*research article*

# Genetic polymorphisms in aquaporin 1 as risk factors for malignant mesothelioma and biomarkers of response to cisplatin treatment

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**Background.** Malignant mesothelioma (MM) is an asbestos related aggressive tumor with poor prognosis. The aim of this study was to investigate if aquaporin 1 (*AQP1*) genetic polymorphisms influence the risk of MM and the response to cisplatin based MM treatment.

**Patients and methods.** The case-control study included 231 patients with MM and a control group of 316 healthy blood donors. All subjects were genotyped for three *AQP1*polymorphisms (rs1049305, rs1476597 and rs28362731). Logistic and Cox regression were used in statistical analysis.

**Results.** *AQP1* rs1049305 polymorphism was significantly associated with MM risk in dominant model adjusted for gender and age (OR = 0.60, 95% CI = 0.37–0.96, $P_{adj}$ = 0.033). This polymorphism was also significantly associated with cisplatin based treatment related anaemia (unadjusted: OR = 0.49, 95% CI = 0.27–0.90, P = 0.021; adjusted: for CRP: OR = 0.52, 95% CI = 0.27–0.99, P = 0.046), with leukopenia (OR = 2.09, 95% CI = 1.00–4.35, P = 0.049) in dominant model and with thrombocytopenia (OR = 3.06, 95% CI = 1.01–9.28, P = 0.048) and alopecia (OR = 2.92, 95% CI = 1.00–8.46, P = 0.049) in additive model. *AQP1* rs28362731 was significantly associated with thrombocytopenia (unadjusted: OR = 3.73, 95% CI = 1.00–13.84, P = 0.049; adjusted for pain: OR = 4.63, 95% CI = 1.13–19.05, P = 0.034) in additive model.

**Conclusions.** *AQP1* may play a role in the risk of MM. Furthermore, *AQP1* genotype information could improve the prediction of MM patients at increased risk for cisplatin toxicity.

Key words: malignant mesothelioma; *AQP1;* polymorphism; cisplatin

## Introduction

It is generally accepted that the risk of developing diseases and an individual's response to the treatment may also depend on their genetic characteristics. In this study, we have focused on malignant mesothelioma (MM), which is a very aggressive cancer associated with the exposure to asbestos.[1-4] Most frequently it arises from pleura or peritoneum, but can also arise from other serous surfaces.[5,6]

In Slovenia, the professional exposure to asbestos occurred mainly in asbestos cement industry, in construction, in manufacture of machinery and insulation materials, in maintenance of various means of transport, in textile industry and in other activities.[7-9] Malignant mesothelioma is associated also with exposure to asbestos outside the workplace.[5,8-10] It is estimated that the incidence of MM will remain stable or will even increase in the near future due to the continuous presence of asbestos in buildings and to the long latent period after exposure to asbestos.[11,12] It is predicted that its incidence in the most industrialized countries will continue to increase until 2020,[5] or even later.[11]

Due to an increasing incidence of MM and its poor prognosis, new prognostic and predictive biomarkers are needed.[13] Symptoms of MM commonly occur only at late stages, therefore novel biomarkers for earlier diagnosis of MM and for establishing the response to treatment might be a promising opportunity for these patients.[14] Several classes of potential biomarkers of MM have been studied so far, from serum peptides to genetic and epigenetic biomarkers, however with limited success. Among serum biomarkers, soluble peptides related to mesothelin (soluble mesothelin-related peptides, SMRP),[15] fibulin-3,[16] survivin[13] have been studied, however none of them had sufficient predictive value as a standalone biomarker. It has been proposed, that biomarkers from two different molecular classes: protein and miRNA could be used in a combination to improve the biomarker sensitivity and specificity.[14]

