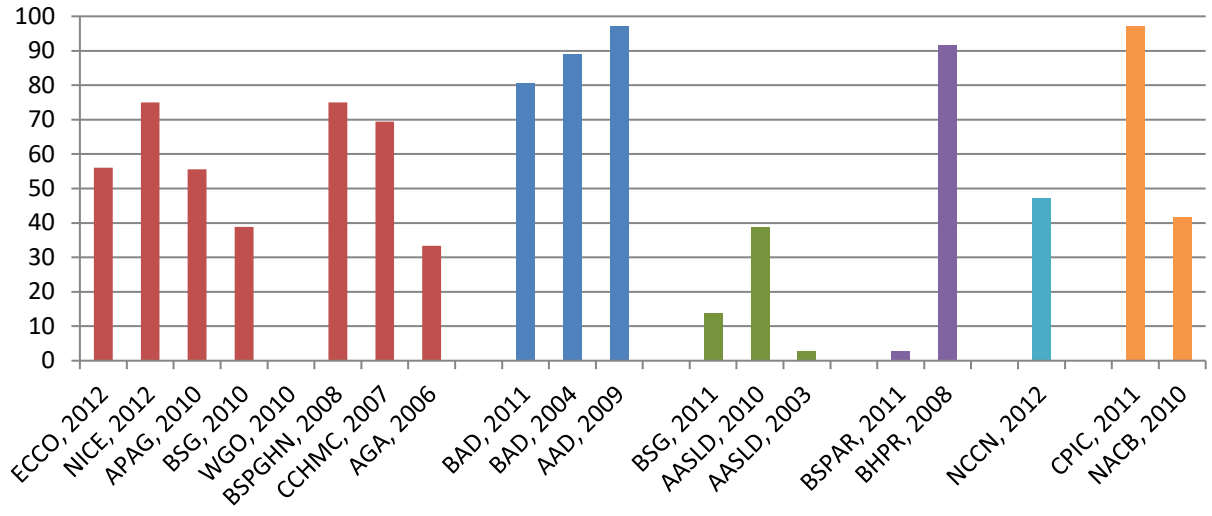
For applicability (domain 5) the highest quality guidance documents were the IBD guidelines produced by NICE[38] and Cincinnati Children's[39] as well as the joint rheumatology guideline produced by the BHPR.[40] (see Figure 4). The mean score for domain 4 was 24.6 (SD 20.8), representing the lowest scoring domain. Low scores were the result of very few guidelines describing how guidelines can be implemented and monitored.

Figure 5: AGREE-II results for domain 5 (applicability)



For editorial independence (domain 6) the highest quality guidance documents were produced by the American Association of Dermatologists (AAD)[49], the BHPR,[40] and the CPIC[56] (see Figure 6). The mean score within domain 6 was 53.0 (SD 32.5), representing the domain with the greatest degree of variation across included guidance documents.

Figure 6: AGREE-II results for domain 5 (editorial independence)



3.2 Genotype vs. phenotype testing

Five CPGs made explicit recommendations for genotype testing prior to the initiation of thiopurine therapy. [41,56, 59, 60,54, 57] The CPIC[49] and National Academy for Clinical Biochemistry (NACB)[57] documents were focused specifically on the use of genotyping technologies (see Table 3). None of the guidelines recommended a specific type of genetic assay. The CPIC guideline recommends phenotype testing in conjunction with genotype testing[56], the 2011 BAD guideline recommends genotyping for patients with intermediate phenotypes[41], and the Children's Oncology Group (COG) protocol recommends genotyping in patients with a history of blood transfusions.[55] The National Comprehensive Cancer Network (NCCN)[54] and NACB[57] CPGs scored low using AGREE in terms of rigor of development, despite the NACB reporting Grade A (good evidence that it improves health outcomes and the benefits substantially outweigh harms), Level I (consistent results from well-designed, well-conducted studies in representative populations) evidence.[57] The NACB recommendation was based on two review articles[61,62] and results from a single retrospective cohort study of 171 kidney transplant patients.[63] The cohort study included 12 patients heterozygous for TPMT status, 58% of whom required AZA dose reductions as a result of leukopenia (compared to 30% of wildtype patients).[63]

A statement in the introduction of the NACB guideline claims that in rapidly evolving fields such as pharmacogenetics, where evidence is uncertain, there is a need for robust recommendations regardless of whether or not rigorous evidence-based approaches can be applied.[57]

Three guidance documents recommend phenotype testing (see Table 4). The COG protocol recommends phenotype testing for patients in whom genotyping was not informative.[55] All phenotyping recommendations were moderate in terms of their score for rigor of development. While several guidelines recommended either genotype or phenotype testing, many failed to specify the type of test. Thirteen guidelines made general statements about the need for TPMT testing, with several recommending either genotyping or phenotyping[39, 42, 43, 45, 47] and others disregarding the test method making vague statements to “test”, “measure”, “check” or “assess” TPMT status (see Table 5).

