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Title

The MMP9 rs17576 A>G polymorphism is associated with increased lumbopelvic pain-intensity in pregnant women

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Abbreviated title

MMP9 genotype and lumbopelvic pain in pregnant women

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Key message

This is a prospective study of 838 Norwegian pregnant women, where the aim was to investigate whether the MMP9 SNP rs17576 A>G may be associated with lumbopelvic pain-intensity. Women with two copies of the G allel reported significantly more pain at 32-36th gestation week.

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Abstract

Background and aims: Matrix metalloproteinase 9 (MMP9) is an enzyme that may affect degradation of several extracellular matrix components in the pelvic ligaments during pregnancy. Previous studies indicate that genetic variations in the gene encoding MMP9 may affect the enzymatic activity. One such genetic variant is a single nucleotide polymorphism (SNP), rs17576 A>G. In this study we investigated whether the MMP9 SNP rs17576 A>G may be associated with increased lumbopelvic pain in 838 pregnant woman. The study was registered with ClinicalTrials.gov (NCT 00476567) on May 21, 2007.

Methods: Lumbopelvic pain-intensity was measured by visual analogue scale (VAS) at 2 time points during pregnancy, T1 (18-22 weeks), T2 (32-36 weeks) and 3 months after delivery. Blood samples were collected at each point and SNP genotyping was carried out using predesigned TaqMan SNP genotyping assays.

Results: The results showed a significant association between the number of G alleles and pain-intensity in the evening at T2. The pain among G/G carriers was higher than among A/G carriers, which in turn was higher than among the A/A carriers. The most pronounced association between the G allele and pain-intensity was observed in primiparae.

Conclusions: We conclude that the MMP9 rs17576 A>G polymorphism is associated with increased lumbopelvic pain-intensity during pregnancy. The present data support the hypothesis that lumbopelvic pain during pregnancy may be related to a relaxin – MMP9 – tissue remodelling mechanism.

Implications: The present findings may be important for future mechanistic studies on how MMP9 rs17576 A>G may affect changes in the extracellular matrix components in pelvic ligaments and lumbopelvic pain-intensity during pregnancy.

Keywords: MMP9, relaxin, lumbopelvic pain, pelvic girdle pain, polymorphism.

Background

Earlier data show that lumbopelvic pain during pregnancy has a prevalence of more than 50 % (1, 2). Such pain interferes with daily activities and accounts for most of the sick leave among pregnant women in Scandinavian countries (3). Often, the onset of pain occurs in early pregnancy and reaches peak intensity between the 24th and 36th gestation week (1, 4). Since the pain often disappears 3 months after birth (5), it has been suggested that lumbopelvic pain may be related to changes in the ligaments in the pelvic joints during pregnancy (6).

During pregnancy, high levels of relaxin is produced by the decidua and placenta (7). A previous study indicate that this hormone contributes to laxity of pelvic joints in pregnancy (8). Relaxin may through collagenases cause remodelling of the extracellular matrix (ECM) of the pelvic ligaments (9). One important collagenase that targets the ECM is matrix metalloproteinase 9 (MMP9). MMP9 is a zinc depended protease, which cleaves collagens of type I, III, IV, V and elastin (10, 11). This MMP9 zinc depended enzyme is expressed in active remodelling tissues (12, 13).

Relaxin has been shown to up-regulate MMP9 expression and to facilitate tissue remodelling both *in-vivo* and *in-vitro* (14, 15). The function of MMP9 may, however, also be influenced by genetic variability; for example the single nucleotide polymorphism (SNP), rs17576 A>G in exon 6. Therefore, ECM remodelling may be influenced by this SNP. The MMP9 SNP, rs17576 A>G has previously been linked to conditions like pelvic organ prolapse, lumbar-disc herniation, endometrioses and metastases in cervical cancer (16-19).

Genetic variability that influences tissue remodelling, degradation or inflammation – including the SNP emphasized above – may possibly also affect pain-sensitivity. Both reduced and increased MMP9 enzymatic activity could have undesirable effects on the tissue and cause pain. Hence, we hypothesized that the MMP9 SNP rs17576 A>G could affect lumbopelvic pain in pregnant woman. The aim of this study was to investigate whether the MMP9 SNP rs17576 A>G may be associated with lumbopelvic pain-intensity in pregnant woman during their pregnancy.

