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SNPs in Matrix metalloproteinase genes and lung cancer chemotherapy response and prognosis

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Short title:
MMP SNPs and lung cancer chemotherapy outcome

Abstract

The prognosis for lung cancer patients treated with chemotherapy is poor. Single nucleotide polymorphisms (SNPs) in Matrix Metalloproteinase genes could influence treatment outcome by altering apoptotic pathways. Eight SNPs with known or suspected phenotypic effect in six genes (*MMP1*, *MMP2, MMP3, MMP7, MMP9 and MMP12)* were investigated.

For 349 Caucasian patients with primary lung cancer receiving first-line chemotherapy three different endpoints were analysed: response after the 2nd cycle (ORR), progression free survival (PFS), and overall survival (OS).
The prognostic value of the SNPs was analysed using multiple logistic regression for all patients and histology-, stage- and treatment-specific subgroups. Hazard ratio estimates for PFS and for OS were calculated using Cox regression methods.
None of the investigated polymorphisms modified response significantly in the whole patient population.
However, tumour stage IIIB variant allele carriers of *MMP2 C-735T* showed a significantly worse response. PFS was significantly prolonged in *MMP1 G-1607GG* variant allele carriers and OS in SCLC patients carrying the *MMP12 A-82G* variant allele.
In conclusion, this study identified SNPs in *MMP1*, *MMP2, MMP7* and *MMP12* for further investigation as possible predictors of chemotherapy outcome in lung cancer patients.

Introduction

With about 1.35 million new cases diagnosed per year lung cancer is the most common cancer worldwide [1]. It is a disease with major morbidity and continuingly bad prognosis. While early stage non small cell lung cancer (NSCLC) can be treated by surgery, late stage NSCLC and small cell lung cancer (SCLC) cases receive chemotherapy as the treatment of choice [2]. Polymorphisms, which reduce or inhibit apoptosis, can cause chemotherapy resistance [3]. Cohort studies are used in order to elucidate correlations between biomarkers in the host genome, shown to be relevant for patient outcome, and therapy response.

Matrix metalloproteinase (MMP) expression is associated with the development of an extensive list of diseases especially various malignant tumours. Their involvement in promotion of metastasis, chemotherapy resistance of cancer and a bad treatment outcome has been shown in previous studies [4,5].

High expression of MMPs is usually associated with all steps of cancer initiation and progression. MMP3, MMP7 and MMP9, have also been reported to influence the Fas/FasL mediated extrinsic as well as the p53/PKC mediated intrinsic apoptotic pathway [6,7]. By cleavage of plasminogen and collagen XVIII, MMP12 is one of the most effective producers of the angiogenesis inhibitors angiostatin and endostatin [8]. In addition it has an influence on the plasmin levels and subsequent activation of MMPs like MMP3 and MMP9 [9]. MMP2, also known as gelatinase A, is involved in migration and invasion processes and also seems to have influence on chemotherapy response. It has been in rat models that platinum- based chemotherapy has higher response rates when the animals are co-treated with prinomastat, a specific MMP2 inhibitor [10]. MMP1 is the most highly expressed interstitial collagenase degrading fibrillar collagen. Overexpression of MMP1 shown in tumour tissues has been suggested to be associated with tumour invasion and metastasis [11].

Polymorphisms in *MMP* genes have been shown to influence the expression pattern of the protein. One polymorphism in the promoter region of *MMP2* (*C-735T*) was reported to lead to a lower expression of the protein due to a disruption of a SP1-binding site [12]. As far as *MMP3* is concerned, the variant allele of the ins/del polymorphism (6A-1171 5A) is associated with a higher transcription rate [13]. For *MMP7* two single nucleotide polymorphisms (SNPs) (*C-153T*; *A-181G*) have been functionally characterised and in both cases the variant alleles lead to an enhanced expression [14]. Another SNP in the promoter of the *MMP9* gene (C-1562T) increases transcription by the disruption of a repressor binding site [12]. Polymorphisms in the *MMP12* gene (*A-82G*; *A1082G*) also have been shown to influence the outcome of cancer patients. The promoter polymorphism is responsible for lowering the transcription by disruption of a AP1 binding site [15], whereas the polymorphism *A1082G* has not been shown to have a phenotypic effect. The GG allele of the functional *MMP1 G-1607GG* polymorphism is associated with higher expression levels of MMP1 and with increased susceptibility to head and neck and lung cancer [16,17].

This study focuses on the relationship between polymorphisms in MMP genes and three defined endpoints of clinical outcome: response to chemotherapy after the 2nd cycle (ORR), overall survival (OS) and progression free survival (PFS). We hypothesised that genetic background, such as polymorphisms in gDNA can influence therapy outcome. We also assumed that this background has a more immediate effect on an early endpoint, such as ORR than the clinically more relevant late endpoints OS and PFS due to tumour specific accumulation of genetic variations by clonal selection during many cycles of therapy.

Eight SNPs in six MMP genes were analyzed in a cohort of 349 lung cancer patients, consisting of 187 NSCLC, 161 SCLC and one patient with a mixed histology, receiving first-line chemotherapy.

