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No association of genetic variants of interleukin 6 and the susceptibility

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Authors:
Dr. Susanne Schulz, Dr. Uta Zimmermann,
University School of Dental Medicine, Department of Operative Dentistry and Periodontology

Prof. Hans-Günter Schaller,
University School of Dental Medicine, Department of Operative Dentistry and Periodontology

Dr. Helmut Machulla, Wolfgang Altermann
Interbranch HLA Laboratory/Department GHATT

Dr. Christiane Gläser
Institute of Human Genetics and Medical Biology

Dr. Stefan Reichert
University School of Dental Medicine, Department of Operative Dentistry and Periodontology,

Martin-Luther-University Halle, Germany

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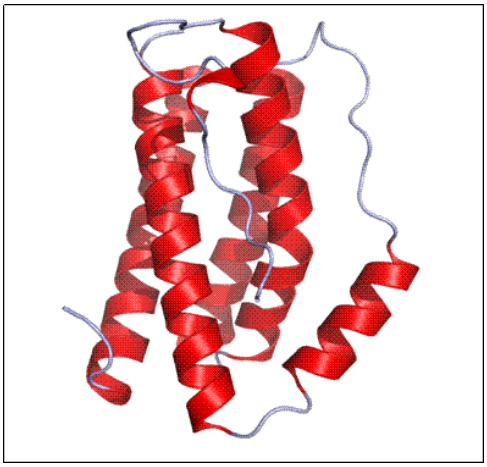
Bonn

Introduction

Interleukin 6 in periodontitis Bacterial inflammation of supporting tissue of teeth --> Production of proinflammatory cytokines including Il-6 -->
1. Imbalance between collagenases and matrix metalloproteinases activity and collagen synthesis
2. Induction of fibroblast and osteoclast activation
3. Bone resorption

Genomic characteristics of interleukin 6
Genomic variants of interleukin 6, including SNPs in the promoter c.-597G>A and c.-174G>C, were shown to trigger the expression of this cytokine. Therefore, the genetic background of interleukin 6 might play an important role in influencing the immune response to periodontopathic bacteria via regulating its expression. Interleukin 6 and its genomic variants may be regarded as markers of the progression and severity of periodontitis as well as indicators of an appropriate response to treatment.



Introduction: Crystal structure of human interleukin 6

Objectives

Objectives:
The aim of the present clinical study was to evaluate the importance of genomic variants (c.-174G>C and c.-597G>A) as well as the corresponding haplotypes of Il-6 for the occurrence of chronic and aggressive periodontitis.

Material and Methods

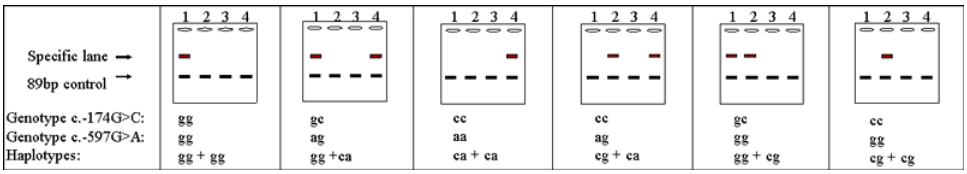
Genomic analyses

DNA-isolation from EDTA-blood
Preparation of genomic DNA from human venous EDTA-blood was carried out using the blood extraction kit (Quiagen). 200µl EDTA-blood and 20 µl protease were mixed in a 1,5 ml tube. After adding of 200 µl denaturation buffer AL and pulse-vortexing for 15 sec the samples were incubated at 56°C for 10 min. 200 µl of ethanol was added to the samples, vortexed and the samples were applied to a QIAamp Spin Column were the DNA is bound. After two washing steps (buffer AW1 and AW2) the DNA bound to the column is dried by centrifugation. 200 µl distilled water is added to the samples, incubated at room temperature for 5 min and then centrifuged. The solved DNA is now in the filtrate. Long-term storage of DNA is possible at -20°C.

