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Title:
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Date:
2018-04-01

Citation:
Conyers, R., Devaraja, S. & Elliott, D. (2018). Systematic review of pharmacogenomics and adverse drug reactions in paediatric oncology patients. Pediatric Blood and Cancer, 65 (4), https://doi.org/10.1002/pbc.26937.

Persistent Link:
https://hdl.handle.net/11343/294096

Title: Systematic review of Pharmacogenomics and Adverse Drug reactions in Paediatric Oncology Patients

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Word Count

Abstract 114

Main Text 3798

Tables 5 (Main text) 7 (Supplemental)

Figures 1

Running Title: Investigating the pharmacogenomics changes associated with chemotherapy toxicity in Paediatric Oncology

Keywords: Pharmacogenomics, Adverse Drug Reactions, Paediatric Oncology, Genome Wide Association Studies, Single-nucleotide polymorphisms

Abbreviations:

ABCB1	ATP binding cassette subfamily B member
ABCC	ATP binding cassette subfamily C member
ACT	Anthracycline cardiotoxicity
ADR	Adverse drug reaction
ALL	Acute Lymphoblastic Leukaemia
ASNS	Asparagine synthetase
CBR3	Carbonyl reductase 3
CCND1	Cyclin D1
CEP72	Centrosomal protein 72
CGA	Candidate gene approach
COMT	Catechol-O-methyltransferase
CTCAE	Common terminology criteria for adverse events
CYP3A	Cytochrome p450
HPRT1	Hypoxanthine Phosphoribosyltransferase 1
GCR	Glucocorticoid receptor
GRIA1	Glutamate ionotropic receptor AMPA type subunit 1
GRIK1	Glutamate ionotropic receptor kainite type subunit 1
GRIN3A	Glutamate receptor subunit 3A
GTSP1	Glutathione S-transferase P
GWAS	Genome-wide association study
IC	Inclusion criteria
MTHFD1	Methylenetetrahydrofolate dehydrogenase gene 1
MTHFR	Methylenetetrahydrofolate reductase

NOS3	Nitric oxide synthase 3
PRISMA	Preferred reporting items for systematic reviews and meta-analysis statement
RARG	Retinoic acid receptor gamma
RFC	Replication factor C
SLC	Solute carrier
SLCO1B1	Solute carrier organic anion transporter family member 1B1
SNP	Single nucleotide polymorphism
6-MP	6 mercaptopurine
TYMS	Thymidylate synthetase
TPMT	Thiopurine methyltransferase

Conflict of Interest The authors have no conflict of interest to declare

Abstract

SYSTEMATIC REVIEW OF PHARMACOGENOMICS AND ADVERSE DRUG REACTIONS IN PAEDIATRIC ONCOLOGY PATIENTS

Many paediatric patients with cancer experience significant chemotherapy side effects. Predisposition to drug reactions is governed by single nucleotide polymorphisms (SNP). We performed a systematic review of the literature from 2006 through 2016. Outcomes of interest included patient characteristics, cancer type, and drug of interest, genes investigated, toxicity identified, and genetic polymorphisms implicated. The primary toxicities studied were neurotoxicity cardiotoxicity, osteonecrosis, and thromboembolism and hypersensitivity reactions. The retrieved studies were grouped according to toxicity reported and SNP associations. This review highlights the discoveries to date in pharmacogenomics and paediatric oncology along with highlighting some of the important limitations in the area.

INTRODUCTION

An individual's genetic makeup is a major contributor to the development of an adverse drug reaction (ADR). Pharmacogenomics is the study of how variations in single nucleotide polymorphisms (SNPs), together with insertion and deletion polymorphisms, contribute to inter-individual differences in efficacy and toxicity of drugs, thereby affecting clinical response (1). This is pertinent in cancer therapy, as most regimens include high dose administration of therapy with highly toxic profiles that may result in a wide variety of complications. ADRs are often a result of direct toxicity in normal healthy cells, due to the low specificity displayed by chemotherapeutic drugs and occur more frequently with treatment intensification. Unsurprisingly, chemotherapy toxicity is a common cause of morbidity and mortality in most paediatric cancer patients, and a frequent cause of mid- and long-term sequelae (2). The gravity of paediatric oncology ADRs is best illustrated by anthracycline cardiotoxicity (ACT). Anthracyclines are a potent chemotherapeutic drug and part of over 50% of curative regimens (3). The ADR most commonly related to anthracycline exposure is severe ACT, affecting more than 7% of patients (4). ACT results in free radical damage and causes cardiomyocyte death(5). It often presents months or years post-treatment, and no screening methods exist to identify at-risk individuals. Whilst current treatment regimens can slow or stabilise this disease, in the event of congestive cardiac failure patients can be predisposed to sudden cardiac death. The cardiovascular mortality rates are > ten fold higher than aged matched controls in paediatric oncology patients necessitating the need to investigate cardiovascular ADR further(6, 7) .

