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Research paper

Polymorphisms in the *TP53-MDM2-MDM4*-axis in patients with rheumatoid arthritis

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ABSTRACT

Background: In addition to being a tumour suppressor, *TP53* is a suppressor of inflammation, and dysfunction of this gene has been related to autoimmune diseases. Patients with autoimmunity, such as rheumatoid arthritis (RA) have an increased risk of certain cancers, like lymphomas, indicating that some underlying mechanisms may modulate risk of both cancers and autoimmunity.

Methods: We genotyped 5 common genetic variants in *TP53* and its main regulators *MDM2* and *MDM4* in a sample of 942 RA patients and 3,747 healthy controls, and mined previously published GWAS-data, to assess the potential impact of these variants on risk of RA.

Results: For the *TP53* Arg72Pro polymorphism (rs1042522), *MDM4* SNP34091 (rs4245739) and *MDM2* SNP285C (rs117039649), we found no association to risk of RA. For *MDM2* SNP309 (rs2279744), the minor G-allele was associated with a reduced risk of RA (OR: 0.87; CI: 0.79–0.97). This association was also seen in genotype models (OR: 0.86; CI: 0.74–0.99 and OR: 0.79; CI 0.63–0.99; dominant and recessive model, respectively), but was not validated in a large GWAS data set. For *MDM2* del1518 (rs3730485), the minor del-allele was associated with an increased risk of RA in the dominant model (OR: 1.18; CI: 1.02–1.38). Stratifying RA cases and controls into phylogenetic subgroups according to the combined genotypes of all three *MDM2* polymorphism, we found individuals with the del158-285–309 genotype del/ins-G/G-T/T to have an increased risk of RA as compared to those with the ins/ins-G/G-G/G genotype (OR: 1.56; CI: 1.18–2.06) indicating opposite effects of the del1518 del-allele and the SNP309 G-allele.

Conclusion: We find a potential association between the *MDM2* del1518 variant and RA, and indications that combinatorial genotypes and haplotypes in the *MDM2* locus may be related to RA.

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disease where the immune system initiates an inflammatory response to the synovial joints leading to joint swelling and inflammation known as arthritis (Klareskog et al., 2009). While RA is a multifactorial disease with a strong heredity (Klareskog et al., 2009), known genetic factors such as the Human Leukocyte Antigen (HLA)-association only explains part of the heritability.

The p53 protein, encoded by the *TP53* gene, is a major tumour suppressor, frequently mutated across a panel of cancer forms (Forbes et al., 2015). Germline mutations in the *TP53* gene is the cause of the Li-Fraumeni syndrome, associated with a strongly elevated risk of multiple cancer forms at young age (Li and Fraumeni, 1969; Malkin et al., 1990). In addition, p53 is known to be a suppressor of inflammation (Gudkov et al., 2011), and p53 dysfunction has been related to autoimmune

Abbreviations: CI, confidence interval; CONOR, cohort of Norway; LD, linkage disequilibrium; OR, odds ratio; SNP, single nucleotide polymorphism; RA, rheumatoid arthritis.

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diseases (Takatori et al., 2014). Further, patients suffering from autoimmunity, such as RA, have an increased risk of certain cancers, like lymphomas (Lim et al., 2019).

The activity of the p53 protein is regulated at the post-translational level. In unstressed cells, the p53 level is kept low mainly through ubiquitination by the MDM2 ubiquitin ligase (Haupt et al., 1997; Honda et al., 1997), in particular in the form of the MDM2-MDM4 heterodimer (Linares et al., 2003; Wang et al., 2011). Multiple studies have related p53 protein activity to polymorphisms not only in the *TP53* gene itself but in the *MDM2* and *MDM4* genes as well (Bond et al., 2004; Wynendaele et al., 2010). Thus, a single nucleotide polymorphism in the *TP53* gene (rs1042522; Arg72Pro) has been linked to differential p53 protein activity, where the Arg-variant is typically proposed to be more effective in inducing apoptosis as compared to the Pro-variant (Dumont et al., 2003), while the Pro-variant is suggested to induce a higher level of G1 cell cycle arrest (Pim and Banks, 2004) and activation of p53-dependent DNA repair genes resulting in reduced genomic instability (reviewed in (Whibley et al., 2009)).

In addition, several germline polymorphisms in the *MDM2* gene promoters have been shown to affect transcription efficacy and to be associated with risk of diverse malignancies. In particular, the *MDM2* SNP309 (rs2279744) G-allele has been linked to increased cancer risk. The functional role of this variant is that it elongates a binding site for the transcription factor Sp1, leading to increased binding of Sp1 to the *MDM2* promoter and subsequently, increased transcription (Bond et al., 2004; Hu et al., 2007; Economopoulos and Sergentanis, 2010). In contrast, the neighboring *MDM2* SNP285 (rs117039649) C-allele has been linked to reduced cancer risk and has been shown to diminish the binding of Sp1 to another Sp1 binding site in the same promoter (Knappskog et al., 2011, 2012). In the alternative P1 promoter of the *MDM2* gene, there is also a potentially functional polymorphism: the del1518 variant (rs3730485) is a 40 bp deletion, that removes a putative TATA motif and this variant has been linked to risk of multiple cancer types (Dong et al., 2012; Gansmo et al., 2016b, 2017)

As for *MDM4,* a SNP (SNP34091 A > C; rs4245739) has been found to have functional a functional role. The SNP is located in the 3′UTR of the *MDM4* transcript and has been found to increase the affinity of the microRNAs miR-191-5p and miR-887. The binding of these microRNAs results in inhibited translation and subsequently lowered MDM4 protein levels (Wynendaele et al., 2010; Stegeman et al., 2015).