Haplotype specific PCR

The detection of genotypes and haplotypes of Il-6 SNPs (c.-174G>C and c.-597G>A) was carried out using the CYTOKINE Genotyping array CTS-PCR-SSP Tray kit of the Collaborative Transplant Study, Department of Transplantation Immunology of the University Clinic of Heidelberg. For every PCR a fragment of 89bp of the human b-globin gene was coamplified as a positive control. The PCRs were performed using sequence specific primers for detection of possible haplotypes prepipetted and lyophilized in thin-walled plastic 96-well PCR trays. For every PCR 10µl of a Mastermix containing 1U Taq-Polymerase (Invitek), 100ng genomic DNA, 5% glycerol, and PCR reaction buffer was added. PCR-program (2 min 94°C; 10 cycles: 15 sec 94°C, 1 min 64°C; 20 cycles: 15 sec 94°C, 50 sec 61°C, 30 sec 72°C) After cycling was completed, the PCR products were loaded onto a 2% agarosegel for electrophoresis. After electrophoresis, the ethidium bromide stained gel is photographed and interpreted.

Lane 1: sequence specific fragment at 427bp: g at pos. -174; g at pos. -597
Lane 2: sequence specific fragment at 426bp: c at pos. -174; g at pos. -597
Lane 3: sequence specific fragment at 428bp: g at pos. -174; a at pos. -597
Lane 4: sequence specific fragment at 428bp: c at pos. -174; a at pos. -597



Materials and Methods: Haplotype specific PCR

Evaluation of periodontopathic bacteria in subgingival pockets

Subgingival sampling
Paper points for collection of subgingival samples were used to bind periodontopathogens of the deepest pocket of each quadrant.

DNA-isolation
Preparation of bacterial DNA was carried out using the QIAamp DNA Mini Kit (Quiagen). The paper points were incubated with 180 µl ATL-buffer and 20 µl proteinase K and incubated at 70°C for 10 min. 200 µl buffer Al was added and the mixture was incubated at 96°C for 5 min. The mixture (without paper points) was applied to a QIAamp Spin Column and washed twice with buffer AW1 and AW2. The DNA was solved in 400 µl AE-buffer and stored at -20°C.

Multiplex-PCR
For specific amplification of Actinobacillus actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), Tannerella forsythensis (Tf) , Treponema denticola (Td) the micro-Ident® test of HAIN-Diagnostik based on alkaline phosphatase mediated staining reaction was used. Mastermix provided in the micro-Ident® test (containing buffer, biotynilated primer, DNA for positive control'), 2U Taq-polymerase (Eppendorf), and 5 µl of isolated bacgterial DNA were mixed. PCR was performed (5 min 95°C; 10 cycles: 30 sec 95°C, 2 min 58°C; 20 cycles: 25 sec 95°C, 40 sec 53°C, 40 sec 70°C; 8 min 70°C) The quality of PCR product was checked by agarosegelelectrophoresis.

Bacteria specific hybridization
20 µl of the PCR product were mixed with 20 µl of the denaturation solution in the well of the tray and incubated at room temperature for 5 min. 1 ml prewarmed (45°C) hybridization buffer was added to the sample and a strip (hybridized with DNA sequences of each bacteria as well as a positive control) was placed in the well of the tray. The tray was incubated at 45°C for 30 min in a shaking water bath. After complete aspiration of hybridization buffer 1 ml of stringent wash solution was added and incubated at 45°C for 15 min. The strip was washed once with 1 ml rinse solution for 1 min and 1 ml of conjugate solution was added (room temperature for 30 min). After washing twice with 2ml rinse solution and once with 1 ml distilled water 1 ml of substrate solution was added. The substrate incubation time varied between 3 and 20 min and the occurrence of bacteria was evaluated visually by means of colored bands. Two positive controls for amplification reaction and for conjugate were included in the test.

Results

1. No significant association between the patient groups and the healthy control group could be proven investigating age, gender and smoking status. As expected, both patient groups showed significant more severe clinical symptoms compared to the control group.
2. As expected, both patient groups showed distinct and mostly significant increase in the occurrence of periodontopathic bacteria. Interestingly, no significant difference in the subgingival colonization with Aa could be shown for patients suffering from CP.
3. For both polymorphisms of interleukin 6 no significant disease specific variation in genotype distribution was evaluated.
4. Investigating the haplotype combinations no significant difference in distribution pattern could be detected comparing patients with chronic or aggressive perio-dontitis with healthy controls.
5. The genetic background of interleukin 6 was not associated with clinical parameters investigated except CAL. In the group of patients suffering from AP carriers of heterozygous genotypes for c.-174C>G and c.-597G>A were significantly associated with increased attachment loss. Furthermore no association of genetic variants and subgingival bacterial colonization was detectable.

