

DISSECTION OF THE X-CHROMOSOMAL MCLEOD LOCUS AND DIRECT GENE SEQUENCING AS TOOLS FOR THE ANALYSIS OF XK GENE DELETIONS AND MUTATIONS

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Background

The X-linked McLeod syndrome (MLS) is a rare disease, which leads to pathognomonic deficiencies of the hematological, neurological, muscular, ocular and cellular immune system, mainly in males. MLS is caused by either direct gene mutations (point mutations) of *XK*, or different (large) deletions on Xp 21.1, hitting *XK*'s and its neighbour genes' integrity. MLS usually becomes apparent by either neurological symptoms, improperly expressed KEL epitopes on red blood cells, hemolytic anemia or the presence of neuro-psychiatric, muscular or ocular symptoms and/or chronic granulomatous disease (CGD) leading to severe bacterial and fungal infections.

Methods

Since males have only one X-chromosome, presence of X-chromosomal mutations can easily be tested by positional PCRs. In this way 32 regularly distanced PCRs, organized in 16 biplexes, were used to test a 8.8 mbp region covering the genes from Dystrophin (DMD, telomeric) to Ornithine Carbamoyltransferase (OTC, centromeric) and flanking the entire *XP* 21.1 locus. Regions of deletions were narrowed by further PCRs until breakpoints could be "bridged" by one PCR, which was the DNA for breakpoint-sequencing. Undeleted *XK* genes with assumed exonic point mutations were assessed by direct sequencing of the 3 exon-specific PCR-fragments of *XK*.

Results

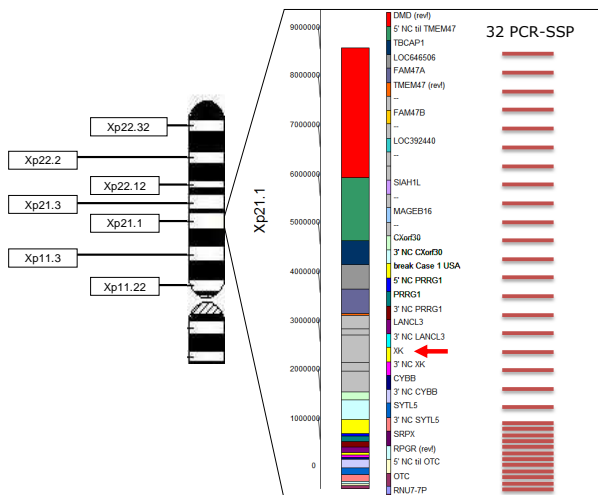
Samples of three unrelated individuals with MLS were found to show no ("Bavarian"), a 151.47 kbp ("Turkish") and a 1.71 mbp ("Finnish") deletion around the *XK* gene, respectively. The "Bavarian" *XK* gene, revealed a *XK* (W257X) stop mutation in exon 3 of *XK*. Related to "A" of the start codon of *XK*, the "Turkish" and "Finnish" sample revealed a deletion of 48.15 kbp upstream to 103.31 kbp downstream and a deletion 1.67 mbp upstream until 0.03 mbp downstream, respectively. Clinically, the "Bavarian" patient suffered of a complex movement disorder, the "Finnish" patient presented with a neuro-acanthocytosis. And the "Turkish" mutation carrier needed treatment for CGD including allogeneic stem cell transplantation.

Conclusion

The presented method allows a reliable and robust approach to the genetic analysis of *XK* gene deletions as well as point mutation in cases of suspected MLS. The precise definition of underlying molecular defect in MLS allows prognostic prediction of disease development and assessment of familial carrier status, including female mutation heterozygosity prone to transfer the disease to their male offsprings.

Figure 1: Short arm of X-chromosome

At the short arm of the X chromosome 32 PCRs around Xp21.1 have been developed. Particular attention was paid to the *XK* region. Figure 1 shows the approximate position of the individual PCRs.



Figures 2: Flowcytometrie

Red blood cells were stained by the KEL-specific monoclonal antibodies BRIC 18, BRIC 68 an BRIC 203 (BRISTOL INSTITUTE FOR TRANSFUSION SCIENCES). The specific binding was visualized by a secondary PE-conjugated F^{ab}-Fragments. Since there are no Anti-Kx antibodies available that are suitable to assess directly the Kx epitope, the BRIC based approach provides a surrogate test strategy to evaluate the phenotypic expression of the KEL/XK Protein complex on the red blood cells of suspected individuals (HH.Jung et al Transfusion 2003, 43:928-38). (*BD FACS Canto II, **BD FACS Calibur)

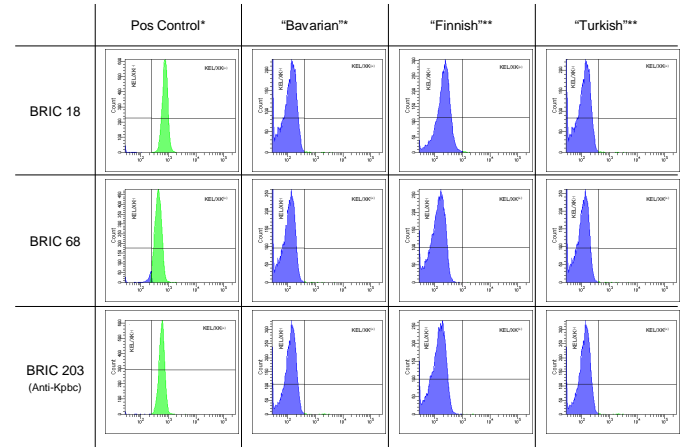


Figure 3: PCR-SSP

The aim of this project was to establish a method to determine the DNA break point in MLS. This should be achieved with a number of regular "position PCRs", confirming the presence or absence of sequential fragments of the X-chromosome. Boxes represent missing PCR fragments around the deletion defect in the respective carriers. The "Bavarian" case showed no deletion defect but was found positive for a stop mutation W257X in exon 3 of *XK*.

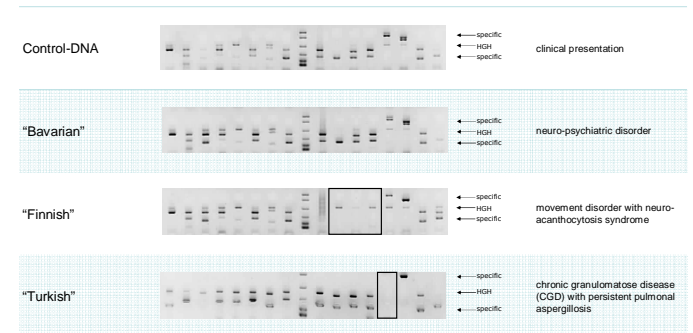


Figure 4: DNA-Sequence

The "Bavarian"- Sample shows a newly observed point mutation in exon 3 of *XK*. This point mutation causes the substitution of tryptophan to a stopcodon (W257X) at position 257 of the *XK* protein. The *XK* protein is therefore not translated properly and the expression of the KEL/XK protein complex in the red cell membrane is hampered.