Contemporary data regarding a potential role of p53 and MDM2 status with respect to rheumatic diseases are conflicting. Experimental data has shown p53 to regulate the Th17/Treg cell balance in RA (Park et al., 2013), and abundant MDM2 protein expression to reduce p53 activity in fibroblast-like human synoviocytes (Taranto et al., 2005). In contrast, MDM2 was found to reduce systemic inflammation and abrogate production of lupus autoantibodies in mice (Allam et al., 2011) while promoting RA activity through activating MAP-kinase (Zhang et al., 2016). Considering potential impact of *MDM2* SNPs on risk of RA, Assman et al (Assmann et al., 2009) reported a reduced OR for RA for individuals harbouring the SNP309G variant allele, while no association between *TP53* Arg72Pro and susceptibility of RA was observed. To the best of our knowledge, a comprehensive overview of the RA risk across polymorphisms in the *TP53-MDM2-MDM4*-axis has not been reported.

In the present study, we genotyped the *TP53* Arg72Pro (rs1042522), *MDM4* SNP34091 (rs4245739) and the three *MDM2* promoter polymorphisms SNP309T > G (rs2279744), SNP285G > C (rs117039649) and del1518 (rs3730485), in a sample of RA patients (n = 942) and compared the distribution of genotypes to a population-based control group (n = 3,747), for assessment of these variants' impact on risk of RA.

2. Materials and methods

2.1. Study populations

A total of 953 RA patients were included in the present study. The inclusion criteria were age above 18 years and fulfillment of the ACR 1987 RA criteria (Arnett et al., 1988). No other inclusion/exclusion criteria were applied. Demographic data are listed in Table 1. All patients were recruited at Diakonhjemmet Hospital, Norway, and were previously included in one or several cohorts of RA patients (Uhlig et al., 1999; Haavardsholm et al., 2008; Syversen et al., 2008; Halvorsen et al., 2009). We genotyped the five investigated polymorphisms (see below) successfully in 942 out of the 953 patients. This set of 942 RA patients was used for subsequent comparisons to healthy controls.

For comparison, we used the genotypes of a sample of 3,749 healthy Norwegian individuals previously analyzed (Gansmo et al., 2015a, 2016b) who were drawn from the population based Cohort of Norway (CONOR) study (Naess et al., 2008). Two samples failed *MDM4* SNP34091 genotyping, as reported previously (Gansmo et al., 2015b, 2016a). Therefore, the set of healthy controls was 3,747 individuals with successful genotyping of all five polymorphisms. Demographic data for the controls are listed in Table 1.

Statistical power was estimated based on reported frequencies (dominant model) for *TP53* Arg72Pro and *MDM2* SNP309 (Assmann et al., 2009). No previous data for RA risk was available for the three remaining polymorphisms. Considering our control group to be 3,747 and applying an Alpha value of 0.05 and a required 1-Beta of 0.8, we found that we needed 756 RA-patients for assessment of *TP53* Arg72Pro and 237 RA-patients for assessment of *MDM2* SNP 309. As such, we found our sample set of > 900 RA patients to be adequate for analyses.

2.2. Genotyping

All samples were genotyped for five polymorphisms: *TP53* Arg72Pro (rs1042522), *MDM4* SNP34091 (rs4245739) and the three *MDM2* promoter polymorphisms SNP309T > G (rs2279744), SNP285G > C (rs117039649) and del1518 (rs3730485). All analyses were performed on DNA extracted from white blood cells as previously described (Knappskog et al., 2007).

The *MDM4* SNP34091 and the *MDM2* SNPs 285 and 309 were genotyped using custom LightSNiP assays (TIB MOLBIOL Syntheselabor GmbH, Berlin, Germany) on a LightCycler 480 II instrument (Roche, Basel, Switzerland) as previously described in detail (Knappskog et al., 2014; Gansmo et al., 2015b).

TP53 Arg72Pro polymorphisms was also genotyped by a custom LightSNiP assay (TIB MOLBIOL Syntheselabor GmbH, Berlin, Germany).

Table 1
Genotype distribution and OR.

RA patients		
Total	*n* (%)	942 (100)
Gender (*n* = 936*)	Female *n* (%) Male *n* (%)	724 (77.4) 212 (22.6)
Age at onset (*n* = 920*)	Average Range	48.4 16–85
Disease duration (yrs) (*n* = 700*)	Average Range	11.0 0–54
Rheumatoid factor (*n* = 878*)	Positive *n* (%) Negative *n* (%)	474 (54.0) 404 (46.0)
Anti-CCP (*n* = 876*)	Positive *n* (%) Negative *n* (%)	542 (61.9) 334 (38.1)
Healthy controls		
Total	*n* (%)	3,747 (100)
Gender (*n* = 3,747*)	Female *n* (%) Male *n* (%)	1870 (49.9) 1877 (50.1)
Age at sampling (*n* = 3,747*)	Average Range	60.7 20–93

**n* with information available.

The reaction mix was similar to that previously used for *MDM4* SNP34091 (Gansmo et al., 2015b), with the exception that the probes used were specific for the *TP53* Arg72Pro variant. The thermocycling conditions for the *TP53* Arg72Pro genotyping were identical to those used for *MDM4* SNP34091 (Gansmo et al., 2015b).