Clinical and demographical characterization

	Chronic periodontitis (CP)	Aggressive periodontitis (AP)	healthy controls	p values vs. controls	
	n =48	n=59	n=40	CP	AP
Mean age (years)	48.1±10.1	41.6.7±9.8	43.9±11.1	n.s.	n.s.
Gender (% male)	33.3	35.6	40	n.s.	n.s.
Smoking (%)	27.1	37.3	30	n.s.	n.s.
Approximal plaque index (%)	58.7±25.9	55.7±30.4	39.9±19.2	**<0.001**	**0.005**
Bleeding on probing (%)	67.7±25.1	77.1±24.4	39.2±20.4	**<0.001**	**<0.001**
Pocket depth (mm)	5.2±1.1	6.6±6.2	2.8±1	**<0.001**	**<0.001**
Pocket depth on microbial test site (mm)	6.8±1.3	7.5±1.6	3.1±0.3	**<0.001**	**<0.001**
Clinical attachment loss in general (mm)	5.7±1.4	6.7±1.6	3.1±1.1	**<0.001**	**<0.001**
Clinical attachment loss on microbial test site (mm)	7.3±1.7	8.4±1.8	3.3±0.5	**<0.001**	**<0.001**

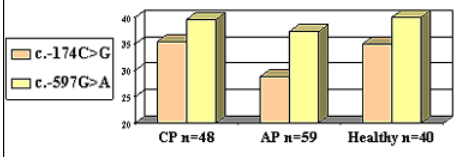
Results and Discussion: Clinical characterization of the patient groups

Microbiological assessment

Actinobacillus actinomycetemcomitans (%)	26.9	45,9	14.6	n.s.	**0.001**
Porphyromonas gingivalis (%)	86.5	83.6	17.1	**<0.001**	**<0.001**
Prevotella intermedia (%)	61.5	67.2	24.4	**<0.001**	**<0.001**
Tannerella forsythensis (%)	96.2	90.2	58.5	**<0.001**	**<0.001**
Treponema denticola (%)	98.1	90.2	65.9	**<0.001**	**0.002**
Pg, Td, Tf (%)	80.8	77.0	17.1	**<0.001**	**<0.001**

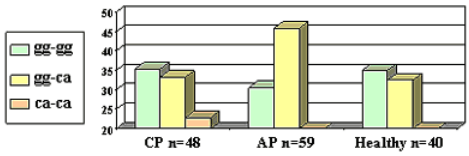
Results and Discussion: Microbiological characterization of the patient groups

Genotype distribution c.-174C>G and c.-597G>A



gg (%)	35.4	28.8	35
cg+cc (%)	37.5+27.1	52.5+18.6	37.5+27.5
gg (%)	39.6	37.3	40
ag+aa (%)	37.5+22.8	50.8+11.9	42.5+17.5

Haplotype combinations of c.-174C>G/c.-597G>A



gg-gg (%)	35.4	30.5	35
gg-ca (%)	33.3	45.8	32.5
ca-ca (%)	22.9	11.9	17.5
cg/ca (%)	4.2	5.1	10
gg-cg (%)	4.2	5.1	5
cg-cg (%)	0	1.7	0

Results and Discussion: Genomic analyses (a)

Association of clinical and microbiological conditions with genomic background

	CP, n=48						AP, n=59						Healthy, n=40					
	c.-174C>G			c.-597G>A			c.-174C>G			c.-597G>A			c.-174C>G			c.-597G>A		
	cc	cg	gg	gg	ag	aa	cc	cg	gg	gg	ag	aa	cc	cg	gg	gg	ag	aa
API (%)	55.1	54.2	64.9	65	52.5	55.7	65.1	52.1	56	56.7	50.9	73.5	34.6	45.2	38.3	37.8	44.1	34.3
BOP (%)	64.8	65.8	71.8	71.8	67.4	61.5	70.3	79.7	77.1	79.5	76.4	72.6	37.4	42.4	37.4	39.7	40.9	34.2
PD (mm)	4.9	5.1	5.4	5.5	5	5	5.2	6.4	8.1	7.9	6.2	4.5	2.7	2.6	2.9	2.9	2.6	2.7
CAL (mm)	5.7	5.6	6	6.1	5.4	5.9	6	7.2	6.2	6.5	7.1	5.4	3	2.8	3.3	3.2	2.9	3.1
							p=0.035			p=0.04								
Aa (%)	45.5	45.2	41.2	45.5	43.3	42.9	15.4	27.8	29.4	31.6	22.2	18.2	18.2	20	0	0	17.6	28.6
Pg (%)	81.8	83.9	88.2	90.9	80	85.7	84.6	88.9	88.2	89.5	88.9	81.8	18.2	20	14.3	12.5	23.5	14.3
Pi (%)	81.8	71	52.9	59.1	73.3	71.4	46.2	55.6	82.4	78.9	55.6	45.5	36.4	26.7	14.3	12.5	35.3	28.6
Tf (%)	100	87.1	88.2	90.9	86.7	100	100	94.4	100	100	94.4	100	54.5	46.7	71.4	68.8	52.9	42.9
Td (%)	100	83.9	94.1	95.5	83.3	100	100	94.4	100	100	94.4	100	45.5	66.7	78.6	75	64.7	42.9