The successful application of pharmacogenetics research to clinical outcome in paediatric oncology, is demonstrated by genetic polymorphisms within the thiopurine methyltransferase (*TPMT*) gene. *TPMT* is the main gene regulator of the active and inactive metabolites of the drug 6-mercaptopurine (6-MP). Intracellularly, 6-MP can either be metabolized by Hypoxanthine phosphoribosyltransferase 1 *HPRT1*, resulting in synthesis of active cytotoxic metabolites that incorporate into DNA or RNA to induce cell death, or be inactivated if methylated by *TPMT* to methyl-MP. Previous studies have shown that *TPMT* activity is highly variable among individuals. Three variant alleles, *TPMT*2* (G238C), *TPMT*3A* (G460A and A719G) and *TPMT*3C* (A719G), account for more than 95% of the inherited variability in TPMT enzyme activity (8). It has become the gold standard of practice to determine the germline genetic variations in *TPMT* among paediatric cancer patients at the start of therapy, so dose adjustments can be anticipated. *TPMT* is now the only pharmacogenetics marker in ALL with guidelines for drug dosing according to one's *TPMT* genotype.

The clinical application of pharmacogenomics within paediatric oncology is a fast moving subspecialty. The primary aim of this review was to evaluate the current understanding of genomic associations across a range of frequent chemotherapy ADR including vincristine neuropathy, methotrexate encephalopathy, anthracycline cardiotoxicity, glucocorticoid-induced osteonecrosis, asparaginase hypersensitivity and thromboembolism.

METHODS

This review was conducted according to the guidelines for Preferred Reporting Items for Systematic Reviews and Meta-Analysis Statement (PRISMA)(9).

Eligibility Criteria

Randomised, non-randomised and cohort studies were included. Studies had to involve paediatric or adolescent patients aged 18 years and below, who had been diagnosed with cancer and had undergone chemotherapy. In addition studies had to investigate genetic polymorphisms in the study participants with the outcome measures including drug-related toxicity. Articles had to be available in full text and published in English no earlier than 2006. Studies were included if > 75% of participants met eligibility criteria. Secondary screening was performed on articles short listed during initial screening. Articles were removed during secondary screening if the identified toxicity included neutropenia, infection or neurocognitive outcomes as these outcomes were thought to have many contributing factors and could confound genetic findings. In addition studies involving TPMT were excluded on secondary screening as this genetic variant screening is already standard of care within the paediatric oncology setting.

Search Methods

Published scientific literature on OvidSP Medline, OvidSP Embase, PubMed, and Cochrane Library, from 2006 to 2016 was considered for inclusion. Limits were applied for English language, and search terms indicative of 'cancer', 'neoplasm' or 'malignancy', with their alternatives and truncations, AND 'toxicity', or 'adverse effect', or 'side effect', with their alternatives, AND 'pharmacogenetics', 'genetic polymorph', 'genotype', or 'genetic variability', with their alternatives AND 'newborn', 'neonate', 'infant', 'pre-schooler', 'child', 'adolescent', 'teen', 'youth', or 'paediatric', with their alternatives were used. Letters, practice guidelines, comments and editorial articles were excluded. Reference lists of included studies and relevant review articles were checked to ensure all studies had been obtained. Citation tracking of included articles was completed using EndNote. All studies were exported to and managed in EndNote Version 7 for Mac. 335 duplicates were removed

using the 'Find Duplicates' function (EndNote), and manual duplicate screening. Screening of the titles and abstracts for relevance assessed eligibility of retrieved papers. From the relevant studies, full-text papers were assessed for inclusion and exclusion criteria independently by two authors.

Article Appraisal

Data was extracted by one reviewer on study design, participant characteristics, setting, sample size, participant numbers, genomic analysis and reported adverse drug reactions (i.e. neurotoxicity, cardiotoxicity, osteonecrosis, and thromboembolism and hypersensitivity reaction). The outcome measures to assess toxicity of ADR were categorised as either compliant with the Common Terminology Criteria for Adverse Events (CTCAE) by the National Cancer Institute (10) or according to the World Health Organisation criteria (11). Furthermore the studies were categorised based on toxicity identified and tumour group. In addition, how participants were enrolled within cohort studies, and the genetic profiling methods that were utilised in studies, have been reported as these are likely to impact the results. A standardised Excel form was used to extract information on study demographics, patient characteristics, cancer type, chemotherapeutic drug of interest, genetic profiling method, genes investigated, toxicity identified, and genetic polymorphisms identified. A second investigator reviewed the extracted data and disagreements were discussed until a consensus was reached.

Data Analysis

The primary outcomes of interest were neurotoxicity (neuropathy and encephalopathy), cardiotoxicity, osteonecrosis, thromboembolism, and hypersensitivity reactions. These toxicities were chosen as they are common in paediatric patients undergoing chemotherapy and formed the basis of further retrospective research undertaken by the author at The Royal Children's Hospital, Australia. Secondary outcomes included: diarrhoea, gastrointestinal

toxicity, glucose metabolism abnormalities, hepatotoxicity (including hyperbilirubinemia), hypertension, mucositis, myelotoxicity (anaemia, granulocytopenia, leukopenia, and thrombocytopenia), nephrotoxicity, pancreatitis, skin toxicity, stomatitis, and vomiting where recorded but not reported upon this review. Initial screening for inclusion criteria (IC) was performed manually by developing groups on EndNote and articles were excluded if they did not meet all the IC. Excluded articles were placed in groups including: (i) adult participants aged over 21 years if < 75% of the study population were paediatric (ii) non-human subjects (iii) not related to cancer (iv) had not undergone chemotherapy (v) descriptive literature reviews (vi) no genetic polymorphisms investigated (vii) no drug-related toxicity investigated

RESULTS

Study selection

The electronic search of OvidSP Medline, OvidSP Embase, PubMed, and the Cochrane Library, identified 1471 citations, of which 1136 studies were identified after excluding duplicates. Of this 1136 studies, 243 articles were identified as being relevant to this review (see Figure 1). Secondary selection reviewed full texts to establish relevance of these 243 articles to study aims. Neutropenia or infection, and neurocognitive outcomes were specifically searched for and these studies were excluded as they had many contributing factors and a clear link with genetic polymorphisms cannot be established. Studies investigating the *TPMT* gene were also excluded as previously addressed. When duplicate reports of the same study were identified, the most recent and/or complete publication was chosen for review. Conference abstracts and posters were excluded as they did not yield sufficient information to fit our IC, and often reported an already included study. After secondary screening 86 articles were deemed relevant and selected for review.