Patients and Methods

Study cohort

349 patients of Caucasian origin with histologically confirmed primary lung cancer eligible for first-line chemotherapy were recruited between 3/1999 and 10/2004 at the Thoraxklinik in Heidelberg. The cut-off date for the follow-up was 3/2005. All patients had never received antineoplastic chemotherapy nor had they previously been diagnosed with another malignancy. Where possible, tumour stage at the time of diagnosis was determined according to the cTNM of the Union Internationale contre le Cancer (UICC) [18] using hospital records. For some SCLC patients the tumour stage was classified as limited or extensive disease based on the Veterans Administration Lung Cancer Study Group (VALG) criteria [19]. For statistical analysis limited and extensive disease were regarded as stage III and IV, respectively (table1).
Tumour response was assessed after the 2nd cycle of first line chemotherapy as complete remission (CR), partial remission (PR), stable disease (SD) or progressive disease (PD) according to the RECIST criteria for solid tumours [20]. Progression free survival (PFS) is defined as the time interval (in days) between start of chemotherapy and documented progression (uncensored observation). In case no progression was documented PFS was calculated until the last progression free examination (censored observation) irrespective of whether that patient was lost to follow up or whether death occurred later. Overall survival (OS) is defined as the time interval (in days) between start of chemotherapy and the documented date of death (uncensored observation) or, when date of death was unknown, the last date when the patient was still alive (censored observation).
Sixty percent of the 187 NSCLC cases died during the observation period, the median OS time was 410 days (95% CI 353–514). The median PFS time was 216 days (95% CI 189–251), and the median observation time was 812 days (95% CI 499–1120). Sixtythree percent of the 161 SCLC patients died during observation, the median OS time was 405 days (95% CI 347–557). The median PFS time was 271 days (95% CI 239–314), and the median observation time was 777 days (95% CI 648–838). All participants signed informed consent forms, and most study subjects completed a self-administered questionnaire with detailed information on pre-treatment, smoking habits and occupational exposure. The study was approved by the ethical committee of the Medical Faculty of the University of Heidelberg (Ref.-Nr.199/2001). Blood samples were collected prior to chemotherapy.

DNA extraction

Buffy coat from 5 ml venous blood in EDTA was archived at –80°C. Genomic DNA was isolated using either the QIAamp DNA blood midi kit, or by an automated DNA extraction protocol on the MagNA Pure System according to the manufacturer's instructions.

Genotyping

For the detection of the polymorphisms *MMP3 6A-1171 5A, MMP12 A-82G* and *MMP12 A1082G* assays using fluorescence-based melting curve analysis (LightCycler480) were designed (shown in Table 2). Each PCR with a final volume of 10µl was performed in 96 well plates (Roche, Mannheim, Germany) with approximately 20ng of genomic DNA as template. After two minutes 95°C initial incubation 45 subsequent cycles of 5s at 95°C, 10s for annealing at 56°C and 15s at 72°C for elongation were carried out. Concentrations for the multiplex reaction for *MMP12 A-82G* and *MMP12 A1082G* genotyping were 1x PCR Buffer, 5mM $MgCl_2$, 200µM dNTPs, 0.5U Qiagen Polymerase, 0.7µM forward primer (*A-82G*), 0.2µM reverse primer (*A-82*G), 0.1µM forward primer (*A1082G*), 0.7µM reverse primer (*A1082G*) and for all four probes 0.2µM each. Concentrations for *MMP3* 6A-1171 5A genotyping were 0.1µM forward primer and 0.5µM reverse primer, 2.5mM $MgCl_2$, 1x PCR

buffer, 200µM dNTPs, 0.5U Taq Polymerase (Eurolone, England) and both probes 0.2µM each. All primers and probes (supplied by TIB MOLBIOL, Berlin, Germany) are detailed in table 2.
For validation and quality control of the LightCycler480 method, PCR Restriction Fragment Length Polymorphism (RFLP) was performed for *MMP12 A-82G* [21]. Another PCR-RFLP analysis, for *MMP12* A1082G was newly developed and carried out in a total volume of 20µl with 400nM of each primer (Forward primer 5`-GATGACAAATACTGGTTAATTAGGA-3`; Reverse primer 5`-CTGGTTATCTACAAAGAAGT-3´), 200µM dNTPs, 1xPCR Buffer, 0.8U Taq-Polymerase and 5mM magnesium. Cycling conditions were as follows: initial denaturation at 95°C for 3 min followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 59°C for 30 sec, elongation at 72°C for 30 sec with a temperature transition rate of 20°C per sec and a final elongation step at 72°C for 2 min. Results were compared for at least 200 samples and showed 100% concordance. All genotyping was carried out blinded before data analysis. 10% of samples were genotyped twice for quality control and results showed no discrepancies. PCR-RFLP methods were employed as previously published for *MMP1 G-1607GG*, *MMP3 6A-1171 5A*, *MMP2 C-735T, MMP9 C-1562T*, *MMP7 C-153T* and *MMP7 A-181G* [12,14,17,22].
Linkage analysis of the polymorphisms situated on the long arm of chromosome 11 was calculated using the HAPReg software. Calculation of haplotypes and the logistic regression for evaluation of haplotypic effect was conducted with the Thesias software [23].

Statistical analysis
The allele frequencies and their standard deviation for the group of all patients were calculated and the genotype distribution was tested for deviations from Hardy-Weinberg Equilibrium (HWE) using the chi-square test. The prognostic value of the SNPs was analysed using multivariable logistic regression. Odds ratios (OR) were calculated by comparing genotype frequencies in responders (CR and PR) and non-responders (SD and PD) after the 2nd cycle of first-line chemotherapy.

