

Pharmacogenetics of chemotherapy treatment response and -toxicities in patients with osteosarcoma: a systematic review of 2010-2020

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Abstract

Background: Osteosarcoma is the most common bone tumor in children and adolescents. Despite multiagent chemotherapy, only 71% of patients survives and these survivors often experience long-term toxicities. The main objective of this systematic review is to provide an overview of the discovery of novel associations of germline polymorphisms with treatment response and/or chemotherapy-induced toxicities in osteosarcoma.

Methods: MEDLINE and Embase were systematically searched (2010-2020). Genetic association studies were included if they assessed >10 germline genetic variants in > 5 genes in relevant drug pathways or if they used a genotyping array or other large-scale genetic analysis. Quality was assessed using adjusted STrengthening the REporting of Genetic Association studies (STREGA)-guidelines. To find additional evidence for the identified associations, literature was searched to identify replication studies.

Results: After screening 1533 articles, fifteen articles met our inclusion criteria. These range from studies focusing on genes in relevant pharmacokinetic pathways to whole genome sequencing. Eight articles reported on doxorubicin-induced cardiomyopathy. For seven genetic variants in CELF4, GPR35, HAS3, RARG, SLC22A17, SLC22A7 and SLC28A3, replication studies were performed, however without consistent results. Four small studies reported on bone marrow, nephro- and/or hepatotoxicity. Six studies included analysis for treatment efficacy. Genetic variants in ABCC3, ABCC5, FasL, GLDC, GSTP1 were replicated in studies using heterogeneous efficacy outcomes.

Conclusions: Despite that results are promising, the majority of associations were poorly reproducible due to small patient cohorts. For the future, hypothesis-generating studies in large patient cohorts will be necessary, especially for cisplatin-induced ototoxicity as these are lacking. In order to form large patient cohorts, national and international collaboration will be essential.

Background

Osteosarcoma is the most common primary bone tumor, that occurs most often in adolescents between the age of 10 and 25 (1, 2). Treatment of osteosarcoma consist of surgical removal of the tumor combined with systematic pre- and postoperative chemotherapy protocols. High-dose methotrexate, doxorubicin and cisplatin form the backbone of this chemotherapy treatment (MAP regimen), combined with ifosfamide or etoposide in some regimens (3). The introduction of this chemotherapeutic treatment in the 1970s has drastically improved survival rates compared to surgery alone (1). However, no major improvements in the treatment protocol have been made since then, with survival rates remaining 71% in the latest publications of the Euramos-1 trial (4).

Despite the positive effect of the MAP regimen on survival rates, patients also develop toxicities that can have a major effect on patients' quality of life, during and after treatment. Moreover, severe toxicities can force oncologists to modify or even discontinue chemotherapy treatment, risking an unfavorable effect on tumor eradication. High-dose methotrexate can lead to bone marrow suppression, liver (hepato-) and renal (nephro-) toxicities (5). The most treatment-limiting side effect of doxorubicin is cardiotoxicity, which can develop during treatment, but most often manifests more than a year after treatment (6). Consequently, pediatric cancer survivors have a 5-15 times higher risk to develop congestive heart failure compared to the general population (7). Lastly, cisplatin can cause acute renal damage and long-term renal insufficiency and above all, cisplatin-induced hearing loss (ototoxicity). This ototoxicity is often irreversible and has a significant impact on quality of life. Approximately 60% of all patients treated with cisplatin will develop some form of ototoxicity, of which the risk increases significantly with exposure and the final cumulative dose (8).

Gaining more insight in the cause and the development of toxicities could lead to more personalized therapies, without compromising on survival rates. Clinical factors, such as age, sex, anthracycline- and cisplatin dose and kidney function, are known to contribute to the risk of developing these different toxicities (7, 9). Metastasis at diagnosis is the best validated predictor for treatment response and used in current protocols for stratification (4, 10, 11). However, this has not led to individualized treatment protocols so far. In addition, these clinical predictors do not explain all interpatient variation. Gaining more insight in genetic risk factors for survival and risk of toxicities (pharmacogenomics) may lead to a better understanding of biological mechanisms behind the different phenotypes and may ultimately lead to improved prediction models for these phenotypes seen in clinical practice.

The main goal of genetic association studies in the field of pharmacogenomics is to identify genetic variants which may explain interpatient variability in drug response, to improve drug efficacy and reduce the risk of drug-induced toxicities (12). In patients with osteosarcoma, pharmacogenomics may also give new opportunities to optimize treatment response and reduce toxicities. Most studies in this field have been focusing on genes that were already known to be involved in the working mechanisms of the treatments used, as described previously by our group in 2016 (13). So far, this has not resulted in the identification of genes that can be used in the clinical setting. Investigating the genetic background of treatment outcome in a non-hypothesis driven manner has the advantage that new unexpected genes might be uncovered. An illustrative example is the association between *ACYP2* and ototoxicity in patients with pediatric brain tumors which was identified in a genome-wide association study (GWAS) (14). This association was replicated in other patients, e.g. osteosarcoma patients (15) and adult testicular cancer patients (16) and these were combined in meta-analyses (17, 18). In all studies, patients with the AA genotype have an increased risk of ototoxicity, however the evidence is not yet strong enough to implement interventions based on this association in clinical practice. With regard to hypothesis-generating studies, osteosarcoma patients are especially suitable, because treatment has been consistent for a long time and is comparable around the world allowing the formation of larger homogenous cohorts in this relatively rare disease. The size of a homogenous patient cohort is an issue for many pediatric pharmacogenetic studies, because cohorts of patients with multiple diseases treated with similar medication are often combined. Discovery of novel associations in osteosarcoma cohorts reduces variance in the data and may accelerate the path towards the implementation to clinical practice.

This current review aims to systematically summarize the findings of hypothesis-generating pharmacogenetic studies that included patients with osteosarcoma. More specifically, we studied literature about the discovery of novel associations of germline polymorphisms with treatment response and/or chemotherapy-induced toxicities (bone marrow, hepato-, nephro-, oto-, and cardiotoxicity) in osteosarcoma patients, published in the last decade. In addition to that, replication studies of these discoveries were summarized to determine the strength of the evidence at this point in time.

Methods

Systematic search

The aim of this systematic review was to assess the currently available literature about the discovery of novel genetic variants involved in treatment response or treatment toxicity in patients with osteosarcoma. MEDLINE and Embase were systematically searched for relevant publications between 2010 and 2020. The search strategy in both electronic databases consisted of three elements connected by AND, as shown in Figure S1. The first element describes the patient group, which are patients with osteosarcoma. To include articles with patient cohorts with multiple diagnoses, a search term is added to describe pediatric patients with cancer, studying toxicities of cisplatin, doxorubicin or methotrexate. It was assessed manually if these mixed cohorts also include patients with osteosarcoma. The second element defines that articles should investigate genetic variation or be genome-wide association studies. The third element defines all outcomes of interest. These include outcomes that describe treatment efficacy, for example overall survival, event-free survival, disease progression or relapse. In addition, search terms are added to include toxicities of interest: ototoxicity, nephrotoxicity, bone marrow toxicity, cardiotoxicity and hepatotoxicity. These toxicities were studied, because patients with these side effects are most at risk for a dose reduction or discontinuation of treatment, which may have negative effects on treatment efficacy. In the final search strategy, all keywords with their synonyms, MeSH terms (Pubmed), emtree terms (Embase) were searched, as described in Table S1. Cochrane was searched manually with similar search words as the other databases. The date of the literature search was 30th of September 2020.