Study characteristics

The systemic review of the 86 studies comprised 71 cohort studies, 3 case reports, 2 systematic reviews, and 2 meta-analyses. Of the cohort studies, 38 were retrospective and 33 were prospective. 49 studies were single-centre, three were dual-centre, and 20 were multi-centre studies. 32 of the cohort studies were conducted in Europe (including one study done across Austria, Germany and Switzerland), 17 in the Americas (North and South), 11 in Asia, 5 in Canada, 3 in Africa, and 1 in Australasia. 62 studies utilised candidate gene approach (CGA) to identify potential genes that confer toxicity susceptibility based on existing literature, while 9 used genome-wide association study (GWAS). 42 studies used a published or adapted version of the Common Terminology Criteria for Adverse Events (CTCAE) by the National Cancer Institute (10) for grading toxicities, while 11 used the World Health Organisation criteria(11) . Other studies used a mix of methods to grade toxicities. The three case reports each described a single patient's toxicity outcomes (12-14). All three studies utilised CGA to identify genes of interest and used descriptive reports of toxicities without clearly defined grading criteria.

The two systematic reviews (15, 16) and two meta-analyses (17, 18) displayed large-scale heterogeneity across various comparator domains, including the number of studies included, tumour type studied, and the toxicity being assessed.

Outcomes

Key outcomes from cohort studies are provided in a tabulated synthesis according to the toxicity identified including neurotoxicity, cardiotoxicity, osteonecrosis, hypersensitivity to Aspariginase and Ototoxicity (Table 1-5 and supplementary tables 1-7). None of the studies to date investigated thromboembolism as a toxicity, although this is a primary outcome of interest for this review.

Neurotoxicity

12 cohort studies and three case reports reported neurotoxicity outcomes across (Table 1 and 2). Four studies looked at cytochrome p450 (*CYP3A)* and its association with neurotoxicity. Three studies found no association (19-21) and one found *CYP3A5* expression was associated with the toxicity (22). Two studies looked at the centrosomal protein 72 (*CEP72)* with variable findings. Diouf et al.(23) using a GWAS in two separate cohorts found rs924607 associated with neurotoxicity, after multivariate analysis and imputation of the GWAS to better define the statistical significance of the SNP. In contrast, Guitierrez-Carnino et al. (24) found no association with the same SNP in a smaller, retrospective study that only assessed toxicity over the first month of therapy. ATP binding cassette subfamily B member (*ABCB1)* was studied in five studies (14, 18, 25-27) with two demonstrating protective effects from rs4728709 and rs10244266(18, 25). Of these, Lopez-Lopez et al. used criteria that investigated association with any neurotoxicity from Grade 1 to 4(28). Two studies used magnetic resonance imaging findings of leukoencephalopathy as a neurotoxicity endpoint (29, 30). Bhojwani et al. observed leukoencephalopathy in 85 of 364 ALL patients, and demonstrated statistically significant association with 347 SNPs (29). Despite Grade 2 leukoencephalopathy being included in analysis as a neurotoxic outcome, none of the SNPs reached significance. The C677T SNP in Methylenetetrahydrofolate reductase (*MTHFR)* was associated with neurotoxicity in three case reports (12-14) although these findings were not replicated across the cohort studies.

Cardiotoxicity

10 cohort studies reported cardiotoxicity outcomes across Europe and North America (Table 3)(3, 31-39). Two studies looked at the ATP binding cassette subfamily C member (*ABCC)* family, with a risk associated with rs7627754 (31) , rs3743527 TT genotype, and in

combination with the rs246221 TC/TT genotype(34). Blanco et al. found the expression of carbonyl reductase 3 (*CBR3)* was associated with cardiotoxicity (36) whilst no association was found in the authors' previous study (35). Visscher et al. conducted two multi-centre, prospective studies in different cohorts in Canada and Holland, looking at the solute carrier (*SLC)* family. The first study (37) revealed three SNPS (rs7853758, rs885004, rs4877847) that were significant for cardiotoxicity risk whilst the second study (38) found 4 additional SNPs (rs4982753, rs4149178, rs12882406, rs12896494) to be associated. The SNPs found in Visscher et al. were validated within the same study using a European cohort (38). Only one study observed a cardio protective effect through rs1799983 in nitric oxide synthase 3 (*NOS3)* (31) and this has not been validated in further studies.

Osteonecrosis

Four cohort studies reported outcomes in osteonecrosis across North America (Table 4). Karol et al. conducted two GWAS studies looking at two distinct chemotherapy regimens in paediatric patients with ALL, one for standard-risk disease (protocol AALL0331) and one for high-risk disease (protocol AALL0232)(40) with differing doses of glucocorticoids between disease groups. The standard risk study involved 369 patients with a validation cohort of 817 patients and demonstrated multiple SNPs (rs77556622, rs76599360, rs1891059, rs115602884, rs74533616, rs80223967, rs17021408, rs61818937, and rs141059755, rs117532069) associated with osteonecrosis. The high risk study rs10989692 and rs2154490 in glutamate receptor subunit 3A (*GRIN3A)* and glutamate inotropic receptor kainite type subunit 1 (*GRIK1)* (40, 41) to be associate with osteonecrosis. Similarly, Kawadia et al. using a GWAS in ALL patients interestingly demonstrated SNP associations that were both annotated to genes and intergenic (42).

