Expression and functional analysis of *EFNB1* mutations in craniofrontonasal syndrome

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Summary (English)

Ephrin-B1 protein forms signalling complexes with EphB receptor expressed in complementary cells. This complex was shown to work as a bi-directional signalling system, and ephrin-B1 was called “receptor-like protein”. Ephrin-B1 is encoded by *EFNB1* gene. Mutations of this gene cause the X-linked disease called craniofrontonasal syndrome (CFNS). This disease shows an unusual phenotypic pattern of inheritance, it affects heterozygous females more severely than hemizygous males although *EFNB1* is located on the X chromosome and has no homologue on the Y chromosome. This inheritance has been explained by random X-inactivation in heterozygous females and the consequences of cellular interference of wild type and mutant *EFNB1*-expressing cell populations with EphB-receptors expressing cells. Several patient derived *EFNB1* mutations have been analysed in this work, which consists of two parts. First one premature termination codon (PTC)-causing mutations were analysed: frameshift mutation c.377_384delTCAAGAAG, frameshift mutation c.614_615delCT (PTC in exon 4) and splice-site mutation c.406+2T>C (PTC in intron 2 or exon 3). All three mutations were predicted *in silico* to result in a truncated, soluble ephrin-B1 protein. Such protein would cause distant interaction with EphB-receptors. In the second part of this work the impact of missense mutations c.161C>T/p.P54L and c.332C>T/p.T111I on cell behaviour and reverse ephrin-B1 cell signalling was investigated. The role of these mutations was analysed in a cell culture model using NIH3T3 fibroblasts. This cell line was chosen because it does not express *Efnb1*, the mouse homologue of *EFNB1* gene. Both missense mutations are located in the extracellular ephrin domain that is involved in Eph-ephrin-B1 recognition and higher order complex formation. Reverse signalling of ephrin-B1 involves phosphorylation of several conserved tyrosine residues of the cytoplasmic tail. Two of them, Tyr324 and Tyr329 in human ephrin-B1, were shown to be the most important for signal transduction. To analyse the impact of missense mutations in ephrin-B1 signalling the phosphorylation of these two amino acids in mutant and wild type ephrin-B1 was monitored after the EphB2-Fc stimulation. It was reported previously, that ephrin-B1 is involved in STAT3 signalling pathway. Also, it is known that STAT3 controls the expression of *TWIST1* gene and *MSX2* is also involved in STAT3 signalling. According to that, the change of expression level of *MSX2* and *TWIST1* due to ephrin-B1 stimulation was monitored.

Mutation c. .377_384delTCAAGAAG showed a strong decrease of the mutant transcript level, mutations c.614_615delCT and c.406+2T>C showed the presence of the mutant RNA but not the mutant protein.

Mutation p.P54L showed no phosphorylation of the Tyr324/329, wild type and p.T111I mutation showed the difference in the phosphorylation timing. Also wild type and p.T111I expressing cells showed patches formation after the EphB2-Fc stimulation whereas p.P54L expressing cells remained to be scattered.

MSX2 and *TWIST1* genes showed only a minor change of the transcription level in response to the ephrin-B1 stimulation with EphB2.

Four from the five investigated *EFMB1* mutations appeared to have rather loss-of-function.