All articles were screened systematically by two independent reviewers (EH and AB) for eligibility of titles. Thereafter, abstracts were reviewed and before selection for full text, a secondary abstract selection was performed to filter for genetic association studies with available full text articles. During full text selection, the number of variants and genes that are genotyped for the publications were recorded to be able to filter out small candidate gene studies or replication studies. Genetic association studies are included if they assess more than 10 germline genetic variants in more than 5 genes in relevant drug pathways or if they use a genotyping array or other large-scale genetic analysis. The aim of the study should be to generate novel hypotheses as to which genes and/or genetic variants play a role in one of our outcomes of interest in patients with osteosarcoma. Exclusion criteria include: no osteosarcoma patients, replication studies, case reports, review articles, animal studies, in vitro studies, tumor DNA is studied or only a conference abstract is available. In case conflicts between reviewers occurred, a third independent reviewer evaluated documents leading to consensus (MC). The same reviewers (EH and AB) also independently collected and reported results (including study design, cohort size, genotyping methods, phenotype, associated variants, effect size with confidence intervals and p-values) of the studies that met the inclusion criteria.

Quality assessment

Quality assessment was performed to assess quality of the articles that were found in this systematic review. The quality assessment form was adapted from van Vugt and colleagues (Table S5), who adapted a previously published tool (19, 20). Briefly, the STrengthening the REporting of Genetic Association studies (STREGA) guidelines for reporting of genetic association studies was adjusted to be more applicable to pharmacogenetic studies (20, 21).

Follow-up research

In order to find additional evidence for the associations that were found in the systematic search, a structured literature search was performed to identify studies that aim to replicate these associations. This included a search of the 91 genetic association studies identified during selection of the articles of the systematic search. In addition, PharmGKB and Pubmed were searched manually using the gene and variant of interest, doxorubicin and/or cardiotoxicity as search words. Furthermore, Web of Science was consulted to assess all articles that cited one of our selected articles. The articles that contained relevant information on replication of the associations found in the systematic search are included in the results.

Results

Literature search

The systematic search in MEDLINE and Embase yielded a total of 1533 publications. The manual search in Cochrane yielded no additional publications. During the screening process, 229 duplicates were removed, and 1053 articles were removed because the title was not applicable to our research question (Figure 1). Consequently, the abstract screening was performed in 2 phases. In the first phase, 113 articles were removed because they were other studies than (pharmaco)genetic association studies, and secondly, all remaining studies were assessed again to make sure the full text was available, and the study included patients with osteosarcoma. In the full-text assessment, an inventory was made of the number of variants and genes studied in each article (Table S2 and S3). Twenty articles, studying >10 variants in >5 genes, passed our inclusion criteria. Of these, two articles were excluded because they were not hypothesis-generating (22, 23), two did not have osteosarcoma patients in the discovery phase of the study (24, 25) and one article only studied methotrexate (MTX) pharmacokinetics, but not one of the outcomes of our interest (26). Table S4 shows an overview of the

articles that were excluded in the last phases of the selection as a result of the pre-defined inclusion criteria, as indicated with an asterisk in Figure 1. Eventually, fifteen articles were explored further in this systematic review.

Quality assessment

The 15 studies that resulted from the systematic search were assessed for their quality of reporting and the results to the questions are presented in Table S6. The reporting of basic characteristics of the patient cohort was sufficient in 13 of 15 studies (87%). As shown in Table S7, these 13 studies all reported on the sex, age and diagnosis of the patients. The reporting of additional characteristics depended on the main outcome that was studied. Of the six articles on treatment efficacy, five reported on metastasis at diagnosis, four on the location of the osteosarcoma (axial or not) and four on the histological subtype of the tumor. The most important characteristics of the eight studies focusing on cardiotoxicity were follow-up time (7/8), radiation involving chest region (6/8) and anthracycline cumulative dose (6/8). Power calculations are lacking in 12 of the 15 studies (80%). Three studies performed a retrospective power calculation to explain why variants that were previously found, were not found in their study. Seven studies contained a validation cohort to replicate their findings.

For genotyping, five studies used a genome-wide array, one study used whole genome sequencing, four studies assessed an array with variants in genes involved in absorption, distribution, metabolism and excretion (ADME) of medicines and one used a chip with genes involved in cardiovascular disease. Four studies did manual genotyping with (multiplex) allelic discrimination assays. All studies that did not analyze their data as a GWAS, had a clear rationale in their introduction or methods regarding the choice of variants (e.g. ADME or cardiovascular disease variants). Regarding quality control of the genotyping data, nine studies reported on the exclusion of variants based on call rates, with cut-offs ranging from 0.85 to 0.99. Hardy Weinberg equilibrium (HWE) calculations were performed in 13 studies and lead to exclusion of variants in 12 of these articles if a variant deviated from HWE. During statistical analyses 12 studies assessed the effects of covariates and corrected for them in their genetic association analysis accordingly. Finally, ten studies used a form of correction for multiple testing, ranging from the strict Bonferroni correction to adjusting to a stricter p-value threshold without further explanation of rationale.

Treatment response

Six of the fifteen studies identified with the systematic review focused on the role of genetic variants in treatment response or treatment efficacy of chemotherapy in patients with osteosarcoma (Table 1). A variation of treatment outcomes was used to describe treatment response, including progression free survival (PFS), event free survival (EFS), overall survival (OS), histological response, relapse and tumor necrosis.

Table 1

Study design, population characteristics and outcomes of hypothesis-generating pharmacogenetic studies investigating the association between genetic variants and treatment response.

Author, year	Study design				Associated phenotype(s)	Associated variant(s)	Ref.
	Study approach	No. of osteosarcoma patients	Ethnicity; nationality	Investigated number of variants and genes			
Bhuvaneshwar <i>et al.</i> , 2019	Whole genome sequencing	INOVA: 15	INOVA: NS; American	Mutation hotspot haplotypes	Relapse	Haplotypes in 26 genes	(41)
		TARGET: 85	TARGET: NS; Canadian/Brazilian				
				4,543 variants in ADME genes	Tumor necrosis and survival	<i>SLC22A1</i> rs4646272*	
						<i>SLC22A8</i> rs2187384*	
						<i>UGT2B15</i> rs34073924*	
						<i>CHST12</i> rs3735099*	
						<i>CHST12</i> rs3735100*	
Caronia <i>et al.</i> , 2011	Pathway approach	102	NS; Spanish	366 variants in 24 metabolism and transporter genes	OS	<i>ABCC3</i> rs4148416	(27)
					EFS	<i>ABCB1</i> rs4148737	
						<i>ABCB1</i> rs1128503	
						<i>ABCB1</i> rs10276036	
Hagleitner <i>et al.</i> , 2015	Pathway approach	Disc.: 126 Repl.: 64	NS; Dutch	381 variants in 54 drug metabolism genes	PFS	<i>FasL</i> rs763110*	(34)
						<i>MSH2</i> rs4638843*	
						<i>ABCC5</i> rs939338*	
						<i>CASP3</i> rs2720376*	
						<i>CYP3A4</i> rs4646437*	
Hattinger <i>et al.</i> , 2016	Pathway approach	126	NS; Italian	47 variants in 31 drug metabolism and transport genes	EFS	<i>ABCC2</i> rs2273697*	(31)
						<i>GGH</i> rs11545078*	
						<i>TP53</i> rs1642785*	
						<i>CYP2B6</i> *6*	
Koster <i>et al.</i> , 2018	GWAS	GWAS: 523	GWAS: >80% European	510,856 variants	OS	<i>GLDC</i> rs3765555	(40)
		Repl.: 109	Repl.: NS; Brazilian			<i>GLDC</i> rs55933544	
Windsor <i>et al.</i> , 2012	Pathway approach	58	Caucasian: 41	35 variants in 21 pharmacological pathway genes of MAP.	PFS	<i>CCND1</i> rs9344 *	(32)
			Afro-Caribbean: 8			<i>RFC</i> rs1051266*	
			Indian/Asian: 9			<i>GSTT1</i> null*	
				<i>GSTP1</i> rs1695*			
				Histological response	<i>ABCC2</i> rs717620*		
					<i>GSTP1</i> rs1695*		
		<i>MTHFD1</i> rs2236225*					