Another approach was to investigate interindividual genetic variability in genes coding for key determinants of molecular pathogenesis of MM as potential biomarkers for prediction of the risk of MM as well as treatment response. Several studies have shown that polymorphisms in the genes involved in xenobiotic and oxidative metabolism or in DNA repair processes may play an important role in aetiology and pathogenesis of MM.[17-19] The most commonly studied *GSTM1* null polymorphism showed an increased risk for MM.[17] Similarly, two variant alleles of *XRCC1* and *XRCC3* were associated with increased risk for MM.[17] The carriers of at least one polymorphic *NQO1* allele (CT and TT genotypes) had an increased risk of MM compared to those with CC genotype.[19] A recent study showed also the association of *FTO* variability with MM susceptibility.[20] On the other hand, *MMP2* polymorphism was suggested to have a protective role in MM.[21] Furthermore, two of the investigated *MMP9* single nucleotide polymorphisms (SNPs) had significant but opposing effect on time to progression (TTP) and overall survival (OS) in MM.[22] *FASL-844* polymorphism could predict progression free survival (PFS) in MM patients receiving platinum based chemotherapy.[23] Polymorphisms in *REV1* and *REV3L* were also associated with the outcome of cisplatin based chemotherapy in MM.[24] The results of the study showed that DNA repair gene polymorphisms *XRCC1* may modify the response to gemcitabine-platinum combination chemotherapy in MM patients.[18] Despite such high numbers of genetic factors investigated, the search for potential novel genetic biomarkers continues.

Aquaporins (AQPs) are small transmembrane proteins, which facilitate an osmotically controlled passage of water. Recent research indicated a key role of AQPs in human carcinogenesis.[25-27] All key processes in cancer cells depend on water in the tumour microenvironment, therefore an enhanced transmembrane transmission of water is stimulated in comparison to normal cells. Overexpression of AQPs in the cell lines of the vascular endothelium and tumour cell lines suggests that AQPs may be closely related to the development and progression of a tumour.[28] In some cancers AQP1 expression was also shown to participate in metastatic processes.[29] In *AQP1*-knockout mice, xenograft tumour growth and angiogenesis were reduced, and significant necrosis occurred in the tumour tissues.[30]

The expression of AQP1 in MM tumour cells has been suggested to be an independent prognostic factor favouring survival in MM patients: higher levels of an AQP1 expression only in tumour cells, but not in vascular cells, predicted a better survival.[31] Higher levels of AQP1 expression were also associated with a better course of the disease in MM, but with worse course of the disease in some other tumours such as breast cancer, melanoma, urothelial and pharyngeal carcinoma.[32-35] AQP1 is of interest as a potential biomarker in MM patients as it was shown to be an independent prognostic factor[11] with high levels of its expression correlating with an increased survival.[29,31,36] AQP1 expression also correlated with improved survival rates in MM with epithelioid component in comparison to AQP1-poor MM.[37] Furthermore, AQP1 is also a possible new target for MM treatment,[5] and there are already AQP1 blockers available which could be used for therapy.[38]

Genetic polymorphisms were reported in *AQP1* gene, however according to our knowledge they have never been investigated in MM. A functional *AQP1* rs1476597 (-783G/C) SNP leading to transcriptional activation of the *AQP1* promoter and increased *AQP1* mRNA expression in C allele carriers was associated with better survival in glioblastoma multiform patients with GG and GC genotype.[39] Other *AQP1* SNPs were studied in a variety of conditions, but not in cancer. Firm evidence suggested that *AQP1* rs1049305 SNP could be involved in genetic susceptibility for development of water retention in patients with liver cirrhosis.[40] The study in marathon runners reported a significant association between *AQP1* rs1049305 and running performance. This study suggested that *AQP1* rs1049305 polymorphism located in 3′-UTR, in interaction with miRNAs could influence

the mRNA expression and AQP1 protein levels.[41] Triathletes who carried *AQP1* rs1049305 C allele had better running performance in comparison to GG genotype. This SNP was not associated with relative body weight change.[41] It has been suggested that *AQP1* rs10244884 could predict the risk of vaso-occlusion in sickle cell patients.[42]

The aim of the present study was to investigate the influence of *AQP1* genetic polymorphisms on the risk of developing MM and response to cisplatin-based treatment.

## Patients and methods

### Study population

The case-control study included patients treated for mostly MM of pleura or also peritoneum at the Institute of Oncology Ljubljana from 2007 to the end of 2016. Control group consisted of blood donors from the Institute of Transfusion Medicine in Ljubljana and were over 40 years old.

The diagnosis of MM was made by means of thoracoscopy or video-assisted thoracoscopic surgery (VATS) in patients with pleural MM and by means of laparoscopy or laparotomy in peritoneal MM. The diagnosis was confirmed histopathologically by an experienced pathologist [15].[15]

Demographic and clinical data (age, gender, smoking, possible other diseases) from patients with MM were obtained from the medical records of the Institute of Oncology Ljubljana.

