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Genetic variants found in paediatric oncology patients with severe chemotherapy-induced toxicity: A case series

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Abstract

Paediatric oncology patients who develop severe chemotherapy-induced toxicity that requires dose reduction, delay or termination of treatment are at risk of decreased treatment efficacy. Previous research has provided evidence that genetic variants in *TPMT, NUDT15, UGT1A1* and *DPYD* are associated with toxicity of anticancer drugs. This led to pharmacogenetic guidelines that are integrated into clinical practice in paediatric oncology. Recently, novel genetic variants have been associated with a higher risk of developing chemotherapy-induced toxicity. In this case series, we selected 21 novel variants and genotyped these in nine patients with excessive chemotherapy-induced toxicity using whole exome sequencing or micro-array data. We observed that six out of nine patients carried at least one variant that, according to recent studies, potentially increased the risk of developing methotrexate- or vincristine-induced toxicity. As patient-derived genetic data are becoming widely accessible in paediatric oncology, these variants could potentially enter clinical practice to mitigate chemotherapy-induced toxicity.

Keywords

Paediatric oncology, precision medicine, pharmacogenomics, chemotherapy, toxicity

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Background

Understanding why some paediatric oncology patients develop severe chemotherapy-induced toxicity is key to improving treatment outcomes. In past decades, discoveries in the pharmacogenetics field revealed that patients carrying genetic variants that lead to decreased activity of metabolizing enzymes of thiopurine methyltransferase (TPMT), nudixhydrolase 15 (NUDT15), UDP Glucuronosyltransferase Family 1 Member A1 (UGT1A1) and dihydropyrimidine-dehydrogenase (DPD) are at risk of chemotherapy-induced toxicity.[1] This knowledge is now well established in evidenced-based pharmacogenetic (PGx) guidelines of thiopurines, irinotecan and fluoropyrimidines, including dose and treatment recommendations, and have been integrated in clinical practice of paediatric oncology in the Netherlands.[2–4] Since 2016, various genome-wide association studies (GWAS) and retrospective case–control studies in paediatric oncology cohorts have discovered novel

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genetic variants in metabolism, transporter and drug-target genes that are associated with a higher risk of developing chemotherapy-induced toxicity due to anthracyclines, methotrexate (MTX), vinca alkaloids or asparaginase (see Table 1).[5–15] As these findings have only recently been discovered, there is not sufficient evidence to support uptake into PGx guidelines, while there is a high need to better predict susceptibility for severe chemotherapy-induced toxicity in paediatric oncology patients. In this case series, we explored the role of these novel genetic variants that have recently been associated with excessive chemotherapy-induced toxicity of anthracyclines, MTX, vinca alkaloids or asparaginase in nine paediatric oncology patients. We genotyped these patients after they developed severe unexplained grade III/IV chemotherapy-induced toxicity. Here, we report the genotypes and chemotherapy-related toxicities of these nine patients and present future perspectives on follow-up studies to determine the relevance of genotyping these variants for clinical practice in paediatric oncology.

Methods

Developing pharmacogenetic test panel

In 2020, we published a review that included recent studies (from 2016 onward) that investigated associations between genetic variants and (any grade) toxicity induced by widely used chemotherapeutic agents in paediatric oncology.[5] In this review, we excluded novel genetic variants that have been associated with study outcomes other than chemotherapy-induced toxicity (e.g. chemotherapy pharmacokinetics, efficacy and/or survival). From the results of this review, we selected 21 genetic variants (see Table 1) that were available in whole exome sequencing (WES) data and have been associated ($p < 0.05$ in univariate analysis) in at least one study with a higher risk of developing chemotherapy-induced toxicity. Novel genetic variants reported in our earlier review article were excluded in this case series in case these could not be extracted from WES data or have been associated with chemotherapy-induced toxicity that was not present in our patients.[5] Details on nucleotide change and minor allele frequencies (MAF) can be found in supplementary material 1.