GWAS: genome-wide association study; INOVA: Inova pediatric group osteosarcoma patients; TARGET: TARGET osteosarcoma dataset; Disc.: discovery cohort; Repl.: replication cohort; NS: Not specified; ADME, absorption, distribution, metabolism and excretion; MAP: Methotrexate – Adriamycin (Doxorubicin) – Cisplatin chemotherapy regimen; OS: overall survival; EFS: event-free survival; PFS; progression-free survival

* Association was not significant after multiple testing correction, but is/are the top hit(s) of the study.

Four of the six studies investigated treatment response in a broad panel of variants in genes involved in metabolism and transport of cisplatin, doxorubicin and methotrexate, as shown in Table 1. In the study by Caronia *et al.*, *ABCC3* rs4148416 was associated with EFS, with a hazard ratio of 6.33 (95%CI 1.79-12.7, p=0.0021)(27). This was the first evidence of the genes' clinical relevance in osteosarcoma treatment response and this was

replicated successfully in two Chinese populations with consistent directions of effects, as shown in Figure 2 and Table S8 (28, 29). In addition, Caronia *et al.* identified three variants in *ABCB1* to be associated with both EFS and OS (rs4148737, rs1128503, rs10276036)(27). The T-allele of the *ABCB1* rs1128503 was significantly associated with improved survival. However, replication studies of this variant yielded contradictory results, namely, one study replicated this significant association with consistent directions of effect (29), two studies found significant associations in which the T-allele was a risk allele instead of a protective allele (28, 30) and two studies found no significant associations with osteosarcoma treatment response whatsoever (31, 32). The second study using a pathway approach was the study by Hattinger *et al.*. They showed that *GGH*rs11545078, *CYP2B6**6 and *TP53* rs1642785 and *ABCC2* rs2273697 are associated with EFS (31). However, none of these four variants retained statistical significance in a multiparametric Cox proportional hazard regression analysis (31). Despite that Windsor *et al.* could not replicate the association of *ABCC2* rs2273697 with EFS, another variant in the *ABCC2* gene, namely rs717620, was identified to be associated with poor histological response (32). However, this association was not confirmed in four replications studies (28, 31, 33, 34). In addition, the presence of the G-allele of *GSTP1* rs1695 was associated with poor histological response and with PFS and a variant in *RFC1/SLC19A1* (rs1051266) was significantly associated to PFS. Three of the six studies that also assessed *GSTP1* rs1695 and one of three studies that studied *RFC1/SLC19A1* rs1051266 in association to EFS or OS, also found a significant association, as shown in Figure 2 and Table S8 (29–31, 33, 35–38). Lastly, the study performed by Hagleitner *et al.* included a discovery cohort of 126 osteosarcoma patients and a replication cohort of 64 patients (34). Five variants were identified to be significantly associated with PFS (Table 1), including *FasL* rs763110, *MSH2* rs4638843, *ABCC5* rs939338, *CASP3* rs2720376, *CYP3A4* rs4646437. Genetic risk scores were generated based on these five variants, using the number of unfavorable alleles patients had for these variants. This risk score was able to distinguish between patients with good and poor outcome, both in patients with and without metastases (34). In the replication cohort by Xu *et al.*, only *FasL* rs763110 and *ABCC5* rs939338 contributed to the risk score to predict treatment outcome (39). Overall, all studies that used a pathway approach found novel genetic variants that may play a role in the response to treatment of osteosarcoma, however, the study by Caronia *et al.* was the only one large enough to correct for multiple testing.

A genome-wide association study (GWAS) including 523 osteosarcoma patients was performed by Koster *et al.* in 2018. They investigated 510,856 genetic variants in relation to OS (40). The variant which had the strongest association with OS, namely rs1030228, could not be replicated in their replication cohort, consisting of 109 osteosarcoma patients. However, in a combined analysis of the discovery cohort and replication cohort, another variant, rs3765555, was inversely associated with OS (HR = 1.76 per copy of the A-allele). Imputation of the region centered around this variant identified a second variant, rs55933544, significantly associated with OS (HR = 1.92 95% CI 1.53-2.41, $p=1.34 \times 10^{-8}$). Lin *et al.* replicated the association of rs55933544 with OS in their patient cohort with an odds ratio of 2.98 (95% CI 1.87-4.96, $p<0.001$). Furthermore, expression quantitative trait locus (eQTL) analysis showed that the T-allele of rs55933544 was significantly associated with a decreased *IL33* expression and lower *IL33* expression was independently associated with worse osteosarcoma patient survival (40). The study by Bhuvaneshwar *et al.* used whole genome sequencing data to identify haplotypes associated with relapse (41). Using both the TARGET and INOVA patient datasets, 231 haplotypes were described of which the variants could be mapped to 26 genes. From the haplotypes, only four variants in *MKI67*, one in *CACNA2D4*, three in *SLC13A2* and two in *PPP1R12C* were associated with relapse in both patient datasets independently. These variants were not previously indicated in osteosarcoma treatment response and no replication studies have been performed yet. Bhuvaneshwar *et al.* also extracted variants in ADME genes from their dataset to investigate the association with tumor necrosis and OS. A total of 281 variants were associated with tumor necrosis and five of these variants, in *SLC22A1*, *SLC22A8*, *CHST12* and *UGT2B15*, were also associated with OS, prioritizing these for future research.

Doxorubicin-induced cardiotoxicity

Eight studies reported on pharmacogenetics concerning doxorubicin-induced cardiotoxicity (Table 2). These range from small studies with limited numbers of patient and small numbers of variants studied to GWASs with larger patient cohorts. In an exploratory study by Windsor *et al.*, 36 genetic variants in 21 genes in the pharmacokinetic pathways of cisplatin, doxorubicin and methotrexate were investigated in relation to multiple outcomes in a patient cohort of 58 osteosarcoma patients (32). With regard to doxorubicin-induced cardiotoxicity, the G-allele of *GSTP1* rs1695 was associated to both early and end-of-treatment cardiotoxicity. This variant was previously mainly indicated in treatment efficacy, as described above, however not in cardiotoxicity. Due to the exploratory nature of this study and the small patient cohort, these associations were not corrected for multiple testing. No replication studies of this association were identified in our search (Table S9). In another study, Hildebrandt *et al.* assesses 12 loci that were previously indicated in hypertension by a GWAS (42). They found that the G-allele of *PLCE1* rs932764 and the G-allele of *ATP2B1* rs17249754 are protective to doxorubicin-induced cardiotoxicity in pediatric cancer survivors. In addition, they showed that doxorubicin exposure to iPSC-cardiomyocytes is associated to decreased *PLCE1* expression and increased *ATP2B1* expression in a dose-dependent manner.