Table 3: Guidelines recommending genotype testing in order to determine TPMT status

Identifier, year	Target condition/field	Recommendation for TPMT testing	Reported strength of recommendation	Rigor of development score (rank)
BAD, 2011	Inflammatory skin disorders	“TPMT genotyping is only required for patients with indeterminate phenotype (i.e. borderline values) or those who have had a recent blood transfusion”	Grade D,Level 4	83.3(2)
COG, 2008	Acute lymphoblastic leukaemia	“TPMT testing should be performed if myelosupression leads to delays in therapy. Genotyping may be preferable to phenotype testing in cases where a history of red cell transfusions would potentially confound assessments of TPMT activity.”	Not reported	52.8 (8)
NCCN, 2012	Acute lymphoblastic leukaemia	"For patients receiving 6-MP, consider testing for TPMT gene polymorphisms, particularly in patients that develop severe neutropenia after starting 6-MP"	Not reported	19.4 (18)
CPIC, 2011	General pharmacogenetictesting	"Genotype tests have a high likelihood of being informative. Complementary phenotype tests can be helpful adjuncts to genotyping tests"	Not reported	59.0 (5)
NACB, 2010	General pharmacogenetictesting	"TPMT genotyping is recommended as a useful adjunct to a regimen for prescribing azathioprine"	Grade A,Level I	27.1 (17)

Table 4: Guidelines recommending phenotype testing in order to determine TPMT status

Identifier, year	Target condition/ field	Recommendation for TPMT testing	Reported strength of recommendation	Rigor of development score (rank)
AAD, 2009	Inflammatory skin disorders	“TPMT levels are generally used to guide dosing”	Not reported	56.9 (7)
BSG, 2010	Inflammatory bowel disease	"All patients should have measurement of TPMT levels before starting thiopurines, mainly to avoid administration to a patient with no functional TPMT”	Grade B, Level 4,	34.0 (12)
APAG, 2010	Inflammatory bowel disease	"Where available, TPMT and thiopurine metabolite testing for 6-thioguanine and 6-methylmercaptopurine may assist dose optimization of AZA/6-MP"	Not reported	43.8 (10)
COG, 2008	Acute lymphoblastic leukaemia	“TPMT genotyping will be informative in all patients, if at least one mutant allele is identified. If not, and myelosuppression continues, send samples for TPMT activity and/or metabolites since TPMT genotyping will miss 5-10% of mutants.”	Not reported	52.8 (8)