Patient inclusion

The nine patients described in this report were treated in the Princess Máxima Center for Pediatric Oncology in the Netherlands. In the Princess Máxima Center, around 600 children per year are diagnosed and treated for their cancer disease. Of these patients, most will experience severe chemotherapy-induced toxicity. We did not select patients for this series, but the nine patients were brought to our attention by paediatric oncologists. These patients developed severe unexplained grade III/IV chemotherapy-induced toxicity (measured according to the Common Terminology Criteria for Adverse Events [CTCAE], version 5) that limited subsequent treatment.[16] At diagnosis, all nine patients gave informed consent to use their data, which were collected during regular care in the clinic, for clinical purposes. For this case series, we retrospectively collected information on diagnosis, treatment, dose interventions and toxicities from the electronic health record system (i.e. HIX) used in the Princess Máxima Center in the Netherlands.

Pharmacogenetic testing

Of the 21 variants that have recently been associated with chemotherapy-induced toxicity, we exclusively extracted the variants that were relevant for the specific drug and adverse event that was observed in the patient. To minimize interventions within our patients, we reused, where possible, whole exome sequencing (WES) or mRNA sequencing data that has been previously collected. WES and mRNA genotyping was performed on skin biopsies or blood and sequenced on the Illumina Novaseq 6000 sequencer. If no sequencing data was available, we genotyped and used microarray data that was obtained with the global diversity array (GDA) from Illumina. Samples analysed for the WES and on the array were assessed for several quality control (QC) parameters (sex, heterozygosity, call rates per single nucleotide polymorphism and sample) and passed all criteria. Most important, the WES data median coverage was 100× and the array call rate was >99.3% for all samples, and there were no indications of swapped samples or contamination.

Case series results

In Table 2, the diagnosis, toxicity and genotype results of nine paediatric patients can be found. Six out of nine patients carried a genetic variant that has previously been associated with an increased risk of developing MTX- or vincristine-induced toxicity. A complete overview of genotype results can be found in supplementary material 2. All patients were treated with standard chemotherapy regimens according to the treatment protocols (see supplementary material 3).[18] In eight out of nine patients a dose intervention (i.e. dose reduction, treatment delay and/or complete termination of chemotherapy) was performed due to the severity of the toxicity. One patient (patient 3) suffered from severe mucositis and eventually died in the intensive care unit due to a septic shock caused by neutropenic enterocolitis. The treatment interventions (i.e. dose reductions/termination) were based on the toxicity profile of the patient, not on the genotype results.

Methotrexate-induced toxicity

In our patients, we identified three variants as potential risk factors for developing MTX-induced toxicity: ATP

Table 1. Genetic variants associated with chemotherapy-induced toxicity.

Drug agent	Gene	rsid	Role gene	Associated with[a]	References
Anthracyclines	*GPR35*	rs12468485	Potential role in cardiac physiology and pathology	More frequent cardiotoxicity	6
Methotrexate	*MTHFR*	rs1801133	Catalyzer in the folic acid cycle	Increased risk of mucositis and leucopenia	7,17
	ABCC2	rs2273697	Drug transporter	Increased risk of myelotoxicity and leucopenia	8
	ABCC2	rs3740066	Drug transporter	Increased risk of vomiting/nausea	9
	ABCC2	rs717620	Drug transporter	Increased risk of leucopenia	10
	miR-1206	rs2114358	Possible effect on miR-1206 expression	Increased risk of mucositis	11
	ARID5B	rs4948496	Regulates transcriptions of target genes	Increased risk of leucopenia	10
Vinca alkaloids	*ITPA*	rs1127354	Catalysator in adenosine metabolism pathway	Increased risk of neurotoxicity and gastrointestinal toxicity	12
	ADORA2A	rs2236624	Modulate TNF-alpha production and hepatic glucogen metabolism	Increased risk of hepatotoxicity	12
	miR- 4481	rs7896283	Regulate genes	Increased risk of neurotoxicity	13
	SYNE2	rs2781377	Linker within cellular cytoskeleton	Increased risk of neurotoxicity	14
	MRPL47	rs10513762	Role in oxidative phosphorylation system	Increased risk of neurotoxicity	14
Asparaginase	*PKD2L1*	rs6584356	Role in cell-cell interactions	Higher risk of thrombosis	14
	SLC39A12	rs62619938	Cofactor for enzymes	Higher risk of thrombosis	14
	MPEG1	rs7926933	Role in innate immune system	Higher risk of thrombosis	14
	RIN3	rs3742717	Interaction-interference proteins	Higher risk of thrombosis	14
	ADAMTS17	rs72755233	Role in maturation of proteins	Higher risk of pancreatitis	14
	IL16	rs11556218	Cytokine involved in immune system	Higher risk of thrombosis	14
	MYBBPIA	rs3809849	Role in cellular processes and developments	Higher risk of hypersensitivity and pancreatitis	14
	PNPLA3	rs738409	Role in triacylglycerol metabolism and signaling	Higher alanine aminotransferase levels	15
	SPEF2	rs34708521	Role in correct axoneme development	Higher risk of thrombosis	14