Hypersensitivity to Asparaginase

Three cohort studies reported hypersensitivity to asparaginase outcomes across Europe and North America (Table 5). Two studies looked at glutamate ionotropic receptor AMPA type subunit 1 (*GRIA1)*, with both finding association with multiple risk variants (rs2055083, rs707176, rs4958351, rs10070447, rs4958676, rs6889909, rs6890057) (43, 44). Interestingly, rs4958351 was found to be both protective (43) and a risk variant (44). Additionally, Ben Tanfous et al. investigated the asparagine synthetase (*ASNS)* and found rs3832526 was associated with asparaginase hypersensitivity (45).

Ototoxicity

Ten cohort studies reported ototoxicity outcomes across Asia, Europe and North America (Supplementary Table S7). While all studies investigated cisplatin regimens in the treatment of solid tumours, there was no continuity across the SNP and gene associations between the cohorts with some contradictory results. A discovery cohort of 54 patients in Canada reported by Ross et al found two SNPS in the genes encoding TPMT and catechol-O-methyltransferase (COMT) were highly associated with cisplatin-induced deafness(46). This finding was further confirmed in a replication cohort of 112 patients from paediatric oncology centres throughout Canada (46). In a study performed by the same group and reported in 2013, a third replication cohort of 155 patients found statistically significant genetic variation in the same three previously report *TPMT* SNPS (rs12201199, rs1142345, rs1800460) (47). Interestingly, in a separate evaluation by Yang et al for children enrolled on the St. Jude Medulloblastoma 96 and 03 protocols, none of the *TPMT* or *COMT* genetic variation was replicated to cisplatin ototoxicity in the same described variants (48). The German study by Lanvers-Kaminsky et al also failed to show replication of significance for both *TPMT* (rs12201199) and *COMT* (rs9332377) as did the initial Dutch cohort described by Hagleitner et al (49). However, in a different Spanish replication cohort described by Hagleitner the

same SNPs were found to be statistically significant. Finally the Rednam et al study found that *GSTP1* (rs1695) was associated with ototoxicity requiring hearing aids in patients with medulloblastoma, however this finding cannot be attributed to cisplatin alone as the patients also received radiotherapy(50).

Myelotoxicity

23 cohort studies reported myelotoxicity outcomes across Asia, Europe, North America and South America (Supplementary Table S6). 15 studies looked at *MTHFR*, with two finding no association (51, 52). Nine studies found the C677T mutation in *MTHFR* contributed to myelotoxicity (53-60). The A1298C SNP was also associated with myelotoxicity in three studies (53, 54, 57) although two studies found this polymorphism to be protective (61, 62). Only a single study associated the G80A SNP with myelotoxicity(63). Two studies investigated the replication factor C (*RFC)* gene. Salazar et al. found the GG genotype was associated with myelotoxicity(64) whilst Karathanasis et al. found no association(52).

Nephrotoxicity

Six cohort studies reported nephrotoxicity secondary to methotrexate, with outcomes reported across Europe and Asia (Supplementary Table S1)). Three studies investigated *MTHFR* and found C677T SNP was associated with nephrotoxicity (55, 64, 65). Of these, two also found an association with A1298C (64, 65). Two further studies investigated solute carrier organic anion transporter family member 1B1 (*SLCO1B1)* with rs11045879 (66) and rs4149035(18) associated with nephrotoxicity.

Gastrointestinal toxicity

17 cohort studies reported gastrointestinal toxicity outcomes across Asia, Europe and North America (Supplementary Table S2). Three studies looked at *MTHFR*, with two finding

C677T associated with toxicity (61, 67). The three studies investigating the *ABCC* gene family each found SNPs associated with gastrointestinal toxicity (66, 68-70). Two studies found the same rs11045879 SNP of *SLCO1B1* associated with gastrointestinal toxicity (66, 71). Furthermore, two studies demonstrated association with the GG genotype of *RFC1* (64, 72). Of the two studies investigating the gene family *SLC*, one study found a protective SNP (73) while the other found a SNP associated with gastrointestinal toxicity (66). Additionally, both studies investigating thymidylate synthetase (*TYMS)* found an associated protective SNP each (56, 74).

Hepatotoxicity

19 cohort studies reported hepatotoxicity outcomes across Asia, Europe, North America and South America (Supplementary Table S3). Seven studies looked at *MTHFR*, with one finding no association(51), four finding an association with C677T (55, 57, 65, 67) and two studies identifying other SNPs (52, 63). Two studies conducted by Eipel et al. showed N363S in glucocorticoid receptor (*GCR)* associated with hepatotoxicity (75, 76). Two studies looked at Cyclin D1 (*CCND1)* with one finding no association (51) while one identified a SNP only in medium-high risk ALL patients (77). Furthermore, two studies identified methylenetetrahydrofolate dehydrogenase gene 1 (*MTHFD1)* with one showing protective effects (78) while the other (79) demonstrated hepatotoxicity. Similarly, *ABCC2* was investigated in two studies with one showing protective effects (80) while the other (18) demonstrated hepatotoxicity.