Hazard ratio estimates (HR) with 95% confidence intervals were calculated using Cox proportional hazard models for progression free survival (PFS) (adjusted for tumour stage) and for overall survival (OS) (adjusted for tumour stage and gender). Kaplan-Meier survival curves were plotted and the log-rank test was used to test for differences, both for PFS and OS. In univariate analysis, tumor stage had a statistically significant influence on the response after the second cycle of NSCLC patients ($p=0.0012$), and on PFS and OS of all lung cancer patients ($p < 0.001$) in this study. Therefore we included stage as adjustment factor in all analyses. Gender had a significant influence on OS ($p = 0.044$) but not on PFS ($p = 0.982$) of all lung cancer patients and also not on chemotherapy response. Therefore, it was included as an adjustment factor only for OS. There was no significant influence of age in all three clinical outcomes ($p_{Response}=0.59$, $p_{OS}=0.88$, $p_{PFS}=0.82$). The same was observed for performance status measured by ECOG ($p_{Response}=0.61$, $p_{OS}=0.81$, $p_{PFS}=0.13$). Thus neither of these two parameters was used as confounding factor.
This analysis was performed for all 349 lung cancer patients. In addition, it was performed separately for the two histological sub-populations of SCLC and NSCLC. The wild type genotype was considered as reference. With explorative aim, we analysed therapy-based subsets including the patients receiving gemcitabine- (143; all NSCLC), etoposide- (161; 158 SCLC, 2 NSCLC and 1 mixed) and platinum-based therapy (256; 117 SCLC and 139 NSCLC). Additionally we analysed early- (IIB+IIIA) and late-stage (IIIB+IV) patients as well as IIIB and IV separately in the histological subgroups.

Allele frequencies were calculated using the website of the International Helmholtz Gemeinschaft (IHG) [24]. All other calculations were done using the statistical software package SAS (SAS Institute, Cary, NC, USA) version 9.1.3.

Results

The total of 349 lung cancer patients, who received first-line chemotherapy were included into this study. Three different endpoints were analysed: response after the 2nd cycle (R), progression free survival (PFS), and overall survival (OS) each for the whole patient group, as well as for the two subgroups SCLC and NSCLC.

The distribution of the genotypes of the SNPs analysed is given in Table 3. For all SNPs the distribution of genotypes is within HWE (*MMP1 G-1607GG* p= 0.65, *MMP2 C-735T* p=0.57; *MMP3 6A-11715A* p=0.39; *MMP7 C-153T* p=0.11; *A-181G* p=0.18; *MMP9 C-1562T* p=0.71, *MMP12 A-82G* p=0.97*, A1082G* p=0.99). The calculated frequencies of the wild-type alleles are *MMP2 C-735T* 0.88 +/-0.015*, MMP3 6A-11715A* 0.49 +/-0.024, *MMP7 C-153T* 0.93 +/-0.012, *A-181G* 0.46 +/-0.022, *MMP9 C-1562T* 0.86 +/-0.016, *MMP12 A-82G* 0.85 +/-0.017, *A1082G* 0.93 +/-0.012. These frequencies are comparable to those previously published for Caucasians by other groups [5,12-14].

While for SCLC, chemotherapy is the treatment of choice for all stages, early stage NSCLC patients are preferentially treated surgically. This is reflected in table 1 where response rates of SCLC patients are also much higher compared to NSCLC patients.
Odds ratios and their 95% confidence interval (CI) describing the risk of being a non-responder for variant allele carriers after multivariate analysis, are shown in Table 3.
In the group of all lung cancer patients none of the examined polymorphisms modified the chemotherapy response after the 2nd cycle significantly; only a borderline significant effect was observed for the *MMP7 C-153T* polymorphism (OR 1.79; 95%CI 0.99-3.20; p=0.05). Among stage IIIB patients the risk of being a non-responder was significantly increased in the aggregated groups of variant allele homozygotes and heterozygotes for *MMP2* C-735T (OR 2.79; 95%CI 1.06-7.41; p=0.02) and *MMP3 6A-1171 5A* (OR 10.37 95%CI 1.32-81.46; p=0.01) (Table 3).
An enhanced risk for being a nonresponder was also found for IIB-IIIA patients among the SCLC cohort carrying the *MMP2* C-735T polymorphism (OR 12.75; 95% CI 1.39-167.39; p=0.03) as well as for stage IIIB NSCLC patients carrying the variant allele of the *MMP3 6A-1171 5A* polymorphism (OR 7.78; 95%CI 1.35-147.84; p=0.03) (tables 4 and 5).
PFS was significantly affected in the group of NSCLC patients, who received gemcitabine as first-line chemotherapeutic drug and are carriers of the variant allele of the *MMP12 A1082G* polymorphism (HR 1.88; 95%CI 1.02-3.46; p=0.04). Individuals with the variant genotype of *MMP9 C-1562T* in the whole cohort among late stage patients (HR 1.36; 95%CI 1.00-1.84; p=0.05, data not shown) and also among late-stage SCLC patients (adjusted HR 1.72 95%CI 1.02-2.89; p=0.04) (Table 7) had a significant shorter PFS than those with the wildtype genotype. The Kaplan Meyer curve describing the progression-free survival for all patients also shows a significant modulation by the *MMP9* polymorphism (Figure 1). Individuals in the whole cohort carrying the GG allele of the *MMP1 G-1607GG* allele had a significantly longer PFS compared to the reference genotype carriers HR 0.72 (95%CI 0.55-0.95; p=0.04) and a longer PFS and OS in early stage SCLC (Table 6 and 7).
SCLC patients, who are carriers of the variant alleles of the *MMP12 A-82G* and homozygote carriers for the *MMP7 A-181G* polymorphism had a significantly longer lifetime after starting chemotherapy. The adjusted HRs were 0.50 (95%CI 0.31-0.78; p< 0.003) for the *MMP12 A-*