Table 2

Study design, population characteristics and outcomes of hypothesis-generating pharmacogenetic studies investigating anthracycline-induced cardiotoxicity in patients with osteosarcoma

Author (year)	Study design				Associated variant(s)	Ref.
	Study approach	No. of osteosarcoma patients	Ethnicity; nationality	Investigated number of variants and genes		
Aminkeng <i>et al.</i> , 2015	GWAS	Stage 1: 11 (of 280)	Stage 1: Caucasian; Canadian	738,432 variants	<i>RARG</i> rs2229774	(54)
		Stage 2: 9 (of 96)	Stage 2: Caucasian; Dutch			
		Stage 3: 5 (of 80)	Stage 3: African, East Asian and Aboriginal			
Hildebrandt <i>et al.</i> , 2017	Hypertension loci	41 (of 108)	White, hispanic or black; American	12 variants in <i>PLCE1</i> , <i>ATP2B1</i> , <i>ARHGAP42</i> , <i>GNAS-EDN3</i> , <i>C10orf107</i> , <i>CSK</i> , <i>BAG6</i> , <i>CACNB2</i> , <i>MTHFR</i> , <i>CACNB2</i> , <i>HFE</i> , <i>NPR3</i>	<i>PLCE1</i> rs932764* <i>ATP2B1</i> rs17249754*	(42)
Ruiz-Pinto <i>et al.</i> , 2017	Exome array with low frequency variants	15 (of 93)	NS; Spanish	247,870 variants	<i>GPR35</i> rs12468485	(48)
Visscher <i>et al.</i> , 2012	ADME panel	Disc.: 11 (of 156)	Disc. and repl.: (non-) European; Canadian	1,931 variants in 220 drug bio-transformation genes	<i>SLC28A3</i> rs7853758	(43)
		Repl.: 10 (of 188)	Repl.2: NS; Dutch			
		Repl.2: 6 (of 96)				
Visscher <i>et al.</i> , 2015	ADME panel	Discover.: 21 (of 344)	NS; Canadian and Dutch	4,153 variants in 300 pharmacokinetics and -dynamics genes	<i>SLC22A17</i> rs4982753* <i>SLC22A7</i> rs4149178*	(49)
		Repl.: 16 (of 218)				
Wang <i>et al.</i> , 2014	Cardiovascular panel: gene – environment interaction	Disc.: 54 ^a (of 287) Repl.: 0 (of 76)	Disc.: Non-Hispanic white; American Repl.: Non-Hispanic white, Hispanic, other; American	34,912 variants in 2,100 genes associated with cardiovascular disease.	<i>HAS3</i> rs2232228	(51)
Wang <i>et al.</i> , 2016	GWAS: gene – environment interaction	Disc.: 96 ^b (of 331) Repl.: 17 (of 54)	Disc.: Non-Hispanic white; American Repl.: Non-Hispanic white, Hispanic, Other; American	709,358 variants	<i>CELFA</i> rs1786814	(52)
Windsor <i>et al.</i> , 2012	Pathway approach	58 (of 58)	Caucasian, Afro-Caribbean, Indian, Asian; UK	35 variants in 21 pharmacological pathway genes of MAP.	<i>GSTP1</i> rs1695*	(32)
NS, not specified; GWAS, genome-wide association study; ADME, absorption, distribution, metabolism and excretion; disc., discovery cohort; repl., replication cohort; MAP: methotrexate, adriamycin (doxorubicin), cisplatin chemotherapy regimen.						
^a Number of bone tumors, osteosarcoma is not specified.						
^b Number of sarcomas, osteosarcoma is not specified.						
* Association was not significant after multiple testing correction, but is/are the top hit(s) of the study.						

In two pharmacogenetic studies by Visscher *et al.*, both studying a broad panel of ADME genes, multiple variants in the Solute Carrier (SLC) family were significantly associated with anthracycline-induced cardiotoxicity. In their patient cohorts, approximately 80% of patients was treated with doxorubicin

and the rest was treated with other anthracyclines, most often daunorubicin. In the first study in 2012, the A-allele of *SLC28A3* rs7853758 was found more often in controls than in cases, and significantly associated in a protective manner (43). Another variant in the same gene (rs4877847) was also significantly associated with anthracycline-induced cardiotoxicity, even after conditioning for rs7853758 thus suggesting an independent effect. The association of the top-hit (rs7853758) was replicated in the same study in an independent patient cohort. In addition, Visscher *et al.* published a validation study in 2013 in which this effect was strengthened in a meta-analysis (22). The additional power of this meta-analysis also gained a novel top-hit association. The A-allele of *UGT1A6* rs17863783 was found to be significantly associated with anthracycline-induced cardiotoxicity (OR (95%CI) = 4.30 (1.97-9.36), $p = 2.4 \times 10^{-4}$). Of the additional nine patient cohorts that attempted to replicate the *SLC28A3* rs7853758 association (44–47), only two cohorts succeeded, as shown in Figure 2 and Table S9 (24, 48). Interestingly, the associations were only found in pediatric patient cohorts and the effect was never found in adults. In their second discovery study, Visscher *et al.* studied a more broad ADME panel, containing 4153 variants in 300 genes. For both *SLC22A17* rs4982753 and *SLC22A7* rs41491178, the minor allele was found more often in controls than in cases and had a protective effect for developing cardiotoxicity after doxorubicin treatment (49). Both variants were not statistically significant after a strict Bonferroni correction, but finding two variants associated with cardiotoxicity within the SLC22 gene family was significantly higher than expected by chance alone. In addition, the associations were successfully replicated in an independent patient cohort in the same study. However, the associations were not found in a subsequent candidate gene study (24) and GWAS (50).

A study by Wang *et al.* focused on genes that were previously associated with cardiovascular disease, since cardiotoxicity risk is influenced by coexistence of cardiovascular disease risk factors like hypertension and diabetes (51). In this study, cumulative anthracycline exposure was calculated by multiplying the cumulative dose of individual anthracyclines with a factor that describes the drug's cardiotoxic potential, but it was not indicated what percentage of the cohort received which anthracycline. Despite that, for this systematic review it was assumed that the majority was treated with doxorubicin. No variants were associated with anthracycline-induced cardiomyopathy, but a gene-environment interaction analysis identified the variant *HAS3* rs2232228. Among patients with the GG genotype for this variant, cardiomyopathy was infrequent and not dose related. However, the AA genotype conferred an 8.9-fold increased cardiomyopathy risk when also exposed to anthracycline doses > 250 mg/m², compared to the GG genotype. In the GWAS that Wang *et al.* executed two years later, they found no variants associated with anthracycline-induced cardiomyopathy (52). Again, they carried out a gene-environment analysis, which identified *CELF4* rs1786814 to have a gene-environment interaction with anthracycline dose. Patients with the *CELF4* rs1786814 GG genotype who were exposed to anthracycline levels greater than 300 mg/m², had a 10.2-fold increased risk of cardiomyopathy compared to patients with the GA/AA genotype and exposure to anthracycline levels of 300 mg/m² or lower. Among other variants, *HAS3* rs2232228 and *CELF4* rs1786814 were studied by Leger *et al.* in hematopoietic cell transplantation survivors treated with anthracyclines. For the association of *CELF4* rs1786814 with cardiomyopathy, the interaction between SNP and anthracycline dose was found, with $p=0.02$ (53). In addition, a significant association was found with an analysis limited to anthracycline doses >300 mg/m² (1-sided $p=0.01$; Table S9). The interaction of *HAS3* rs2232228 AG genotype and anthracycline dose had a 1-sided $p=0.01$ in this study. When restricting the analysis to anthracycline doses >250 mg/m², a significant association was found (Table S9). However, no significant association was found when studying the main effects of the variants in a complete patient cohort containing all dosages with short- or long term cardiomyopathy, for neither *HAS3* rs2232228 and *CELF4* rs1786814.