Other toxicities

Five cohort studies reported toxicity outcomes including pancreatitis, cytotoxicity, glucose abnormalities and hypertension across Europe and North America. Two studies undertaken by Eipel et al. looked at *GCR* with both finding an association to glucose abnormalities (75,

76). Chen et al. identified 11 SNPs associated with cytotoxicity in patient-derived cell lines through GWAS(81).

Systematic reviews and meta-analyses

Four studies contributed secondary literature to this review. Both meta analyses found no association between the *MTHFR* (18) and *RFC1* (17) genes respectively with multiple toxicities. Of the systematic reviews, one study (15) identified multiple SNPs associated with cardiotoxicity while the other found reduced expression related to neurotoxicity.

DISCUSSION

This systematic review was conducted to evaluate the genetic polymorphisms associated with chemotherapy toxicity in paediatric oncology patients. Literature on this topic is conflicting with great heterogeneity among the genes and subsequent SNPs investigated. The approach to define toxicity was also variable which may have further influenced the relevance of SNP findings.

Previous discoveries supports the clinical utility of genetic predisposition to drug toxicity. This systematic review clearly shows that there are a plethora of studies currently being undertaken to elucidate genetic associations across a range of common and uncommon toxicities following cancer therapy in paediatrics. However, the generalisation of these results either inter- or intra- toxicity is limited by small sample sizes. The scope of available data is also incomplete with a lack of uniform approach to defining causative genetic associations and limited approaches to integrating already performed studies. Some larger cohorts have endeavoured to confirm the genetic association findings through validation cohorts (37, 46) but the cumulative numbers of patients still remains significantly low (<500 patients). To attain statistically significant results for any genome wide association study, thousands of

patients are often required. One such publication by Aminkeng et al went so far as to recommend upfront screening for the SNP associated in their studies with ACT, which has created controversy within the sub specialty. Aminkeng et al(3) recommended genomic testing for RARG rs2229774 (S427 L), SLC28A3 rs7853758 (L461 L) and UGT1A6 rs17863783 (V209 V). This was challenged by Craig et al(82) who raised the point that SLC28A3 rs7853758 and UGT1A6 rs17863783 in particular require further validation since neither alter protein-coding for these genes, and UGT1A6 rs17863783 is intronic in some alternate transcripts. Craig et al reflect that other potential explanations for an apparent contribution to ACT may be that the SNP represents a haplotype that includes variants that do not alter function. Controversially it was noted that neither SLC28A3 nor UGT1A6 are expressed in the heart. The controversy raised by recommending guidelines for ACT on the back of pharmacogenomic studies highlights the need for prospective studies to be performed prior to recommendations being proffered and appropriate functional models to be sufficiently investigated prior to these recommendations.

Furthermore supporting Craig et al reluctance to bring routine screening directly into clinic is the conflicting GWAS results that can exist amongst cohorts screening for the same toxicity. This is demonstrated for the studies investigating cisplatin ototoxicity. Three separate cohorts have been described in which the SNPS associated with *TPMT* (rs12201199, rs1142345 and rs1800460) were found to be associated with ototoxicity (46) (47). Interestingly, in the American large multi-institute St Jude Medulloblastoma 96 and 03 protocols, none of the previously described variants in TPMT or *COMT* were found to be significant (48). This lack of association for *TPMT* (rs12201199) and *COMT* (rs9332377) was mirrored in the German study by Lanvers-Kaminsky(83) and the initial Dutch cohort described by Hagleitner et al (49). This example illustrates the need for collaborative multi-institute trials but also the requirement for vigorous functional validation of any described variants to ensure their

contribution to the associated toxicity.

Only one prospective pharmacogenomic study in paediatric oncology has found an inherited polymorphism that resulted in an increased risk and severity of an ADR with satisfactory functional validation that allowed for inclusion in prospective Phase III trials. The prospective study by Diouf et al found that the polymorphism in the promoter region of *CEP72* was associated with an increased risk and severity of vincristine associated neuropathy. The benefit of this study over many others in the area, beyond its prospective approach, was the uniformity of the cohorts enrolled for analysis treated either by St Jude Children's Research Hospital or the Children's Oncology Group (COG) according to contemporary institutional review board-approved Acute Lymphoblastic Leukaemia (ALL) protocols. This study allowed for the phenotype to be well documented prospectively and the causality upheld. As a result of this study, the *CEP72* polymorphism is now screened for in the current St Jude's paediatric ALL protocol and provides a good example of how pharmacogenomics can be translated directly to clinical care.

Our results of all described studies show that there is limited functional validation efforts made once a SNP is found. The possibility of using pluripotent stem cells (PSC) from individuals who have suffered adverse drug reactions is one proposed way to functionally validate a genomic finding (84, 85). In the Diouf et al(23) study, the clinically findings were corroborated by multiple lines of laboratory evidence using human pluripotent stem cell neurons showing *in vitro* sensitivity to the drug. This functional analysis, required to prove causation between pharmacogenomics and clinical phenotype further strengthened this study and expedited the transition of this screening into current trials.

Whilst there is preliminary evidence for a range of SNP associated with chemotherapy ADR, clear recommendations to evaluate these ADR formally within clinical trials are lacking.