Table 5: Guidelines recommending TPMT testing without specification of test type

Identifier, year	Target condition	Recommendation for TPMT testing	Reported strength of recommendation	Rigor of development score (rank)
BAD, 2011	Inflammatory skin disorders	“TPMT activity should be checked in all patients prior to receiving azathioprine” “TPMT testing only identifies a proportion of individuals at increased risk of haematological toxicity, hence the continued need for regular monitoring of blood counts irrespective of TPMT status”	Grade A, Level 1+ Grade B, Level 2++	83.3(2)
ECCO, 2012	Inflammatory bowel disease	“The determination of TPMT genotype or phenotype, if available, is encouraged to identify patients at greater risk for early profound myelosuppression”	Not reported	30.6 (16)
NICE, 2012	Inflammatory bowel disease	"Assess TPMT activity before offering AZA or 6-MP"	Not reported	86.8 (1)
WGO, 2010	Inflammatory bowel disease	"Before starting AZA or 6MP measuring TPMT by phenotype (enzyme levels) or genotype will help direct dosing"	Not reported	5.6 (20)
BSPGHN, 2008	Inflammatory bowel disease	"TPMT should be checked prior to initiating treatment and is probably best done at diagnosis"	Not reported	42.4 (11)
CCHMC, 2007	Inflammatory bowel disease	"It is recommended that TPMT genotype or phenotype be determined prior to initiation of 6-MP or AZA"	1 large prospective study, 1 retrospective study, expert opinion and consensus	79.2 (3)
AGA, 2006	Inflammatory bowel disease	"Individuals should have TPMT genotype or phenotype assessed before initiation of therapy with AZA or 6-MP"	Grade B	31.9 (14)
BAD, 2004	Inflammatory skin disorders	"Pre-treatment TPMT measurement should be performed in all patients prescribed AZA”	Not reported	61.8 (4)
BSG, 2011	Autoimmune hepatitis	"TPMT measurement should be considered to exclude homozygous TPMT deficiency and is recommended in patients with pre-existing leucopenia"	Grade B2, Level II-iii	34.0 (12)
AASLD, 2010	Autoimmune hepatitis	"Azathioprine therapy should not be started in patients with known complete deficiency of TPMT activity"	Class 3, Level C	32.6 (13)
AASLD, 2003	Autoimmune hepatitis	"Pre-treatment testing for TPMT is a reasonable precaution, and it should be considered in all patients, especially those with pretreatment cytopenia”	Not reported	30.6 (15)
BHPR, 2008	Rheumatic disease	“Perform TPMT assay prior to treatment with AZA”	Not reported	56.9 (6)
BSPAR, 2011	Paediatric rheumatic disease	“ Pre-treatment testing: TPMT activity”	Not reported	9.7 (19)

3.3 Dose adjustments

A total of 13 guidelines included dosing recommendations based on TPMT status. The majority of dosing recommendations were statements to avoid AZA or 6-MP in patients who are homozygous mutant or have extremely low or absent TPMT activity,[38, 40, 45-47, 50-52] and to reduce thiopurine doses in patients who are heterozygous or who have intermediate TPMT activity.[38, 40, 46] A total of 5 guidelines included dose adjustments based on TPMT status, including a recommended adjusted dose or a percentage of the normal dose for each of the TPMT genotypes or phenotypes.[39, 41, 48, 55, 56] The specific dosing recommendations are summarized below for azathioprine, 6-mercaptopurine and 6-thioguanine in Tables 6, 7 and 8, respectively.

Overall, consistency was observed in recommended AZA dosing for patients with full, intermediate or low TPMT activity. The only exception was the CPIC guideline which considered a 10-fold reduction with titration based on tolerance in patients with low or absent TPMT activity. All other guidelines recommend avoiding AZA in homozygous mutant patients. For 6-MP, slight variation was observed in dose adjustments, with COG[55] recommending 30-50% of a normal dose and CPIC[**56**] recommending 30-70% in patients with intermediate activity. For patients with low or absent TPMT activity COG[55] recommends 10-20mg/m^2 daily while CPIC[**56**] recommends a 10-fold reduction in non-cancer patients. The Cincinnati Children's[39] CPG recommends avoiding 6-MP in patients with paediatric IBD. Only CPIC provided dosing recommendations for 6-thioguanine based on TPMT status and advised a 10-fold dose reduction with dose adjustment based on tolerance and disease-specific guidelines.[**56**]

The CPIC[56] and 2011 BAD[41] guidelines acknowledged that alternative treatments should be administered in non-malignant patients with low TPMT activity, with the BAD guideline providing a list of alternative treatments to AZA. The CPIC guideline recommended dose reductions and did not recommend alternatives to 6-MP and thioguanine for patients with malignancy.

Table 6: Dosing recommendations for azathioprine based on TPMT status

Identifier, year	CCHMC, 2007[39]	BAD, 2011[41]	AAD, 2009[49]	BAD, 2004[48]	CPIC, 2011[56]
Target condition	Inflammatory bowel disease (paediatric)	Inflammatory skin disorder	Inflammatory skin disorder	Inflammatory skin disorder	None, general TPMT testing
Normal (functional) activity (wildtype)	2.5 mg/kg daily	Conventional dose (2-3 mg/kg daily)	TPMT < 19U: 2.5 mg/kg	1-3 mg/kg daily	Normal dose (2-3 mg/kg daily), adjust based on disease disease-specific guidelines, allow 2 weeks to reach steady state
Intermediate activity (heterozygous)	1.5 mg/kg daily and if labs are ok, advance over 4 weeks to 2.5 mg/kg daily	Lowered dose, 1-1.5 mg/kg daily*	TPMT 5-13.7U: 0.5 mg/kg (max) TPMT 13.7-19U: 1.5 mg/kg (max)	Do not prescribe or, if used, dose of 0.5-1 mg/kg daily with more frequent monitoring**	30-70% of target dose and titrate based on tolerance, allow 2-4 weeks to reach steady state
Low or absent activity (homozygous mutant)	Do not use AZA	Do not prescribe AZA	TPMT < 5U: Do not use AZA	Alternative therapies recommended	Consider an alternative therapy, or, if using, reduce dose by 10-fold and titrate based on tolerance and disease-specific guidelines, allow 4-6 weeks to reach steady state
Reported strength of recommendation	NR	Grade A, Level 2+	NR	Grade A, Level II-ii	Strong
Rigor of development score (rank)	79.2 (3)	83.3 (2)	56.9 (6)	61.8 (4)	59.0 (5)