[a]Study design, odds ratios and *p*-values can be found in supplementary material 1 of Bernsen et al. 2020.[5]

binding cassette subfamily C member 2 (*ABCC2*) (rs717620, C > T), methylenetetrahydrofolate reductase (*MTHFR*) (rs1801133, G > A) and microRNA(miR)-1206 (rs2114358, G > A).[7,10,11,17]

Patients 2 and 8 carried a heterozygote variant in *ABCC2* (rs717620, C > T) that has been associated with the developed MTX-induced haematological toxicity.[10] *ABC* genes encode for proteins that facilitate active efflux transport of drugs such as methotrexate.[20] Recently, there has been an increase in number of publications studying associations between genetic variants in *ABCC2* and efficacy and toxicity of MTX.[21–23] MTX toxicity is a complex process and is in most cases related to prolonged (low concentration) MTX exposure or persistent high MTX levels.[24,25] An interesting approach is adopted by Grželj et al. who showed a possible association between MTX efficacy and *ABCC2* polymorphism (rs717620) in patients diagnosed with psoriasis who received low-dose MTX therapy.[26] In this study, the authors demonstrated that MTX drug survival has been significantly longer in patients carrying a T allele in *ABCC2* (rs717620, C > T) than patients carrying *ABCC2* (rs717620, C > T) CC genotype who discontinued their treatment due to a lack of treatment response (hazard ratio, 0.606; 95% confidence interval, 0.380–0.967; $p = 0.036$). However, in paediatric oncology it is common to administrate high-dose (HD) MTX (dose >500 mg/m^2) that could potentially lead to prolonged MTX exposure in paediatric patients carrying an *ABCC2* (rs717620, C > T) variant. A recent study showed that heterozygote or homozygote *ABCC2* (rs717620, C > T) genotypes lead to higher MTX blood concentrations in a paediatric ALL cohort.[21] However, Simon et al. showed that there is an association with lower MTX plasma concentrations and *ABCC2* (rs717620, C > T), yet in a small sample size ($n = 50$).[23] Also, in a recent systematic review, Taylor et al. (2021) studied genetic variants that influenced elimination and pharmacokinetics (PK) of HD-MTX in paediatric cancers. They found consistent evidence that the solute carrier organic anion transporter family member 1B1 (*SLCO1B1)* has an effect on MTX PK and exposure, but not for *ABCC2* (rs717620, C > T).[27,28] In particular, *SLCO1B1* (rs4149056, T > C) has repeatedly been associated with an increased MTX exposure and presumably a lower MTX clearance. In this study, Taylor et al. (2021) excluded pharmacogenetic studies investigating associations between genetic variants and MTX-induced toxicity as an outcome (hence the reason this variant was not included in this report), as this was not the

Table 2. Patient characteristics, toxicity and pharmacogenetic results.

