# Foxi1, an important gene for hearing, kidney function and male fertility

AKADEMISK AVHANDLING

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av

**Hilmar Vidarsson**

Fakultetsopponent:
Professor Mats Ulfendahl
Centrum för hörsel- och kommunikationsforskning
Karolinska Universitetssjukhuset, Solna

Avhandlingen baseras på följande delarbeten:

**I.** Tao Yang, **Hilmar Vidarsson**, Sandra Rodrigo-Blomqvist, Sally S. Rosengren, Sven Enerbäck, and Richard J.H. Smith (2007) Transcriptional Control of SLC26A4 Is Involved in Pendred Syndrome and Non-syndromic Enlargement of Vestibular Aqueduct (DFNB4). *Am. J. Hum. Genet.*, 80:1055-1063

**II.** Blomqvist SR, **Vidarsson H**, Fitzgerald S, Johansson BR, Ollerstam A, Brown R, Persson AE, Bergstrom G G, Enerback S (2004) Distal renal tubular acidosis in mice that lack the forkhead transcription factor Foxi1. *J Clin Invest.* 113:1560-70.

**III.** Blomqvist SR*, **Vidarsson H***, Soder O, Enerback S (2006) Epididymal expression of the forkhead transcription factor Foxi1 is required for male fertility. *EMBO J.* 25:4131-41.
* authors contributed equally

**IV.** **Vidarsson H**, Blomqvist SR and Enerback S. The transcription factor Foxi1 is a master regulator of vacuolar H+-ATPase proton pump subunits in the inner ear, kidney and epididymis. *Manuscript 2007*

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## ABSTRACT

**Hilmar Vidarsson**

Department of Biomedicine, the Sahlgrenska Academy
Göteborg University

When mice lacking the winged helix transcription factor Foxi1 were compared to their wt littermates in their response to acidic load, we discovered that Foxi1 deficient mice develop distal renal tubular acidosis (dRTA). Moreover, during breeding we observed the inability of Foxi1-/- males to generate offspring, indicating male infertility. In the light of these observations, together with our previous results from the inner ear of Foxi1-/- mice, we set out to examine the biological importance of Foxi1 in the inner ear, kidney and the epididymis. In this thesis we assess the expression pattern of Foxi1 and its potential downstream target genes in these three different tissues by using Northern blot analyses, *in situ* hybridization and immunohistochemistry. We demonstrated that Foxi1 is exclusively expressed in cells positive for the "kidney specific" vATPase subunits B1and a4, as well as the anion exchangers AE1 and pendrin, all characteristics for acid/base regulating cells of the inner ear, kidney and epididymis, called "mitochondria rich" cells. We present evidence that Foxi1 is required for the expression of these genes. Furthermore, in transfection experiments and in electrophoretic mobility shift assays (EMSA) we established direct transcriptional activation of promoter constructs by Foxi1. This activation was abolished when specific Foxi1 *cis*-elements on the promoters were mutated.

Previously we have shown that mice with target disruption of the Foxi1 locus exhibit severe cochlear and vestibular malformation and that Foxi1 is expressed in endolymphatic epithelial cells important for regulation of volume and composition of endolymph fluid. We have also shown that pendrin is missing from endolymphatic epithelium in Foxi1 deficient mice, making human FOXI1 a potential transcriptional activator of the gene coding for pendrin, the *SLC26A4* gene. While recessive mutations in the *SLC26A4* gene are known to be responsible for Pendred syndrome (PS) and non-syndromic hearing loss associated with enlarged vestibular aqueduct (EVA), a large percentage of patients with this phenotype lack mutations in the *SLC26A4* coding region in one or both alleles. We have identified and characterized a key transcriptional regulatory element in the *SLC26A4* promoter that binds FOXI1. Moreover, we have identified six PS or non-syndromic EVA patients with mutations in FOXI1 that inhibits its activation of *SLC26A4* transcription

In summary, we present molecular data showing that Foxi1 is required for the expression of some of the most important transporter molecules of acid-base regulation in the inner ear, kidney and epididymis, establishing Foxi1 as an essential regulator of fluid homeostasis in these organs. Hence, mice deficient for Foxi1, beside their early onset deafness, develop dRTA and male infertility. We also demonstrate that mutations in the human FOXI1 gene are involved in pathogenetic mechanisms underlying human deafness. Finally, these results together with our earlier findings in mice support the hypothesis that mutations in the human FOXI1 gene might prove to cause a sensorineural deafness syndrome with distal renal tubular acidosis and male infertility.

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