The following clinical indicators were used to evaluate the efficacy of treatment: response to treatment according to the modified criteria RECIST (Response Evaluation Criteria in Solid Tumours),[43] PFS and overall survival (OS). The toxicity of the treatment was assessed according to NCI criteria (National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0).[44]

### Ethical approval

The study was approved by the Republic of Slovenia National Medical Ethics Committee (41/02/09) and was carried out according to the Helsinki Declaration. All the subjects included in the study have signed the written informed consent.

### Genotyping methods

DNA samples from 26 patients were isolated from peripheral venous blood with commercially available reagent sets (QIAamp DNA Mini Kit and Flexigene DNA Kit (Qiagen, Hilden, Germany)). For all other patients and controls DNA was already isolated from peripheral venous blood samples during the course of the previous studies.[18,45-48]

Based on the bioinformatics analysis, we selected the following SNPs: *AQP1* rs1049305 G> C in 3'-untranslated region that may affect the binding of miRNA [41], *AQP1* rs1476597 G> C in the 5'-regulatory region that may affect the binding of the transcription factors[49] and *AQP1* rs28362731 G> A that may affect splicing.

All the polymorphisms were genotyped using competitive allele specific PCR (KASPar) according to the manufacturer's instructions (LGC Genomics, UK).

### Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics version 21.0 (IBM Corporation, Armonk, NY, USA). With the usual descriptive statistics we first described the characteristics of each variable separately. In order to assess the causal relationship between MM and the individual variables, we first used a univariate logistic regression. Both additive and dominant models were used to assess the effect of the selected *AQP1* polymorphisms. Analysis was followed by the multivariate statistical modelling, taking into account the selected *AQP1* polymorphisms and possible confounders such as age, gender, and smoking and significant clinical parameters. Hazard ratio (HR), 95% confidence interval (95% CI) and P-value were determined by Cox regression and median survival was determined by the Kaplan-Meier method.

In order to test the interactions between the selected *AQP1* polymorphisms we introduced the logistic regression models with dummy variables.

## Results

The clinical characteristics of MM patients are shown in Table 1. Among all 231 patients whose median (25%–75% range) age was 66 (58–73) years, men represented 73.6%. Epithelioid MM was present in 72.3% of patients. ECOG performance status 1 (48.1%) and 2 (39.0%) prevailed. Exposure to asbestos was confirmed in 73.8% of patients. Among all patients, 46.7% were smokers. In total 194 patients were treated with cisplatin based therapy.

In addition, 316 healthy blood donors, 235 men and 81 women, whose median (25%–75% range)

age was 49 (45-55) years were also included in the molecular-genetic part of the study.

The genotype frequency distribution for the investigated polymorphisms in 231 MM patients and in 316 controls, their minor allele frequencies (MAF) and the risk of developing MM are shown in Table 2. The genotypes' distribution was in Hardy-Weinberg equilibrium (HWE), except for the distribution of *AQP1* rs1476597 in MM patients and also in healthy controls that were not consistent with HWE and therefore we excluded this polymorphism from further statistical analysis.

In univariate analysis no polymorphism was associated with the risk of developing MM (Table 2). Higher age was associated with a higher risk of developing MM (OR = 1.21, 95% CI = 1.17–1.25, P < 0.001) but gender (OR = 1.04, 95% CI = 0.71–1.53, P = 0.838) was not. *AQP1* rs1049305 polymorphism was significantly associated with the risk of developing MM when adjusted for age and gender (OR = 0.59, 95% CI = 0.35–0.97, $P_{adj}$ = 0.039 in additive model; OR = 0.60, 95% CI = 0.37–0.96, $P_{adj}$ = 0.033 in dominant model). *AQP1* rs28362731 was not significantly associated with the risk of developing MM even when adjusted for age and gender (Table 2).

Clinical characteristics of MM patients treated with cisplatin based chemotherapy are presented in Table 3. The majority (68.0%) of patients were treated with gemcitabine in combination with cisplatin. In chemotherapy response a third (32.8%) of patients responded with partial response (PR) and only in few patients (3.2%) the response was complete (CR). A half (49.5%) of patients had stable disease (SD) and a few (14.5%) of them had progressive disease (PD). Median progression free survival (PFS) was 7.8 months, median overall survival (OS) 18.1 months and median follow-up from the start of chemotherapy 49.2 months.