Patient characteristics					Toxicity				Pharmacogenetic results verifying previous studies[a]		
No	Age	Diagnosis	Treatment protocol	Developed grade III/IV toxicity[b]	Symptoms	Onset of symptoms	Most likely induced by	Dose intervention due to toxicity	Gene variant associated with toxicity (rsid)	Genotype patient[c]	European genotype frequency (in%)[19]
1	17	ALL	ALL11	Hepatotoxicity	bilirubin >67 µmol/L (3–21 µmol/L) ASAT >40 U/L (0–30 U/L) ALAT >160 U/L (0–35 U/L) triglycerides >78 mmol/L (0.0–2.0 mmol/L)	Induction phase	Pegasparaginase	Postponed pegasparginase treatment for five weeks	None	na	-
				Neurotoxicity	Constipation, muscle weakness, neuropathy	Induction phase	Vincristine	Dose reduction to 50% with no effect, where after vincristine treatment was terminated	None	na	-
2	16	Osteosarcoma	EURAMOS	Gastroinstestinal	Oral mucositis	Neoadjuvant phase	Methotrexate	20% dose reduction	None	na	-
				Haematotoxicity	Febrile neutropenia				*ABCC2* (rs717620)	CT	32
3	17	Osteosarcoma	EURAMOS	Gastroinstestinal	Oral and rectal mucositis, death due to neutropenic enterocolitis	Induction and adjuvant phase	Methotrexate	-	*MTHFR* (rs1801133)	GA	44
									miR-1206 (rs2114358)	GA	48
4	13	AML	NOPHO-DBH AML 2012	Infectious	Probable pulmonary aspergillus, fever, aplasia	After first and second course	Cytarabine, etoposide	Complete treatment termination after two courses	None	na	-
				Cardiotoxicity	LVEF 22% FS 46%	After first course	Mitoxantrone				
				Haematotoxicity	thrombocytes >10×10^9/L (150×10^9/L-450×10^9/L)	After first course	Cytarabin, etoposide				
5	14	ALL	ALL Together	Neurotoxicity	Muscle weakness, myopathy	Induction phase	Vincristine	50% vincristine dose reduction, but after three months complete termination of planned treatment due to untreatable pulmonary infection	None	na	-
				Hepatotoxicity	bilirubin 162 µmol/L (3-21 µmol/L) ammonia 72 µmol/L (0-35 µmol/L) ASAT 1036 U/L (0-30 U/L) ALAT 2108 U/L (0-35 U/L) NT-pro BNP 806 pg/ml (0-125 pg/ml) an extended prothrombin		Vincristine		*ADORA2A* (rs2236624)	TC	38

(continued)

Table 2. Continued.

Patient characteristics					Toxicity				Pharmacogenetic results verifying previous studies[a]		
No	Age	Diagnosis	Treatment protocol	Developed grade III/IV toxicity[b]	Symptoms	Onset of symptoms	Most likely induced by	Dose intervention due to toxicity	Gene variant associated with toxicity (rsid)	Genotype patient[c]	European genotype frequency (in%)[19]
					time of 14.0 s (10.0-13.0 s) APTT 37 s (24-34 s) albumin 18.9 g/L (35.0-50.0 g/L)						
				Cardiotoxicity	Hypertrophy FS 23%		Doxorubicin		None	na	-
				Infectious	Difficult to treat probable aspergillus		Unknown		None	na	-
6	22	Neuroblastoma	NBL2009	Neurotoxicity	Vocal cord paresis	After first administration of vincristine	Vincristine	Termination vincristine treatment	miR-4481 (rs7896283)	AG	50
7	18	ALL	ALL Together	Hepatotoxicity	Thrombosis right atrium hypertriglyceridemia >120 mmol/L (0.0–2.0 mmol/L)	Induction phase	Pegasparaginase	Replacement with asparaginase	None	na	-
8	17	Osteosarcoma	EURAMOS	Haematotoxicity	Persistent thrombocytopenia 4×10^9/L (150×10^9/L-450×10^9/L)	Neoadjuvant phase	Methotrexate	Complete termination of planned treatment	*MTHFR* (rs1801133)	GA	44
									ABCC2 (rs717620)	CT	32
9	5	Wilms tumour	UMBRELLA 2016	Neurotoxicity	Constipation, polyneuropathy, facial weakness, bilateral ptosis, foot drop paresis	Induction phase	Vincristine	77% dose reduction without effect, complete termination of vincristine treatment	*ITPA* (rs1127354)	CA	13

ALL: acute lymphatic leukaemia; EURAMOS: European and American Osteosarcoma Study; NOPHO-DBH: cooperative protocol including Nordic Society for Paediatric Haematology and Oncology and Dutch Belgian Estonia Hong Kong NBL Neuroblastoma; UMBRELLA: the International Society of Paediatric Oncology (SIOP) Renal Tumour Study Group (RTSG); ASAT: aspartate aminotransferase; ALAT: alanine aminotransferase; LEVF: left ventricular ejection fraction; FS: fractional shortening; NT: N-terminal pro b-type natriuretic peptide; BNP: brain natriuretic peptide; APTT: activated partial thromboplastin clotting time.

[a]The pharmacogenetic results represent earlier findings for a possible association between the chemotherapy-induced toxicity in the patient and the pharmacogenetic result.

[b]Toxicity is graded according to the CTCAE.