82G and 0.55 (95%CI 0.31-0.99; p=0.05) for the *MMP7 A-181G* polymorphism (Table 6). Linkage analysis for the *MMP1*, *MMP3, MMP7* and *MMP12* polymorphisms showed a high linkage for *MMP1*, *MMP3* and *MMP12* and the two *MMP7* polymorphisms. Haplotype analysis of the combination of the two highly linked *MMP7* polymorphisms showed a significantly increased risk for carriers of the GT haplotype to be a non-responder after the 2nd cycle of chemotherapy OR 1.86 (95%CI 1.01-3.47; p<0.05) in the whole cohort.

Discussion

The choice of therapy for the treatment of lung cancer therapy is currently dependent on stage, histology and performance status of the patient. Given that there are a number of chemotherapy treatments to choose from, host genetic polymorphisms which affect therapy success should be taken into account. Genotyping prior to therapy would be feasible because it is a quick, reliable and minimally-invasive procedure as only a blood sample is required. Individually tailored treatment could improve patient survival and quality of life and is thus very desirable for the future.

Therefore, we genotyped 7 polymorphisms in 5 different matrix metalloproteinase genes in a hypothesis-driven approach and analysed their impact on three endpoints: chemotherapy response after the 2nd cycle, progression free survival and overall survival. Response rates in SCLC and NSCLC are known to differ strongly and are also dependent on therapy received. Therefore we stratified data for histology, therapy protocol and stage. Stage has been shown to correlate strongly with MMP expression.

A particular strength of this study lies in the analysis of three endpoints, chemotherapy response, PFS and OS. Numbers are adequate for the separate analysis of SCLC and NSCLC. A big limitation for this study is the lack of adequate sample numbers in the stage subgroups. This is illustrated by the fact that certain ORs and HRs are not calculable for the MMP3 polymorphism in the SCLC cohort due to a lack of wild-type carriers in the non-responder group. Small sample sizes in stage subgroups also lead some to inconsistencies in the results between the whole cohort and the histological subgroups e.g. the non-significant point estimate for chemotherapy response for carriers of the MMP2 variant allele points towards a protective effect among NSCLC stage IIIA patients, but is associated with a significant risk of non-response among all Stage IIB and IIIA and Stage IIIB patients. However a number of effects appear consistent, if not significant, across subgroups.

MMP1 G-1607GG variant allele carriers have a longer PFS than individuals homozygous for the G allele. This result is not in concordance with the hypothesis that the GG allele, representing higher MMP1 expression, has harmful effects during therapy and in the later endpoints. We observed a significant protective effect in the whole cohort and especially in early stage SCLC. This result has to be validated in further studies of preferably larger cohorts.

The variant allele of *MMP2* correlates significantly with a poorer response in IIIB and SCLC early-stage patients. and the variant allele of *MMP3* correlates significantly, with a poorer response in IIIB patients, especially NSCLC, but not stage IV patients after the 2nd cycle of chemotherapy.

Regarding *MMP2*, the result does not reflect the hypothesized benefit of the T allele, which is expected to result in low expression. This is not in accordance with Liu *et al.* [10], where rats treated with platinum-based therapy in combination with prinomastat, a specific MMP2 inhibitor, had a higher response. Why this effect is seen in the logistic regression analysis is not clear and should be confirmed in other studies.

The significant results for MMP3 and MMP7 show large confidence intervals, which means the point estimate of the effect size is much less precise. However, they are in accordance with

a publication by Blons *et al* [6] which suggests modulation of chemotherapy response by altered MMP3 expression. The reason for this could be enhanced Fas ligand cleavage from the cell surface by *MMP7* and *MMP3* [25], leading to inhibition of the extrinsic apoptotic pathway.

A possible explanation for why this effect, in the case of the *MMP7C-153T* polymorphism is observed only in early-stage patients is that the genotype-dependent modulation of treatment outcome is expected to be stronger before extensive metastasis. Both MMP3 and MMP7 are well known for their involvement in metastasis. In stage IV patients where metastasis has already occurred other resistance mechanisms which are not specific for *MMP3* and *MMP7* could be more important for response after the 2^{nd} cycle of chemotherapy. The results presented here, which will require confirmation in further pharmacogenetic studies, suggest a genotype specific modulation of therapy protocol in earlier stages of lung cancer.

The Kaplan-Meier estimate for all lung cancer patients shows a significant harmful effect of the T allele situated in the promoter region of *MMP9* and representing high expression [26], on the progression free survival. This is in accordance with previous publications, which have shown high MMP9 expression as relevant to metastasis and patient outcome. However, this result could not be confirmed by corresponding hazard ratios for the *MMP9 C-1562T* analysis.

