A novel Next Generation Sequencing (NGS) panel to facilitate the diagnostic process of X-linked hypophosphataemia (XLH) and other genetic disorders of renal phosphate wasting

Susanne Thiele*, Ralf Werner*, AnniKA Stubbe*, Wolfgang Höppner**, Olaf Hiort**

*Division of Paediatric Endocrinology, Department of Pediatrics, University of Luebeck; e-mail: Susanne.Thiele@uksh.de; **Bioglobe GmbH, Hamburg

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Conclusion

- In this study, we created a new NGS tool for an easy, fast, and reliable diagnostic process of XLH caused by inactivating mutations in the PHEX gene and including mosaicisms in PHEX.
- In addition, our approach further enables to reveal molecular changes in ten other candidate genes leading to related disorders of renal phosphate wasting as differential diagnosis to XLH.
- Molecular genetic proven diagnosis of a disorder of renal phosphate wasting allows not only a certain genetic counselling, but also to initiate the most promising therapeutic approach.

Background

X-linked hypophosphataemia (XLH) is the most common genetic disorder of renal phosphate wasting. It is caused by inactivating mutations in PHEX (located at Xp22.1), encoding fibroblast growth factor 23 (FGF23)-cleavage enzyme, which regulates the phosphaturic secretion. Affected individuals present with a broad phenotypic spectrum, ranging from isolated hypophosphataemia up to severe symptoms, such as rickets with extreme lower limb deformities, bone pain, distinct tooth problems, pseudo fractures, and disproportion short final height.

One side, early treatment has a strong impact on the long-term outcome; but on the other side, the diagnosis of this rare disorder is often delayed. The clinical and laboratory based diagnosis in XLH is hampered by related disorders with an overlapping phenotype, but caused by other gene defects than in PHEX. Therefore, molecular confirmation of the diagnosis is strongly recommended (1). The importance of molecular diagnosis has risen dramatically, since there is a new treatment with a selective licensure for XLH with an FGF23 antibody as alternative to the conventional therapy.

In the past, genetic testing of PHEX (composed of 22 exons) has been done by Sanger sequencing, being both expensive and time consuming, followed by MLPA analysis. Our goal was to develop an easy, fast, and reliable tool for XLH and related disorders.

Patients and Methods

First, a NGS panel was developed in silico (see table 1 for technical details) including PHEX and the ten other genes: FGF23, DMP1, ENPP1, SLC34A3, CLCN5, SLC34A1, SLC9A3R1, FAM20C, FGF1R, and KLF.

Results

In all 50 samples the known PHEX mutation and in two the known polymorphism have been detected by the panel. All together, 42 different mutations were found, including nonsense (n=16), missense (n=12), and splice site mutations (n=4); further small deletions (n=6), small insertions (n=2), small duplications (n=1), and a large duplication (n=1) (see table 2).

Table 2: Results of the sequencing of the NGS panel: The patient samples are numbered from PHEX1-55. On the left side the different mutations, the kind of mutation and the concerned exon (and intron 21) of PHEX is demonstrated. Since the males only have one X-chromosome, they appear to be homozygous for the mutation, while the females appear to be heterozygous (with one exception). The blue underlined sample has been investigated in addition by MLPA, in the green one we detected a mosaicism in PHEX het: heterozygous, hom: homozygous

In sample 55 we detected a mosaicism mutation in PHEX with the NGS panel (see figure 2). Based on this result, this patient could be treated by the new FGF23 antibody Burosumab.

For validation of the panel we analysed fifty-five DNA-samples from patients, which have been sent to our laboratory for molecular genetic testing under the suspicion of XLH, based on clinical and laboratory changes. In all (but five) we had detected a proven mutation in PHEX by Sanger sequencing, in two only a known polymorphism. In three samples (53-55), no mutation had been found in the past. Samples were sent anonymized and blinded to our partner company.

Reference

2) FGF23 and its role in X-linked hypophosphataemia-related morbidity. Beck-Nielsen et al. Orphanet Journal of Rare Diseases 2019