MDM2 del1518 was genotyped by a previously described amplicon size assay (Gansmo et al., 2016b). In brief, the region of *MDM2* promoter P1 harbouring the del1518 indel locus was amplified by PCR, and the alleles were separated and visualized by electrophoresis in a 3% agarose gel pre-stained with GelRedTM Nucleic Acid Gel Stain (BIOTIUM: Fig. 1).

2.3. Mined validation data

For potential validation of findings, we mined the publicly available dataset from a large GWAS-study, holding allele frequency and odds ratio data from a comparison of > 29,000 RA cases and > 73,000 controls (Okada et al., 2014). In this data set, summary statistics was available for *TP53* Arg72Pro (rs1042522), *MDM4* SNP34091 (rs4245739) *MDM2* SNP309T > G (rs2279744), and SNP285G > C (rs117039649). Data for *MDM2* del1518 (rs3730485) was not available since this variant is an insertion-deletion variant, not a single nucleotide polymorphism (SNP).

2.4. Statistics

Potential associations between the polymorphisms and RA were evaluated by estimating the Odds Ratios (ORs) and application of the Fisher's exact test. Potential associations between genotypes and ACPA-status were assessed by Chi-square tests. Linkage disequilibrium was assessed by calculations of D' and r^2 as previously described (Helwa et al., 2016). ORs are given with 95% confidence intervals (CIs), and p-values are given as two-sided. P-values from Fisher's exact tests are given as two-sided and cumulative.

All statistical analyses were performed using the IBM SPSS statistics (version 22) software package.

3. Results

3.1. Distribution of TP53-MDM2-MDM4-axis polymorphisms

Out of 953 patients with rheumatoid arthritis (RA) included in the present study, we successfully genotyped the five polymorphisms *TP53* R72P (rs1042522), *MDM4* SNP34091 (rs4245739) and the three *MDM2* promoter polymorphisms SNP309T > G (rs2279744), SNP285G > C (rs117039649) and del1518 (rs3730485; Fig. 2a-c) in 942. Among these, 77.4% were females and 22.6% were males. The average age at onset was 48.4 years (range 16–85), while the average disease duration was 11.0 years (range 0–54; Table 1).

The genotype distributions of the five polymorphisms are listed in Table 2. Demographics and clinical data, including data on anti-citrullinated protein antibodies (ACPA) status was available for most (n = 876) patients. The distribution of genotypes and alleles did not differ between ACPA+ (n = 542) and ACPA- (n = 334) RA patients for any of the five polymorphisms (p > 0.2 for all comparisons). Therefore, all RA patients were included in further analyses without stratifying for autoantibody status.

Applying the Hardy-Weinberg principle for each of the five polymorphisms individually, we found no significant skewness of genotypes for any of them, neither when assessed in healthy controls (previously reported in (Gansmo et al., 2015a, 2015b, 2016b)), nor in RA patients, (p > 0.1 for all comparisons, Supplementary Table S1a.

The three *MDM2* SNPs were previously found to be in strong linkage disequilibrium (LD) to each other (Knappskog et al., 2011; Gansmo et al., 2016b) and seem to have occurred in a distinct order through evolution (depicted in Fig. 3a). Similar results were observed among the RA patients analyzed in the present study: the three *MDM2* variants were in strong LD with each other (D' > 0.99 for all comparisons; r^2 ranging from 0.02 to 0.4; Fig. 3b; Supplementary Table S1b–d). As such, the inferred haplotype structures were restricted to five different haplotypes, one of which was observed in one RA patient and one healthy control only, and therefore likely to have occurred by a rare rearrangement (Fig. 3a). Notably, the minor alleles of del1518 (del) and SNP285 (C) resides on different alleles of the SNP309 (T and G, respectively; Fig. 3a).

3.2. TP53-MDM2-MDM4-axis polymorphisms and risk of RA

Comparing the distribution of the *TP53* Arg72Pro polymorphism between RA patients and healthy controls we found no association between genotype and risk of RA neither applying a dominant nor a recessive model (OR: 1.00; CI: 0.87–1.15 and OR: 1.05; CI 0.81–1.35, respectively; Table 2; Fig. 4). Similarly, we observed no association between *MDM4* SNP34091 status and risk of RA neither applying a dominant nor a recessive model (OR: 0.96; CI: 0.84–1.11 and OR: 1.05; CI 0.80–1.38, respectively; Table 2; Fig. 4). In addition to our own data, we mined the allele distribution of these SNPs and their potential association to RA in a large GWAS-based data set holding information from >29,000 RA cases and >73,000 controls (Okada et al., 2014). In this mined data set, we found no allelic association between *MDM4* SNP34091 and risk of RA, while *TP53* Arg72Pro was weakly associated with increased risk of RA (Supplementary Table S2). However, this

