Clinica Chimica Acta 411 (2010) 1487–1490

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Analysis of MMP2 promoter polymorphisms in patients with pseudoxanthoma elasticum

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ARTICLE INFO

Article history:
Received 26 March 2010
Received in revised form 27 May 2010
Accepted 3 June 2010
Available online 10 June 2010

Keywords:
Matrix metalloproteinase
MMP2
Pseudoxanthoma elasticum
PXE
ABCC6

ABSTRACT

Background: Pseudoxanthoma elasticum (PXE) is a rare hereditary disorder predominantly affecting the skin, the eyes and the cardiovascular system. The disease is caused by mutations in the *ABCC6* gene and characterized by ectopic calcification and extracellular matrix (ECM) alterations. Matrix metalloproteinases (MMPs) play a pivotal role in the process of ECM remodeling and are likely implied in PXE pathology. The aim of the present study was to investigate the association of single nucleotide polymorphisms (SNPs) in the promoter of the *MMP2* gene, and PXE.
Methods: We evaluated the allelic distribution of five SNPs in the *MMP2* promoter in DNA samples from 168 German patients affected by PXE and in 168 healthy, age- and sex-matched control subjects using restriction fragment length polymorphism analysis.
Results: The alleles c.-1575G, c.-1306C, and c.-790T were more abundant in the PXE patients' group. Furthermore, the haplotype GCTCG was significantly associated with PXE (OR 1.56, 95% CI 1.14–2.12, $P_{corrected} = 0.026$).
Conclusions: Our results may indicate an involvement of MMP2 in the pathology of PXE. The promoter polymorphisms associated with PXE may lead to increased *MMP2* expression and thereby contribute to the elevated proteolytic activity observed in PXE *in vitro* and *in vivo*.

1. Introduction

The autosomal recessive heritable disease pseudoxanthoma elasticum (PXE) is characterized by extensive connective tissue alterations [1,2]. These alterations include progressive calcification and fragmentation of elastic fibers and accumulation of proteoglycans in the extracellular matrix (ECM) of the skin, the Bruch membrane in the retina and the vessel walls. PXE is caused by mutations in the *ABCC6* gene [3]. The protein encoded by this gene is a member of the superfamily of ATP-binding cassette (ABC) transporters and belongs to the multidrug resistance-associated protein (MRP) subfamily, which is involved in multidrug resistance [4]. The precise physiological function of ABCC6 and its physiological substrate are yet unknown, although it has been suggested that it might serve as an export pump facilitating the removal of certain metabolites from hepatocytes. *ABCC6* is mainly expressed in the liver and the kidneys, while little or no protein is found in the tissues affected by PXE. Based on this finding, it was concluded that PXE is a metabolic disease [5].

The clinical picture of PXE is highly variable, with age at disease onset and the number and magnitude of its symptoms differing considerably among the patients [6]. This is even true in the case of affected siblings bearing the same *ABCC6* genotype [7]. Based on these observations, it has been assumed that other genes, called modifier genes, as well as environmental factors might contribute to the expression and severity of PXE. We were able to confirm the relevance of this approach by the identification of modifier genes for PXE in recent years [8–11]. Those genes are involved in established or assumed pathomechanisms of PXE, namely the biosynthesis of glycosaminoglycans, regulation of biological calcification, response to oxidative stress, and the initiation of pathological neovascularization in PXE-associated retinal disease. Further evidence for the importance of modifier genes comes from recent experiments with $Abcc6^{-/-}$ mice conducted by Li and coworkers [12]. Their results impressively demonstrated that the genetic background exerts influence on the phenotype also in an animal model of PXE.

Extensive alterations of the ECM are the histopathological hallmark of PXE. Hence, the molecular mechanisms participating in ECM remodeling in PXE are of particular interest. It is well known that fibroblasts from PXE patients have increased proteolytic potential [13,14]. In particular, elevated expression of matrix metalloproteinase 2 (MMP2) in PXE fibroblasts has been shown, both on the mRNA and

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doi:10.1016/j.cca.2010.06.006

the protein level [15]. Our group could recently demonstrate increased serum concentrations of MMP2 and MMP9 in PXE patients [16]. These findings point to an involvement of MMPs in PXE pathogenesis.

While elevated production of MMP2 in PXE fibroblasts and increased levels of MMP2 and MMP9 in serum from PXE patients have been shown, the cause of these findings remains unknown. Increased MMP expression may at least partly result from genetic variation. Certain alleles of MMP2 and MMP9 were shown to be associated with altered transcriptional activity of the genes and a variation of *in vivo* levels due to genetic differences can be anticipated [17,18]. In order to examine a possible role for MMP2 as genetic cofactor for PXE, we conducted an analysis of polymorphisms in the promoter of the MMP2 gene in PXE patients and healthy control subjects.

