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RESEARCH ARTICLE

Impact of rs12917 MGMT Polymorphism on [^{18}F]FDG-PET Response in Pediatric Hodgkin Lymphoma (PHL)

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Abstract

Purpose: The enzyme O6-methylguanine-DNA methyltransferase (MGMT) is an important component of the DNA repair machinery. MGMT removes O6-methylguanine from the DNA by transferring the methyl group to a cysteine residue in its active site. Recently, we detected the single nucleotide polymorphism (SNP) rs12917 (C/T) in the MGMT sequence adjacent to the active site in Hodgkin lymphoma (HL) cell line KM-H2. We now investigated whether this SNP is also present in other HL cell lines and patient samples. Furthermore, we asked whether this SNP might have an impact on metabolic response in 2-deoxy-2-[^{18}F]fluoro-D-glucose positron emission tomography ([^{18}F]FDG-PET), and on overall treatment outcome based on follow-up intervals of at least 34 months.

Stefanie Kewitz-Hempel and Lars Kurch contributed equally to this work.

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Procedures: We determined the frequency of this MGMT polymorphism in 5 HL cell lines and in 29 pediatric HL (PHL) patients. The patient cohort included 17 female and 12 male patients aged between 4 and 18 years. After characterization of the sequence, we tested a possible association between rs12917 and age, gender, Ann Arbor stage, treatment group, metabolic response following two courses of OEPA (vincristine, etoposide, prednisone, and doxorubicin) chemotherapy, radiotherapy indication, and relapse status.
Results: We detected the minor T allele in four of five HL cell lines. 11/29 patients carried the minor T allele whereas 18/29 patients showed homozygosity for the major C allele. Interestingly, we observed significantly better metabolic response in PHL patients carrying the rs12917 C allele resulting in a lower frequency of radiotherapy indication.
Conclusion: MGMT polymorphism rs12917 seems to affect chemotherapy response in PHL. The prognostic value of this polymorphism should be investigated in a larger patient cohort.

Key words: Pediatric Hodgkin lymphoma, MGMT, rs12917, [^{18}F]FDG-PET, qPET, Deauville score

Introduction

Cure rates of pediatric Hodgkin lymphoma (PHL) patients are excellent if poly-agent chemotherapy followed by radiotherapy is administered [1–3]. However, treatment-related late effects, mostly due to radiotherapy, threaten life quality and life expectancy which is especially problematic in young Hodgkin lymphoma (HL) survivors [4]. Since more than 10 years, 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG)-positron emission tomography (PET) is regularly applied in the largest European-wide PHL studies (i. e., EuroNet-PHL-C1 or -C2 trials) to tailor therapy: in case of adequate metabolic response in [^{18}F]FDG-PET following two cycles of OEPA (vincristine, etoposide, prednisone, and doxorubicin) chemotherapy, radiotherapy is omitted. Yet, depending on the applied [^{18}F]FDG-PET response criteria, about 30–50 % of all PHL patients show inadequate metabolic response [3]. However, the mechanisms that explain why PHL respond differently to the initial chemotherapy are not clarified in detail at the moment. Moreover, it is not yet known why some patients remain relapse-free despite a poor metabolic response. One explanation could be that the gene expression profile of the lymphoma is different. Steidl et al. [5] and Scott et al. [6] investigated the gene expression profile of patients with therapy failure and patients with therapy success. They found a plethora of genes which were differentially expressed between both groups. Beside the differentially expressed genes, more general mechanisms could also play an essential role regarding the therapy response. An important candidate is the DNA repair machinery. One component of this machinery is the O6-methylguanine-DNA methyltransferase (MGMT). This protein was originally described to be crucial for the removing of methyl groups which were added to the DNA by alkylating cytostatic drugs [7]. If the methyl group cannot be removed, the methylated guanine pairs in the next replication round with thymidine. This O6-methylguanine-T pair is thermodynamically suboptimal and is recognized by the mismatch repair machinery, which cut off the thymidine from the DNA. Then again, a thymidine is incorporated to the opposite of the O6-methylguanine and the repair machinery starts again and finally will lead to a transition from a GC pair to an AT pair [8]. This can result in cell cycle arrest, chromosomal aberrations, or apoptotic cell death [9, 10]. MGMT removes the methyl group from guanine by transferring it to a cysteine residue in its active site. Thereby, the enzyme is inactivated by itself. Thereafter, the inactive MGMT is ubiquitinated and degraded by the proteasome [7, 11, 12]. Every MGMT molecule can remove a methyl group only once [7]. HL cell lines showed different expression levels of the MGMT gene. Cell lines with high MGMT expression are more resistant against the cytostatic drug dacarbazine than cell lines without MGMT expression [13]. MGMT has also been shown to affect the resistance of cancer cells against non-alkylating drugs like doxorubicin [14]. In our initial study [13], we have already observed a single nucleotide polymorphism (SNP) in the cell line KM-H2 which might affect the activity of MGMT. In the present study, we determined the frequency of this SNP in additional HL cell lines and tumor samples of PHL patients. Moreover, we investigated the influence of this MGMT polymorphism on [^{18}F]FDG-PET response following two cycles of doxorubicin-containing chemotherapy (OEPA).