[c]Complete genotype results can be found in supplementary material 2; None = we did not find a genetic variant that has been associated with the chemotherapy-induced toxicity present in the patient; na = not applicable, we did not profile all genetic variants within each patient. This was either because the patient did not experience toxicity due to the chemotherapy associated with the genetic variants or the sequencing data had a low read and coverage at the chromosome location.

objective of their review. Although this study contributes to a better insight into genes involved in MTX PK and exposure and can help to create practical recommendations including MTX dose adjustments, it potentially overlooks genetic indicators such as variants in *ABCC2* that could be predictive for MTX-induced toxicity, but lack a clear effect on MTX PK. However, the role of *ABCC2* (rs717620, C > T) in MTX toxicity is uncertain as shown by the conflicting study results. Notably, both patients 2 and 8 had high MTX plasma concentrations after MTX infusion (T = 48, >0.2–1.0 µmol/L, reference value 0–0.2 µmol/L), which recovered quickly after rescue medication.

We found two patients (patients 3 and 8) with a heterozygote variant in *MTHFR* (rs1801133, G > A) that has previously been associated haematological toxicity.[7,17] The homozygote genotype of *MTHFR* (rs1801133, G > A) has been significantly associated with MTX-induced mucositis. There are a considerable number of studies investigating *MTHFR* (rs1801133, G > A) associations with MTX-induced toxicity.[29] The MTHFR enzyme is involved in the metabolism of folate to active folate moieties. The homozygote genotype of *MTHFR* (rs1801133, G > A) leads to an impaired MTHFR function with a reduced capacity of around 50%, which could lead to slower folate metabolism and cell repair.[30,31] Given that MTX also acts as an inhibitor in the folate pathway, there is a potential synergistic effect in patients with an *MTHFR* (rs1801133, G > A) variant. Patients 3 and 8 developed extreme toxicities with drastic consequences as patient 3 eventually died of a septic shock caused by neutropenic enterocolitis. Patient 8 experienced such severe and persistent thrombocytopenia that chemotherapy treatment was prematurely terminated.

Patient 3 additionally carried a heterozygote variant in miR-1206 (rs2114358, G > A) that has earlier been associated with MTX-induced mucositis. MicroRNAs are short non-coding RNAs that regulate gene expression.[32,33] Since the discovery of microRNAs, it has been suggested that they also play a role in the regulation of pharmacokinetic and pharmacodynamics genes and drug toxicity.[34] Three recent studies assessed the role of miR-1206 (rs2114358, G > A) in MTX-induced mucositis.[11,35,36] These studies have shown a possible role of miR-1206 (rs2114358, G > A) in MTX-induced mucositis but could not replicate the significant finding found in earlier studies.[32,33]

Notably, two patients (patients 3 and 8) carried two heterozygote pharmacogenetic variants that have previously been associated with toxicity due to MTX. Likely, the metabolism and transport of MTX are affected by multiple genes and variants in these pharmacogenes could potentially contribute to an overall higher risk of developing MTX-induced toxicity.

Vinca alkaloid-induced toxicity

We identified three variants as potential risk factors for developing vincristine-induced hepatotoxicity and neurotoxicity seen in our patients: adenosine A2A receptor (*ADORA2A*) (rs2236624, T > C), miR-4481 (rs7896283, A > G) and inosine triphosphatase (*ITPA*) (rs1127354, C > A).[12,13]

Patient 5 carried a heterozygote variant in *ADORA2A* (rs2236624, T > C), but only the homozygote wild type genotype (TT genotype) has been significantly associated with vinca alkaloid-induced hepatotoxicity.[12] *ADORA2A* genes are expressed in various tissues such as the brain, liver, bone marrow and lymph nodes.[37,38] The ADORA2A receptor is a guanine nucleotide-binding protein (G protein)-coupled receptor (GPCR) and plays an important role in the activation of cellular responses and the cyclic adenosine monophosphate (cAMP) signal pathway and is suggested to play a role in liver injury. One study showed a possible association between the homozygote TT genotype of *ADORA2A* (rs2236624, T > C) and vinca alkaloid-induced hepatotoxicity. However, the association was not significant after multiplicity correction and it was difficult to identify the chemotherapeutic agent causing hepatotoxicity.[12] We encountered the same difficulties in patient 5. This patient developed multiple toxicities, including severe neurotoxicity and hepatotoxicity during treatment with vincristine and pegasparaginase. In the literature, there are no studies investigating the link between genetic variants and pegasparaginase-induced hepatotoxicity.[39]

