Characterization of X-Chromosomal Deletions in Two Patients with McLeod Syndrome by Array Comparative Genomic Hybridization (CGH)

Kantonsspital Aarau

*Hergersberg M[1], Ottiger C[1, 2], Frey BM[2], Jung HH[3], Reichenbach J[4], Seger R[4], Udd B[5], Bruni J[1], Huber AR[1], Röthlisberger B[1]

[1]Center for Laboratory Medicine, Kantonsspital Aarau, 5001 Aarau, Switzerland; [2]Immunohematology, Blutspendedienst SRK, Zürich; [3]Neurologische Klinik, Universitätsspital Zürich; [4]Universitätskinderspital Zürich; [5]Neurology Department, Tampere University Hospital and Medical School, Tampere, Finnland

*martin.hergersberg@ksa.ch Corresponding author: Prof. Dr. med. Andreas R. Huber, Center for Laboratory Medicine, Kantonsspital Aarau AG, 5001 Aarau, andreas.huber@ksa.ch

Introduction

McLeod syndrome (MLS) results from mutations in the XK gene on Xp21.1 The syndrome affects haematology and the central and peripheral nervous systems in middle-aged men. In addition to 32 point mutations described to date, at least 8 large multi-gene deletions encompassing the XK locus have been identified (e.g., Peng et al 2007). Deletion of several adjacent genes can result in the comorbidity for two different syndromes. Several families with both MLS and chronic granulomatous disease (CGD) have been described (Bertelson et al 1988). The gene mutated in X-linked CGD is located 10 kb adjacent to the XK gene and encodes a subunit of cytochrome b(-245), which itself is a subunit of the phagocytic NADPH-oxidase. The description of the phenotype of the carriers of multi-gene deletions could eventually give insight into the function of the deleted genes.

Patient 1

DNA was from a 58-year old man from Finland diagnosed with MLS. In addition to neurological symptoms, he was found in immunohematological analysis to have the McLeod blood group phenotype.

Patient 2

DNA was from a 9-year old boy diagnosed with CGD. In addition to the immunodeficiency, he was found in immunohematological analysis to have the McLeod blood group phenotype.

Methods

Diagnosis of MLS was concluded from the lack of Kx-antigens on the surface of erythrocytes but presence of all relevant Kell antigens (immunhaematological, flowcytometrical and PCR methods). Exon-specific PCR of the XK gene in both patients initially identified a deletion encompassing the XK gene. PCR of markers spanning approximately 2 Mb between 35,5 Mb to 37,5 Mb of the X chromosome was used for preliminary characterization of the deletions. By array CGH applying the X chromosome chip from NimbleGen® the size of the deletion was estimated with an accuracy of +/- 4 kb. The information from the CGH experiment was used to design a series of primers to narrow down the breakpoints and eventually determine the breakpoint sequence.

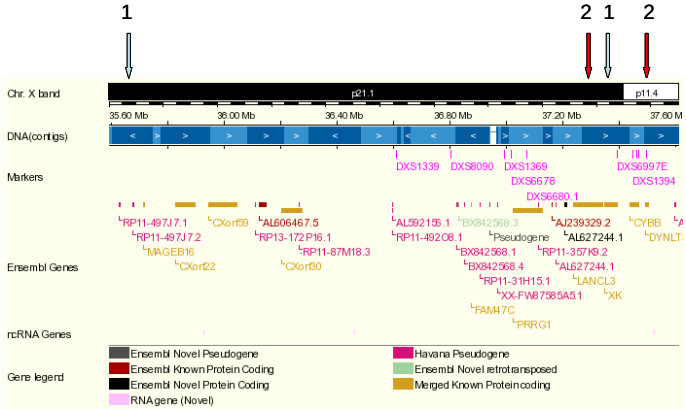
Results

Patient 1: PCR of the exons of the XK gene suggested a deletion spanning the whole XK gene. Testing of 13 markers on Xp21.1 gave evidence for a deletion including the region between stFSX915 and XK, excluding CYBB. The CGH experiment confirmed a deletion from app. 35'710'000 bp to 37'462'000 bp (Figure 1). Further primers were used to narrow down the proximal and distal breakpoints to several 100 bp.

Patient 2: Testing of 13 markers on Xp21.1 suggested a deletion spanning the XK gene, but not extending beyond the XK gene and the CYBB gene. The CGH experiment confirmed a deletion from app. 37'381'000 bp to 37'533'000 bp (Figure 1). With this information, the design of primers amplifiying and sequencing the deletion breakpoint was possible (Figure 2).

