Genetic diversity of the heat labile (LT) and heat stable (ST) toxins of human enterotoxigenic *Escherichia coli* (ETEC)

New insights into polymorphism, regulation, and gene transcription

Akademisk avhandling

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av

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Avhandlingen baseras på följande arbeten:

I. **Allele variants of enterotoxigenic *Escherichia coli* heat-labile toxin are globally transmitted and associated with colonization factors.**
Joffré E, von Mentzer A, Abd El Ghany M, Oezguen N, Savidge T, Dougan G, Svennerholm AM, and Sjöling Å.
J Bacteriol 2015;197:392-403

II. **Identification of new heat-stable (STa) enterotoxin allele variants produced by human enterotoxigenic *Escherichia coli* (ETEC)**
Joffré E, von Mentzer A, Wiklund G, Iniguez V, Svennerholm AM, and Sjöling Å.
Manuscript

III. **The LT1 and LT2 variants of enterotoxigenic *Escherichia coli* (ETEC) heat labile toxin (LT) are associated with major ETEC linages**
Joffré E and Sjöling Å.
Submitted for publication

IV. **RNA-seq transcriptome, transcription factor, and metabolome analysis of enterotoxigenic *Escherichia coli* (ETEC) indicate a transient transcription phase during early stationary phase.**
Xiao X, Joffré E, Nookaew I, Wang Z, Klena J, Zhu B, and Sjöling Å.
Manuscript

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Genetic diversity of the heat labile (LT) and heat stable (ST) toxins of human enterotoxigenic *Escherichia coli* (ETEC): New insights into polymorphism, regulation, and gene transcription

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Department of Microbiology and Immunology, Institute of Biomedicine, University of Gothenburg, Göteborg, Sweden 2015.

Abstract
Infection with enterotoxigenic *Escherichia coli* (ETEC) is a leading cause of diarrhea in children in developing countries and travelers to endemic regions. ETEC is a diverse pathogen, with a wide range of virulence factors including enterotoxins and more than 25 identified colonization factors (CFs). ETEC infection causes varying symptoms (mild to profuse, watery, cholera-like diarrhea) as a result of the colonization of the small intestine via CFs, secretion of heat labile (LT) and/or heat stable enterotoxins (STp and STh).

To expand the knowledge about the complexity of ETEC pathogenesis we studied the genetic diversity of the LT and ST toxins, using a clinical ETEC strains collection isolated worldwide during three decades. By genomic sequencing we found high diversity in the toxin amino acid sequences, especially in LT where 20 amino acid variants were identified. The LTA subunit was highly polymorphic while the LTB subunit was more conserved. The most common LT variants were LT1 and LT2. ST was less heterogeneous, including 3 ST alleles found in STp and 3 in STh. Phylogenetic analysis of the toxins revealed worldwide distribution of the different variants, and an association with specific CF profiles. The most frequent toxin variants belonged to ETEC linages that have disseminated globally over decades. We also found that main variants differed in ability to produce and secrete the toxins. The STp variant STa5 was linked to disease in adults while the STh variant STa3/4 was associated with disease in children.

The gene expression levels of LT (*eltAB)*, and ST (*estA*) were analyzed by qPCR. We found significantly lower levels of *eltAB* in presence of glucose in LT1 strains. No polymorphisms were found at the CRP binding sites at *eltAB* promoter. ST alleles were also significantly downregulated by glucose while bile supplementation favored STp expression.

Finally, we performed an RNA-transcriptome study, which showed a dramatic change in global gene expression at the onset of stationary phase. During a specific transient phase we observed up- and down-regulation of genes involved in mechanisms related to virulence, such as biofilm formation, indole induction, iron uptake, fucose catabolism, and the putrescine pathway. The expression levels of the toxins and CFs remained high during this phase.

Altogether, this study highlights the diversity within the ETEC population and its virulence factors. We propose that certain combinations of virulence genes influence strain specific responses to host factors that may impact the pathogenesis and severity of ETEC infections.

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