The *MMP7 A-181G* polymorphism did not show a synergetic effect with *C-153T* in the logistic regression. SCLC patients carrying the variant allele even have a longer lifetime after starting chemotherapy. This is not in accordance with the assumption, that both polymorphisms have similar effects on the transcription [14]. However, haplotypic analysis shows a harmful effect of the GT haplotype, which is due to a combination of the high expression alleles. The reason for this is not clear and further studies are required to validate this result.

Because of the antiangiogenic features of MMP12 [27] it was predicted that the hypothetically low expressing G allele has a harmful effect on overall survival and progression free survival. This could not be confirmed by our data. In contrast, it showed a significant protective tendency in the analysis for the overall survival for SCLC patients.

Low expression level also represent a higher plasmin level and a consequently higher activation rate of MMP9 in a MMP3 dependent manner, which might promote tumour growth and shorten PFS and survival [28]. Several studies indicate a benefit of MMP12 expression in various cancers [8,29]. However it was also shown that NSCLC patients who have high MMP12 expression have earlier relapse [30]. A reason for this effect could be that there is a link between high MMP12 expression, especially in the macrophages in the lung [31], COPD, inflammation and cancer progression [32-34] and that a lower expression indicates a better outcome for the patients. This would also indicate a tissue dependency especially in men, where cases of COPD appear more frequently.

The function of the exon polymorphism of *MMP12* is still unknown, but in this study it associated with bad outcome for NSCLC patients who received gemcitabine as chemotherapy. An explanation for this effect remains speculative but a change in the amino acid sequence of the hemopexin-like domain could alter its affinity or specificity.

Of course the overall survival and the PFS are parameters which are most clinically relevant. However, in the long run studies which measure the effect of genotypes on chemotherapy response could if the results are confirmed by larger independent study cohorts, contribute to better treatment protocols. To our knowledge this is the first pharmacogenetic study on MMPs and lung cancer that complements analysis of tumour progression and patient survival with an evaluation of the early tumour response. The results prioritise *MMP1, MMP2*, *MMP7, MMP9* and *MMP12* as candidate genes to be further investigated as possible predictors of the clinical outcome of chemotherapy in lung cancer patients.

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Table 1: Main clinical characteristics for the NSCLC and SCLC patient groups

	NSCLC		SCLC	
	n=187	%	n=161	%
Gender				
Male	126	67.4	117	72.7
Female	61	32.6	44	27.3
Age				
<60	112	59.9	70	43.5
>=60	75	40.1	91	56.5
Stage[a]				
IIB	0		5	3.8
IIIA	16	8.6	19	14.6
IIIB	57	30.8	41	31.5
IV	112	60.5	65	50.0
Response after 2nd Cycle				
CR+PR	101	54.0	128	79.5
SD+PD	86	46.0	33	20.5
Patients treated with				
Etoposide	2	1.1	158	98.1
Gemcitabine	143	76.5	0	
Platinum based drugs	138	73.8	117	72.7
Other drugs	60	32.1	59	36.6
Treatment after 2nd cycle [b]				
Chemotherapy only	135	72.2	59	36.6
Chemo- and Radiotherapy	43	23.0	101	62.7
Chemotherapy and Surgery	6	3.2	0	0.0
Chemotherapy, Radiotherapy, and Surgery	3	1.6	1	0.6
No of cycles administered				
2	52	27.8	17	10.6
3	42	22.5	15	9.3
4	15	8.0	52	32.3
5	21	11.2	20	12.4
6	52	27.8	49	30.4
>6	5	2.7	8	5.0

One patient with mixed histology is not included in this table.

[a]) For 2 NSCLC patients (1.1%) Stage is unknown. For 31 SCLC patients the tumour stage was only classified as limited or extensive disease based on the VALG criteria. Limited and extensive disease were then regarded as stage II - III and IV, respectively.

[b]) Patients with Surgery or radiotherapy prior to the 2nd cycle of chemotherapy were not included in the study.

Table 2: Primers and probes

Gene	Primers/ Probes	Primer sequence (5'-3')	Annealing Temp. (°C)
MMP12	Forward	TGCTAATTGATCCATTGTCG/	57
A-82G	Reverse	GAGCTCCAGAAGCAGTGG/	57
	Anchor	AGCCCTTAGTCCGGGTTCTGTGAA-FL	
	Sensor mut	610-TGAATCCTATGAGTGACTCA**C**AGTTGAT-PH	
MMP12	Forward	TGGGAACCATAGAAAAGAGACTA/	57
A1082G	Reverse	GGTCCTATAAAAACGTGGGT/	57
	Anchor	670-GCCAAATTATCCCAAGAGCATACATTCTT-PH	
	Sensor wt	ACTGGTTAATTAGCA**A**TTTAAGACCAG-FL	
MMP3	Forward	GAGCTGCCACAGCTTCTACA/	55
6A-11715A	Reverse	CTCAACCTCTCAAAGTGCTAGGAT/	55
	Anchor	640-CCATCAAAGGAATGGAGAACCATAGAATAC	
	Sensor wt	AAGACATGG**TTTTTT**CCCCX	

Bold letters show where the polymorphism is situated; 610, 640, 670 indicate the fluorescent dye
FL: Fluorescein
PH: Phosphate

Table 3: Association of matrix metalloproteinase SNPs and response after the 2nd cycle of chemotherapy for all lung cancer patients