IBD = inflammatory bowel disease; mg = milligram; kg = kilogram; AZA = azathioprine; NR = not reported; U = units; max = maximum

* Strength of evidence for heterozygous dosing is Grade C, Level 2+
** Strength of evidence for heterozygous dosing is Grade B, Level III

Table 7: Dosing recommendations for 6-mercaptopurine based on TPMT status

Identifier, year	CCHMC, 2007[39]	COG, 2008[55]	CPIC, 2011[56]
Target condition	IBD (paediatric)	ALL (paediatric)	None, general TPMT testing
Normal activity (wildtype)	1.5 mg/kg daily	Normal dose	Normal dose (1.5mg/kg daily) allow 2 weeks to reach steady state
Intermediate activity (heterozygous)	0.75-1 mg/kg daily and if labs are ok, advance over 4 weeks to 1.5 mg/kg daily	30-50 of normal dose	30-70 of target dose and titrate based on tolerance and disease-specific guidelines, allow 2-4 weeks to reach steady state
Low or absent activity (homozygous mutant)	Do not use 6-MP	< 10 of normal dose – reduce normal dose by 10-20mg/m^2 daily	*Non-malignant condition*: consider alternative therapy; *Malignancy*: reduce daily dose 10-fold and frequency to weekly instead of daily, allow 4-6 weeks to reach steady state;
Reported strength of recommendation	NR	NR	Strong
Rigor of development score (rank)	79.2 (3)	52.8 (8)	59.0 (5)

IBD = inflammatory bowel disease; ALL = acute lymphoblastic leukemia; mg = milligram; kg = kilogram; NR = not reported

Table 8: Dosing recommendations for 6-thioguanine based on TPMT status

Identifier, year	CPIC, 2011[56]
Target condition	None, general TPMT testing
Normal activity (wildtype)	Normal dose, adjust along with other myelosuppressive agents as needed
Intermediate activity (heterozygous)	30-50 of target dose, adjust based on tolerance and disease-specific guidelines, allow 2-4 weeks to reach steady state*
Low or absent activity (homozygous mutant)	*Non-malignant conditions*: consider alternative therapy; *Malignancy*: reduce daily dose 10-fold and thrice weekly, adjust dose based on tolerance and disease-specific guidelines, allow 4-6 weeks to reach steady state
Reported strength of recommendation	Strong
Rigor of development score (rank)	59.0 (5)

4 DISCUSSION

Evidence-based and consensus-based clinical guidance is important for guiding the safe and effective use of drug treatments.[64] The application of pharmacogenetics to further personalize therapy should not be overlooked in the development of treatment recommendations. As healthcare delivery becomes more patient-based, healthcare professionals require guidance on selecting the most appropriate test and how the test results should be interpreted to improve patient care. However, recommendations must be supported by high quality evidence and developed using rigorous methods. The present review reveals gaps in the evidence and a lack of methodological rigor in guidance documents for TPMT testing.

4.1 Gaps in the evidence

The clinical guidance documents included in this systematic review varied not only in scope, but also in terms of the recommendations for the type of TPMT testing. Inconsistencies in the quality of guidance documents were also observed. Only a few of the included documents scored high across more than three AGREE-II domains. Guidance documents that paired

recommendations with dose adjustments tended to provide more details on the methods used to generate recommendations, with most describing a systematic literature review. Unfortunately, the inclusion of a systematic literature review in the guideline development process did not always result in a recommendation based on high quality evidence. For example while the 2006 AGA[47] assigned a high level of evidence to recommendations for TPMT testing (grade B), the reference associated with the statement was the FDA drug label warning[3] which recommends "TPMT genotyping or phenotyping (red blood cell TPMT activity) can identify patients who are homozygous deficient or have low or intermediate TPMT activity." The FDA warns about the use of phenotype tests in patients who have received recent blood transfusions and the need for regular complete blood cell count monitoring.