Without functional validation using appropriate modelling (i.e. pluripotent stem cells, zebra fish, mouse models, *in vitro* cell line work) and large international collaborative studies ensuring statistical significance, the benefit of pharmacogenomic studies will be diluted. This is demonstrated by only one of the 86 studies resulting in screening for the identified SNP in a paediatric oncology trial. Our systematic review highlights the need for more concerted and collaborative approaches to ensure that the discovery phase of pharmacogenomics leads to clinical change.

The main strength of this review is the far reaching scope of the inclusion criteria and the comprehensive summary of pharmacogenomics studies to date. The collation of all these contributing studies may, in and of itself, lend itself to collaborations. To date a centralised report of pharmacogenomics and chemotherapy toxicity in paediatric patients has not been published. The broad focus across a number of ADR is another strength, and whilst not all studies were particularly large or homogenous, their description leads the reader to recognise areas for improvement and growth. There are several limitations that exist across paediatric pharmacogenomic research. Firstly, small sample size poses a significant challenge. This is especially pertinent for GWAS, with some literature advocating 10, 000 cases to achieve sufficient statistical power to detect a causative association through meta-analyses and data pooling(86). Demonstrative of this error, 62% of the included studies had less than 200 affected individuals each. Secondly, selection bias is problematic with studies in this review. The near majority of the literature (54%) used a retrospective approach, often recruiting by convenience sampling with patients still engaged in care within hospital systems, further limiting selection to participants who are still alive. Additionally, patient cohorts are often heterogeneous with respect to disease, drug dosing, drug route and administration, all which have the potential to confound toxicities related to the drug of interest. Finally, the methods used to assess and grade toxicities were varied. Retrospective studies involve review of

patient medical records and are wholly dependent on the meticulousness of medical providers in reporting toxicity signs and symptoms. While versions of the CTCAE and WHO grading was used in 53 cohort studies (75%), there is conflicting evidence on the effectiveness of these tools in some toxicity assessments. Future studies should thus utilise uniform grading methods relevant to each toxicity outcome to achieve more consistency in comparing reported literature.

This systematic review has provided a thorough approach to identifying pharmacogenetics associations related to chemotherapy toxicities in paediatric cancer patients. Whilst a total of 86 studies were identified, only 18 (20%) demonstrated good methodology to confirm SNP associations across multiple cohorts. The single largest limitation of paediatric pharmacogenetics research relates to the limited patient numbers. This is true of pharmacogenetics research in general. Well-designed, sufficiently-powered prospective studies utilising GWAS with an internationally recognised toxicity-grading approach are thus needed to further confirm the findings of SNP associations in paediatric cohorts.

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Tables and Figures

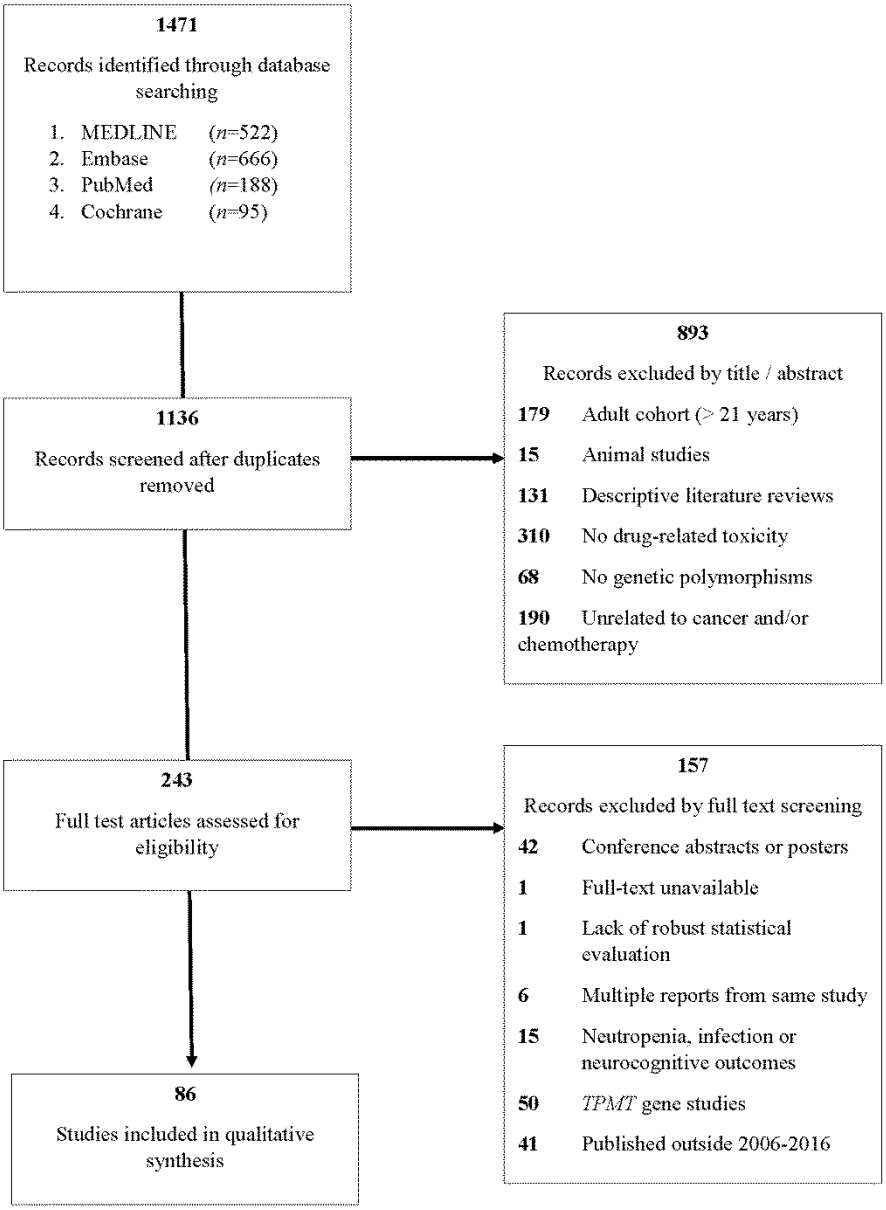

Figure 1: PRISMA Flow chart of study selection

Table 1 Single Nucleotide Polymorphisms associated with Neurotoxicity associate with Vincristine, (b) not associated with Vincristine