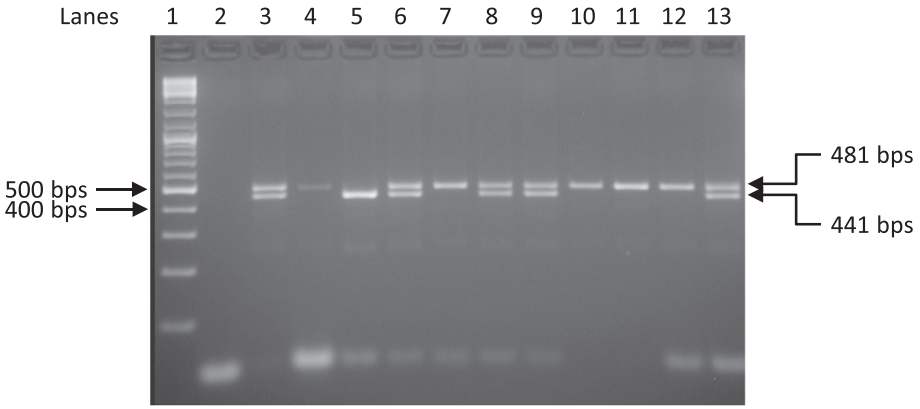

Fig. 1. Representative image of samples scored as genotypes ins-ins (lane 4, 7, 10, 11 and 12) ins-del (lane 3, 6, 8, 9 and 13) and del-del (lane 5). Theoretical sizes for PCR products were 481 and 441 bp for the ins-allele and the del-allele, respectively. Lane 1; ladder (GeneRuler DNA Ladder mix; Thermo Fisher Scientific). Lane 2; Negative control (ddH_2O instead of DNA template).

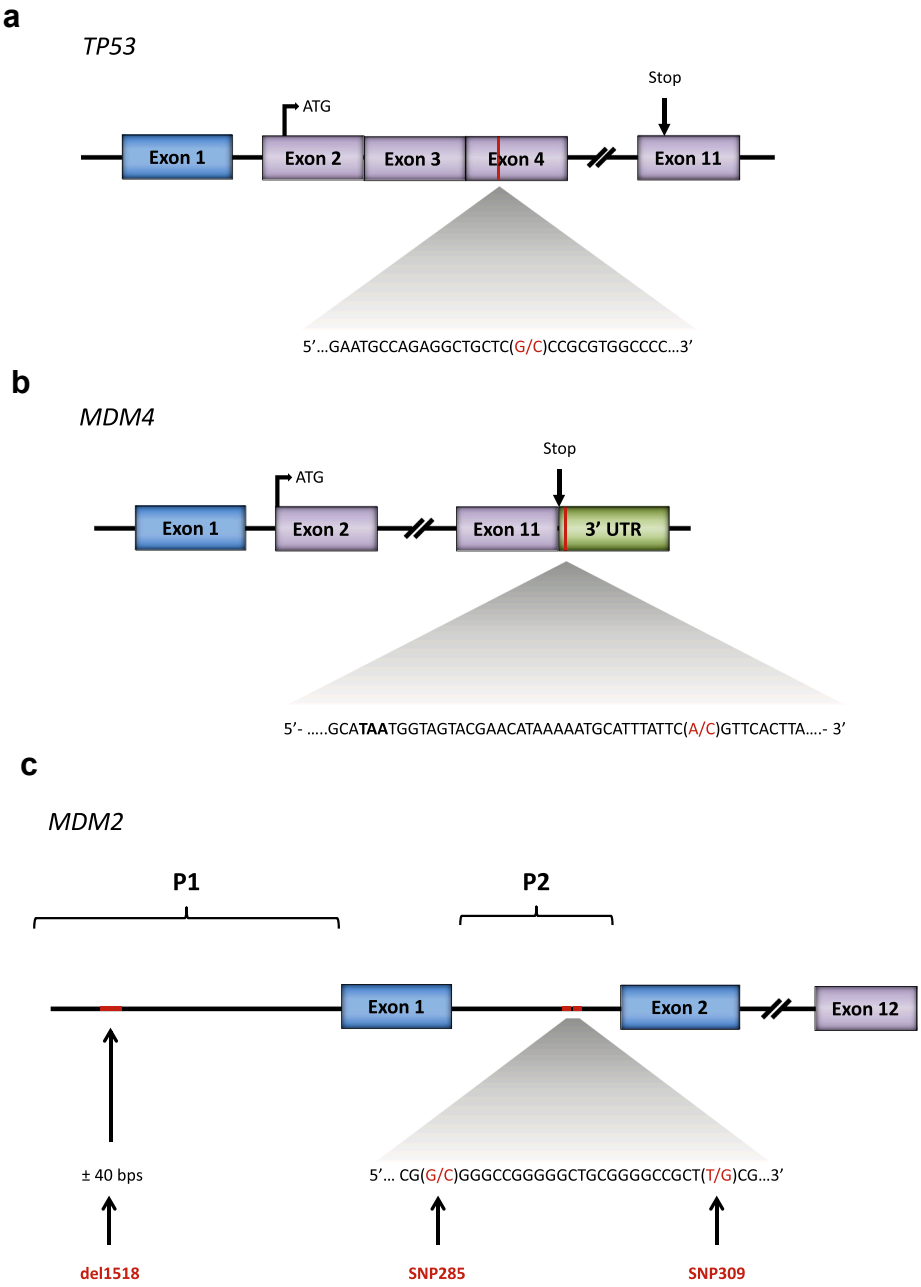

Fig. 2. Schematic representation of locations for the five studied polymorphisms. A. In the *TP53* gene. B. In the *MDM4* gene. C. In the *MDM2* gene.

association was not significant when corrected for multiple testing.