OR - Chemotherapy response after the 2nd cycle								
Polymorphism	**Genotypes**	**Numbers of individuals**	**All (349)**	**SCLC (161)**	**NSCLC (187)**	**Stage IIB+IIIA (n=40)**	**Stage IIIB (n=98)**	**Stage IV (n=177)**
MMP1								
G-1607GG	**G/G**	n=96	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
	G/GG+ GG/GG	n=253	0.9(0.5-1.4)	0.9-(0.3-2.1)	1.0(0.5-1.9)	0.65 (0.13-3.31)	1.03(0.37-2.83)	0.8(0.4-1.4)
MMP2								
C-735T	**CC**	n=266	1(ref)	1(ref)	1(ref)	**1(ref)**	**1(ref)**	1(ref)
	CT+TT	n=81	1.5 (0.9-2.6)	2.1(0.9-4.8)	1.5(0.7-3.0)	**6.0(1.1-31.3)[a]**	**2.8(1.1-7.4)[b]**	1.0(0.5-2.1)
MMP3								
6A-1171 5A	**6A 6A**	n=90	1(ref)	1(ref)	1(ref)	1(ref)	**1(ref)**	1(ref)
	6A 5A + 5A 5A	n=259	1.4(0.8-2.4)	1.4(0.6-3.4)	1.2(0.6-2.3)	1.1(0.20-8.48)	**10.4(1.3-81.5)[c]**	0.9(0.5-1.6)
MMP7								
C-153T	**CC**	n=287	1(ref)	1(ref)	1(ref)	**1(ref)**	1(ref)	1(ref)
	CT+TT	n=60	1.8(1.0-3.2)	2.2(0.9-5.4)	1.7(0.8-3.9)	**6.0 (1.1-31.3)[d]**	1.54(0.52-4.70)	1.4(0.6-2.9)
A-181G	**AA**	n=66	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
	AG+GG	n=283	1.0(0.6-1.8)	1.0(0.4-2.6)	1.1(0.5-2.2)	1.3 (0.1-14.1)	1.39(0.41-4.62)	0.7(0.3-1.5)
MMP9								
C-1562T	**CC**	n=261	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
	CT+TT	n=87	0.9(0.5-1.5)	1.2 (0.5-2.8)	0.7(0.4-1.4)	2.0 (0.4-10.8)	0.6 (0.1-2.1)	1.0(0.5-1.9)
MMP12								
A-82G	**AA**	n=254	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
	AG+GG	n=94	0.9(0.5-1.5)	0.8(0.3-1.8)	1.3(0.7-2.7)	0.9(0.2-5.3)	1.0(0.3-2.6)	0.8(0.4-1.5)
A1082G	**AA**	n=308	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
	AG+GG	n=40	0.7(0.3-1.4)	0.4(0.1-1.7)	1.0(0.4-2.7)	0.5 (0.0-4.1)	N/A	1.1(0.5-2.7)

Genotype results are missing for *MMP2 C-735T*, *MMP7 C-153T* (each 2 patients), *MMP9 C-1562T*, *MMP12 A-82G*, *MMP12 A1082G* (each 1 patient)
ORs were adjusted for stage ; [a] p=0.03, [b] p=0.02, [c] p=0.01, [d] p=0.03

Table 4: **Association of matrix metalloproteinase SNPs and response after the 2nd cycle of chemotherapy for SCLC patients**

Polymorphism	Genotypes	No. of ind	StageIIB+IIIA	No. of ind	Stage IIIB+IV	No. of ind	Stage IIIB	No. of ind	Stage IV
MMP1									
G-1607GG	**G/G**	6	1(ref)	28	1(ref)	12	1(ref)	16	1(ref)
	G/GG+ GG/GG	18	0.40(0.05-3.27)	78	1.1(0.4-3.1)	29	1.3(0.2-7.6)	49	1.0(0.3-4.0)
MMP2									
C-735T	**CC**	19	1(ref)	77	1(ref)	27	1(ref)	50	1(ref)
	CT+TT	5	**12.8(1.4-67.4)** [a]	27	2.1(0.8-5.5)	13	2.6(0.5-12.5)	14	2.0(0.5-7.0)
MMP3									
6A-1171 5A	**6A 6A**	2	1(ref)	30	1(ref)	9	1(ref)	21	1(ref)
	6A 5A + 5A 5A	22	N/A	76	1.0(0.4-2.6)	32	N/A	44	0.5(0.2-1.7)
MMP7									
C-153T	**CC**	20	1(ref)	84	1(ref)	35	1(ref)	49	1(ref)
	CT+TT	4	5.7(0.6-57.2)	21	1.5(0.5-4.3)	6	0.8(0.1-8.0)	15	1.7(0.5-6.1)
A-181G	**AA**	3	1(ref)	16	1(ref)	6	1(ref)	10	1(ref)
	AG+GG	21	0.5(0.0-6.6)	90	0.9(0.3-3.0)	35	1.25(0.1-12.5)	55	0.7(0.2-3.2)
MMP9									
C-1562T	**CC**	20	1(ref)	82	1(ref)	35	1(ref)	47	1(ref)
	CT+TT	4	5.7(0.5-66.7)	24	1.6(0.5-4.3)	6	0.8(0.0-6.1)	18	1.9(0.5-6.1)
MMP12									
A-82G	**AA**	20	1(ref)	68	1(ref)	29	1(ref)	39	1(ref)
	AG+GG	3	2.0(0.1-28.0)	38	0.5(0.2-1. 5)	12	0.8(0.1-4.5)	26	0.4(0.1-1.5)
A1082G	**AA**	21	1(ref)	94	1(ref)	37	1(ref)	57	1(ref)
	AG+GG	2	<0.1(<0.0-999)	12	0.7(0.0-2.7)	4	<0.1(<0.0-999)	8	1.0(0.2-5.7)