The observed lack of high quality evidence to support TPMT recommendation statements may be a result of the view that pharmacogenetic testing is unique from other treatment or disease management interventions and should not be required to show improvements in health outcomes in order to be implemented. Altman[65] proposes that pharmacogenetic testing is ready for clinical implementation on the basis of non-inferiority; a concept that requires that a new intervention (i.e. TPMT genotyping) to not be worse than a comparator (i.e. TPMT phenotying or standard monitoring). Altman also believes that the cost-effectiveness of pharmacogenetic tests should not be considered prior to initial implementation since the cost of genotyping is rapidly decreasing.[65] This view fails to consider the relationship between test performance and frequency of gene variants. In the case of very rare variants, such as a homozygous TPMT gene mutation, the false positive rate may exceed the true positive rate, resulting in unnecessary costs and risk to the patient.[66] Moreover, it's critical to assess the cost-effectiveness of pharmacogenetic testing by weighing the added costs of the new intervention compared to standard care against any added health benefits to the patients. Failing to do so may result in inappropriate allocation of health care resources in health care systems facing fixed budget constraints. Thus incremental cost-effectiveness is another useful criteria for inclusion in guidance documents.

4.2 Implications for paediatric patients

Physiological factors, including age, sex, and disease states are known to contribute significantly to individual variations in the pharmacokinetic and pharmacodynamics properties of administered drugs[67]. Researchers in the fields of pharmacogenetics and pharmacology

propose that there may be important differences in the genetic characterization of patients across disease classes and age groups which may be indicative of treatment response. TPMT status is no exception, and the 2011 recommendations by the Agency for Healthcare Research and Quality (AHRQ)[68] state that there is currently insufficient evidence to support the clinical validity and utility of TPMT testing across the board in patients with any auto-inflammatory disease. Only one of the included guidance documents referred to the AHRQ report in their recommendations.[56]

Aside from the six guidance documents focused on paediatric populations,[42, 46, 53, 54, 60, 69] none of the other documents considered or discussed age in the context of TPMT testing. The CPIC addressed the issue of age in a 2013 update[70] of the original 2011 guideline. The update included five new studies and concluded that "the original dosing recommendations can be used in both the adult and paediatric populations." The authors justified this statement based on the fact that a large proportion of the evidence used to support the original recommendations were focused on studies of children and the fact that dosing recommendations were presented in units of mg/m^2 and mg/kg.[70] It's also important to consider that genotyping in children is associated with ethical concerns related to obtaining consent and also to testing of other family members.[71]

4.3 Validity of recommendation statements

In terms of the comparability of recommendations within each disease group, variation was observed by test type (phenotype enzyme activity or genotype) as well as the magnitude of dose adjustment. For example, COG recommends reducing doses by 30%-50% in patients taking 6-mercaptopurine (a maintenance therapy for ALL)[55] while the CPIC recommends 30-70% of a normal dose.**[56]** The applicability and relevance of wide-scoping recommendations, like those produced by the CPIC are yet to be determined. It is likely that in the absence of advanced tools or algorithms to assist with clinical implementation, medical specialties (such as paediatric oncologists) will continue to follow clinical guidance produced by their own specialized medical bodies (such as COG).

Reasons for the observed differences in recommendations across common disease categories could be a result of changes in clinical practice over time which may or may not be driven by improved evidence to support the development of recommendations. These differences may

also reflect the perspectives of the individuals/organizations producing or endorsing the guidelines. It is known that different medical specialties have different risk-benefit perspectives.[72, 73] Alternatively the differences observed could reflect variation in the evidence used to support recommendation statements or variation in the methods of guideline development. For example, when comparing 2004[41] and 2011[48] BAD recommendations for the treatment of dermatologic conditions, the most recent guideline scored much higher in terms of rigor of development according to the AGREE domain.