Gene designation	Gene name
MAGEB16	melanoma antigen family B, 16
CXorf22	X chromosome ORF 30
CXorf59	X chromosome ORF 59
Q5JQZ6	obsolete
CXorf30	X chromosome ORF 30
ENSG00000197337	retrotransposon/pseudogene
FAM47C	
ENSG00000214071	pseudogene
PRRG1	proline-rich Gla (G-carboxyglutamic acid) polypeptide 1
FTHL19	ferritin, heavy polypeptide-like 19
ENSG00000174678	
LanC l	antibiotic synthetase component C-like 3 (bacterial)
CYBB	Cytochrome b-245 beta subunit

Table 1 List of the genes and pseudogenes located in the region from 35'710'000 bp to 37'450'000 bp



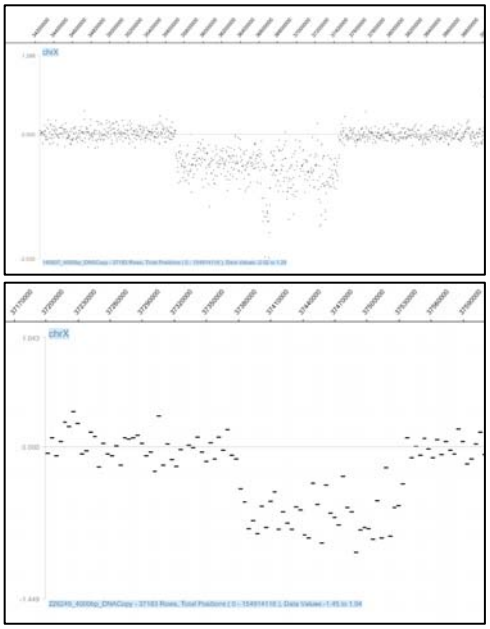


Figure 1
Intensity of the hybridization signal of each oligonucleotide spotted along the X chromosome sequence. Top, patient 1. Bottom, patient 2

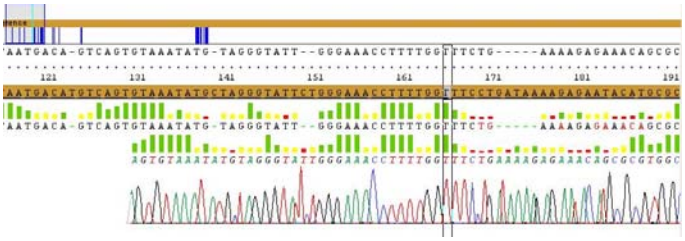


Figure 2
Sequence covering the breakpoints of the deletion in patient 2.

Conclusion

Patient 1: At least 10 genes of unknown function, as well as 3 pseudogenes are deleted in this patient (Table 1, Figure 3). Primers bridging the breakpoint have not been identified yet, probably because additional sequences are included between the two breakpoints. Recently, a MLS patient with a deletion of 1,12 Mb encompassing the XK gene and 7 additional other genes was described (Peng et al. 2007). Analysis of such patients allows the conclusion that the genes located in the deleted region have only an indirect and subtle effect on the phenotype. Because the patient belongs to an extended family, the design of primers amplifying the breakpoint region is important for proper carrier diagnosis of female relatives.

Patient 2: The deletion encompasses the complete XK gene and the first three (of 13) exons of the CYBB gene. Sequencing of the breakpoint region allowed the determination of the breakpoints at 37'381'984 +/- 1 bp and 37'533'472 +/- 1 bp, respectively. The PCR product covering the breakpoint is in this family also essential for the identification of female deletion carriers.

References

Peng J, Redman CM, Wu X, Song X, Walker RH, Westhoff CM, Lee S (2007) Insights into extensive deletions around the XK locus associated with McLeod phenotype and characterization of two novel cases. Gene 392:142-150

Bertelson CJ, Pogo AO, Chaudhuri A, Marsh WL, Redman CM, Banerjee D, Symmans WA, Simon T, Frey D, Kunkel LM (1988) Localization of the McLeod locus (XK) within Xp21 by deletion analysis. Am J Hum Genet 42:703-711.

Figure 3
Screenshot of the deleted chromosomal region of the X chromosome (Ensemble, http://www.ensembl.org/index.html). The locations of the breakpoints of patients 1 and 2 are shown by the corresponding arrowheads (1 blue, 2 red) above the sequence.