Materials and Methods

Patient Population and Treatment Regime

Twenty-nine pediatric patients with classical HL (17 females, 12 males, aged between 4 and 18 years) who were diagnosed in the interim phase of the EuroNet-PHL-C1 and -C2 trial received treatment according to the latest version of the EuroNet-PHL-C1 study protocol [4] (Table 1). Patients were stratified into one of three treatment groups (TG) depending on the respective Ann Arbor stage, presence of E lesions, B symptoms, the bulk volume ($\geq$200 ml), or an

Table 1. Summary of patient characteristics. Presented are all assessed parameters: qPET value (qPET), Deauville Score (DS), International Harmonization Project Criteria (IHP), MGMT genotype (MGMT), gender, age (in years), stage, treatment group (TG), radiotherapy (RT), relapse status, and the follow-up time (in months) of the investigated patients.

No.	qPET	DS	IHP	MGMT	Gender	Age	Stage	TG	RT	Relapse	Follow-up
1	0.9	2–3	–	C/C	Female	16	IV B	3	No	No	58
2	0.92	2–3	–	C/C	Female	14	III A	2	No	No	58
3	1.11	3	+	C/C	Female	16	II B	2	Yes	No	59
4	0	1	–	C/C	Male	17	II A	1	No	No	58
5	1.05	3	+	C/C	Male	12	IV A	3	Yes	No	58
6	1.03	3	+	C/C	Female	16	II A	2	Yes	No	57
7	0.95	2–3	–	C/C	Male	10	II B	2	No	No	55
8	0	1	–	C/C	Male	17	II A	1	No	Yes	52
9	0	1	–	C/C	Female	17	II A	1	No	No	54
10	1.4	3–4	+	C/C	Female	13	IV BE	3	Yes	No	47
11	5.44	5	+	C/C	Female	10	IV B	3	Yes	No	44
12	1.33	4	+	C/C	Male	17	IV BE	3	No	No	42
13	0.87	2	–	C/C	Male	12	II B	2	No	No	48
14	0	1	–	C/C	Female	12	IV B	3	No	No	61
15	0.81	2	–	C/C	Female	16	III B	3	No	No	40
16	1.09	3	+	C/C	Female	17	IV AE	3	Yes	No	46
17	0	1	–	C/C	Male	8	III A	2	No	No	34
18	0.72	2	–	C/C	Male	15	II A	1	No	No	34
19	3.39	5	+	C/T	Female	17	IV AE	3	Yes	No	58
20	1.11	3	–	C/T	Male	18	III BE	3	No	No	59
21	2.22	5	+	C/T	Female	4	III A	2	Yes	No	57
22	0.97	2–3	–	C/T	Female	14	II A	1	No	No	51
23	1.46	3–4	+	C/T	Male	18	IV B	3	Yes	No	52
24	4.63	5	+	C/T	Female	17	II A	2	Yes	No	56
25	1.35	4	+	C/T	Male	17	II B	2	Yes	No	48
26	1.75	4	+	C/T	Male	8	II A	1	Yes	No	40
27	0.89	2	–	C/T	Female	17	II A	1	No	No	39
28	1.45	4	+	C/T	Female	18	II A	2	Yes	No	41
29	1.18	2–3	–	T/T	Female	14	II A	1	No	No	42

elevated erythrocyte sedimentation rate (≥ 30 mm/h). Each TG started with two cycles of OEPA chemotherapy followed by [^{18}F]FDG-PET for metabolic response evaluation. Patients in TG2 and TG3 received 2 and 4, respectively, additional courses of COPDAC (cyclophosphamide, vincristine, prednisone, and dacarbazine) chemotherapy. In case of inadequate response in [^{18}F]FDG-PET, radiotherapy of all initially involved regions except for bone marrow lesions was applied after all chemotherapy cycles were completed. In adequate metabolic responders, radiotherapy was omitted.

We tested the association between the MGMT polymorphism and clinical data at initial staging (age, gender, stage, and therapy group), [^{18}F]FDG-PET response, and follow-up data. The clinical, treatment, response to treatment, and outcome data of all the patients are summarized in Table 1. The time of follow-up was between 34 and 61 months. This study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. All patients and their guardians gave their written informed consent. The study was approved by the ethical committee of the Medical Faculty of the Martin Luther University Halle-Wittenberg.