In a GWAS by Aminkeg *et al.* an association was found which was also replicated in their two replication patient cohorts (54). In all cohorts, the majority of patients was treated with doxorubicin, followed by daunorubicin and epirubicin. The SNP, *RARG* rs2229774, was associated with anthracycline-induced cardiotoxicity for both patients receiving a low to moderate anthracycline dose and patients receiving a high anthracycline dose. Overall, rs2229774 carriers (AA or AG genotype) had significantly increased odds of developing cardiotoxicity in comparison to non-carriers after doxorubicin treatment (OR (95%CI) = 4.7 (2.7-8.3), $p = 4.3 \times 10^{-11}$). The only other replication study that found a significant association, was the study by Schneider and colleagues in 2017 (50). However, they found that the A-allele causes a decreased risk rather than an increased risk for cardiotoxicity (Table S9). They argue that difference in direction of the effect may be due to the general heterogeneity between studies.

The genome-wide analysis of Ruiz-Pinto *et al.* focused on low frequency exome variants, as they used an exome array that is enriched with low frequency variants (80% of variants with minor allele frequency (MAF) $\leq 1\%$) (48). No variant showed a significant association with chronic anthracycline-induced cardiotoxicity after correction for multiple testing, but a novel significant association for *GPR35* was identified by gene-based testing. The SNP rs12468485 made the greatest contribution toward the observed association. The T-allele was almost exclusively found in cases, and 67% of cases carrying the CT genotype had an extreme chronic cardiotoxicity phenotype. Up to now, no replications of this association have been attempted.

Cisplatin-induced ototoxicity

In this systematic review, no publications were identified that studied the association of genetic variants with cisplatin-induced ototoxicity in patients with osteosarcoma. Windsor *et al.* planned on including ototoxicity in the genetic association study described above (32). However, this analysis was not performed because of incomplete data.

Bone marrow- hepato- and nephrotoxicity

Four studies were identified that focused on nephrotoxicity, hepatotoxicity and/or bone marrow toxicity in the systematic search. Table 3 shows that studies by Hattinger *et al.*, Hegyi *et al.*, and Windsor *et al.* are among the smallest studies in this review, as they studied 45, 29 and 36 variants in cohorts of 57, 59 and 58 osteosarcoma patients, respectively (31, 32, 55). Whereas significant associations were found, none of these studies corrected for multiple testing and that was also reflected by many replication studies with negative results. The results of the *ABCC2* gene is a clarifying example for

this. rs2273697 was associated with hepatotoxicity and thrombocytopenia by Hattinger *et al.*, however, as Figure 2 indicates, six studies that also related this variant to hepatotoxicity did not find a significant association (31–33, 55–58), negative results were also found in four studies that related this variant to thrombocytopenia (31, 32, 56–58). In addition, rs2273697 was found to be associated with leukopenia according to both Hattinger *et al.* and Hegyi *et al.*, but this association was not found in 4 other cohorts (31, 32, 55–58). Lastly, Hegyi *et al.* and Windsor *et al.* found that *ABCC2* variants rs3740066 and rs17222723, respectively, were associated with leukopenia. However these associations were not replicated in any of the replication studies (31, 32, 55, 58). Altogether, multiple replication studies were performed of the initial findings, but none of the replication studies could confirm these (Table S10).

Table 3

Study design, population characteristics and outcomes of hypothesis-generating pharmacogenetic studies investigating bone marrow- hepato- or nephrotoxicity in patients with osteosarcoma

Author, year	Study design					Associated phenotype(s)	Associated variant(s)	Ref.
	Study approach	No. of osteosarcoma patients	Ethnicity; nationality	Investigated number of variants and genes	Investigated phenotypes			
Hattinger et al., 2016	Pathway approach	57	NS; Italian	45 variants in 31 drug metabolism and transport genes	Leukopenia, thrombocytopenia, red blood cell transfusion, platelet transfusion, hepatotoxicity	Leukopenia	<i>ABCC2</i> rs2273697 *	(31)
							<i>MTHFR</i> rs1801131 *	
						Thrombocytopenia	<i>ABCC2</i> rs2273697 *	
							<i>XPD</i> rs1799793 *	
						Hepatotoxicity	<i>ABCB1</i> rs1128503 *	
							<i>ABCC2</i> rs2273697 *	
	<i>GGH</i> rs1800909 ^{HW} *							
Hegyí et al., 2017	Pathway approach	59	NS; Hungarian	29 variants in <i>ABCB1, ABCC1, ABCC2, ABCC3, ABCC10, ABCG2, GGH, SLC19A1, NR1I2</i>	Leukocyte/ neutrophil granulocyte count	Myelotoxicity	<i>ABCC2</i> rs2273697 *	(55)
							<i>ABCC2</i> rs3740066 *	
							<i>NR1I2</i> rs3732361 *	
							<i>NR1I2</i> rs3814058 *	
							<i>NR1I2</i> rs6785049 *	
						Hepatotoxicity	<i>NR1I2</i> rs3732361 *	
	<i>NR1I2</i> rs3814058 *							
	<i>NR1I2</i> rs6785049 *							
Hurkmans et al., 2020	ADME panel	113	Caucasian; Dutch, Spanish, Australian	1936 variants in 231 ADME genes	Creatinine, ALAT, ASAT, hemoglobin, thrombocyte, leukocyte and neutrophil counts	Thrombocyte counts	<i>CYP2B6</i> rs4803418	(58)
							<i>CYP4F8</i> rs4808326	
							<i>CYP2B6</i> rs4803419	
Windsor et al., 2012	Pathway approach	58	Caucasian: 41	36 variants in 21 pharmacological pathway genes of MAP	Anemia, leucopenia, myelo-suppression, GFR, infection	Leucopenia	<i>ERCC1</i> rs3212986 *	(32)
			Afro-Caribbean: 8			<i>GSTP1</i> rs1695 *		
			Indian/Asian: 9			<i>ABCC2</i> rs17222723 *		
						Anemia	<i>MTHFD1</i> rs2236225 *	
						<i>MTHFR</i> rs1801131 *		
	<i>CYBA</i> rs4673 *							

Author, year	Study design		Ethnicity; nationality	Investigated number of variants and genes	Investigated phenotypes	Associated phenotype(s)	Associated variant(s)	Ref.
	Study approach	No. of osteosarcoma patients						
						Infection	<i>XPC</i> rs2228001*	
						Nephrotoxicity	<i>ERCC2</i> rs13181*	
							<i>MTHFR</i> rs1801133*	
NS, Not specified; ADME, absorption, distribution, metabolism and excretion; MAP: Methotrexate – Adriamycin (Doxorubicin) – Cisplatin chemotherapy regimen; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase								
* Association was not significant after multiple testing correction, but is/are the top hit(s) of the study.								