In our original data, two out of the three tested polymorphisms in the *MDM2* promoter region were found associated with risk of RA. For *MDM2* SNP309, the minor G-allele (previously linked to increased risk of cancer (Bond et al., 2004)) was associated with a *reduced* risk of RA (OR: 0.86; CI: 0.74–0.99 and OR: 0.79; CI 0.63–0.99, applying a dominant and recessive model, respectively; Table 2; Fig. 4). Importantly, in the allele model, this finding was not validated in the mined GWAS data set (Supplementary Table S2) and, as such, our data must be interpreted with great caution.

For *MDM2* del1518, the minor del-allele (previously linked to reduced risk of cancer (Linares et al., 2003)) was associated with an *increased* risk of RA in a dominant model (OR: 1.18; CI: 1.02–1.38). However, applying a recessive model, no significant association was observed (OR: 0.97; CI 0.80–1.17; Table 2; Fig. 4), indicating that the effect of the minor del-allele on risk of RA is restricted to the dominant setting. Unfortunately, this association could not be tested in the GWAS data set, since the *MDM2* del1518 is an indel-variant, not a SNP.

For *MDM2* SNP285C (previously linked to reduced risk of cancer (Knappskog et al., 2011)), as expected (Knappskog et al., 2012, 2014) we observed no individuals, either among healthy controls or RA patients, harbouring the homozygous C/C-genotype. As such, risk estimates based on the recessive model were not feasible. Applying the dominant model, we found no significant associations between SNP285 status and risk of RA (OR: 0.85; CI: 0.63–1.15; Table 2, Fig. 4).

Notably, the above calculations were performed regarding the *MDM2* polymorphisms as independent of each other. Given the close proximity (24 bp) and strong LD of SNP309 and SNP285 as well as the fact that these two SNPs have opposing effect on binding of the same transcription factor (Sp1) (Knappskog et al., 2011), we performed stratified sub-analyses. First, we re-assessed SNP309 after removing individuals harbouring the less frequent and counteracting SNP285C-allele. Here, the OR related to RA was similar to the overall assessments, although the CIs were slightly wider due to a lower number of

Table 2
Genotype distribution and OR.

Controls/Cases	Genotype n (%)			OR (95% CI)	Fisher exact	OR (95% CI)	Fisher exact
				Dominant model		**Recessive model**	
***TP53* R73P**	**G/G**	**G/C**	**C/C**				
Healthy Controls	1932 (51.6)	1502 (40.1)	313 (8.4)				
RA patients	486 (51.6)	374 (39.7)	82 (8.7)	1.00 (0.87–1.15)	1.000	1.05 (0.81–1.35)	0.743
***MDM4* SNP34091**	**A/A**	**A/C**	**C/C**				
Healthy Controls	2042 (54.5)	1439 (38.4)	266 (7.1)				
RA patients	522 (55.4)	350 (37.2)	70 (7.4)	0.96 (0.84–1.11)	0.634	1.05 (0.80–1.38)	0.724
***MDM2* del1518**	**ins/ins**	**ins/del**	**del/del**				
Healthy Controls	1284 (34.3)	1776 (47.4)	687 (18.3)				
RA patients	288 (30.6)	486 (51.6)	168 (17.8)	1.18 (1.02–1.38)	0.034	0.97 (0.80–1.17)	0.741
***MDM2* SNP285**	**G/G**	**G/C**	**C/C**				
Healthy Controls	3493 (93.2)	254 (6.8)	0 (0.0)				
RA patients	887 (94,2)	55 (5.8)	0 (0.0)	0.85 (0.63–1.15)	0.339	na	na
***MDM2* SNP309**	**T/T**	**T/G**	**G/G**				
Healthy Controls	1464 (39.1)	1782 (47.6)	501 (13.4)				
RA patients	403 (42.8)	437 (46.4)	102 (10.8)	0.86 (0.74–0.99)	0.041	0.79 (0.63–0.99)	0.038