Cell Lines, Cell Line Identification, and Cell Culture

HL cell lines [15–19] were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Brunswick, Germany. All HL cells were cultured in RPMI-1640 medium (Invitrogen, Karlsruhe, Germany), supplemented with 10 % fetal calf serum, 100 U/ml penicillin, and 100 µg/ml streptomycin at 37 °C in a humidified atmosphere with 5 % CO_2. Identity of the used cell lines was verified by polymerase chain reaction (PCR) with short tandem repeat (STR)-specific primers [20]. The following STR-specific primers were used: D13S317: 5'-ACA GAA GTC TGG GAT GTG GA-3', 5'-GCC CAA AAA GAC AGA CAG AA-3'; D16S539: 5'-GAT CCC AAG CTC TTC CTC TT-3, 5'-ACG TTT GTG TGT GCA TCT GT-3'; D5S818: 5'-GGG TGA TTT TCC TCT TTG GT-3', 5'-TGA TTC CAA TCA TAG CCA CA-3'; D7S820: 5'-TGT CAT AGT TTA GAA CGA ACT AAC G-3', 5'-CTG AGG TAT CAA AAA CTC AGA GG-3'.

DNA and RNA Isolation and Polymerase Chain Reaction

DNA isolation, RNA isolation, and polymerase chain reaction were performed essentially as described [13]. In short, the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) was used for DNA isolation from HL cell lines at early passages. For amplification of MGMT, we used the following primer combination: 5-GAA TCA GGG GAC TGC AGG TA-3′, 5′-AAG TGT TGG AGT GGG TGG AG-3′. Polymerase chain reaction was performed in a total

volume of 25 µl containing 5 µl Green Go Taq Buffer (Promega, Mannheim, Germany), 16.8 µl water, 0.5 µl of 10 mM dNTPs (Fermentas, St. Leon Roth, Germany), 0.25 µl of each primer combination, 0.5 µg DNA, and 0.2 µl Go Taq polymerase (Promega). After an initial denaturation step at 95 °C for 5 min, amplification was performed with 35 cycles (94 °C, 30 s; 60 °C, 30 s; 72 °C, 45 s). PCR was finished by a terminal elongation step at 72 °C for 5 min. Thereafter, the product was visualized by agarose (1.5 %) gel electrophoresis in the presence of ethidium bromide.

In order to isolate RNA from the blood of patients with HL, blood was collected with Paxgene Blood RNA Tubes (BD, Heidelberg, Germany) and RNA was isolated with the PAXgene Blood RNA Kit according to manufacturer's information (Qiagen). Trizol (peglab, Erlangen, Germany) was used for isolation of RNA from HL cell lines. RNA from blood or cell lines was transcribed into cDNA with Superscript II reverse transcriptase essentially as described [13]. However, we used an Oligo (dT) primer (Fermentas) for reverse transcription and the incubation time was shorted to 60 min at 42 °C. After cDNA synthesis, polymerase chain reaction (30 cycles) was performed as described above using the following primer combinations: actin beta (ACTB): 5′-GGC ATC GTG ATG GAC TCC G-3′, 5′-GCT GGA AGG TGG ACA GCG A-3′; MGMT: 5′-CCG GAT ATG CTG GGA CAG-3′; 5′-AGG GCT GCT AAT TGC TGG TA-3′. The product was visualized by agarose (1.5 %) gel electrophoresis in the presence of ethidium bromide. Products were eluted from agarose gels and sequenced as described previously [13] using the MGMT forward primer.

[18F]FDG-PET Data, Analyses, and its Impact for Patient Management

According to the EuroNet-PHL-C1 protocol, every patient received [18F]FDG-PET for initial staging (IS) and for response assessment (RA) following two cycles of OEPA chemotherapy [21]. The [18F]FDG-PET datasets were acquired in 8 different PET centers during the period from April 2013 to July 2015. These datasets were uploaded to a central image data server [22]. For an adequate performance of the whole body [18F]FDG-PET scans, the "Guidelines for [18F]FDG/PET and PET/x-ray computed tomography imaging in pediatric oncology" of the European Association of Nuclear Medicine" [23] and the EANM dosage calculator was recommended. Blood glucose levels were measured in all patients before [18F] FDG was administered and were within the normal limits. In addition, none of the patients had a history of diabetes. Patients' body weight ranged from 29 to 94 kg. For the initial staging, body mass adjusted [18F]FDG dosages between 75 and 473 MBq (mean 211.3 ± 16.54) and for the RA between 88 and 326 MBq (mean 210.1 ± 12.31) were administered. Time from tracer application until start of scanning reached from 49 to 141 min (mean 84.9 ± 17.0) at initial staging and from 54 to 141 min (mean 86.8 ± 21.9) at RA. On average, the respective time intervals between IS and RA differed by 10.6 % (± 11 %).