The association of *MTHFR* variant rs1801131 with anemia that was identified by Windsor *et al.*, was conformed in two cohorts of acute lymphatic leukemia patients, but the association was not significant in five other studies (32, 59–65). In addition, Hattinger *et al.* showed that this variant is also associated to leukopenia. Whereas ten studies also assessed this association, there was only one study with a significant result, however with an opposite direction of effect (31, 59–61, 64–70). The only association that shows consistent replication is the association of *ERCC2* variant rs13181 with nephrotoxicity, that was identified by Windsor *et al.*. In four of six patient cohorts treated with cisplatin-based treatment, the association of the AC or CC genotype was associated with increased risk to develop nephrotoxicity was confirmed, with odds ratios ranging from 3.16 to 4.4 (32, 71–75).

Finally, the study by Hurkmans *et al.* assessed a panel of 1936 variants in 231 genes involved in absorption, distribution, metabolism and excretion of medicines for multiple toxicities (58). Three variants in the Cytochrome P450 family were significantly associated with thrombocyte count, namely *CYP4F8* rs4808326, *CYP2B6* rs4803418 and *CYP2B6* rs4803419, and these remained significant after Bonferroni correction for multiple testing. The two variants in *CYP2B6* were in high linkage disequilibrium, and thus likely represent the same locus. Regarding *CYP4F8* rs4808326, carriers of the A-allele had higher thrombocyte counts after methotrexate infusion compared to carriers of the G-allele. The gene has not been linked to methotrexate or thrombocyte count before. The underlying mechanisms of the associations with all three variants are still unclear (58). These associations are not replicated in other patient cohorts yet, as the publication was recent.

Discussion

This systematic review provides an overview of hypothesis-generating pharmacogenetic studies in osteosarcoma patients of the last 10 years. In addition, replication studies of all top-hit associations of the studies were identified in a structured manner to give a more complete idea of the evidence that is present. Treatment response and doxorubicin-induced cardiotoxicity are the most extensively studied phenotypes. Chemotherapy-induced nephrotoxicity, hepatotoxicity and bone marrow toxicity were examined, but only in small patient cohorts. The influence of genetic variants on cisplatin-induced ototoxicity was not investigated in any of the hypothesis-generating studies.

The only GWAS that was performed in relation to treatment response to chemotherapeutic treatment in osteosarcoma patients was the GWAS by Koster *et al.* (40). In that study, two variants in the *GLDC* gene were found to be associated to overall survival. As previously mentioned, Lin *et al.* successfully replicated the association of rs55933544 with decreased overall survival in their patient cohort (76). Noteworthy, the TT genotype of this variant was not related to *GLDC* expression, but it was associated to lower expression of interleukin-33 (*IL33*) (40, 76). On top of that, Kang and colleagues genotyped common variants in the *IL33* gene and found that the A-allele of rs1048274 was associated to survival in a osteosarcoma patient cohort of Chinese ancestry (77). The patient cohort of Koster *et al.* consisted of European and Brazilian subjects and they did not identify statistically significant associations with common variants in *IL33*. Differences in linkage disequilibrium structures between populations allowed for different variants on the same locus to be associated to survival of patients with osteosarcoma, indicating that not *GLDC*, but *IL33* is causal for decreased survival through the variant. This emphasizes that studying populations of different ethnicities helps in fine mapping the genetic background that causes a phenotype. *IL33* was previously associated to prognosis in other cancers (78–80) and it is known to have pro- and anti-tumorigenic properties mediated through immune cells (81). In osteosarcoma, *IL33* plays a role in osteosarcoma cell viability in *in vitro* experiments mediated through the PI3K/AKT pathway (82, 83). However, the exact role and the effects of genetic variants remains to be found.

A pathway approach including genes linked to osteosarcoma treatment was used by most of the included studies, so consequently members of the ABC transporter family were included in the pharmacogenetic investigations and sometimes found to be associated to the outcomes of interest. These genes code for membrane-bound proteins which participate in the movement of most drugs and their metabolites across cell surface and cellular organelle membranes. Defects in these genes can be important in terms of cancer therapy and pharmacokinetics (84). As indicated in the results of this review, *ABCC2* variants were repeatedly associated to toxicities in these studies, however Figure 2 shows that these associations were scarcely replicated. Doxorubicin and methotrexate are both transport substrates for *ABCC2*, which caused these variants to be studied, but this does not necessarily explain the causative functional background of the association that is observed. On the other hand, in the pathway approach study by Caronia *et al.* it was

found that per T-allele of the *ABCC3* rs4148416 variant, patients have an 8-fold higher risk of death, and 6 times lower risk on event-free survival, and this association was consistently replicated in two other cohorts (27–29). *ABCC3* codes for multidrug resistance protein 3 (MRP3) and is an important transporter of bile salts, but is also involved in efflux of methotrexate from liver and kidney cells (85, 86).

The necessity of routine MTX plasma concentration measurement during treatment with MTX has allowed several research groups to study genetic variation involved MTX pharmacokinetics. This was not one of the clinical outcome measures of interest of this systematic review, and therefore these studies were excluded as shown in Figure 1, however, genetic variants that are associated to high MTX plasma levels may also give increased risk for toxicity and genetic variants that are associated to low MTX plasma levels may also predispose to a suboptimal treatment response. Lui *et al.* found three variants localized in *ABCG2* to be associated with methotrexate clearance in patients with osteosarcoma, namely rs13120400, rs13137622, rs12505410 (26). Rs13120400 was the most significant variant, and the CC genotype of this variant was previously also associated to increased response to methotrexate in psoriasis patients (87). In addition, in the study by Hegyi *et al.*, which included 59 osteosarcoma patients, *ABCG2* rs2231142 was found to be significantly associated with a longer half-life time of methotrexate (55). However, this variant is not in LD with variants identified by Lui *et al.* *ABCG2* codes for the breast cancer resistance protein (BCRP) and has an important role in the transport of methotrexate out of the liver and kidney and knockdown of *ABCG2* increased the bioavailability of methotrexate in mice (86, 88). Both *ABCC3* and *ABCG2* may be of interest for further investigation with a larger patient cohort to relate it to both pharmacokinetic parameters and clinical outcomes of treatment.