Results and Discussion: Genomic analyses (b)

Conclusions

Investigating possible associations of the occurrence of chronic and/or aggressive periodontitis and the genetic background of the proinflammatory cytokine interleukin 6 (c.-174C>G, c.-597G>A) no difference in genotype, or haplotype frequency could be demonstrated. However, a significant association between clinical attachment loss representing a symptom of periodontitis and both SNPs could be shown for patients suffering from aggressive periodontitis. Since no correlation between genetic variability and risk markers of periodontitis including smoking, gender, age, approximal plaque index, and subgingival bacterial colonization was demonstrated in this study further more comprehensive investigations are necessary for evaluation of the impact of interleukin 6 on the occurrence of periodontitis. According to the results from this study, the two promoter polymorphisms c.-174C>G and c.-597G>A of interleukin 6 could not be describes as independent risk factors for chronic or aggressive periodontitis.

This Poster was submitted by Dr. Susanne Schulz.

Correspondence address:
Dr. Susanne Schulz
University School of Dental Medicine
Department of Operative Dentistry and Periodontology
Martin-Luther-University Halle
Harz 42
06097 Halle
Germany

Poster Faksimile:

No association of genetic variants of interleukin 6 and the susceptibility to periodontitis

S Schulz [1], J Klapproth [1], U Zimmermann [1], HO Schaller [1], HKO Machulla [2], W Albermann [3], C. Glöser [3], S Reichert [1]

1 University School of Dental Medicine, Department of Operative Dentistry and Periodontology, Martin-Luther-University Halle, Germany
2 Interbranch HLA Laboratory/Department GHATT, Martin-Luther-University Halle, Germany
3 Institute of Human Genetics and Medical Biology, Martin-Luther-University Halle, Germany

Introduction

Interleukin 6 in periodontitis

Bacterial inflammation of supporting tissue of teeth

⇩

Immune response

⇩

Production of proinflammatory cytokines including IL-6

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1. **Imbalance between collagenases and matrix metalloproteinases activity and collagen synthesis**
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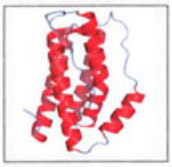
Crystal structur of human interleukin 6



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Genomic variants of interleukin 6, including SNPs in the promoter c.-597G>A and c.-174G>C, were shown to trigger the expression of this cytokine.

Therefore, the genetic background of interleukin 6 might play an important role in influencing the immune response to periodontopathic bacteria via regulating its expression.

Interleukin 6 and its genomic variants may be regarded as markers of the progression and severity of periodontitis as well as indicators of an appropriate response to treatment.

Objectives:

The aim of the present clinical study was to evaluate the importance of genomic variants (c.-174G>C and c.-597G>A) as well as the corresponding haplotypes of IL-6 for the occurrence of chronic and aggressive periodontitis.

Materials and Methods

Genomic analyses

DNA-Isolation from EDTA-blood

Preparation of genomic DNA from human venous EDTA-blood was carried out using the blood extraction kit (Qiagen).
200µl EDTA-blood and 20 µl protease were mixed in a 1,5 ml tube.
After adding of 200 µl denaturation buffer AL and pulse-vortexing for 15 sec the samples were incubated at 56°C for 10 min.
200 µl of ethanol was added to the samples, vortexed and the samples were applied to a QIAamp Spin Column were the DNA is bound.
After two washing steps (buffer AW1 and AW2) the DNA bound to the column is dryed by centrifugation.
200 µl distilled water is added to the samples, incubated at room temperature for 5 min and then centrifuged. The solved DNA is now in the filtrate.
Long-term storage of DNA is possible at –20°C.