Every PET image underwent central reference review. During central reference, [18F]FDG-PET reading the glucose metabolism of every lymphoma lesion at staging was evaluated visually towards its alteration following 2 courses of OEPA. Every lymphoma residual was graded according to the International Harmonization Project (IHP) criteria [21, 24]. Thereafter, inadequate metabolic response was stated if the glucose metabolism of the residual(s) < 2 cm exceeded the local background or if the glucose metabolism of the residual(s) > 2 cm exceeded the uptake of the mediastinal blood pool [24]. In case of inadequate metabolic response radiotherapy to all initially involved sites, except for bone marrow lesions, was recommended [21]. However, for register and non-study patients, recommendations of the central reference reading were not obligatory.

For the 29 patients analyzed here, additionally, the Deauville score (DS) was determined and the qPET value was calculated for the hottest lymphoma residual in the response assessment [18F]FDG-PET. This was done because currently, active PHL study protocols do no longer use IHP criteria, but DS and/or qPET [3, 25]. To assess the DS, the uptake of the residual lymphoma was visually compared with the uptake of the mediastinal blood pool and the liver (Table 2). qPET calculation was performed by dividing the mean standardized uptake value (SUVmean) measured within the four hottest connected voxels inside the lymphoma residual by the SUVmean of the liver [3] (Fig. 1). So, for qPET calculation, no quantitative data are necessary from the IS. It was shown that qPET can be translated in DS [3]. The cutoff between DS 2 and 3 was at a qPET value of 0.95, between DS 3 and 4 at a qPET value of 1.3, and between DS 4 and 5 at a qPET value of 2.0 [3]. The correspondence between DS, qPET, and IHP criteria is presented in Table 2.

Statistical Analysis

We tested the association between the presence of the minor T allele and qPET values (T allele absent: 8 patients with qPET ≥ 0.95 and 10 patients with qPET < 0.95; T allele present: 10 patients with qPET ≥ 0.95 and 1 patient with qPET < 0.95), gender (T allele absent: 10 females and 8 males; T allele present: 7 females and 4 males), age (T allele absent: median age 14.16 years; T allele present: 14.72 years), stage (T allele absent: stage I: 0, II: 8, III: 3, IV: 7; T allele present: stage I: 0, II: 7, III: 2, IV: 2), treatment group (T allele absent: TG1: 4, TG2: 6, TG3: 8; T allele present: TG1: 4, TG2: 4, TG3: 3), extranodal lesions (T allele absent: 2 yes, 16 no; T allele present: 2 yes, 9 no), B symptoms (T allele absent: 9 yes, 9 no; T allele present: 3 yes, 8 no), and radiotherapy (T allele absent: 6 yes, 12 no; T allele present: 7 yes, 4 no) using Fisher exact test (http://

Table 2. Correspondence of Deauville score, qPET values, and IHP criteria

Deauville score	qPET	IHP criteria
1 = No residual uptake	qPET below detection limit	- = Residual < 2 cm and uptake below background
2 = Residual uptake < mediastinal blood pool	0 < qPET < 0.95	OR residual > 2 cm and uptake below mediastinal blood pool
3 = Residual uptake >/= mediastinal blood pool	0.95 ≥ qPET < 1.30	+ = residual < 2 cm and uptake above background
4 = Residual uptake > liver	1.30 ≥ qPET < 2.00	OR residual > 2 cm and uptake above mediastinal blood pool
5 = Residual uptake >>> liver	qPET ≥ 2.00	

www.socscistatistics.com/tests/fisher/Default2.aspx). For all statistical analyses, a p value of 0.05 was set as cutoff for significance.

Results

Analysis of the Polymorphism rs12917 in HL Cell Lines

In our previous work, we observed that HL cell lines L-1236 and L-428 expressed MGMT whereas HL cell lines L-540 and HDLM-2 showed no MGMT expression. Expression was regulated by promoter methylation. The HL cell line KM-H2 showed expression of MGMT despite the presence of a methylated promoter. Sequencing analysis revealed that this cell line harbors a gene fusion between Proline-Rich Coiled-Coil 2B (PRRC2B) and MGMT. During this analysis, we detected the presence of the rs12917 polymorphism in the MGMT sequence in KM-H2 cells [13]. We investigated this SNP in the two other MGMT expressing HL cell lines (L-1236 and L-428). We found that L-428 cells also carry the minor T allele whereas L-1236 cells have both the T and the C allele. To investigate the polymorphism in all HL cell lines, we analyzed the MGMT sequence at the genomic DNA level. Table 3 summarizes the results from all HL cell lines. According to databases [26], the genotype frequency for C/T is 23.3 % in the European population and the T/T genotype has a frequency of only 2.2 % (https://www.ensembl.org/Homo_sapiens/Variation/Population?db=core;g=ENSG00000170430;r=10:129467184-129768007;t=NST00000306010;v=rs12917;vdb=variation;vf=12269#373520_tablePanel; last visited: 10. Jan. 2019). Although the T allele seems to be relatively rare, it was present in four of five HL cell lines. This surprising observation prompted us to analyze whether the minor T allele might be associated with a higher risk for