Six variants were found to be associated with doxorubicin-induced cardiotoxicity in a cohort containing osteosarcoma patients and were replicated minimally once in an independent patient cohort, namely *CELF4* rs1786814, *HAS3* rs2232228, *RARG* rs2229774, *SLC22A17* rs4982753, *SLC22A7* rs4149178, *SLC28A3* rs7853758. The associations with *HAS3* rs2232228 and *CELF4* rs1786814 identified by Wang *et al.*, consisted of gene-environment interactions, which means the variant effect is larger in patients that received a higher dose of doxorubicin. This emphasizes the importance of doxorubicin dose in the development of cardiotoxicity and in the effect size of genetic variant. CUGBP Elav-like family member 4 (*CELF4*) is involved in splicing of *TNNT2*, which codes for cardiac troponin T (cTnT). cTnT plays a role in Ca²⁺ signaling and contraction of the heart muscle and is a biomarker for myocardial damage (89). Whereas the embryonal *TNNT2* splicing variant, carrying an additional exon 5, is usually downregulated in adults, patients with *CELF4* rs1786814 GG genotype express both the adult and embryonal *TNNT2* splicing variant. This results in a temporally split myofilament response to calcium, decreasing the ventricular pumping efficiency, and thereby increasing the risk on dilated cardiomyopathy and cardiotoxicity (52, 90). In addition, pathogenic variants in *TNNT2* are an established cause of hypertrophic and dilated cardiomyopathy (91). Adult and pediatric patients with cancer who developed chemotherapy-induced cardiomyopathy have an increased prevalence of pathogenic variants in sarcomere genes compared to controls, indicating that genetics involved in susceptibility to cardiomyopathy, such as mutations in sarcomere genes, may also be of importance in doxorubicin-induced cardiotoxicity (92). *HAS3* encodes for hyaluronan which is a component of the extracellular matrix and is involved in tissue remodeling after cardiac damage. In addition, hyaluronan reduces cardiac injury caused by ROS, which is an important element of doxorubicin-induced cardiac damage. Furthermore, the *RARG* gene codes for retinoic acid receptor gamma and binds to the topoisomerase II β (Top2b) promoter to repress its expression. Top2b is a target of doxorubicin mediated DNA damage, and if Top2b expression is low in cardiac tissue due to repression by *RARG*, the tissue is less susceptible to damage caused by doxorubicin (54). Despite that replication studies of the association of the *RARG* variant with cardiotoxicity were inconsistent, a functional study in iPSC-derived cardiomyocytes showed that the variant *RARG* increases sensitivity to doxorubicin-induced cardiomyopathy (93). Lastly, three variants in genes of the solute carrier transporter family were associated to doxorubicin-induced cardiomyopathy. A variant downstream of *SLC22A17* was associated to cardiotoxicity. *SLC22A17* is ubiquitously expressed, also in the heart, and plays a role in iron transport and homeostasis. Accumulation of iron in mitochondria can cause doxorubicin-induced cardiotoxicity, however the exact role of *SLC22A17* in this process is not studied (94). Secondly, *SLC22A7* encodes for the organic anion transporter 2 (OAT2), which is highly expressed in liver and kidney and is known to play an important role in clearance of medicines but is not previously indicated in transport of cisplatin, doxorubicin or methotrexate. Lastly, a variant in *SLC28A3* was associated with cardiotoxicity. *SLC28A3* codes for the sodium-coupled nucleoside transporter 3 (CNT3). Only for CNT3, it was established to transport doxorubicin, indicating it may play a role in doxorubicin pharmacokinetics (95). Despite that this was not shown for OAT2, it does have considerable overlap in substrates with CNT3 and transports several nucleoside-based drugs, for example 5-fluorouracil and zidovudine (96).

While cisplatin-induced ototoxicity is one of the most prevalent adverse effects of cisplatin treatment, it was not investigated in any of the studies that were identified in this systematic review. The most recent GWAS on cisplatin-induced ototoxicity that included patients with osteosarcoma, was performed in 2009 and was therefore excluded from this review (97). In this study, Ross *et al.* identified genetic variants in the *TPMT* and *COMT* gene that are associated to cisplatin-induced hearing loss in pediatric patients with cancer. However, replication studies are very contradictory, as shown in a meta-analysis by Thiessen *et al.* in 2018 (17, 18). Whereas this was the only GWAS that included patients with osteosarcoma, work from other patient cohorts treated with cisplatin may be applicable to patients with osteosarcoma too. Xu *et al.* identified that the A-allele of *ACYP2* rs1872328 gives an increased risk to cisplatin-induced ototoxicity in a pediatric brain tumor cohort (14). In 2020, Clemens *et al.* showed in a meta-analysis of 5 studies with a total of 1418 pediatric patients that this association was also found in cohorts containing patients with osteosarcoma (OR (95%CI) = 3.94 (1.04-14.93), p = 0.04). In addition, they showed a significant association of *SLC22A2* rs316019 with cisplatin induced hearing loss in a meta-analysis of 4 studies (OR (95%CI) = 1.46 (1.07-2.00), p = 0.02). Whereas these results are significant, the heterogeneity between studies remained an obstacle ($I^2=66\%$ for *ACYP2* rs1872328 and 44% for *SLC22A2* rs316019). In conclusion, in order to find variants associated with cisplatin-induced ototoxicity, the priority would be to perform a GWAS with large patient cohorts to find reliable results. In the meantime, *ACYP2* rs1872328 and *SLC22A2* rs316019 could be studied further to find out their true potential for clinical practice.

In general, the quality of reporting data in the included studies was good, as shown with the quality assessment using STREGA guidelines. However, there was minimal reporting on follow-up and missing data. All data in the studies was collected retrospectively and there was no prospective follow-up and, therefore, it is sensible that nothing was reported on patients that were lost to follow-up. It was also not reported how missing data was handled in statistical analysis or what kind of analysis was used for the main comparison regarding the missing data. However, it is assumed that these studies did a complete case analysis, because that is most conventional in retrospective genetic association studies. Therefore, this does not compromise the quality of these articles.

The aim of this review was to identify variants that were discovered to be associated to a phenotype in a hypothesis-free manner and might form the basis for future pharmacogenomic studies in osteosarcoma. The most evident method to identify articles that describe these variants would be to limit the systematic search to GWA studies, as they are the textbook example to hypothesis-free research. However, the number of GWASs is limited in pediatric oncology cohorts and even more so in osteosarcoma cohorts. In addition, when a broad range of ADME genes is studied, it does not restrict itself to only genes that are previously indicated in the pathway of a drug. In order to include all literature with a hypothesis-free component, a boundary has been set to articles that assess more than 10 variants in more than 5 genes. The smallest studies in this review, by Hattinger *et al.*, Hegyi *et al.*, and Windsor *et al.* (45, 29 and 36 variants in cohorts of 57, 59 and 58 osteosarcoma patients), showed the poorest reproducibility (Table S8, S9, S10) (31, 32, 55). Possibly, due to the small number of patients in these studies, the power was too low to perform multiple testing correction causing the authors to report false-positive findings.

Not only in the case of small studies, but also in larger studies, replication remains laborious. Our structured search shows that many replication studies have been performed but the majority do not confirm the findings from the discovery study with significant results (see figure 2). Obvious explanations for this are the general heterogeneity between cohorts, different ethnicities, phenotypes and treatment regimens. In addition, power in discovery studies is often too low to correct for multiple testing, leading to false-positive findings. Insufficient power in replication studies may also stand in the way of confirming true-positive findings. As a solution for that, meta-analysis of the discovery and replication cohorts could increase the total power, however in this review there was too much heterogeneity between studies perform reliable meta-analyses. To study treatment response different outcome parameters, such as overall survival, event-free survival, disease-free survival, progression-free survival, histological response and tumor necrosis were used, making it impossible to combine in a meta-analysis. For toxicity outcomes, there were large differences in grading systems and the exact definitions for which patients are considered cases. In pediatric patient cohorts, cardiotoxicity is defined in as fractional shortening below a limit that varies among studies. In adults, cardiotoxicity is defined as a decrease of ejection fraction below the lower limit of normal or a large absolute reduction in ejection fraction, making it impossible to combine pediatric and adult patient cohorts in meta-analysis. The introduction of more sensitive imaging tools may allow for earlier detection of cardiac dysfunction and homogenize phenotypes, for example using global longitudinal strain (GLS), 3D volumetric echocardiographic or MRI. Lastly, confidence intervals of discovery and replication studies in this review do not always overlap, and therefore, it is already to be predicted that meta-analyses will be very heterogenous. In the future, the consistency of phenotypes would be improved if research groups with similar interests would collaborate to clearly define the phenotypes together, in order to perform powerful analyses in larger patient cohorts.