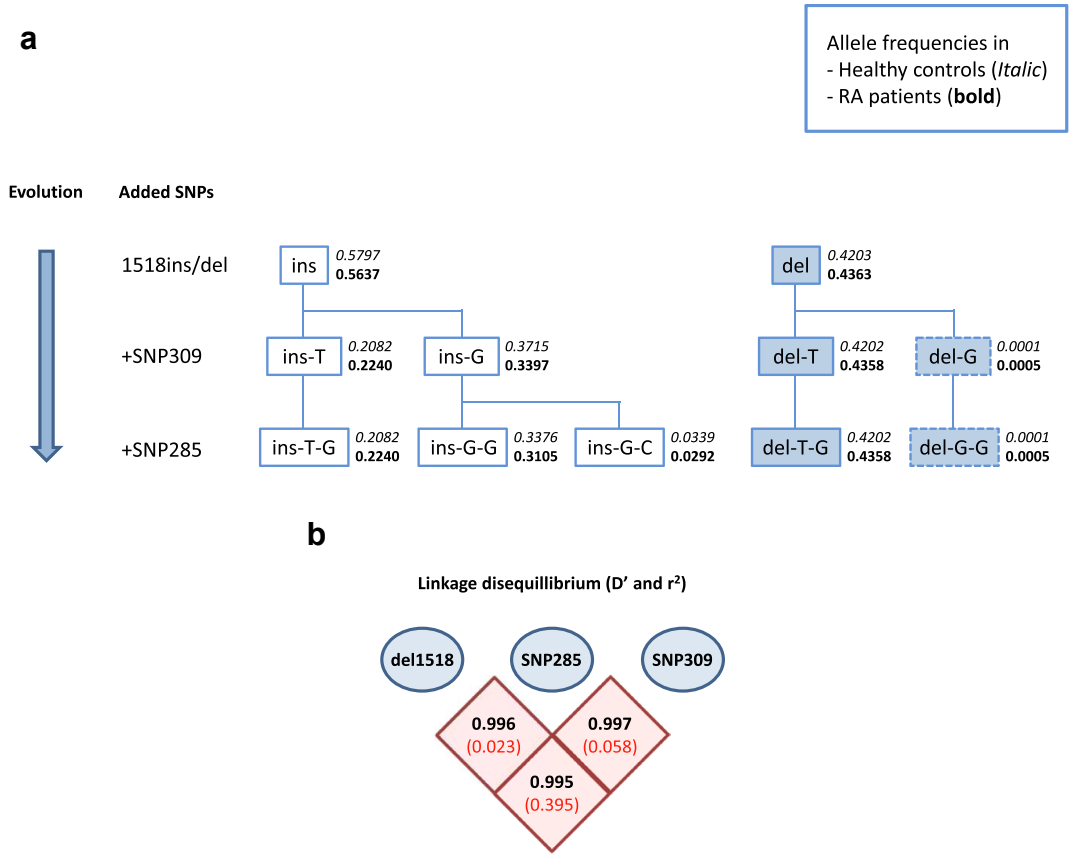

Fig. 3. A. Haplotype structure within the *MDM2* promoter region with respect to the three polymorphisms del1518 (rs3730485), SNP285 (rs117039649) and SNP309 (rs2279744). The order (from top to bottom) is by evolutionary time (time of origin for each of the polymorphisms). For order of positions on DNA, see Fig. 1c. Numbers indicate fraction of alleles in the total population (fractions per added polymorphism) for healthy controls (italic) and RA patients (bold). B. Pairwise linkage disequilibrium (LD) between *MDM2* del1518, SNP285 and SNP309. D′ (in bold) and r2 (in red) were calculated among the 942 analyzed RA patients. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

individuals included in the calculations (OR: 0.87; CI: 0.75–1.01 and OR: 0.80; CI 0.63–1.03, dominant and recessive model, respectively). Secondly, we re-assessed the effect of SNP285C, restricted to those individuals harbouring the SNP309G-allele on which SNP285C originated phylogenetically (i.e. removed individuals with SNP309TT genotype). Here, the OR was also similar to the overall assessment (OR: 0.91; CI: 0.67–1.24).

3.3. Combined MDM2 genotypes and haplotypes

Given the observed potential effects of the minor alleles of del1518 and SNP309 on RA and the generally strong LD between all the three *MDM2* polymorphisms, we went on to perform refined sub-analyses comparing the effects of all the different observed combined genotypes (del1518-SNP285-SNP309). Overall, among the individuals

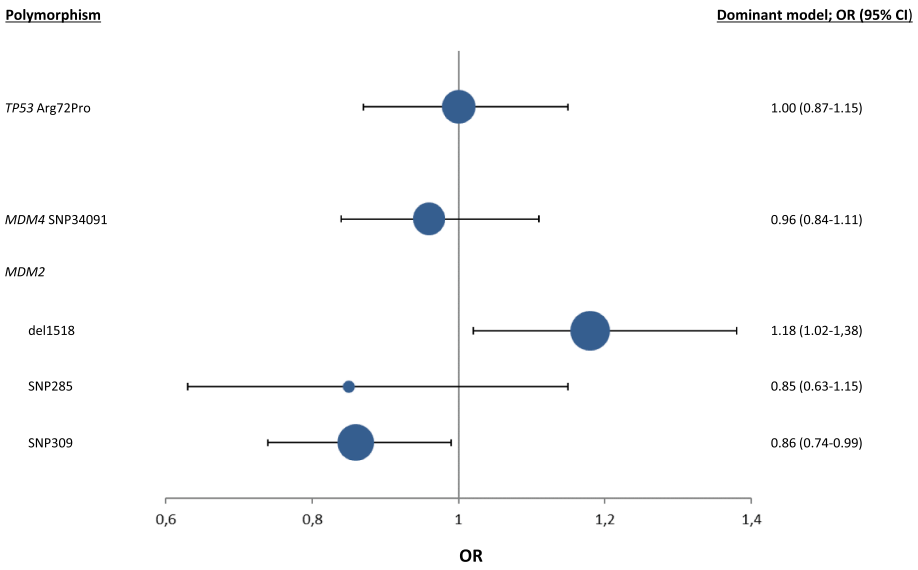

Fig. 4. Forrest plot illustrating odds ratios (ORs) for rheumatoid arthritis related to the minor allele genotypes (dominant model) for polymorphisms in *TP53*, *MDM4* and *MDM2*.

analyzed, we observed 10 different genotypes (Supplementary Table S3a). One of these combined genotypes (del/del-G/G-T/G) was observed in two individuals only (one RA patient and one healthy control). Therefore, we excluded these two individuals and restricted statistical comparisons to the nine remaining genotype combinations. We found the ins/del-G/G-T/T genotype to be significantly over-represented in RA patients as compared to most of the other genotype combinations (Supplementary Table S3b). Setting the most common genotype combination (ins/del-G/G-T/G) as reference, the aforementioned ins/del-GG-TT genotype was found associated with RA (OR: 1.34; CI: 1.09–1.65; Fig. 5, upper panel; for data on all possible genotypes, see Supplementary Fig. S1).