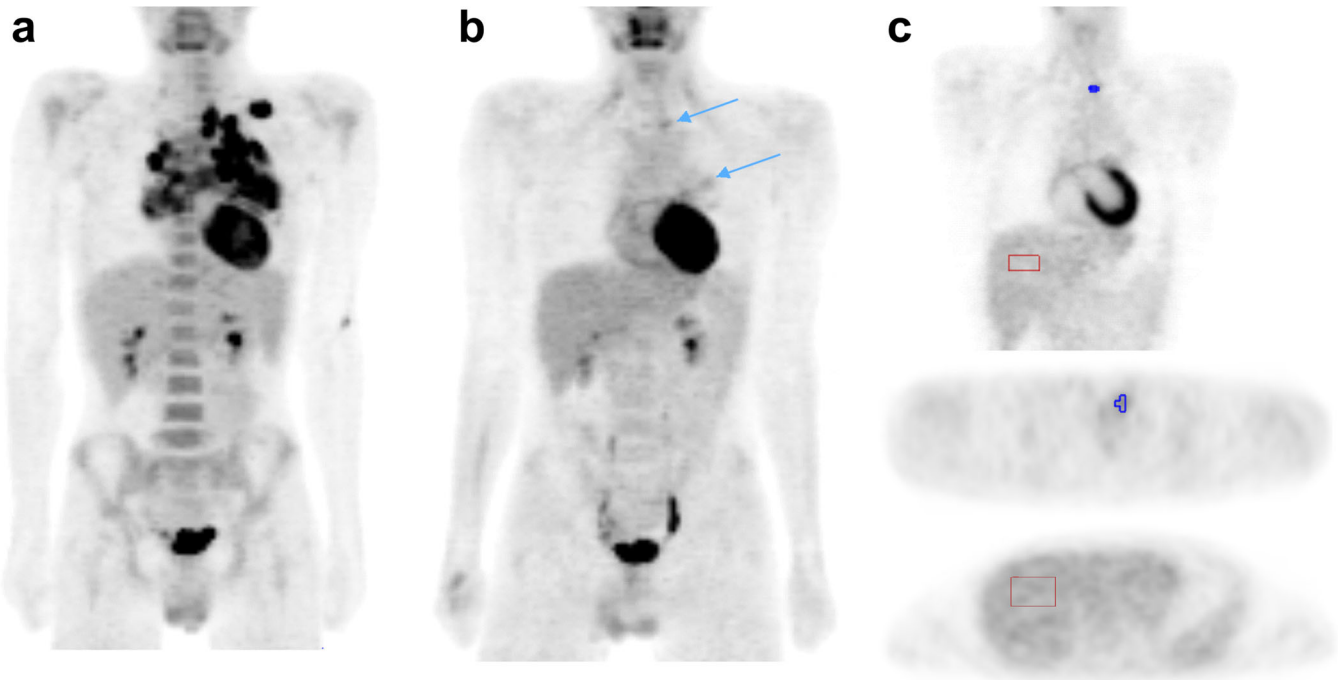

Fig. 1. Example of a qPET analysis. **a** [^{18}F]FDG-PET at initial staging of a patient with C/T genotype (maximum intensity projection). Lymphoma manifestations were detectable on the left supraclavicular fossa and in the mediastinum. **b** [^{18}F]FDG-PET of the same patient following 2 courses of OEPA chemotherapy (maximum intensity projection). Lymphoma residuals (Deauville score 3, qPET 1.35, PET + according to IHP) are still detectable in the mediastinum (blue arrows). **c** qPET calculation (coronal and transversal slices). The four hottest connected voxels of the least responsive region (SUVmean = 2.26) were divided by the SUVmean of a 30 ml VOI in the liver (red cuboid VOI; SUVmean = 1.67), resulting in a qPET value of 1.35.

Table 3. Analysis of SNP rs12917 in HL cell lines at DNA and cDNA level

Cell line	DNA level	cDNA level*	Reference
L-1236	C and T	C and T	–
L-428	T	T	–
L-540	C	–	–
KM-H2	C and T	T	13
HDLM-2	C and T	–	–

Presented are the results of the sequencing analysis of rs12917 in HL cell lines. The analysis was performed at DNA and cDNA level
*For the analysis, RNA was isolated and cDNA synthesis was performed. The HL cell lines L-540 and HDLM-2 showed a methylated MGMT promoter and no MGMT expression was detectable [13]

developing HL, or an increased risk for treatment failure because all the established cell lines are derived from patients with resistant disease. Therefore, we analyzed the distribution of the rs12917 SNP in PHL patients.