Methods

Pregnant women booking for routine ultrasound scans at Trondheim University Hospital and Stavanger University Hospital were recruited during the period from 2007 to 2009. This is a sub study of an earlier RCT, where a total of 855 pregnant women were randomized to an exercise intervention or a control group, for more details see (20). The two hospitals are serving large geographical catchment areas, and approximately 12000 women had routine ultrasound scans in the inclusion period. However, only women from geographical areas within 30 min. drive from the hospitals were eligible. The inclusion criteria were age ≥ 18 years, a singleton live fetus and a normal pregnancy with low risk for developing any complications. The characteristics of the subjects are shown in table 1.

Exclusion criteria were: high-risk pregnancy, placenta previa, diabetes, any chronic disease, high blood pressure (>140/90 before week 20), drug misuse and alcoholism, non-European-Caucasian ethnicity or poor Norwegian language, missing blood samples, insufficient DNA isolated and nondetectable genotype.

In all, 875 women consented to participate, 23 women were excluded due to not meeting the inclusion criteria and five miscarried (Fig. 1). About 15% became dropouts during the follow up. The women received written information and signed informed consent forms. The study was performed in accordance with the Helsinki Declaration, approved by the Regional Committee for Medical and Health Research Ethics (REK 4.2007.81) and registered with ClinicalTrials.gov (NCT 00476567).

Clinical measures

Data collection was done at inclusion 18-22 weeks of pregnancy (T1), at follow-up 32-38 weeks of pregnancy (T2) and 3 months after delivery (T3). At each time point, the women were asked: “Do you have pain in the pelvic and/or lumbar area?” (Yes/No). Moreover, at each time point, the women were further asked to rate the worst pain in the evening on a 100 mm visual analog scale (VAS) with endpoints “no pain” and “worst pain”.

DNA extraction and SNP genotyping

Genomic DNA was extracted from whole blood cells using QIAamp 96 DNA Blood kit (Qiagen, Hilden, Germany). SNP genotyping was carried out using predesigned TaqMan SNP genotyping assays (Applied Biosystems) for *MMP9* rs17576 A>G.

Approximately 10 ng genomic DNA was amplified in a 5 µl reaction mixture in a 384-well plate containing 1x universal TaqMan master mix and 1x assay mix, the latter containing the respective primers and MGB-probes. The probes were labeled with the reporter dyes FAM or VIC at 5'end to distinguish between the two alleles. The reactions were performed on an ABI 7900HT sequence detection system (Applied biosystems) at the following program: After initial denaturation and enzyme activation at 95°C for 10 min, the reaction mixture was subjected to 95°C for 15 s and 60°C for 1 min. Negative controls containing water instead of DNA were included in every run. Genotypes were determined using the SDS 2.2 software (Applied Biosystems). Approximately 10% of the samples were re-genotyped and the concordance rate was 100%.

Statistical analysis

Linear mixed models were used to estimate the influence of the MMP9 genotype on VAS pain score for each time point. An allele dependent model was assumed, such that the effect of the genotype G/G was expected to be twice the effect of the genotype A/G when compared to the genotype A/A (adjusted for covariates age, parity, BMI and smoking). Random intercepts were added for subjects. In addition, the effect of genotype for primiparae versus other subjects at T2 were analysed by linear regression (adjusted for covariates age, BMI and smoking).

Results

In total 838 healthy pregnant women were included to the present study (Figure 1). As expected, an increase of lumbopelvic pain-intensity was observed from 18-22 (T1) to 32-36 (T2) weeks of pregnancy followed by an after-birth (T3) recovery. Still, at all time points, a G allele dependent effect of the SNP was observed. Thus the A>G polymorphism was associated with lumbopelvic pain-intensitiy (Table 2). The pain among G/G carriers was higher than among A/G carriers, which in turn was higher than among the A/A carriers (Fig. 2). No deviation from the Hardy-Weinberg equilibrium was observed.