Country	Study characteristics	No. of patients	Mean age years	Ethnic origin	Drug	Method	Gene/ genotype	Associated SNP[a]	Ref
LIQUID TUMOURS									

CAN	Single-centre, ret	339	N/A	Cauc	Vincristine	CGA	*ACTG1; CAPG; ABCB1*	rs1135989 G>A; rs2229668 G>A, rs377010 C>A; rs4728709 C>T	26
USA	Dual-centre, prospective	222	6.0	Cauc	Vincristine	GWAS	*CEP72; ETAA1; MTNR1B; TMEM215; NDUFAF6*	rs924607; rs17032980; rs12786200; rs4463516; rs7818688. Only CEP72 rs924607 had genome-wide significance when adjusted for genetic ancestry and cumulative vincristine dose.	24
USA	Dual-centre, prospective	99 107	11.4 N/A	Cauc	Vincristine	GWAS CGA	*CEP72;* *ETAA1; MTNR1B; TMEM215; NDUFAF6* *CYP3A5*	rs924607; rs17032980; rs12786200; rs4463516; rs7818688. CYP3A5 expressors	24
USA	Single-centre, ret			Cauc	Vincristine	CGA	*CYP3A5* *ABCB1; ABCG2*		23
Spain	Multi-centre (3), ret	152	5.49	Cauc	Vincristine	CGA	*ABCC1; ABCC2; ABCB1*	6 SNPs; 5 SNPs; 1 SNP with all 12 SNPs showing a dominant protective effect of minor allele. ABCC2 rs3740066 and rs12826 assoc. with neurotoxicity.	28
USA	Single-centre, prospective	53	N/A	Cauc	Vincristine	CGA	*CYP3A*	No association found.	22

MIXED TUMOURS									
France	Single-centre, prospective	24	8.8	Cau	Vincristine	GWAS	*CYP3A4; CYP3A5; ABCB1*	No association found.	20
Aus	Single-centre, ret	43	16.5	Cauc	Vincristine	CGA	*CYP3A5*	No association found.	21

Table 2 Single Nucleotide polymorphisms associated with non-vincristine neurotoxicity

Country	Study characteristics	No.	Mean age	Ethnic origin	Drug	Method	Gene/ genotype	Assoc SNP	Ref
LIQUID TUMOURS									
USA	Single-centre, prospective	364	N/A	N/A	MTX	GWAS	148 genes assoc. with leukoencephalopathy; 103 genes assoc. with clinical neurotoxicity	347 SNPs assoc. with leukoencephalopathy; 206 SNPs assoc. with clinical neurotoxicity	29
USA Hun	Multi-centre ret	275	4.5	Cauc	Vincristine, MTX, cytarabine	CGA	*ABCB1; ABCG2*	ABCB1 3435T>C TT genotype; ABCB1 T3435C and ABCG2 C421A combination genotype	27
Italy	Multi-centre ret	508	5	Cauc	MTX GLU thiopurine, ANTH	CGA	*ABCC1*	rs246240	28
Spain	Single-centre, ret	124	5.1	Cauc	Vincristine, DAUNO PNL CYCLO ASP	CGA	*CEP72*	No association found for rs924607 TT genotype.	25
USA	Case report	1	12	Cauc	MTX	CGA	*MTHFR*	C677T homozygosity	13

Spain	Case report	1	5	Chinese	MTX	CGA	*MTHFR; SHMT; ABCG2; DHFR; TS; MTRR; RFC1; ABCB1; ABCB2*	C677T; C1420T; C421A; ins/del 19bp; 2R/3R and UTR-6bp ins/del; A66G; G80A; C1236T; IVS 23+56T>C	15
Hun	Case report	1	10	N/A	MTX	CGA	*MTHFR*	C677T homozygosity	14
MIXED TUMOURS									
Japan	Single-centre, ret	56	5[b]	Japanese	MTX	CGA	*ADORA2A*	rs2298383 CC genotype	31

Abbreviations: Ret; retrospective: Aus; Australia, Hun; Hungary: Can; Canada: Cauc; Caucasian, VCR; vincristine, Dauno; Daunorubicin, ANTH; anthracycline, PNL; prednisolone, Cyclo; Cyclophosphamide, ASP; asparaginase, MTX; Methotrexate