Notably, we also performed secondary analyses, seeking the extreme differences between the combined genotypes, with respect to RA. Based on the findings when assessing single polymorphisms (excluding SNP285C due to low number of observations) one may expect combined genotypes harbouring the del1518-del-allele and the SNP309T-allele to be the extreme opposite of those harbouring the del1518-ins-allele and the SNP309G-allele. We found individuals carrying the combined del/ins-G/G-T/T genotype to have an increased risk of RA as compared to those with the ins/ins-G/G-G/G genotype (OR: 1.56; CI: 1.18–2.06; Fig. 5, lower panel).

Next, based on the genotype observations and the strong LDs between the *MDM2* polymorphisms, we inferred the presence of five

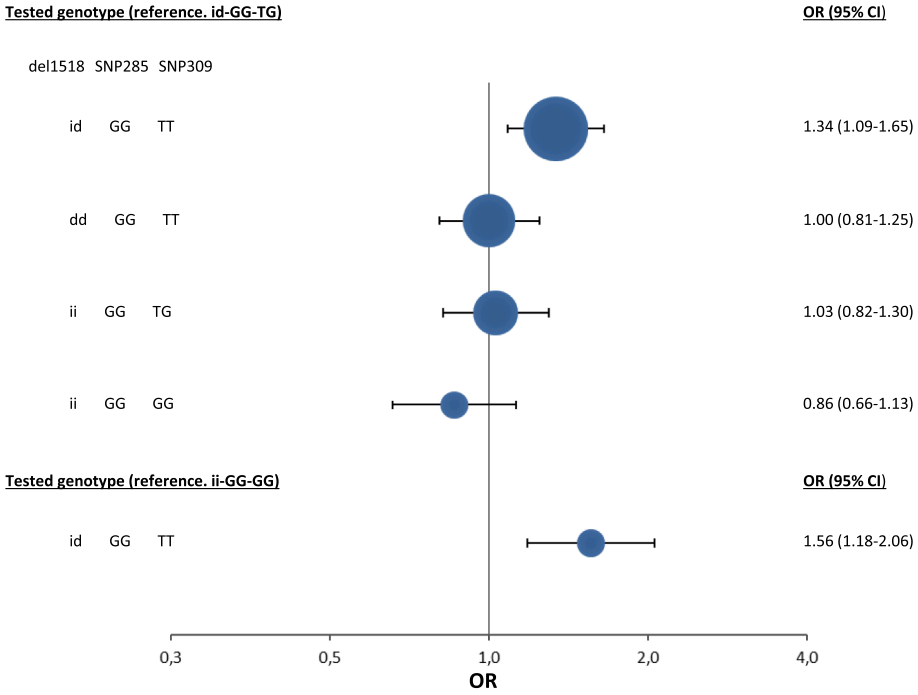

Fig. 5. Forrest plot illustrating odds ratios (ORs) for rheumatoid arthritis related to the combined genotypes of the *MDM2* polymorphisms del1518 (rs3730485), SNP285 (rs117039649) and SNP309 (rs2279744). Upper panel: The most common genotype is set as reference. For clarity, the plot is restricted to the four following most common genotypes (for all genotypes, see Supplementary Figure S1). Bottom panel: comparison of the two most extreme genotype combinations.

del1518-SNP285-SNP309 haplotypes in the dataset (Supplementary Table 4a). One of these, most probably being the result of a rare recombination, was found in two individuals only (del-G-G haplotype; present in the two aforementioned individuals with del/del-G/G-T/G genotype). Thus, our further assessments were restricted to four haplotypes. In line with the findings above, the two haplotypes with SNP309G were underrepresented in RA patients (Supplementary Table S4b). Setting the most common haplotype (del-G-T) as reference, both haplotypes harbouring the SNP309G variant (ins-G-G and ins-C-G) had a relatively low prevalence in RA patients (OR: 0.89; CI: 0.79–1.00 and OR: 0.83; CI: 0.61–1.12, respectively), though these associations did not reach statistical significance, probably due to low number of observations (Supplementary Fig. 2).

4. Discussion

In the present study, we assessed the genotype status of polymorphisms in the p53 network in RA patients. While we found no association with RA for key polymorphisms in *TP53* and *MDM4*, the minor del-allele of *MDM2* del1518 was associated with an increased risk of RA and importantly, individuals with the combined *MDM2* del1518-SNP285-SNP309 genotype del/ins-G/G-T/T to have an increased risk of RA, indicating opposite effects of the del1518 del-allele and the SNP309 G-allele.

The p53 protein regulates a number of cellular processes including growth arrest, apoptosis and senescence in addition to several other functions, including metabolism (Toledo and Wahl, 2006). Thus, any alterations in p53 function, including activity of its key regulators MDM2 and MDM4 may be assumed to influence several different pathogenic processes.