In particular, at 32-36 (T2) weeks of pregnancy, the G allele seemed to be associated with pain-intensity. The most pronounced association between the MMP9 G allele and pain-intensity was observed in primiparae (Fig. 3). In this subgroup, the effect of the MMP9 genotype G/G was twice the effect of the genotype A/G when compared to the genotype A/A. The estimated regression beta coef. at 32-36 weeks of pregnancy revealed that the VAS effect-size of the G allele in primiparae was 4.04 (Table 3) indicating an AA versus GG difference in VAS of 8.08.

Discussion

As in previous studies, an increase of lumbopelvic pain-intensity was observed during pregnancy. For the first time, however, we here show evidence that individual genetic variability, possibly important for remodelling of the extracellular matrix (ECM) and increased joint laxity, may be associated with such pain.

In accordance with our hypothesis, the MMP9 rs17576 A>G polymorphism was associated with lumbopelvic pain-intensity during pregnancy. In particular, at week 32-36 of the first pregnancy, the G allele was associated with increased pain-intensity. An allele dependent effect, such that the effect of the genotype G/G was twice the effect of the genotype A/G when compared to the genotype A/A, was observed.

The MMP9 rs17576 A>G polymorphism resides within the catalytic domain of the enzyme. This domain is highly conserved, and consists of gelatinase-specific fibronectin type II repeats, which plays an important role in substrate binding and substrate cleavage (21). The MMP9 rs17576 A>G polymorphism leads to a substitution of an uncharged amino acid (glutamine) by a positive charged amino acid (arginine) in exon 6 (22). Therefore, the SNP may change the enzymatic properties.

Previous studies support that this SNP is functional and may affect tissue remodelling. For example, evidence exists that the MMP9 rs17576 A>G in a G allele dependent manner promote cancer invasion and metastasis (19, 23). However, the present results do not show any causal relationship between the MMP9 rs17576 A>G, the MMP9 enzymatic activity and tissue remodelling in the pelvic ligaments.

During pregnancy, high levels of relaxin is produced by the decidua and placenta (7). Thus, relaxin may through collagenases including MMP9 cause remodelling of the extracellular matrix (ECM) of the pelvic ligaments (9). Although this may be a natural process important for increased joint laxity (7) before birth, it could also in some subjects have other less desirable effects – for example induce tissue degradation that in turn activate nociceptors and cause pain.

In the synovial joint and pubic symphysis fibocartilaginous tissues, the relaxin-mediated alterations in extracellular matrix composition causing joint laxcity, appear to be caused by increased degradative responses and changes in the collagen content (24, 25). The induction of MMP9 by relaxin occurs via the relaxin receptor, RXFP1. The activation of the RXFP1 involves

the P13K, ERK, Akt and PKC-ζ pathways, including the Elk-1 and c-fos transcriptional factors, which in turn leads to transcription of the gene encoding MMP9 (26).

Conclusions

As described above, the relaxin – MMP9 – tissue remodelling mechanism is complex, and also involves many molecules important for activation of nociceptive primary afferent nerve fibers. Although the main effect may be increased joint laxity, a secondary effect may be pain. We conclude that the MMP9 rs17576 A>G polymorphism is associated with development of lumbopelvic pain-intensity during pregnancy.

Study limitations

The estimated regression beta coef. at 32-36 weeks of pregnancy revealed that the VAS effect-size of the MMP9 rs17576 G allele in primiparae was 4.04. This indicate an AA versus GG difference in VAS of 8.08. Moreover, other SNPs in linkage disequilibrium with MMP9 rs17576 G could also be involved. How the MMP9 genotype interferes with daily activities during pregnancy may be debated. Still, a clear G allele dependent effect, in particular in primiparae, was observed. Hence, the present data suggest that MMP9 genotype may be associated to the pain mechanism during pregnancy.

Authors' contributions

AKM, AM, SNS, SM and JG performed the research. AKM, AM, SNS, SM and JG analyzed the data. AKM, AM, SNS, SM, HSR, NKV, KS and JG drafted the manuscripts. AKM and JG wrote the paper. HSR, NKV, SM and JG designed the study. All authors read and approved the final manuscript.