4.4 Quality of guidance documents

While AGREE-II allowed for the methodological rigor and transparency of each included document to be assessed across the items and domains included, it did not allow for commenting on the quality of the evidence cited to support the testing recommendations. Not all of the guidance documents that scored high in terms of rigor of development were based on high quality evidence. Some of the guidance documents provided references for review articles or case-studies to support TPMT testing recommendations (e.g. BSPR, 2011[53]) while others presented evidence that clearly contradicted recommendation statements. For example the 2010 BSG guideline describes in detail the fact that TPMT deficient IBD patients may not be at the same risk as ALL patients with regard to myelotoxicity and then go on to recommend testing for all patients. The 2010 BSG guideline also addresses the fact that evidence to support testing is limited and the decision to test for TPMT status is controversial. The AGREE-II tool does not explicitly capture the quality of evidence accompanying recommendation statements. A critical appraisal of evidence linked to recommendation statements was beyond the scope of this systematic review. It is also important to note that recommendations for TPMT testing were not the primary objective of the guidance documents included (with the exception of the CPIC document). However, the rigor of development domain of AGREE was applied only to TPMT recommendations (see shaded items in Appendix 3).

4.5 Legitimacy of sources for recommendations

Variation in recommendations, in particular differences between pharmacogenetics organizations such as CPIC and clinical bodies such as the BAD and COG raises the question of who should be responsible for guiding the use of pharmacogenetic testing and whether a single authoritative source is appropriate. The development of high quality clinical practice guidelines in pharmacogenetic testing is not a simple undertaking and unlike clinical therapeutic

guidelines, requires interdisciplinary collaboration between experts in the fields of genetics, pharmacology and the clinical disciplines responsible for administering the test-treatment combinations. Clinical and academic societies could play a crucial role in this process by actively sharing evidence and promoting joint guideline development and endorsement. A consensus approach may also be favorable in cases where evidence is lacking, or to address the reality that data linking genetic test results to health outcomes is rarely available from randomized controlled trials. Systematic reviews of available evidence can be used to identify gaps in the literature which can help inform judgments about the value of a test in particular clinical treatment paradigms, as well as identify areas for future research.

4.6 Uptake of TPMT pharmacogenetic testing

The availability of high quality evidence-based guidelines is not the only requirement to improve the uptake of TPMT pharmacogenetic testing. Evidence to support the cost-effectiveness of tests and the value for money in terms of health gains achieved is essential for reimbursement in private and public health care systems. As with guidelines, economic evaluations require high quality evidence of healthcare costs and outcomes and must use data that are relevant for the health care jurisdiction and target population. Thus the findings from the study by Donnan et al. (described previously) evaluating the cost-effectiveness of TPMT testing for children with ALL[66] cannot be applied to the use of TPMT testing in IBD. Uptake of testing strategies is also hampered by a lack of technology, not in laboratory testing methods, but in terms of electronic networks with which data can be stored, interpreted, and shared with clinicians and patients. Guidance documents are most useful when they are accessible through point-of-care devices and when test results are readily available through data-sharing technology such as centralized electronic e-health records.

The lack of strong consensus in recommendations covered in the present review suggests that it may be premature to issue universal recommendations for TPMT testing across patient target populations, and testing practice may evolve more rapidly in some clinical domains compared to others. Regardless, there is a need to establish a single or multiple authoritative trusted sources for guidance of a technology that has the potential to span multiple patient populations and clinical applications. A call for action has been issued by the CPIC[74] and a number highly regarded researchers in the field[7, 75] that pharmacogenetic clinical practice guidelines should go beyond making recommendations regarding clinical utility and address optimal treatment dosing

for specific genotypes. As new research deepens our understanding of the genetic basis of response to therapy, increasingly detailed guidelines will be needed to clarify which genetic variants that relate to a patient should be considered with respect to a given treatment and which of them do not add critical information.

In summary, recommendations are only as strong as the evidence available to support them and more evidence on the clinical validity and utility of genetic tests, such as those available for TPMT testing is required before definitive recommendations can be issued.