Conclusion

To conclude, in this systematic review, fifteen articles were found that aimed to identify novel genetic variants involved in treatment toxicity or treatment response in patients with osteosarcoma. Most research was done on doxorubicin-induced cardiomyopathy and for seven genetic variants in *CELF4*, *GPR35*, *HAS3*, *RARG*, *SLC22A17*, *SLC22A7* and *SLC28A3*, replication studies were performed without consistent results. Genetic variants in *ABCC3*, *ABCC5*, *FasL*, *GLDC* and *GSTP1* were repeatedly associated to osteosarcoma treatment outcome, using very heterogeneous efficacy outcomes. Studies reporting on bone marrow, nephro- and/or hepatotoxicity were small and had poor reproducibility. Moreover, none of the articles assessed cisplatin-induced ototoxicity. Despite that these results are promising and may have great potential for the future, replications often remain contradictory. Therefore, hypothesis-generating studies in large patient cohorts will be necessary to confirm these variants and to discover novel associations. Large initiatives, for example Euramos-1 or the Children's Oncology Group, could liaise with other research groups around the world with similar interests to boost the discovery of pharmacogenetic variants. Thereafter, functional studies are important to elucidate the mechanism behind the association and, ultimately, interventions should be established that make use of these associations to give patients with osteosarcoma a treatment that fits the needs of the individual best.

Abbreviations

MAP	Methotrexate, adriamycin (doxorubicin), cisplatin chemotherapy regimen
GWAS	Genome-wide association study
MeSH	Medical subject headings
STREGA	STrengthening the REporting of Genetic Association
MTX	Methotrexate
ADME	Absorption, distribution, metabolism and excretion

PFS	Progression free survival
EFS	Event free survival
OS	Overall survival
CYP	Cytochrome P450

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All relevant information on data generated or analyzed during this study are included in this published article and its supplementary information files. Other intermediate datasets are available from the corresponding author on reasonable request.

Competing interests

All authors declare no competing interests.

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Authors' contributions

EH, AB, JV, ML and MC wrote the manuscript; EH, AB, ML and MC designed the research; EH and AB performed the research

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Figures

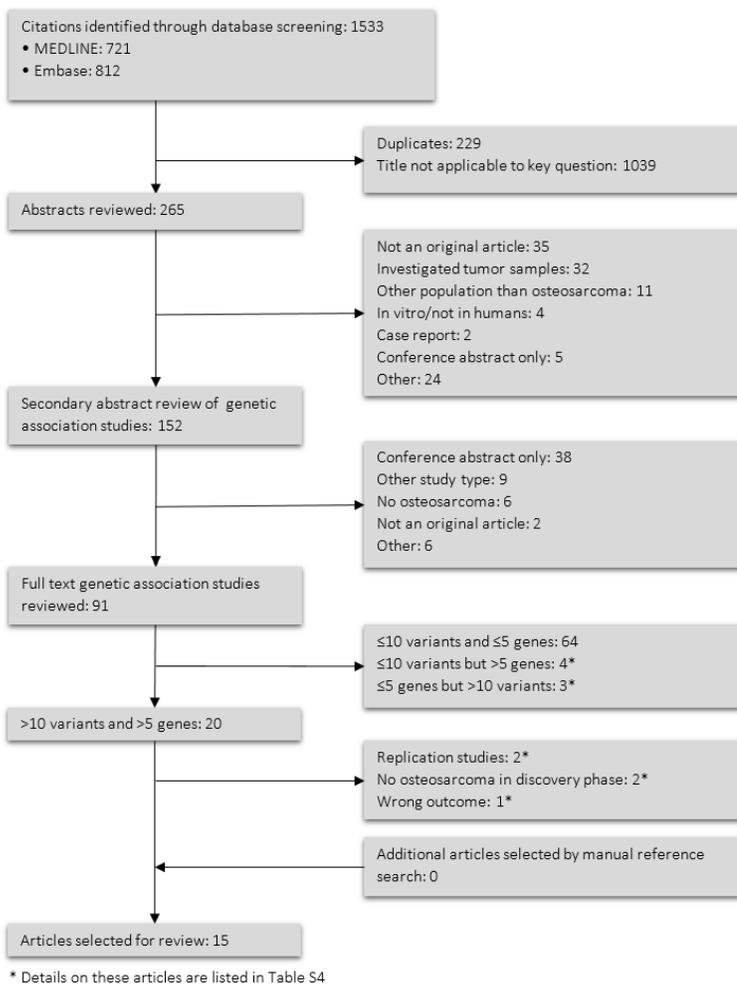


Figure 1

Flowchart for selection of articles.

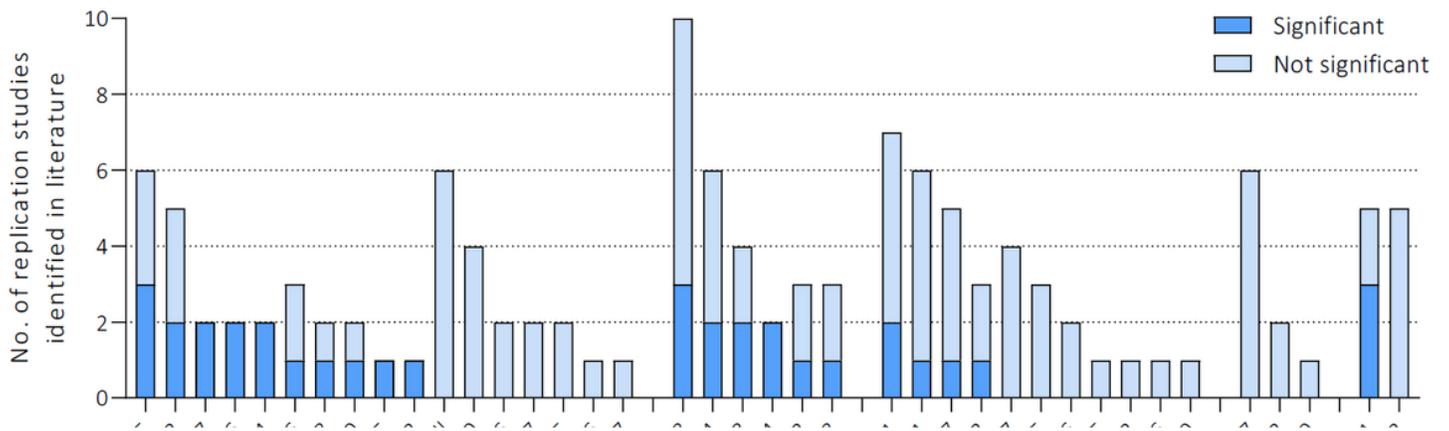


Figure 2

Replication studies of the association of genetic variants with treatment response and toxicities that were identified in literature. Associations that are indicated as significant also showed consistent direction of effect with the discovery study. Table S8, S9 and S10 elaborate more on phenotypes, effect sizes and significance of these replication studies.

Supplementary Files

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