All values are crude odds ratios; [a] p=0.03

Table 5: **Association of matrix metalloproteinase SNPs and response after the 2nd cycle of chemotherapy for NSCLC patients**

Polymorphism	Genotypes	Numbers of individuals	Stage IIIA	Numbers of individuals	Stage IIIB+IV	Numbers of individuals	Stage IIIB	Numbers of individuals	Stage IV
MMP1									
G-1607GG	**G/G**	2	1(ref)	52	1(ref)	17	1(ref)	35	1(ref)
	G/GG+	14	0.8(0.0-14.6)	118	0.9(0.5-1.8)	40	0.9(0.3-3.2)	78	0.9(0.4-2.1)
	GG/GG								
MMP2									
C-735T	**CC**	13	1(ref)	134	1(ref)	42	1(ref)	92	1(ref)
	CT+TT	3	0.3(0.0-4.4)	36	1.2(0.6-2.5)	15	3.2(0.9-11.5)	21	0.9(0.3-2.2)
MMP3									
6A-1171 5A	**6A 6A**	5	1(ref)	38	1(ref)	15	1(ref)	23	1(ref)
	6A 5A + 5A 5A	11	1.3(0.1-10.7)	122	1.3(0.6-2.6)	42	**7.8(1.4-147.8)**[a]	90	0.6(0.2-1.6)
MMP7									
C-153T	**CC**	12	1(ref)	140	1(ref)	44	1(ref)	96	1(ref)
	CT+TT	4	6.0(0.5-77.8)	29	1.3(0.6-2.9)	13	1.9(0.5-6.9)	16	1.4(0.5-4.3)
A-181G	**AA**	1	1(ref)	35	1(ref)	14	1(ref)	21	1(ref)
	AG+GG	15	N/A	135	1.0(0.5-2.2)	43	1.6(0.4-7.9)	92	0.7(0.3-1.9)
MMP9									
C-1562T	**CC**	13	1(ref)	124	1(ref)	46	1(ref)	78	1(ref)
	CT+TT	3	0.6-(0.0-8.2)	56	0.6(0.3-1.2)	11	0.5(0.1-2.3)	35	0.8(0.4-1.7)
MMP12									
A-82G	**AA**	12	1(ref)	131	1(ref)	40	1(ref)	91	1(ref)
	AG+GG	4	0.3(0.0-4.2)	39	1.3(0.6-2.6)	17	1.1(0.3-3.8)	22	1.9(0.7-5.2)
A1082G	**AA**	13	1(ref)	153	1(ref)	51	1(ref)	102	1(ref)
	AG+GG	3	0.6(0.0-8.2)	17	1.0(0.4-2.8)	6	N/A	11	2.3(0.6-10.9)

All values are crude odds ratios; [a] p=0.03

Table 6: Association of matrix metalloproteinase SNPs and progression free or overall survival

Polymorphism	**Genotypes**	**Number of individuals**	HR - Progression free Survival					HR – Overall Survival				
			All (349)	**SCLC (161)**	**NSCLC (187)**	**Etoposide (157)**	**Gemcitabine (143)**	**All (349)**	**SCLC (161)**	**NSCLC (187)**	**Etoposide (157)**	**Gemcitabine (143)**
MMP1												
1G-16072G	G/G	n=96	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
	G/GG+ GG/GG	n=253	**0.7(0.6-1.0)[a]**	0.7(0.5-1.1)	0.7(0.5-1.0)	0.7-(0.5-1.1)	0.7-(0.4-1.1)	0.9(0.7-1.2)	0.7(0.5-1.1)	0.9(0.6-1.4)	0.8(0.5-1.2)	0.9(0.6-1.5)
MMP2												
C-735T	**CC**	n=266	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
	CT+TT	n=81	1.0(0.8-1.4)	0.9(0.6-1.4)	1.1(0.7-1.6)	0.9(0.6-1.4)	1.1(0.6-1.7)	1.0(0.8-1.4)	0.9(0.6-1.5)	1.1(0.7-1.7)	1.0(0.6-1.5)	0.9(0.5-1.6)
MMP3	**6A 6A**	n=90	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
6A-1171 5A	6A 5A + 5A 5A	n=259	0.9(0.7-1.3)	1.2(0.8-1.7)	0.8(0.6-1.3)	1.2(0.8-1.8)	0.9(0.5-1.4)	0.9(0.7-1.3)	1.1(0.7-1.8)	0.8(0.5-1.3)	1.2(0.8-1.9)	0.7(0.4-1.2)
MMP7												
C-153T	**CC**	n=287	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
	CT+TT	n=60	1.0(0.7-1.4)	1.4(0.9-2.3)	0.8(0.5-1.2)	1.5(0.9-2.4)	0.8(0.4-1.3)	1.0(0.7-1.5)	1.2(0.7-2.0)	0.8(0.5-1.4)	1.2(0.7-2.1)	1.0(0.5-1.8)
A-181G	**AA**	n=66	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
	AG+GG	n=283	0.9(0.7-1.2)	0.7(0.5-1.1)	1.0(0.6-1.5)	0.7(0.5-1.1)	0.9(0.6-1.4)	1.0(0.7-1.4)	0.6(0.3-1.0)[b]	1.3(0.8-2.2)	0.6(0.4-1.0)	
MMP9												
C-1562T	**CC**	n=261	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
	CT+TT	n=87	1.2(0.9-1.6)	1.3(0.9-2.0)	1.1(0.8-1.7)	1.4(0.9-2.0)	0.9(0.6-1.4)	1.1(0.8-1.6)	1.2(0.8-2.0)	1.1(0.7-1.7)	1.2(0.8-2.0)	1.0(0.6-1.7)
MMP12												
A-82G	**AA**	n=254	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	**1(ref)**	1(ref)	1(ref)	1(ref)
	AG+GG	n=94	0.9(0.7-1.2)	0.7(0.5-1.1)	1.1(0.7-1.6)	0.7(0.5.1.0)	1.0(0.7-1.6)	0.7(0.5-1.0)	**0.5(0.3-0.8)[c]**	1.1(0.7-1.7)	**0.5(0.3-0.8)**	1.1(0.7-1.8)
A1082G	**AA**	n=308	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
	AG+GG	n=40	1.3(0.9-1.9)	1.4(0.8-2.3)	1.3(0.7-2.3)	1.4(0.8-2.4)	**1.9(1.0-3.5)[d]**	1.4(0.9-2.0)	1(ref) 1.5(0.9-2.7)	1.3(0.7-2.3)	1.5(0.9-2.7)	1.8(0.9-3.5)