4.7 Study limitations

There are a number of limitations to this systematic review. The AGREE-II tool is intended to be applied to CPGs and not consensus statements, medical position statements, and clinical protocols. The evaluation of non-CPG documents warrants caution in the interpretation of quality scores and ranks as well as comparisons across study types. For example, not all of the AGREE-II domains could be applied to the COG protocol, limiting our ability to compare the quality of this document in terms of stakeholder involvement, applicability and editorial independence. Similarly, medical position statements are often brief and direct in comparison to CPGs and as such, fail to provide details on the process of development. The appraisal process did not account for any relationship between the type of guidance document and quality. Another limitation is that AGREE-II is intended to appraise CPG documents as a whole, not just a section of interest (e.g. drug administration and safety sections that include recommendations related to TPMT). Many of the included guidance documents were focused on both diagnosis and treatment of the conditions of interest and as a result only sections that referred to TPMT testing were appraised in terms of rigor of development. Guidance documents that scored high in terms of rigor of development included evidence on TPMT testing in the development of recommendations for drug administration and/or safety monitoring. It is important that quality appraisal tools retain flexibility for application to a wide range of, guidance documents, including clinical practice guidelines, care maps, treatment algorithms and increasingly, electronic disease management tools.

Another limitation is that guidance documents were excluded from the systematic review if they did not include a statement about TPMT testing even if they did consider evidence on TPMT testing in the development process. Also, non-English guidance documents were not included

in this review. Finally, the field of pharmacogenetics is rapidly evolving and as such guidance documents that include statements to test for TPMT status will continue to evolve over time. This will require updates to this systematic review as new guidelines become available.

4.8 Conclusions

Clinical guidance on the use of pharmacogenetics is required to assist healthcare professionals with decisions regarding which test to order and how test results can be used to improve patient care. The present review revealed wide variation in recommendations for TPMT testing reflecting a lack of clear evidence to support the clinical validity and utility of test options as well as a lack of rigor in the methods used to develop recommendation statements. The development of high quality guidance for pharmacogenetic testing requires interdisciplinary collaboration between experts in the fields of genetics, pharmacology and the clinical disciplines responsible for administering the test-treatment combinations. Systematic reviews of available evidence can be used to identify gaps in the literature which in turn can help inform judgments about the value of a test, as well as set research agendas.

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APPENDIX 1: LITERATURE SEARCH STRATEGIES

CINAHL

1. (PT Practice Guidelines) OR (TI guideline*) OR (TI guidance*) OR (TI (position paper or position stand)) OR (TI statement*) OR (TI recommendation*) OR (TI consensus) OR (TI practice parameter*) OR (TI standards)
2. KW(TPMT OR “thiopurine methyltransferase” OR “thiopurine methyl transferase” OR “thiopurine” OR “azathioprine” OR “mercaptopurine” OR “6-mercaptopurine” OR “thioguanine” OR “6-thioguanine”)
3. MH (Azathioprine) OR (MH 6-Mercaptopurine)
4. 2 OR 3
5. 1 AND 4

MEDLINE

1. exp Practice Guideline/ or exp Guideline/ or exp Clinical Protocols/ or exp Critical Pathways/ or exp Decision Support Systems, Clinical/
2. (guideline or guidance or "clinical protocol" or "care pathway" or "pathway of care" or "care map*" or "decision support" or "clinical information system" or "medical pathway" or "clinical annotation" or "recommendation" or "clinical recommendation*" or "clinical consensus" or "consensus statement")
3. 1 or 2
4. (TPMT* or "thiopurine methyltransferase*" or "thiopurine s-methyltransferase*" or "thiopurine methyl-transferase*" or "thiopurine s-methyl-transferase*" or "thiopurinemethyltransferase*")
5. exp 6-mercaptopurine/ or exp azathioprine/ or exp thioguanine/
6. 4 or 5
7. 3 and 6

EMBASE

1. exp Practice Guideline/ or exp clinical protocol/ or exp clinical pathway/ or exp decision support system/ or exp consensus/
2. (guideline or guidance or "clinical protocol" or "care pathway" or "pathway of care" or "care map*" or "decision support" or "clinical information system" or "medical pathway" or "clinical annotation" or "recommendation" or "clinical recommendation*" or "clinical consensus" or "consensus statement")
3. 1 or 2
4. (TPMT* or "thiopurine methyltransferase*" or "thiopurine s-methyltransferase*" or "thiopurine methyl-transferase*" or "thiopurine s-methyl-transferase*" or "thiopurinemethyltransferase*")
5. exp thiopurine methyltransferase/
6. 4 or 5
7. 3 and 6