Analysis of the Minor Allele of rs12917 in PHL Patients and Association with qPET Values

In order to investigate the frequency of the rs12917 SNP in PHL patients, we used blood from PHL patients and isolated RNA. After cDNA synthesis, PCR was performed (Fig. 2), and the SNP was characterized by sequencing of the PCR products (Fig. 3). We investigated 29 patients aged between 4 and 18 years. Most of the patients ($n = 20$) were young adults (14–18 years). The study included 17 female and 12 male patients. Eight patients were treated in treatment group 1, 10 in treatment group 2, and 11 in treatment group 3. Thus, 21 of 29 (72 %) patients received 2 or 4 additional cycles of COPDAC chemotherapy following the first two courses of initial OEPA chemotherapy. Thirteen patients received radiotherapy and 16 did not (Table 1).

Depending on the presence of the minor T allele, patients were divided into two groups. Group one included patients with genotype C/C, group two included patients with C/T and T/T genotypes. Of the investigated 29 patients, we found 11 patients with the T allele. The frequency of the T allele in our study population was higher than expected: 37.9 % of the patients carried the C/T or T/T genotype. The other 18 patients had the homozygous C/C genotype. Because the investigated polymorphism affects an amino acid in proximity to the active site of the MGMT enzyme, the functionality of the enzyme could be influenced [27]. Therefore, we tested the possible association between the SNP rs12917 and clinical data. We found no differences in age, gender, therapy group, B symptoms, extranodal lesions, stage, or indication for radiotherapy between the two groups (Table 4).

However, we found that the T allele was associated with the degree of [^{18}F]FDG-PET response following two courses of OEPA chemotherapy (Table 4). Patients with the T allele showed significantly more often qPET values ≥ 0.95 compared with patients with the C/C genotype (Table 4), indicating that the therapy response as measured in [^{18}F]FDG-PET of patients with T allele is more frequently delayed and therefore, indication for radiotherapy was more frequent compared with the patients with C/C genotype. Radiotherapy recommendation based on the IHP criteria was followed in 13 of 14 patients. In a total of two patients, the recommendations regarding radiotherapy differed between the qPET method (cutoff 0.95) and the IHP rating (Table 1).

Discussion

In our study, we investigated the SNP rs12917 in HL cell lines and patient samples. Only the HL cell lines L-1236 and L-428 expressed normal MGMT transcripts [13]. The HL cell line L-1236 is heterozygous for the rs12917 polymorphism whereas L-428 is homozygous for the T allele. The HL cell line KM-H2 expressed a fusion-transcript between PRRC2B and MGMT and is heterozygous for the rs12917

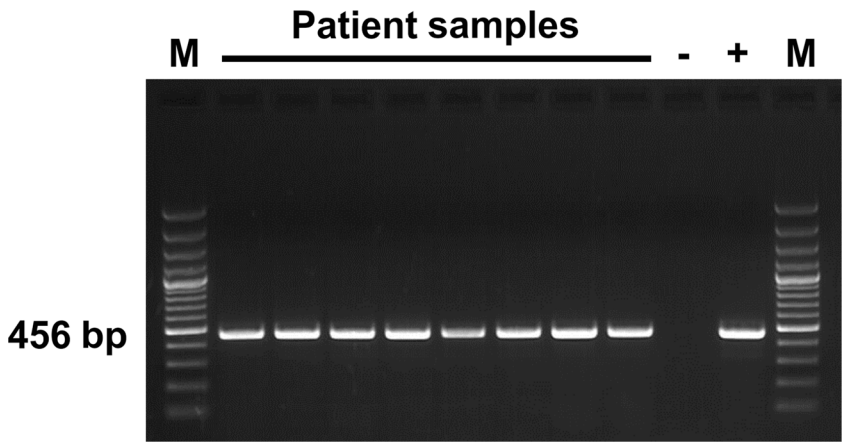

Fig. 2. Example of MGMT PCR results from PHL patients. Presented is a representative result of a PCR with MGMT specific primers. The product with the specific primers for MGMT has a size of 456 base pairs. PCR with L-428 cDNA served as positive control (+) and PCR without template served as negative control (–). M: DNA size marker.