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Availability of data and materials

All data underlying the findings may be available upon request. Requests for the data should be addressed to Director General Pål Molander or Director of Communication Sture Bye at National Institute of Occupational Health (NIOH), Norway: postmottak@stami.no.

Ethical issues

The study was performed in accordance with the Helsinki Declaration and approved by the Regional Committee for Medical and Health Research Ethics (REK 4.2007.81). The participants received written information and signed informed consent forms.

Consent for publication

Not applicable.

Competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could lead to potential conflicts of interest.

Abbreviations

MMP9 (Matrix metalloproteinase 9), extracellular matrix (ECM), single nucleotide polymorphism (SNP).

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Figure legends

Figure 1

Flow chart of the study population. The study was based on 847 pregnant women of which 838 were examined with regard to MMP9 genotype and pain-intensity.

Figure 2

The time course for lumbopelvic pain development in pregnant subjects grouped by genotype. The data are presented as means ± SEM.

Figure 3

Lumbopelvic pain in pregnant subjects grouped by genotype at 32 – 36 weeks. A) Women who are having their first child. B) Women who are having their second or third child. The data are presented as means ± SEM.

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Figure 1:

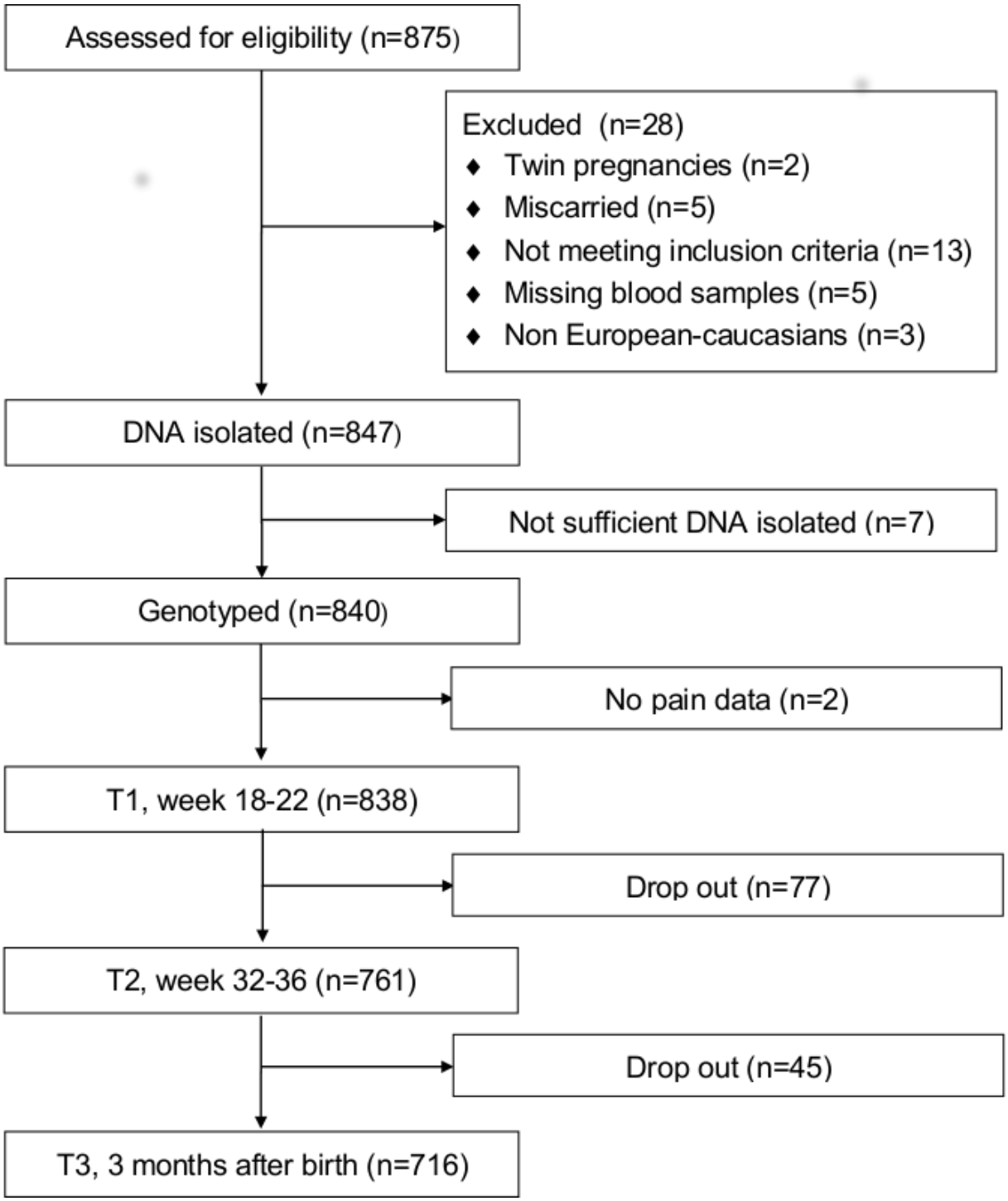

Figure 2:

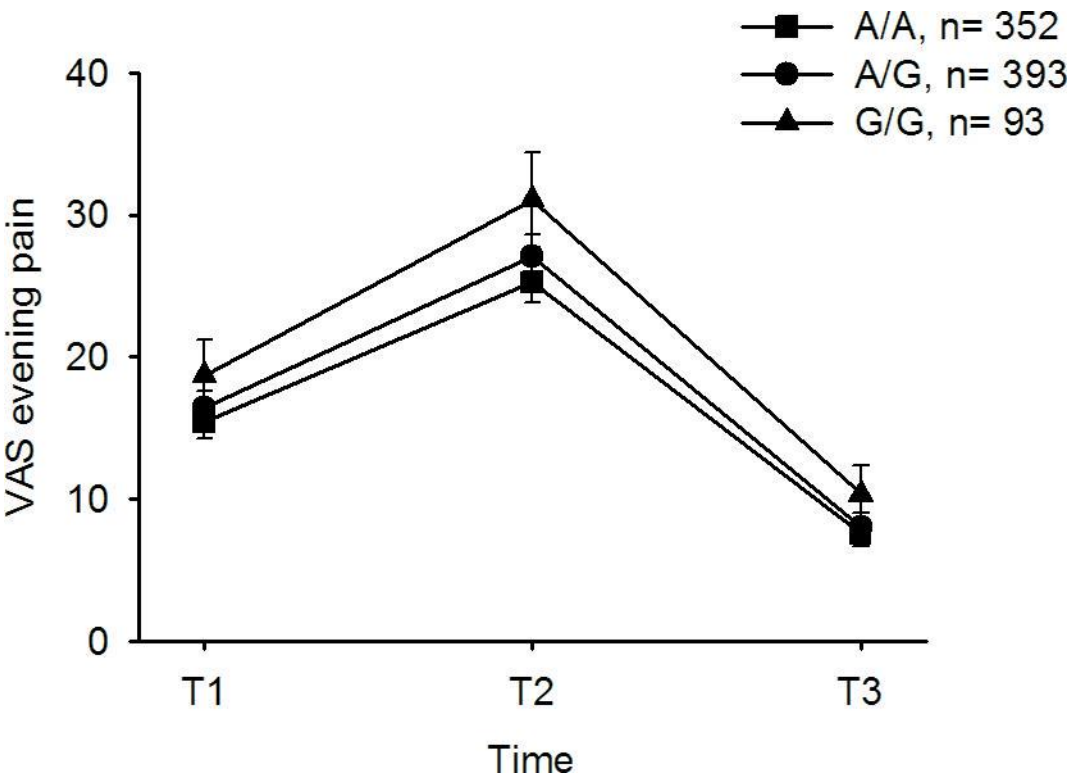
A/A, n= 352
A/G, n= 393
G/G, n= 93
VAS evening pain
40
30
20
10
0
T1
T2
T3
Time

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Figure 3: