Klinikum Stuttgart

Mechanisms of bone regeneration: The influence of PADI4 gene polymorphisms on the progression of Charcot osteoarthropathy

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Introduction:

Wound and fracture healing are significantly influenced by immunological processes. The protein PADI4 plays a central role in this, regulating the citrullination of histones and the formation of neutrophil extracellular traps (NETs) in neutrophils. NETs consist of DNA, histones and antimicrobial peptides and contribute to the defence against pathogens. At the same time, they are associated with chronic inflammatory processes. In diabetic foot ulcers in particular, elevated levels of NET components in the blood and increased PADI4 levels in neutrophils are observed, leading to enhanced NETosis. Furthermore, genetic variants of the PADI4 gene influence enzyme activity and could therefore play a role in impaired wound healing. A specific form of diabetic foot syndrome is Charcot osteoarthropathy (DNOAP). It is characterised by non-infectious, progressive destruction of the bones and joints of the foot and occurs in association with peripheral neuropathy.

Research question:

The aim of this study was to investigate whether the minor variant of the PADI4 haplotype is associated with a more unfavourable clinical course of active Charcot foot.

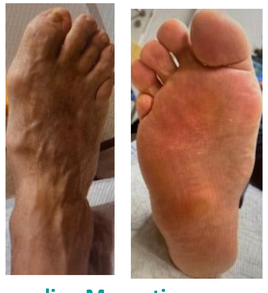
Methodology:

Forty-four diabetic patients with acute Charcot foot were included (Ethics Committee approval 843-2020BO2). The activity of Charcot foot was assessed clinically and by MRI (at study inclusion and 4 months later). After four months, disease activity, the extent of foot deformity and wound healing under immobilisation were evaluated in particular. DNA samples were collected via buccal swabs. PADI4 polymorphisms were detected using the ARMS-PCR test. The distribution of haplotypes was compared with that of the general population; subsequently, a possible correlation between the healing process of acute Charcot foot and the various PADI4 polymorphisms was analysed.

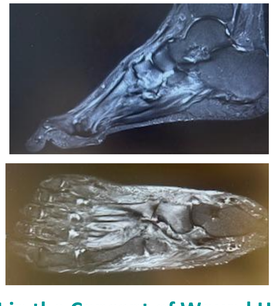
	SNP 163	SNP 245	SNP 335
Reference	rs11203366	rs11203367	rs874881
Base pair change	A>G	C>T	C>G
MAF (Ensembl)	47.5%	46.7%	47.8%
Amino acid mutation	Gly55Ser	Val82Ala	Gly112Ala

Charcot osteoarthropathy

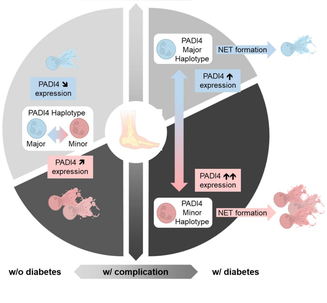
Clinical presentation activated DNOAP



Corresponding Magnetic resonance imaging



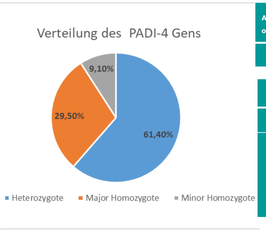
PADI4 in the Concept of Wound Healing



Results:

Sex distribution of the alleles		Alleles: Minor homozygote	Heterozygote	Major homozygote	Total	P-value
Gender	Male	4	15	12	31	p=0.02
		100.0 %	55.6%	92.3%	70.5%	
	Female	0	12	1	13	
		0 %	44.4%	7.7%	29.5%	
		4	27	13	44	

HbA1c and duration of diabetes Distribution across all patients (n=44)		Minor Homozygous	Heterozygous	Major Homozygous	p
15.4 ± 11.9 (13; 1–50)	Duration of diabetes in years	11.33 ± 11.06	13.67 ± 10.09	20.75 ± 15.38	0.32
7.42% ± 1.24 (7.1; 5.4 – 12.5)	HbA1c %	7.10 (1.50)	7.10 (1.60)	7.30 (1.10)	0.97



Age distribution of alleles	Minor Homozygous	Heterozygous	Major Homozygous	p
Age	70.75 ± 15.31	63.30 ± 9.41	62.92 ± 9.70	0.362

		Alleles: Major	Minor	Total	p
Foot deformity	Better/ Same	37 69.8%	19 54.3%	56 63.6%	0.176
	Poor/surgical case	16 30.2%	16 45.7%	32 36.4%	

		Alleles: Major	Minor	Total	p
MRI activity trend	Better	32 65.3%	22 66.7%	54 65.9%	n.s.
	Schechter/surgical case	17 34.7%	11 33.3%	28 34.1%	

		Alleles: Major	Minor	Total	p
Wound healing disorder	No	38 71.7%	24 68.6%	62 70.5%	0.814
	Yes	15 28.3%	11 31.4%	26 29.5%	

Discussion:

70.5% of patients were male, 29.5% female. A significant gender difference was observed in the distribution of haplotypes: men were more frequently homozygous, whilst women were more frequently heterozygous.

52.3% of patients had an HbA1c > 7.1% (poorly controlled), whilst 47.7% were well controlled. The mean age was 63.9 ± 10.1 years, the duration of diabetes was 15.4 ± 11.9 years, and the HbA1c was 7.42 ± 1.34%.

No significant associations were found between age, duration of diabetes or HbA1c and the PADI4 alleles investigated (p > 0.05).

In the analysis of the collected data, the percentage of minor homozygotes was 9.1%. In the cohort of Charcot's disease patients studied, there was no significant shift in allele frequencies compared with the population data described in the Ensembl database.

No increased prevalence of the minor variant could be demonstrated. The majority of patients were heterozygous for the SNPs investigated.

The majority of patients showed a decline in Charcot foot activity, regardless of their PADI4 haplotype. A further 29.5% of patients also exhibited impaired wound healing.

No significant difference could be demonstrated between the different PADI4 alleles. With regard to foot deformity and overall outcomes, patients who were homozygous for the major variant of the PADI4 haplotype tended to show higher rates of improvement; however, these differences did not reach statistical significance.

Conclusion:

This study provides evidence of a possible influence of the PADI4 polymorphism on the clinical outcome of Charcot osteoarthropathy. However, studies involving a larger number of patients are required to draw definitive conclusions.

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