Patient 6 carried a heterozygote variant in miR-4481 (rs7896283, A > G), yet only the homozygote GG genotype has been significantly associated with vinca alkaloid-induced neurotoxicity.[13] This patient developed a vocal cord paresis after a single vincristine administration. As mentioned before, it has been suggested that microRNAs play a role in the regulation of genes.[34] However, the target genes of miR-4481 that are involved in pharmacokinetics and pharmacodynamics of vinca alkaloids are unknown. In the literature, there are no further studies addressing miR-4481. In the study by Gutierrez-Camino et al., the significance of this association was lost after false discovery rate (FDR) correction.[13] Patient 9 developed severe neurotoxicity with constipation grade III, facial weakness and peroneal nerve paralysis for which the vincristine treatment was eventually discontinued completely because a dose reduction to 60% was still intolerable. This patient carried a heterozygote variant in *ITPA* (rs1127354, C > A) that has been associated with vinca alkaloid-induced neurotoxicity.[12] The ITPA enzyme plays a role in the metabolism and synthesis of purines.[40] The heterozygote genotype has been reported to lead to a 75% reduction in enzyme activity of ITPA.[41] One study showed a possible association between *ITPA* and vinca alkaloid-induced neurotoxicity (and gastrointestinal toxicity).[12] However, this association was not significant after multiplicity correction.

Discussion

Recent studies revealed novel genetic variants that have been associated with a higher risk of developing chemotherapy-induced toxicity in paediatric oncology patients beyond the widely recognized pharmacogenes

(i.e. *TPMT, NUDT15, UGT1A1* and *DPYD*). In this case series, we explored the possible role of these newly discovered genetic variants in nine paediatric oncology patients with unexplained severe chemotherapy-induced toxicity (Table 2). Six out of nine patients carried at least one genetic variant that potentially serves as a risk factor for developing MTX- or vincristine-induced toxicity: *ABCC2* (rs717620, C > T), *MTHFR* (rs1801133, G > A), miR-1206 (rs2114358, G > A), *ITPA* (rs1127354, C > A), *ADORA2A* (rs2236624, T > C) and miR-4481 (rs7896283, A > G).

Although we observed potential risk variants for toxicity in our patients, the clinical relevance of these variants included in this report (Table 1) is not fully established. *ABCC2* (rs717620, C > T), *MTHFR* (rs1801133, G > A), miR-1206 (rs2114358, G > A), *ITPA* (rs1127354, C > A), *ADORA2A* (rs2236624, T > C) and miR-4481 (rs7896283, A > G) lack consistent evidence to be implemented into clinical practice as predictors for developing toxicity. For the majority of these variants, associations between variant and chemotherapy-induced toxicity have not been replicated in a prospective setting and validated in independent cohorts.[5–15] Also, homozygote genotypes of *MTHFR* (rs1801133, G > A), *ADORA2A* (rs2236624, T > C) and miR-4481 (rs7896283, A > G) have been significantly associated with MTX- or vincristine-induced toxicity in earlier studies, leading to uncertainties if heterozygote genotypes of patient 3, 5 and 6 can also be identified as risk factors for toxicity.[7,12,13,17] Notably, the variants *ITPA* (rs1127354, C > A), *ADORA2A* (rs2236624, T > C) and miR-4481 (rs7896283, A > G) that have previously been associated with vincristine-induced toxicity lost significance after multiplicity correction, indicating that these associations could be either weak or underpowered studies to show a significant effect.[12,13]