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For every PCR a fragment of 109bp of the human β-globin gene was coamplified as a positive control.
The PCRs were performed using sequence specific primers for detection of possible haplotypes prepacked and lyophilized in thin walled plastic 96-well PCR trays.
For every PCR 10µl of a Mastermix containing 1U Taq-Polymerase (Invitek), 100ng genomic DNA, 5% glycerol, and PCR reaction buffer was added.
PCR-program (2 min 94°C, 10 cycles: 15 sec 94°C, 1 min 64°C; 20 cycles: 15 sec 94°C, 50 sec 61°C, 30 sec 72°C).
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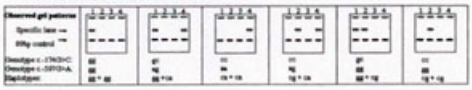

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The paper points were incubated with 180 µl ATL-buffer and 20 µl proteinase K and incubated at 70°C for 10 min.
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The mixture (without paper points) was applied to a QIAamp Spin Column and washed twice with buffer AW1 and AW2.
The DNA was solved in 400 µl AE-buffer and stored at –20°C.

Multiplex PCR

For specific amplification of Actinobacillus actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), Tannerella forsythensis (Tf) , Treponema denticola (Td) the micro-IDent®-test of IMMD Diagnostik based on alkaline phosphatase mediated staining reaction was used.
Mastermix provided in the micro-IDent®-test (containing buffer, biotinylated primer, DNA for positive control*), 2U Taq-polymerase (Sigmaaldrich), and 5 µl of isolated bacterial DNA were mixed.
PCR was performed (3 min 95°C, 10 cycles: 30 sec 95°C, 2 min 58°C, 20 cycles: 25 sec 95°C, 45 sec 55°C, 45 sec 70°C, 8 min 70°C)
The quality of PCR product was checked by agarosegelelectrophoresis.

Bacteria specific hybridization

10 µl of the PCR product were mixed with 20 µl of the denaturation solution in the well of the tray and incubated at room temperature for 5 min.
1 ml prewarmed (60°C) hybridization buffer was added to the sample and a strip (hybridized with DNA sequences of each bacteria as well as a positive control) was placed in the well of the tray.
The tray was incubated at 63°C for 30 min in a shaking water bath.
After complete aspiration of hybridization buffer 1 ml of stringent wash solution was added and incubated at 45°C for 15 min.
The strip was washed once with 1 ml rinse solution for 1 min and 1 ml of conjugate solution was added (room temperature for 30 min).
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The substrate incubation time varied between 3 and 10 min and the occurrence of bacteria was evaluated visually by means of colored bands.
Two positive controls for amplification reaction and for conjugate were included in the test.

Results and Discussion

Clinical characterization of the patient groups

Clinical and demographical characterization

	Chronic periodontitis (CP)	Aggressive periodontitis (AP)	healthy controls	p values vs. controls	
	n=68	n=59	n=43	CP	AP
Mean age (years)	48.1±10.1	41.4.7±9.8	43.9±11.1	n.s.	n.s.
Gender (% male)	33.3	35.6	40	n.s.	n.s.
Smoking (%)	27.1	37.3	30	n.s.	n.s.
Approximal plaque index (%)	58.7±25.9	55.7±30.4	20.9±19.2	<0.001	0.000
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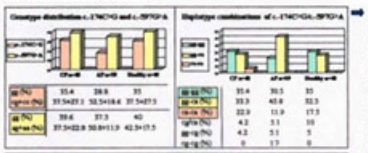
Pg, Td, Tf (%)	80.6	77.0	17.1	<0.001 <0.001

As expected, both patient groups showed distinct and nearly significant increase in the occurrence of periodontopathic bacteria. Interestingly, no significant difference in the subgingival colonization with Aa could be shown for patients suffering from CP.

Genomic analyses



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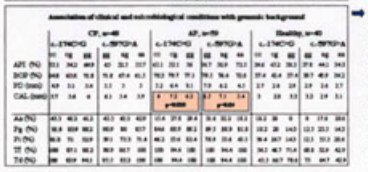

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