In the survival analysis, *AQP1* rs28362731 and *AQP1* rs1049305 were not significantly associated with PFS or with OS when patients were treated with cisplatin based chemotherapy (Table 4). Even when adjusted for histological type of MM, smoking, weight loss and CRP, *AQP1* polymorphisms were not significantly associated with PFS. Likewise associations with OS remained insignificant after adjustment for the histological type of MM, smoking and CRP (data not shown). In the chemotherapy response, *AQP1* rs28362731 and *AQP1* rs1049305 were not significantly associated with response rate when patients were treated with cisplatin in combination with either gemcitabine or pemetrexed (Table 4). These associations remained insignificant when adjusted for loss of weight and CRP (data not shown).

The association between SNPs and side effects in cisplatin based treatment is shown in Tables 5 and 6. *AQP1* rs1049305 was significantly associated with anemia grade ≥ 2 both in additive and dominant genetic model (additive model for genotype GC: OR = 0.40, 95% CI = 0.20–0.78, P = 0.007; dominant model OR = 0.49, 95% CI = 0.27–0.90, P = 0.021). The associations remained significant also when adjusted for CRP (OR = 0.46, 95% CI = 0.23–0.92, P = 0.029 in additive model; OR = 0.52, 95% CI

**TABLE 1.** Description of all malignant mesothelioma (MM) patients (N = 231) and MM patients treated with cisplatin based chemotherapy (N = 194)

| | | All MM patients | MM patients treated with cisplatin based chemotherapy |
|---|---|---|---|
| **Characteristic** | **Characteristic type** | **N (%)** | **N (%)** |
| Age | Median (25%–75%) | 66 (58–73) | 65 (58–71.3) |
| Gender | Men | 170 (73.6) | 146 (75.3) |
| | Women | 61 (26.4) | 48 (24.7) |
| MM stage | I | 18 (7.8) | 15 (7.7) |
| | II | 57 (24.7) | 48 (24.7) |
| | III | 69 (29.9) | 62 (32.0) |
| | IV | 66 (28.6) | 50 (25.8) |
| | Peritoneal MM | 20 (8.7) | 18 (9.3) |
| | Undefined | 1 (0.4) | 1 (0.5) |
| Histological type | Epithelioid | 167 (72.3) | 147 (75.8) |
| | Biphasic | 26 (11.3) | 21 (10.8) |
| | Sarcomatoid | 24 (10.4) | 21 (10.8) |
| | Undefined | 14 (6.0) | 5 (2.6) |
| ECOG performance status | 0 | 15 (6.5) | 15 (7.7) |
| | 1 | 111 (48.1) | 100 (51.5) |
| | 2 | 90 (39.0) | 76 (39.2) |
| | 3 | 15 (6.5) | 3 (1.5) |
| Exposure to asbestos | No | 59 (26.6)[a] | 45 (23.3)[c] |
| | Yes | 166 (73.8) | 148 (76.7) |
| Smoking | No | 120 (53.3)[a] | 101(52.6)[d] |
| | Yes | 105 (46.7) | 91(47.4) |
| Treatment | Gemcitabine/ Cisplatin | 132 (60.0)[b] | 132 (68.0) |
| | Pemetrexed/Cisplatin | 62 (28.2) | 62 (32.0) |
| | Without chemotherapy | 16 (7.3) | - |
| | Other forms of chemotherapy | 10 (4.5) | - |

Data are missing for: [a] 6 patients, [b] 11 patients, [c] 1 patient and [d] 2 patients. ECOG = Eastern Cooperative Oncology Group

**TABLE 2.** Distribution of *AQP1* genotypes in MM patients and controls and risk of MM

| | | | Patients | | | Controls | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SNP** | **Role** | **Genotype** | **N (%)** | **MAF** | **$P_{HWE}$** | **N (%)** | **MAF** | **$P_{HWE}$** | **OR (95% CI)** | **P** | **OR (95% CI)$_{adj}$** | **$P_{adj}$** |
| rs28362731[a] | p.Gly165Asp | GG | 210 (92.1) | 0.039 | 0.535 | 288 (91.7) | 0.041 | 0.444 | Ref. | - | Ref. | - |
| | | GA | 18 (7.9) | | | 26 (8.3) | | | 0.95 (0.51–1.78) | 0.871 | 0.94 (0.38–2.30) | 0.885 |
| rs1049305[b] | c.*578G>C | GG | 107 (46.5) | 0.337 | 0.082 | 128 (40.8) | 0.373 | 0.288 | Ref. | - | Ref. | - |
| | | GC | 91 (39.6) | | | 138 (43.9) | | | 0.79 (0.55–1.14) | 0.207 | 0.59 (0.35–0.97) | **0.039** |
| | | CC | 32 (13.9) | | | 48 (15.3) | | | 0.80 (0.48–1.13) | 0.390 | 0.63 (0.32–1.27) | 0.199 |
| | | GC+CC | 123 (53.5) | | | 186 (59.2) | | | 0.79 (0.56–1.12) | 0.181 | 0.60 (0.37–0.96) | **0.033** |
| rs1476597[c] | c.-783G>C | GG | 157 (68.0) | 0.255 | <0.001 | 220 (70.1) | 0.247 | <0.001 | | | | |
| | | GC | 30 (13.0) | | | 33 (10.5) | | | | | | |
| | | CC | 44 (19.0) | | | 61 (19.4) | | | | | | |