All results for PFS were adjusted for stage. For overall survival additional adjustment for gender was conducted. [a]p=0.04, [b]p=0.05, [c]p=0.0026, [d]p=0.04

Table 7: Association of matrix metalloproteinase SNPs and progression free or overall survival in early and late stage SCLC and NSCLC

Polymorphism	**Genotypes**	HR - Progression free Survival				HR - Overall Survival			
		SCLC		NSCLC		SCLC		NSCLC	
		Stage IIB+IIIA (n=24)	**Stage IIIB+IV (n=106)**	**Stage IIIA (n=16)**	**Stage IIIB+IV (n=169)**	**Stage IIB+IIIA (n=24)**	**Stage IIIB+IV (n=106)**	**Stage IIIA (n=16)**	**Stage IIIB+IV (n=169)**
MMP1									
1G-16072G	**G/G**	1(ref)	1(ref)	1(ref)	1(ref)	**1(ref)**	1(ref)	1(ref)	1(ref)
	G/GG+ GG/GG	0.4(0.1-1.2)	0.9(0.5-1.5)	0.3(0.0-3.2)	0.7(0.5-1.1)	**0.1(0.0-0.60)[a]**	0.8(0.5-1.4)	0.7(0.1-6.7)	1.0(0.7-1.5)
MMP2									
C-735T	**CC**	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
	CT+TT	1.0(0.4-3.4)	0.7(0.4-1.1)	0.6(0.1-4.9)	0.9(0.6-1.4)	0.6(0.1-4.9)	0.9(0.6-1.4)	0.9(0.1-8.7)	1.0(0.6-1.6)
MMP3									
6A-1171 5A	**6A 6A**	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
	6A 5A + 5A 5A	1.7(0.2-13.1)	1.3(0.8-2.2)	1. 6(0.3-8.2)	0.9(0.6-1.4)	1.6(0.2-13.2)	1.1(0.7-1.9)	2.0(0.2-18.0)	0.9(0.6-1.5)
MMP7	**CC**	1(ref)	**1(ref)**	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
C-153T	CT+TT	1.9(0.6-5.9)	**1.8(1.0-3.1)[b]**	1.2(0.2-6.1)	0.7(0.4-1.1)	1.5(0.3-7.3)	1.6(0.9-2.8)	0.9(0.1-8.1)	0.7(0.4-1.2)
A-181G	**AA**	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
	AG+GG	0.7(0.2-2.5)	1.0(0.5-1.8)	N/A	1.0(0.7-1.5)	1.7(0.2-13.0)	0.8(0.4-1.5)	N/A	1.0(0.6-3.1)
MMP9									
C-1562T	**CC**	1(ref)	**1(ref)**	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
	CT+TT	0.3(0.1-1.5)	**1.7(1.0-2.9)[c]**	1.7(0.3-8.8)	1.2(0.8-1.8)	0.2(0.0-1.7)	1.4(0.8-2.6)	0.5(0.1-4.0)	1.2(0.8-1.9)
MMP12									
A-82G	**AA**	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	**1(ref)**	1(ref)	1(ref)
	AG+GG	0.3(0.07-1.44)	0.80(0.5-1.3	0.5(0.1-4.4)	1.1(0.7-1.6)	N/A	**0.6(0.3-1.0)[d]**	0.5(0.1-4.6)	1.1(0.7-1.7)
A1082G	**AA**	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
	AG+GG	0.9(0.2-3.9)	1.4(0.7-2.7)	0.8(0.1-6.8)	1.2(0.7-2.3)	1.0(0.1-8.4)	1.2(0.6-2.6)	N/A	1.2(0.7-2.2)

Hazard Ratios for Overall Survival are adjusted for gender, [a]p=0.001, [b]p=0.04, [c]p=0.04, [d]p=0.04

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Figure 1