2. Patients, materials and methods

2.1. Patient characteristics

The study cohort comprised 168 (48 male, 120 female) German patients with PXE from 168 non-consanguineous families with apparently autosomal recessive or sporadic mode of inheritance of the PXE phenotype. Mean age and mean age at disease onset (years ± SD) were 49.1 ± 15.5 and 31.8 ± 16.3, respectively. The diagnosis of PXE in all patients was consistent with the reported consensus criteria [19]. The status of the PXE patients was determined by the presence of ocular findings and dermal lesions and histologically confirmed by observation of calcified elastic fibers in skin biopsies after von Kossa staining. The biopsy samples were taken from lesional skin. All participants of the study were thoroughly questioned about their personal diseases, organ involvements and family history by one medical specialist in order to minimize inter-observer variability. Blood samples from 168 age- and sex-matched Westphalian blood donors served as controls for polymorphism analysis. The study was approved by the institutional review board; all patients gave their informed consent. The study conformed to the principles of the World Medical Association's Declaration of Helsinki.

2.2. DNA preparation

Genomic DNA was extracted from 200 µl EDTA blood using the QIAamp blood kit (Qiagen) according to the manufacturer's instructions.

2.3. Polymerase chain reaction

PCR primers were designed using the published sequence (GenBank Accession No. NG_008989). PCR was performed as previously described [10]. The primer sequences used were (5′–3′): CAGCCAAGGTTTGTCACT and GCTGGAGGGGTCAGTAA for c.-1575G>A, AAACTTTCTTCTCCAGTGCC and ACCTGAAGAGCAAAAGAGGT for c.-1306C>T, TAGCGTAAAATG-AGCAGTGAGGATG and AGATAGAAATTGGGCAAGACTGGTTTACTA for c.-790T>G, AGCGTAAAATGAGCAGTGCC and CCTTGGGAAATAGAGCAG for c.-735C>T, and finally TAGTACCGCTGCTCTCTAA and CCTGAGG-AAGTCTGGAT for c.-168G>T. The annealing temperature was 56 °C for all SNPs with the exception of c.-790T>G where 63 °C was applied.

2.4. Restriction fragment length polymorphism analysis

The obtained DNA-fragments were digested with 5 U of either Nla III (c.-1575G>A), CspC I (c.-1306C>T), Dde I (c.-790T>G), Ava II (c.-735C>T) or BsaJ I (c.-168G>T) overnight at the temperature recommended by the supplier and subsequently separated on a 1.5% agarose gel. All restriction endonucleases were purchased from New England Biolabs.

2.5. Statistical analysis

Allele and haplotype frequencies were compared between cases and controls using Fisher's exact test. Correction for multiple testing was performed using the Bonferroni method. The chi-square test was used to examine whether the genotype distributions were within the Hardy–Weinberg equilibrium by comparing observed and expected genotypes. P-values of less than 0.05 were considered significant. All tests were performed using SPSS 15.0 (SPSS Inc.).

2.6. Linkage disequilibrium structure and identification of haplotype blocks

Determination of linkage disequilibrium (LD) and haplotype blocks and frequencies was performed using Haploview 4.1 [20]. Haplotype blocks were defined according to the “spine of LD” setting in the Haploview software, on the basis of each end marker of a block having a D′ value of >0.8.

3. Results

3.1. Association of single markers with PXE

The single nucleotide polymorphisms c.-1575G>A (rs243866), c.-1306C>T (rs243865), c.-790T>G (rs243864), c.-735C>T (rs2285053) and c.-168G>T (rs17859829) in the promoter of the *MMP2* gene were genotyped in DNA samples from 168 patients affected by PXE and in 168 healthy controls. The allelic distribution for the polymorphisms is shown in Table 1. The c.-1575G allele, the c.-1306C allele and the c.-790T allele were significantly associated with PXE ($p<0.05$ each). However, the association did not remain significant after correction for multiple testing

Table 1
Allele frequencies of *MMP2* promoter polymorphisms in PXE patients and controls.

Polymorphism	rs number	Allele	Cases ($N=336$)	Controls ($N=336$)	OR (95% CI)	$P_{corrected}$
c.-1575G>A	rs243866	G	273 (81.3)	246 (73.2)	1.59 (1.10–2.28)	0.065
		A	63 (18.7)	90 (26.8)		
c.-1306C>T	rs243865	C	273 (81.3)	247 (73.5)	1.56 (1.08–2.25)	0.082
		T	63 (18.7)	89 (26.5)		
c.-790T>G	rs243864	T	272 (81.0)	245 (72.9)	1.58 (1.10–2.27)	0.122
		G	64 (19.0)	91 (27.1)		
c.-735C>T	rs2285053	C	300 (89.3)	295 (87.8)	1.16 (0.72–1.86)	1.000
		T	36 (10.7)	41 (12.2)		
c.-168G>T	rs17859829	G	313 (93.2)	310 (92.3)	1.14 (0.64–2.04)	1.000
		T	23 (6.8)	26 (7.7)		