Data are missing for: [a]2 controls and 3 patients, [b]2 controls and 1 patient, [c]2 controls. $_{adj}$ = adjusted by gender and age; MAF = minor allele frequency; $P_{HWE}$ = P for Hardy-Weinberg equilibrium; Ref. = reference genotype

**TABLE 3.** Clinical characteristics of MM patients treated with cisplatin based chemotherapy (N = 194)

| **Characteristic** | | **N (%)** |
|---|---|---|
| Chemotherapy type | Gemcitabine and cisplatin | 132 (68.0) |
| | Pemetrexed and cisplatin | 62 (32.0) |
| Chemotherapy response[a] | Complete response (CR) | 6 (3.2) |
| | Partial response (PR) | 61 (32.8) |
| | Stable disease (SD) | 92 (49.5) |
| | Progressive disease (PD) | 27 (14.5) |
| Progression of disease[b] | No | 20 (10.5) |
| | Yes | 171 (89.5) |
| Death | No | 58 (29.9) |
| | Yes | 136 (70.1) |
| PFS | Median (25%–75%) (month) | 7.8 (5.3–13.8) |
| OS | Median (25%–75%) (month) | 18.1 (9.4–28.7) |
| Follow-up from the start of chemotherapy | Median (25%–75%) (month) | 49.2 (18.9–75.5) |
| CRP | Median (25%–75%) | 20.5 (9–58) |
| LDH | Median (25%–75%) | 2.67 (2.26–3.11) |
| Pain[b] | No | 79 (41.4) |
| | Yes | 112 (58.6) |
| Weight loss[c] | No | 68 (35.8) |
| | Yes | 122 (64.2) |

Data are missing for: [a]8 patients, [b]3 patients, [c]4 patients. CRP = C reactive protein; LDH = lactate dehydrogenase; OS = overall survival; PFS = progression free survival

= 0.27–0.99, P = 0.046 in dominant model). *AQP1* rs1049305 was also significantly associated with thrombocytopenia in additive model for genotype CC (OR = 3.06, 95% CI = 1.01–9.28, P = 0.048), but not in dominant model. *AQP1* rs1049305 was also significantly associated with the risk of leukopenia (additive model for genotype CC: OR = 3.03, 95% CI = 1.10–8.38, P = 0.033; dominant model OR = 2.09, 95% CI = 1.00–4.35, P = 0.049). Furthermore, there was a significant association of *AQP1* rs1049305 with alopecia in additive model for genotype CC (OR = 2.92, 95% CI = 1.00–8.46, P = 0.049), however, this SNP was not associated with neutropenia, nephrotoxicity or nausea and/or vomiting.

*AQP1* rs28362731 GA genotype was significantly associated with thrombocytopenia (OR = 3.73, 95% CI = 1.00–13.84, P = 0.049). This association remained significant when adjusted for pain at diagnosis (OR = 4.63, 95% CI = 1.13–19.05, P = 0.034).

The investigated polymorphisms did not statistically significantly influence neutropenia grade ≥ 2, nephrotoxicity or nausea and/or vomiting (Tables 5 and 6).

Multiplicative interaction analysis did not show any interactions between *AQP1* rs28362731 and *AQP1* rs1049305 polymorphisms and the risk of developing MM (OR = 1.22, 95% CI = 0.33–4.56, P = 0.771). Additionally, interactions between rs28362731 and rs1049305, rs28362731 and smok-

**TABLE 4.** Influence of *AQP1* SNP on survival and chemotherapy response in MM patients

| | | Progress free survival | | | Overall survival | | | Chemotherapy response | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SNP | Genotype | PFS median (25%–75%) month | HR (95% CI) | P | OS median (25%–75%) month | HR (95% CI) | P | Poor response N (%) | Good response N (%) | OR (95% CI) | P |
| rs28362731 | GG | 7.7 (5.2–13.6) | Ref. | - | 18.1 (9.1–28.0) | Ref. | - | 112 (65.1) | 60 (34.9) | Ref. | - |
| | GA | 11.1 (7.0–14.7) | 0.72 (0.39–1.33) | 0.299 | 26.5 (14.4–47.8) | 0.56 (0.26–1.19) | 0.130 | 6 (54.5) | 5 (45.5) | 1.56 (0.46–5.31) | 0.481 |
| rs1049305 | GG | 7.9 (5.4–12.1) | Ref. | - | 18.1 (9.0–26.8) | Ref. | - | 55 (64.7) | 30 (35.3) | Ref. | - |
| | GC | 7.8 (5.2–15.0) | 0.80 (0.58–1.11) | 0.187 | 22.1 (10.1–29.7) | 0.72 (0.50–1.05) | 0.091 | 43 (58.1) | 31 (41.9) | 1.32 (0.70–2.51) | 0.394 |
| | CC | 7.4 (4.8–14.1) | 0.92 (0.59–1.46) | 0.736 | 13.3 (8.1–25.4) | 1.10 (0.67–1.80) | 0.712 | 20 (76.9) | 6 (23.1) | 0.55 (0.20–1.52) | 0.248 |
| | GC+CC | 7.8 (4.9–15.0) | 0.83 (0.62–1.13) | 0.233 | 18.2 (9.5–28.7) | 0.81 (0.58–1.14) | 0.220 | 63 (63.0) | 37 (37.0) | 1.08 (0.59–1.97) | 0.810 |

SNP = single nucleotide polymorphisms; OS = overall survival; PFS = progression free survival; Ref. = reference genotype

**TABLE 5.** Association between *AQP1* SNPs and haematological side effects of cisplatin based treatment (N = 176)

| SNP | Genotype | Anemia grade ≥ 2[a] | | | | | Thrombocytopenia[b] | | | | | Leukopenia grade ≥ 2[c] | | | Neutropenia grade ≥ 2 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | N (%) | OR (95% CI) | P | OR (95% CI)$_{adj1}$ | $P_{adj1}$ | N (%) | OR (95% CI) | P | OR (95% CI)$_{adj2}$ | $P_{adj2}$ | N (%) | OR (95% CI) | P | N (%) | OR (95% CI) | P |
| rs28362731 | GG | 79 (49.4) | Ref. | - | Ref. | - | 21 (13.3) | Ref. | - | Ref. | - | 39 (25.2) | Ref. | - | 59 (36.4) | Ref. | - |
| | GA | 3 (27.3) | 0.38 (0.10–1.50) | 0.169 | 0.53 (0.13–2.12) | 0.370 | 4 (36.4) | 3.73 (1.00–13.84) | **0.049** | 4.63 (1.13–19.05) | **0.034** | 2 (18.2) | 0.66 (0.14–3.19) | 0.606 | 3 (27.3) | 0.66 (0.17–2.56) | 0.543 |
| rs1049305 | GG | 46 (56.8) | Ref. | - | Ref. | - | 10 (12.5) | Ref. | - | Ref. | - | 14 (17.5) | Ref. | - | 25 (30.9) | Ref. | - |
| | GC | 23 (34.3) | 0.40 (0.20–0.78) | **0.007** | 0.46 (0.23–0.92) | **0.029** | 8 (11.8) | 0.93 (0.35–2.52) | 0.892 | 0.71 (0.24–1.08) | 0.529 | 18 (27.7) | 1.81 (0.82–3.99) | 0.144 | 26 (37.1) | 1.32 (0.67–2.60) | 0.416 |
| | CC | 13 (52.0) | 0.82 (0.34–2.03) | 0.674 | 0.74 (0.28–1.94) | 0.536 | 7 (30.4) | 3.06 (1.01–9.28) | **0.048** | 2.18 (0.69–6.94) | 0.185 | 9 (36.1) | 3.03 (1.10–8.38) | **0.033** | 11 (45.8) | 1.90 (0.75–4.81) | 0.178 |
| | GC +CC | 36 (39.1) | 0.49 (0.27–0.90) | **0.021** | 0.52 (0.27–0.99) | **0.046** | 15 (16.5) | 1.38 (0.58–3.28) | 0.463 | 1.07 (0.43–2.69) | 0.885 | 27 (30.7) | 2.09 (1.00–4.35) | **0.049** | 37 (39.4) | 1.45 (0.78–2.72) | 0.242 |

Data are missing for: [a]2 patients, [b]4 patients, [c]7 patients. $_{adj1}$ = adjusted by CRP; $_{adj2}$ = adjusted by pain at diagnosis; SNP = single nucleotide polymorphisms

**TABLE 6.** Associations between *AQP1* SNPs and non-haematological side effects of cisplatin based treatment (N = 176)

| SNP | Genotype | Alopecia[a] | | | Nephrotoxicity[b] | | | Nausea/Vomiting[c] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | N (%) | OR (95% CI) | P | N (%) | OR (95% CI) | P | N (%) | OR(95% CI) | P |
| rs28362731 | GG | 60 (45.8) | Ref. | - | 74 (46.8) | Ref. | - | 73 (53.7) | Ref. | - |
| | GA | 5 (55.6) | 1.48 (0.38–5.76) | 0.572 | 3 (27.3) | 0.43 (0.11–1.66) | 0.219 | 5 (55.6) | 1.08 (0.28–4.19) | 0.913 |
| rs1049305 | GG | 30 (46.2) | Ref. | - | 35 (43.8) | Ref. | - | 36 (52.9) | Ref. | - |
| | GC | 20 (35.7) | 0.65 (0.31–1.35) | 0.246 | 34 (50.0) | 1.29 (0.67–2.46) | 0.448 | 26 (44.8) | 0.72 (0.36–1.46) | 0.364 |
| | CC | 15 (71.4) | 2.92 (1.00–8.46) | **0.049** | 10 (43.5) | 0.99 (0.39–2.52) | 0.982 | 15 (71.4) | 2.22 (0.77–6.41) | 0.140 |
| | GC+CC | 35 (45.5) | 0.97 (0.50–1.89) | 0.934 | 44 (48.4) | 1.20 (0.66–2.20) | 0.547 | 41 (51.9) | 0.96 (050–1.84) | 0.900 |

Data are missing for: [a] 33 patients, [b] 4 patients and [c] 28 patients. SNP = single nucleotide polymorphisms

**TABLE 7.** Influence of interactions on the risk of occurrence of side effects

| Side effect | Interaction 1rs28362731 -rs1049305 OR (95% CI) | $P_1$ | Interaction 2 rs28362731 - smoking OR (95% CI) | $P_2$ | Interaction 3 rs1049305 - smoking OR (95% CI) | $P_3$ |
|---|---|---|---|---|---|---|
| Anemia grade ≥ 2[a] | 1.84 (0.10–32.37) | 0.676 | - | 0.999 | 0.34 (0.10–1.16) | 0.085 |
| Leukopenia grade ≥ 2[b] | 0.95 (0.04–23.07) | 0.974 | - | 0.999 | 0.92 (0.21–4.02) | 0.915 |
| Neutropenia grade ≥ 2 | 0.55 (0.03–9.76) | 0.686 | 7.55 (0.39–145.1) | 0.180 | 0.67 (0.19–2.35) | 0.526 |
| Thrombocytopenia[c] | 1.73 (0.11–26.38) | 0.693 | 3.06 (0.20–46.56) | 0.422 | 0.95 (0.16–5.66) | 0.955 |
| Nephrotoxicity[c] | 0.68 (0.04–11.98) | 0.794 | - | 0.999 | 1.01 (0.30–3.43) | 0.982 |
| Alopecia[d] | 2.06 (0.11–40.01) | 0.633 | - | 0.999 | 0.60 (0.16–2.29) | 0.453 |
| Nausea/Vomiting[e] | 2.12 (0.11–40.98) | 0.620 | 6.83 (0.35–132.4) | 0.204 | 0.71 (0.19–2.64) | 0.608 |

Data are missing for: [a] 2 patients, [b] 7 patients, [c] 4 patients, [d] 33 patients and [e] 28 patients. Interaction 1: interaction between rs28362731 and rs1049305. Interaction 2: interaction between rs28362731 and smoking. Interaction 3: interaction between rs1049305 and smoking.

ing and rs1049305 and smoking did not significantly influence the risk of occurrence of side effects (Table 7).