Table 3 Single Nucleootide polymorphisms associated with Cardiotoxicity

Country	**Study characteristics**	**No. of cancer patients (of total)**	**Mean age**	**Ethnic origin**	**Drug**	**Genetic profiling method**	**Gene/ genotype**	**Associated SNP**	**Ref**
LIQUID TUMOURS									
Canada	Single-centre, ret	251	N/A	Cauc	Doxo	CGA	*ABCC5; NOS3*	rs7627754; rs1799983[d]	32
USA	Single-centre, pro	184	6.3	N/A	Doxo	CGA	*HFE*	C282Y heterozygosity	33
Slovenia	Single-centre, ret	76	6.3	Cauc	Anth	CGA	*CAT*	rs10836235 CC genotype	34
Hungary	Multi-centre ret	235	5.7	N/A	Doxo Daun	CGA	*ABCC1*	rs3743527 TT genotype, rs3743527 TT + rs246221	35

								TC/TT combination genotype	
MIXED TUMOURS									
Canada	Multi-centre pros	280	0-18 years	Cauc	Anth	GWAS	*RARG*	rs2229774	3
USA	Multi-centre, retr	145	10.3	Cauc	Anth	CGA	*CBR3*	N/A	36
USA	Multi-centre retro	487	17.6	Cauc	Anth	CGA	*CBR3*	rs1056892 GG genotype	37
Canada & Holland	Multi-centre, pro	562	N/A	N/A	Anth	CGA	*SLC22A17; SULT2B1*	rs4982753, rs4149178, rs12882406, rs12896494; rs10426377	38
Canada & Holland	Multi-centre, pros	440	0-18 years	N/A	Anth	CGA	*SLC28A3*	rs7853758, rs885004, rs4877847	39
USA	Multi-centre, pros	112	7.5	Cauc	Anth	GWAS	*CELF4*	rs1786814 GG genotype	40

Abbreviations: Ret; retrospective: Pro; prospective; Anth: Anthracycline unspecified; Doxo: Doxorubicin; Daun: Daunorubicin ; Cauc; caucasian

Table 4 Single Nucleotide Polymorphisms associated with Osteonecrosis

Country	**Study**	**No.**	**Mean age (years)**	**Ethnic origin**	**Drug**	**Method**	**Gene/ genotype**	**Associated SNP[a]**	**Reference**
LIQUID TUMOURS									
USA	Single-centre, pro	615	4.9	Non-Hisp	Mixed: MTX ASP GLU	CGA	*TS*	2R/2R homozygosity for <10 years, and	41

								bone fractures for ≥10 years	
USA	Dual-centre, pro	250 (2285)	0-20	Cauc	Protocol AALL0232 for HR-ALL	GWAS	*GRIN3A; GRIK1*	rs10989692; rs2154490	40
USA	Single-centre, pro	82 (369)	0-10	Cauc	Protocol AALL0331 for SR-ALL	GWAS	*BMP7; PROX1-AS1; LINC00251; DOK5*	3271 SNPs identified. Top replicated SNPs from discovery cohort replicated in validation cohort: rs77556622, rs76599360 rs1891059, rs115602884 rs74533616, rs80223967, rs17021408, rs61818937; rs141059755 rs117532069	40
USA	Single-centre, pro	364	1-18	N/A	GLU	GWAS	*Many genes*	423 SNPs identified (196 gene associated; 227 intergenic). Top 4 SNPs in SH3YL1-ACP1 locus: rs4241316, rs12714403, rs10167992, rs10193882	42

Abbreviations: Pro; prospective, No; number of patients: Cauc; caucasian ; MTX: Methotrexate, ASP; Aspariginase, GLU; Glucocorticoids; Non-Hisp: Non Hispanic

Table 5 Single Nucleotide polymorphisms associated with allergy to aspariginase

Country	Study characteris	No.	Mean age	Ethnic	Drug	Method	Gene	Associated	Reference

	tics		(years)	origin			SNP[a]	
LIQUID TUMOURS								
Canada	Single-centre, ret	285	N/A	Cauc	ASP (E.Coli)	CGA	*ASNS*	rs3832526	39
Hungary	Multi-centre (9), pro	505	6.2	N/A	ASP (E.Coli)	CGA	*GRIA1*	rs2055083, rs707176, rs4958351 AA/AG genotype[d] in T-cell ALL	37
MIXED TUMOURS								
Slovenia	Multi-centre (13), pro	146	7.2	Cauc	ASP (E.Coli)	CGA	*GRIA1*	rs4958351rs10070447 rs4958676 rs6889909, rs6890057	38

Abbreviations: ASP; Aspariginase: No; Number of patients; Ret; retrospective; Pros; prospective

Table 1 Single Nucleotide Polymorphisms associated with Neurotoxicity associate with Vincristine, (b) not associated with Vincristine

Table 2 Single Nucleotide Polymorphisms associated not associated with Vincristine

Table 3. Single Nucleotide Polymorphisms associated with Cardiotoxicity

Table 4 Single Nucleotide Polymorphisms associated with Osteonecrosis

Table 5: Single Nucleotide Polymorphisms associated with Hypersensitivity to Asparaginase

Supplementary Table S1: Single Nucleotide Polymorphisms associated with Nephrotoxicity

Supplemental Table S2: Single Nucleotide Polymorphisms associated with Gastrointestinal toxicity

Supplemental Table S3: Single Nucleotide Polymorphisms associated with Hepatotoxicity

Supplemental Table S4: Single Nucleotide Polymorphisms associated with Other Toxicities

Supplemental Table S5: Single Nucleotide Polymorphisms described in Systematic Reviews and Meta-analysis

Supplemental Table S6: Single Nucleotide Polymorphisms associated with Myelotoxicity

Supplemental Table S7: Single Nucleotide Polymorphisms associated with Myelotoxicity