Loss of p53 function through mutations or other alterations have been strongly associated with cancer risk as well as tumour biology (Toledo and Wahl, 2006) and all the five polymorphic variants analysed here are previously linked to cancer risk in several reports. While the p53 Arg72Pro variant is linked to apoptosis and cell cycle regulation (Dumont et al., 2003; Pim and Banks, 2004), the *MDM4* SNP34091 has been associated with ovarian (Gansmo et al., 2016a) and breast cancer (Gansmo et al., 2015b). For *MDM2*, the SNP309G variant has been associated with increased risk of multiple cancers (Bond et al., 2004; Economopoulos and Sergentanis, 2010), while SNP285C has been associated with reduced risk of ovarian (Knappskog et al., 2011) and endometrial cancer (Knappskog et al., 2012). The del1518del variant has been found associated with a reduced risk of endometrial cancer (Gansmo et al., 2017) and a moderately increased risk of colon cancer (Gansmo et al., 2016b).

Although the p53-network's role in cancer is well studied, p53 function also plays a role to other pathophysiological conditions, like inflammation (Gudkov et al., 2011). Such roles are also emerging for the p53 regulator MDM2, leading to preclinical studies on MDM2 inhibition by nutlin-3a treatment revealing promising results in terms of reduced joint damage and arthritis in mouse models (Zhang et al., 2016). Whether this may point to MDM2 as a potential target for future treatment of RA remains to be seen through further studies.

Regarding *MDM2* genetics and the risk of RA, the *MDM2* SNP309GG genotype has been found associated with a higher apoptotic activity compared to the 309TT genotype in synoviocytes from RA patients (Heyne et al., 2012), and Assmann and colleagues found the 309GG and 309TG genotypes to be associated with a reduced incidence of RA (Assmann et al., 2009). Similar to the previous study by Assmann and colleagues analyzing 221 RA patients and 521 healthy controls (Assmann et al., 2009), we here found the 309G allele to be associated with a moderately reduced risk of RA. Although we here confirm their findings, in a larger sample set, it is important to note that this association was not confirmed when mining a previous GWAS data set (Okada et al., 2014).

We found an increased OR associated with the del1518del variant. While to the best of our knowledge no previous study has addressed the OR for RA in respect to this variant, interestingly Salimi and colleagues found the same variant to be associated with a reduced OR for Systemic lupus erythematosus (SLE) (Salimi et al., 2017). Unfortunately, since this variant is a deletion and not a SNP, this finding could not be tested in a GWAS data set.

Conflicting evidence has suggested an increased risk of certain cancer forms, like lymphomas, among patients diagnosed with an RA, indicating potential common risk-factors. Our findings of the *MDM2* del1518del variant to be potentially associated with an elevated risk of RA, point in the opposite direction of what has been recorded for the same variant in respect to cancer risk and for the other polymorphisms, no conclusive association were found. Taken together, our findings in the present study does not suggest a common risk factor for RA and solid malignant diseases with respect to variants affecting p53 function. It should however, be noted that the effect of polymorphisms in the p53 network has been found to be tissue specific (Ortiz et al., 2018) and it may be that the observed differences between impact on risk of some cancer types and the risk of RA are more related to tissue specific differences than disease specific differences.

Our observation of no association between *TP53* Arg72Pro and susceptibility to RA is in concordance with a meta-analysis including 550 RA cases and 622 controls (Lee et al., 2012). Another study showed an association between *TP53* Arg72Pro and joint erosion (Macchioni et al., 2007), which may indicate a role for this variant in terms of disease severity. In the present analysis, we did not have data available to perform such sub-analyses.

A limitation in our study is that one of the key findings, relating the *MDM2* del1518del variant to risk of RA, could not be assessed in our validation approach based on SNP-data from a GWAS study. Given the nature of this variant (a deletion not detected by SNP-arrays), validation may need to be performed in sample set specifically genotyped for this purpose. It is also worth noting that our study was performed exclusively in the Norwegian population, while the validation GWAS set was a mix of different nationalities. Further it is a limitation in our study that we selected the polymorphisms to assess based on current knowledge of biological and functional relevance. This knowledge is mostly related to cancer research and cancer risk assessment. As such, it may be that other polymorphism in the *TP53-MDM2-MDM4* axis, not studied here, could also affect risk of RA.

Although we here have focused on the role of *MDM2* in the p53-network, it is important to underline that a role of *MDM2* with respect to RA, may be independent of p53. Several lines of evidence have indicated that *MDM2* may have severe impact on different diseases through functions not mediated by p53. This also includes RA, where both p53-dependent and p53-independent functions are reported (reviewed in(Wang et al., 2020)).

5. Conclusion

In conclusion, we find a potential association between the *MDM2* del1518 variant and RA, and indications that combinatorial genotypes and haplotypes in the *MDM2* locus should be further investigated in relation to risk of RA.

6. Ethics

All analyses were executed according to the Norwegian guidelines for research on human material. Each sample donor involved in this study has provided written informed consent to use the samples for research purpose. The study and biobanks were approved by the Norwegian Regional Committees for Ethics in Medical Research (REK Midt-Norge and REK Sør-Øst).

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CRediT authorship contribution statement

Liv B. Gansmo: Conceptualization, Investigation, Data curation, Validation, Formal analysis, Writing - original draft. **Benedicte A. Lie:** Data curation, Writing - review & editing. **Marthe T. Mæhlen:** Data curation, Writing - review & editing. **Lars Vatten:** Data curation. **Pål Romundstad:** Data curation. **Kristian Hveem:** Data curation. **Per E. Lønning:** Project administration, Conceptualization, Validation, Writing - review & editing. **Stian Knappskog:** Project administration, Conceptualization, Investigation, Validation, Formal analysis, Writing - original draft, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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