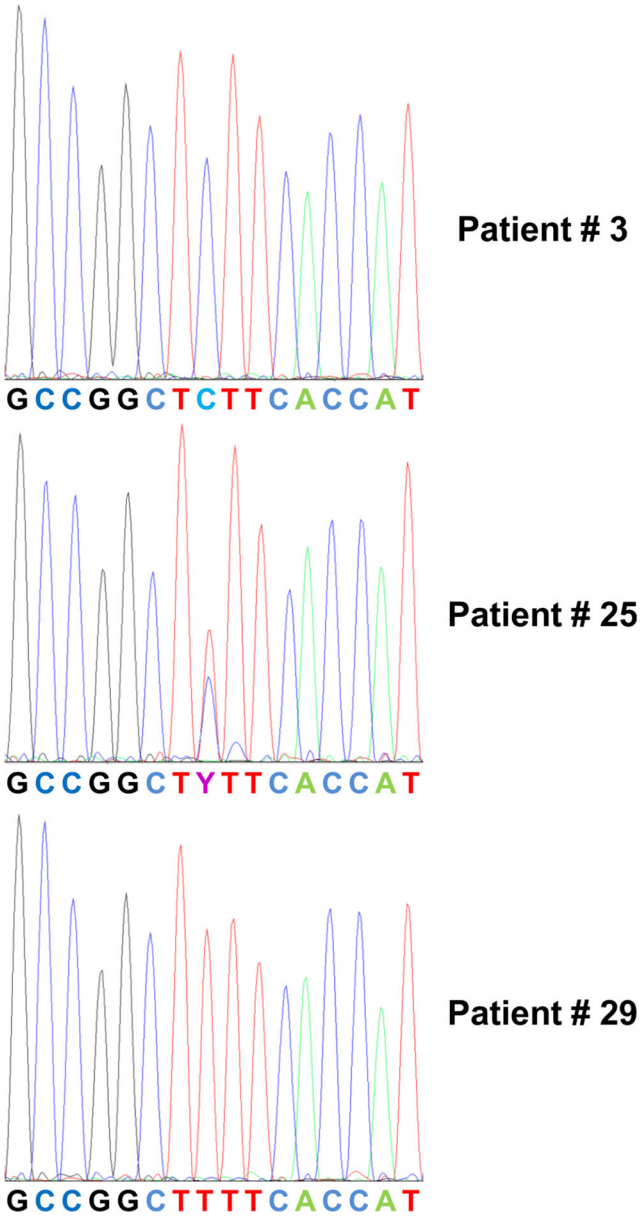

Fig. 3. Examples of sequencing chromatograms from PHL patients. Presented are the sequencing results for the SNP rs12917 in MGMT at cDNA level from 3 PHL patients. Patient # 3 showed the homozygous C/C genotype, patient # 25 the heterozygous C/T genotype, and patient # 29 the homozygous T/T genotype.

polymorphism [13]. Transcription was only observed from the fusion gene [13]. The HL cell lines L-540 and HDLM-2 showed a methylated promoter and no MGMT expression was detectable in these cells [13]. At the DNA level, L-540 cells were homozygous for the C allele and HDLM-2 cells heterozygous for the MGMT rs12917 polymorphism. Altogether, four of five HL cell lines showed presence of the minor rs12917 T allele.

Therefore, we investigated the frequency of the polymorphism in PHL patients. In our study, 11 of 29 patients showed the presence of the minor allele in the MGMT transcript. These are more than 37 % of the study group, but we had no matched control group and, therefore, we cannot conclude association between the SNP and HL risk. Many other studies investigated the association between the MGMT polymorphism rs12917 and the risk of cancer. The minor T allele was associated with a higher risk for some cancers [28–34] and decreased risk for other cancers [28, 35–38].

The investigated SNP rs12917 changes an amino acid in proximity to the active side of MGMT where the zinc binding is coordinated [39, 40]. Some studies found that the polymorphism rs12917 had no effect on the MGMT functionality regarding the repair of O6-methylguanine [41–43]. Other studies suggested that the minor T allele leads to a suboptimal and reduced repair of O6-methylguanine lesions and a higher DNA adduct level [44–46]. The OEPA containing therapy given to our patients did not consist of components which generate O6-methylguanine. However, this chemotherapy includes doxorubicin. Chekhun et al. demonstrated an impact of MGMT on resistance against doxorubicin [14]. In addition, other studies described that MGMT promoter methylation is a prognostic factor for patients that have not been treated with alkylating drugs [47]. It was shown that MGMT inhibition influences the expression of several cancer-related genes [48]. MGMT interacts with proteins involved in cell cycle and binds to tubulin; both were targets of the given OEPA therapy [49, 50]. These observations suggest that MGMT has additional function independent of repair of alkylated DNA. Furthermore, Remington et al. showed that MGMT with the T allele at rs12917 showed an increased degradation when benzylguanine is added [51]. The SNP might lead to a faster degradation of the enzyme before DNA repair is completed. Such effects might also explain the high frequency of the minor T allele in HL cell lines. Notably, all these cell lines are derived from patients with therapy failure. On the other hand, many other factors might play a role in the survival of HL cells, e.g., glutathione-S-transferases, ABC transporters, p53, or the BCL2 pathway. However, at this point, it is also important to consider that 21 of the 29 analyzed patients received two or four additional chemotherapy cycles of COPDAC following OEPA. COPDAC includes dacarbazine which is an alkylating drug and important for an effective PHL treatment [2]. Thus, in case of an inadequate response to OEPA, resistance to dacarbazine due to MGMT could become particularly important.

