Exotoxins of *Aggregatibacter actinomycetemcomitans* and periodontal attachment loss in adolescents

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Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av odontologie doktorsexamen framläggs till offentligt försvar i Hörsal Betula, Unod L, plan 0, Norrlands Universitetssjukhus, Fredagen den 31 januari, kl. 09:00.

Avhandlingen kommer att försvaras på engelska.

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*Aggregatibacter actinomycetemcomitans* is an oral bacterium that is mainly associated with aggressive forms of periodontitis, which most often starts at an early age. Amongst the virulence factors of *A. actinomycetemcomitans*, two exotoxins, the leukotoxin (LtxA) and the cytolethal distending toxin (Cdt), are suggested to play an important role in the pathogenicity of aggressive periodontitis. There is also a genetic diversity of the different strains of *A. actinomycetemcomitans*, and a variation in the ability of different strains to express and release exotoxins has been suggested. Of the different genotypes of *A. actinomycetemcomitans*, the highly leukotoxic JP2 genotype, which is prevalent in individuals of African origin, seems to be the genotype that is most strongly associated with localized aggressive periodontitis.

This thesis is built upon studies of a West African adolescent population. The aim was to study the virulence characteristics of *A. actinomycetemcomitans* genotypes with a specific focus on the LtxA and the Cdt in relation to the progression of attachment loss (AL). The specific aim was first, to investigate cross-sectionally the presence of the JP2 and non-JP2 genotypes of *A. actinomycetemcomitans* in relation to the prevalence of AL and then prospectively to assess the progression of AL in a Ghanaian adolescent population. Second, in clinical isolates of *A. actinomycetemcomitans* obtained from the participants of the study, the serotypes and the virulence characteristics related to the two exotoxins were studied and associated with the progression of AL at the individual level.

In **Paper I**, based on the study population consisting of 500 adolescents (mean age 13.2 years; SD ±1.5), it was shown that the overall carrier rate of *A. actinomycetemcomitans* was high (54.4%) and that the presence of this bacterium was associated with AL ≥ 3 mm. The JP2 genotype was prevalent (8.8%) in this population. In **Paper II**, 397 (79.4%) of the study participants were periodontally examined again at a 2-year follow-up. The presence of the JP2 genotype of *A. actinomycetemcomitans* in subgingival plaque was strongly associated with the progression of AL. This study also provided support for an enhanced estimated risk (odds ratio, OR=3.4), though less pronounced, for the progression of AL in individuals positive for the non-JP2 genotypes of *A. actinomycetemcomitans*.

In **Paper III**, all three *cdt* genes (*a*, *b* and *c*) were detected in 79% of the examined *A. actinomycetemcomitans* isolates, all of which expressed an active toxin. The distribution of the *cdt* genes showed a serotype-dependent pattern. In particular, the presence of the b serotypes (both JP2 and non-JP2 genotypes) was associated with the disease progression, whereas the expression of Cdt was not particularly related to the disease progression. In **Paper IV**, it was shown that the presence of of *A. actinomycetemcomitans* isolates with high leukotoxicity, also those of the non-JP2 genotypes of *A. actinomycetemcomitans*, were associated with an increased risk of the progression of AL in relation to the reference group. The main proportion of the serotype b isolates was distributed in the category of highly leukotoxic isolates. The analyses of the non-JP2 genotypes of serotype b indicated a diversity linked to the level of leukotoxicity.

In conclusion, *A. actinomycetemcomitans* in general was associated with the progression of AL. Individuals with an increased risk of developing progression of AL mainly harboured isolates of *A. actinomycetemcomitans* with a high leukotoxicity, which suggests that the LtxA is an important virulence factor. Of the two exotoxins, the pathogenic potential was mainly associated with the LtxA, while the role of the Cdt is unclear.

**Keywords** *Aggregatibacter actinomycetemcomitans*, periodontal attachment loss, JP2 genotype, non-JP2 genotypes, LtxA, Cdt.