Haplotypes *AQP1* GG, GC and AG (5′ → 3′: rs28362731, rs1049305) were not significantly associated with the risk of developing MM even when adjusted for gender and age (Supplementary table).

## Discussion

In the present study we investigated the influence of *AQP1* genetic polymorphisms on the risk of developing MM as well as the associations with response to cisplatin based treatment. The important novel finding of our study is that the *AQP1* genetic variability might contribute to the risk of developing MM. Furthermore, we have shown the associations with the development of side effects of cisplatin based treatment.

*AQP1* rs1049305 polymorphism was significantly associated with the risk of developing MM, but only after adjustment for gender and age. *AQP1* rs1049305 GC heterozygotes had significantly lower risk of developing MM in the additive model, as well as the carriers of at least one polymorphic C allele in the dominant model in comparison to GG wild type. This polymorphism is located in the 3′-untranslated region, therefore it could affect the binding of miRNA and *AQP1* expression levels, however, the functionality of this polymorphism remains to be determined. On the other hand, *AQP1* rs28362731 was not significantly associated with the risk of developing MM in our study.

The statistical analyses have shown that the genotype distribution for the third investigated polymorphism *AQP1* rs1476597 was not in accordance with HWE, so it had to be excluded from further analysis. In this polymorphism, the substitution of G for C was associated both with increased transcriptional-activation of the *AQP1*-promoter and with increased *AQP1* mRNA expression.[39] This is the only *AQP1* polymorphism that has been investigated in cancer so far and was associated with survival-time in glioblastoma multiforme patients.[39] This study used a pyrosequencing approach and reported that genotype distribution for *AQP1* rs1476597 was in accordance with HWE.[39] We have checked that there was no genotyping error, so deviation from HWE could be interpreted either as a potential influence of the fact, that this polymorphism may be triallelic (G/C/A) or that the polymorphisms in the proximity could affect the binding of our primers from the reaction mixture.

We have also assessed the impact of *AQP1* haplotypes, but they were not significantly associated with the risk of developing MM, not even when adjusted for age and gender.

Our study also showed that *AQP1* rs1049305 and *AQP1* rs28362731 were not significantly associated with PFS, OS or response rate when patients were treated with cisplatin in combination with either gemcitabine or pemetrexed. However, it has been suggested that AQP1 may be an independent prognostic factor in MM, and that higher expression of AQP1 in tumor cells, but not in vascular cells was significantly associated with better survival.[31] It has also been shown that AQP1 expression significantly influenced the course of MM, regardless of the therapy or prognostic factors including histologic subtype, pathologic stage, gender, and age at time of diagnosis.[31]

We have also observed that *AQP1* rs1049305 was significantly associated with some of the treatment side effects such as anemia, leukopenia, thrombocytopenia and alopecia, but not with neutropenia, nephrotoxicity or nausea and/or vomiting. On the other hand, *AQP1* rs28362731 was significantly associated only with thrombocytopenia. Multiplicative interaction analysis did not show any interaction between *AQP1* rs28362731 and *AQP1* rs1049305 polymorphisms and the risk of occurrence of treatment related side effects. Similarly, side effects were not influenced by interactions between either of the studied polymorphism and smoking.

The major limitation of our study was that we had no information on asbestos exposure in healthy controls. Furthermore, MM patients were older than controls, as blood donors can only be up to 65 years old, however we accounted for that with adjustment for age in the statistical analysis. Despite the limited number of patients included in our study, all patients were monitored in the same institution and by the same oncologists, so there were no differences in the clinical assessments. Furthermore, all the patients and controls came from an ethnically homogeneous Slovenian population, so there were no differences due to genetic heterogeneity.[50,51]

Our study brings novel findings of the associations between *AQP1* genetic variability and the risk of developing MM that has not been previously investigated. Furthermore, it shows the impact of *AQP1* polymorphisms on the development of cisplatin treatment related side effects. It needs to be determined if the addition of these polymorphisms to previously described clinical-pharmacogenetics models could improve the prediction of treatment related side effects in MM patients [48]. Better understanding of pharmacogenetic polymorphisms would allow an individualized approach and better outcomes of cisplatin treatment in patients with MM.

In conclusion, our study suggests that the investigated *AQP1* polymorphisms may contribute to the risk of developing MM and cisplatin treatment related side effects, however our findings need to be validated in independent MM patient cohorts and in other cancers.

## Supplementary material

Supplementary table: The association between *AQP1* haplotypes and the risk of MM development.

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