This is to the best of our knowledge, the first publication where the SNP rs12917 was investigated in PHL patients. Whereas no significant difference could be detected for items evaluated on initial staging, metabolic response to OEPA chemotherapy determined by [^{18}F]FDG-PET revealed a significant difference between the two patient groups: in the patients with minor T allele, metabolic response was more often delayed and therefore inadequate. Hence, radiotherapy following chemotherapy was more

Table 4. Associations between rs12917 polymorphism and clinical data

	T allele absent (C/C genotype)	T allele present (C/T and T/T genotypes)	*p* value (Fisher exact test)
qPET value ≥ 0.95	8/18 (44 %)	10/11 (91 %)	0.019
Gender	10 female 8 male	7 female 4 male	0.717
Age	range 8–17 years mean 14.16 ± 2.89	range 4–18 years mean 14.72 ± 4. 62	0.72
Stage	I: 0; II: 8; III: 3; IV: 7	I: 0; II: 7; III: 2; IV: 2	0.6725
Treatment group	TG1: 4; TG2: 6; TG3: 8	TG1: 4; TG2: 4; TG3: 3	0.5934
Extranodal lesions	3/18 (17 %)	2/11(18 %)	1
B symptoms	9/18 (50 %)	3/11 (27 %)	0.2732
COPDAC after OEPA	14/18 (78 %)	7/11 (64 %)	0.4327
Radiotherapy	6/18 (33 %)	7/11 (64 %)	0.1426
Relapse	1	0	1

frequently applied in these patients (64 %) compared with those patients which carriers the C/C genotype (33 %). It seems unlikely that differences in the glucose metabolism or some kind of metabolic/biochemical "stunning" are responsible for differences between qPET values from patients with T allele and patients without T allele. Such effects should be present not only in tumor tissue but also in other tissues. However, we have no evidence for differences in, e.g., liver glucose metabolism. One could hypothesize that an altered OEPA chemosensitivity due to MGMT is the explanation for the frequently persisting glucose metabolism in patients with C/T or T/T genotype. However, qPET values up to 0.95 imply a low degree of residual glucose metabolism as it may also occur in unspecific inflammation. Unspecific inflammation may result in a low positive-predictive value of response [^{18}F]FDG-PET. In order to increase the positive-predictive value, the threshold to differentiate between an adequate and an inadequate metabolic treatment response has been shifted in recent years. Accordingly, a Deauville score of 3 or qPET values of up to 1.3 are currently regarded as an adequate response in PHL [25, 52]. Further adjustments up to a Deauville score of 5 or qPET values up to 2.0 might be conceivable in the future [53]. In this respect, it seems desirable to reapply the same study design as presented here to larger study population that received response-adapted treatment based on more specific [^{18}F]FDG-PET response criteria. Within such a PHL cohort, a significantly lower proportion of patients would receive radiation therapy. Due to the lack of radiotherapy, the effects of different genotypes (C/C versus C/T or T/T) on the efficacy of COPDAC chemotherapy should become more apparent. If a strong correlation between C/T or T/T genotype and chemotherapy response could be confirmed, these patients would need either chemotherapy adjustment or regular irradiation.

In the present study, we have only investigated the SNP rs12917. We have not analyzed SNPs or mutations in other genes. Whether rs12917 is a functional SNP in the context of PHL or whether this SNP is only a tag SNP has to be clarified. However, there are no reported linkage disequilibria between rs12917 and other SNP outside the MGMT locus. Another limitation of our study is to be seen in the analyzed FDG/PET data. These were acquired in different PET centers and primarily under clinical, not scientific aspects.

Conclusion

Taken together, in the present study, the SNP rs12917 in the MGMT transcript was investigated. It turned out that the minor T allele is presented in four of five investigated HL cell lines. The SNP was also detected in 11 of 29 PHL patients. It could be demonstrated that patients with the T allele had a significantly higher rate of inadequate early treatment response determined by [^{18}F]FDG-PET than patients with C/C genotype. However, whether MGMT polymorphisms can be used to tailor PHL treatment requires further investigations with larger patient numbers and more specific [^{18}F]FDG-PET response criteria.

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Competing Interests

The authors declare that they have no competing interests.

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