To place these findings in a clinical context, it should be noted that the genotypes of the six patients in this report are common in the European population; 1/3 of the population carries a heterozygous variant of *ABCC2* (rs717620, C > T) and *ADORA2A* (rs2236624, T > C), and half of the population carries a heterozygote variant of *MTHFR* (rs1801133, G > A), miR-1206 (rs2114358, G > A) and miR-4481 (rs7896283, A > G).[19] A slightly less common variant, *ITPA* (rs1127354, C > A), is present in 13% of the population as a heterozygote genotype.[19] Due to these high allele frequencies, it was expected to find heterozygote genotypes in our patients, which make it difficult to know what the actual contribution of these variants is on the toxicity seen in our patients. Nevertheless, the role of common variants (MAF >1%) in developing toxicity should not be underestimated. For example, common variants in *UGT1A1* are leading to pre-emptive pharmacogenetic screening of *UGT1A1* variants to mitigate the risk of developing irinotecan-induced toxicity.[42] Another important aspect is that chemotherapy-induced toxicity is a complex system with most likely multiple contributing factors. As the patients in this report (and in paediatric oncology in general) are not treated with one, but with multiple chemotherapeutic agents simultaneously, each of these agents will probably contribute to the toxicity seen in the patient. For some toxicity, such as neurotoxicity, it is widely recognized that this is most likely induced by vincristine. Haematotoxicity, however, could be induced by all chemotherapeutic agents. This makes it difficult to investigate the clear effects of one (or more) genetic variants on chemotherapy-induced toxicity.

As more paediatric oncology centres are collecting genetic data upon diagnosis, we expect that future clinical practices in paediatric oncology will use pharmacogenetic screening as a tool to pre-emptively intervene in cancer treatments (beyond thiopurines, irinotecan and fluoropyrimidines) to reduce the risk of severe toxicity. A good example is shown in a recent case report by Loucks et al.[43] The authors genotyped *UGT1A6* (rs17863783, G > T), retinoic acid receptor gamma (*RARG*) (rs2229774, G > A) and solute carrier family 28 member 3 (*SLC28A3*) (rs7853758, G > A) in three paediatric oncology patients. They used this information to reduce the risk of anthracycline-induced cardiotoxicity (ACT) by pre-emptively intervening in anthracycline therapy (i.e. avoid anthracycline treatment, dexrazoxane administration or continue treatment). A gap in knowledge for the novel variants included in this report is the lack of data on the required treatment intervention for patients with risk genotypes. A possible solution is to research the genotype–phenotype correlation of these novel variants. For example, the discovery of lower enzyme activity of *TPMT* genetic variants supported decision-making regarding dose reductions of thiopurines that were necessary for patients carrying genetic variants in *TPMT*.[44] Most of these correlations are performed in *in vitro* studies, but new tools such as *in silico* and neural network-based model AlphaFold has been developed that predicts the function and structure of proteins. These tools could give new insights for suitable drug and treatment interventions based on the activity of the affected protein and can highly contribute to the interpretation of GWAS and case–control studies results.[45,46]

Despite uncertainties of the exact role of *ABCC2* (rs717620), *MTHFR* (rs1801133), miR-1206 (rs2114358), *ADORA2A* (rs2236624), miR-4481 (rs7896283) and *ITPA* (rs1127354) in MTX- or vincristine-induced toxicity, these variants could potentially support decision making in clinical practice to better predict susceptibility for chemotherapy-induced toxicity in patients. As an enormous quantity of genetic data (e.g. whole exome sequencing and whole genome sequencing data) is collected in paediatric oncology, we will use this data to further examine the role of these and other variants, including *UGT1A6* (rs17863783, G > T), *RARG* (rs2229774, G > A) and *SLC28A3* (rs7853758, G > A) that have been associated with ACT and *SLCO1B1* (rs4149056, T > C) that has been associated with an increased MTX exposure, in new

paediatric oncology cohorts.[28,43] In particular, *SLCO1B1* (rs4149056, T > C) has repeatedly been associated with an increased MTX exposure and presumably a lower MTX clearance. We did not screen *SLCO1B1* (rs4149056, T > C) in our patients as this variant has been associated with MTX pharmacokinetics and, to our knowledge, not (yet) with MTX-induced toxicity, but will include this and other novel variants that have a possible effect on pharmacokinetics of chemotherapy in future pharmacogenetic studies. Furthermore, to include complex interactions between metabolism, transport and other genes that are involved in chemotherapeutic agents' pharmacokinetics and/or pharmacodynamics, we want to explore new study approaches in pharmacogenetics such as polygenic risk scores and *in silico* tools that contribute to sophisticated predicting models for developing chemotherapy-induced toxicity in paediatric oncology. In this way, we can hopefully mitigate and hopefully prevent the risk of developing severe toxicity seen in the examples in this case series.

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Authors' contribution

EB collected patient data and literature and wrote the first draft of the manuscript. BT and MvdL were involved in the genetic data extraction (methods). EB, LHanff, LHaveman and MD reviewed and edited patient results and interpretation. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

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