APPENDIX 2: GREY LITERATURE SOURCES

Guideline sources	Website
General guideline sources	
Guidelines International Network	http://www.g-i-n.net/
National Guideline Clearinghouse	http://guideline.gov/
NICE guidance (UK)	http://guidance.nice.org.uk/
Nice pathways (UK)	http://pathways.nice.org.uk/
Scottish Intercollegiate Guidelines Network (SIGN)	http://www.sign.ac.uk/
New Zealand Guidelines Group	http://www.nzgg.org.nz/
Guidelines Advisory Committee (GAC) - Ontario	http://www.gacguidelines.ca/
SUM2 - University of Kansas School of Medicine & Medical Center	http://sumsearch.org/
Canadian Medical Association Infobase	http://www.cma.ca/index.php/ci_id/54316/la_id/1.htm
BC guidelines	http://www.bcguidelines.ca/
eGuidelines (UK) (requires login)	http://www.eguidelines.co.uk/
Medical Journal of Australia Guidelines	https://www.mja.com.au/journal/guidelines
Pharmacogenomics-related sources	
The Pharmacogenomics Knowledgebase	http://www.pharmgkb.org
Disease specific (cancer)	
Children's Oncology Group	http://www.childrensoncologygroup.org/
SAGE Inventory of Cancer Guidelines	http://cancerguidelines.ca/Guidelines/inventory/search.php
Disease specific (gastroenterology)	
The American Gastroenterological Association	http://www.gastro.org/practice/medical-position-statements
Canadian Association of Gastroenterology	http://www.cag-acg.org/guidelines
World Gastroenterology Organization	http://www.worldgastroenterology.org/global-guidelines.html
British Society for Gastroenterology	http://www.bsg.org.uk/clinical/general/guidelines.html
American College of Gastroenterology	http://gi.org/clinical-guidelines/clinical-guidelines-sortable-list/

Pediatric specific	
Cincinnati Children's Hospital: Evidence-based guidelines	http://www.cincinnatichildrens.org/service/j/anderson-center/evidence-based-care/default/
Great Ormond Street Hospital Trust (NHS Foundation Trust) (UK)	http://www.gosh.nhs.uk/health-professionals/clinical-guidelines/
The Royal Children's Hospital (Australia)	http://www.rch.org.au/clinicalguide/cpg.cfm
Auckland District Health Board (New Zealand) – Newborn guidelines	http://www.adhb.govt.nz/newborn/Guidelines.htm
Royal College of Paediatrics and Child Health (UK)	http://www.rcpch.ac.uk/supported-guidelines

APPENDIX 3: AGREE-II INSTRUMENT

Domain	Item	AGREE II Rating						
		1 *Strongly Disagree*	2	3	4	5	6	7 *Strongly Agree*
Scope and purpose	1. The overall objective(s) of the guideline is (are) specifically described.							
	2. The health question(s) covered by the guideline is (are) specifically described.							
	3. The population (patients, public, etc.) to whom the guideline is meant to apply is specifically described.							
Stakeholder involvement	4. The guideline development group includes individuals from all the relevant professional groups.							
	5. The views and preferences of the target population (patients, public, etc.) have been sought.							
	6. The target users of the guideline are clearly defined.							
Rigor of development (shaded items focused on TPMT recommen-dations)	7. Systematic methods were used to search for evidence.							
	8. The criteria for selecting the evidence are clearly described.							
	9. The strengths and limitations of the body of evidence are clearly described.							
	10. The methods for formulating the recommendations are clearly described.							
	11. The health benefits, side effects and risks have been considered in formulating the recommendations.							
	12. There is an explicit link between the recommendations and the supporting evidence.							
	13. The guideline has been externally reviewed by experts prior to its publication.							
	14. A procedure for updating the guideline is provided.							
Clarity of presentation	15. The recommendations are specific and unambiguous.							
	16. The different options for management of the condition or health issue are clearly presented.							
	17. Key recommendations are easily identifiable.							
Applicability	18. The guideline describes facilitators and barriers to its application.							
	19. The guideline provides advice and/or tools on how the recommendations can be put into practice.							
	20. The potential resource implications of applying the recommendations have been considered.							
	21. The guideline presents monitoring and/ or auditing criteria.							
Editorial independence	22. The views of the funding body have not influenced the content of the guideline.							
	23. Competing interests of guideline development group members have been recorded and addressed.							
Overall Guideline Assessment	1. Rate the overall quality of this guideline.	1 *Lowest possible quality*	2	3	4	5	6	7 *Highest possible quality*
	2. I would recommend this guideline for use.	*Yes*	*Yes, with modifications*					*No*