Diagnostics and blood management of an infant with McLeod contiguous gene deletion syndrome

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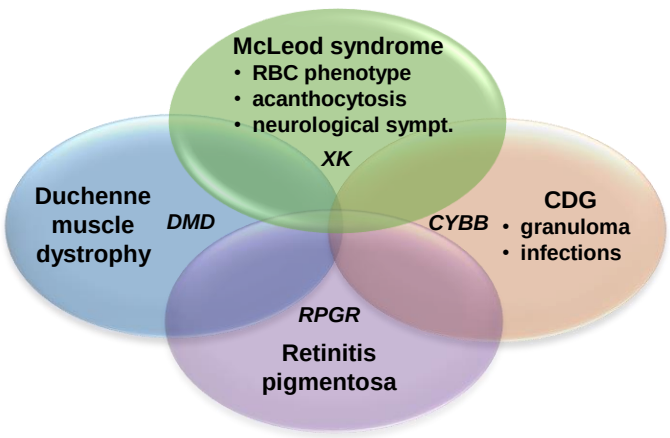
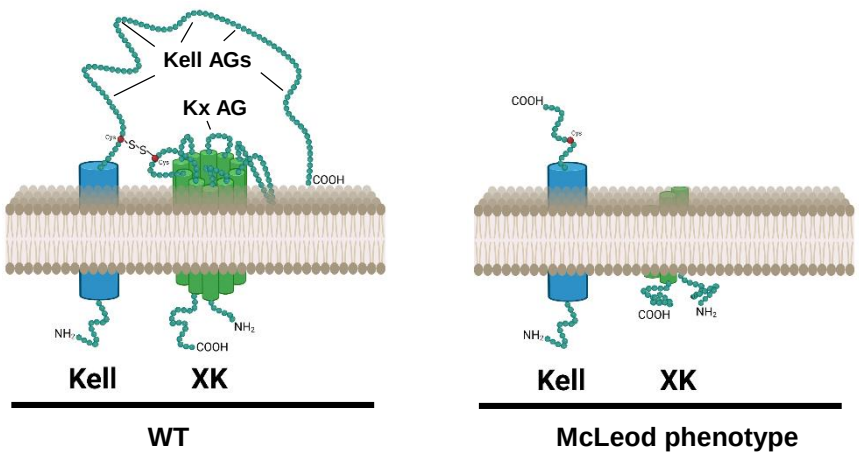
Introduction

X-linked chronic granulomatous disease **(XL-CGD**) is an inborn error of immunity characterized by **malfunction of neutrophils and macrophages** due to reduced or absent activity of the NADPH oxidase subunit (microbicidal system)[1]. Patients suffer from recurrent **bacterial and fungal infections**, **granuloma formation** and **inflammatory complications**. Sporadic cases are in two thirds of x-chromosomal inheritance of **mutations in *CYBB*** (cytochrome B beta subunit; g91^{phox}). In rare cases larger **deletions of the X-chromosome affect nearby genes**, which is referred to as **contiguous gene deletion syndrome**[2]. Depending on the individual deletion genes for **McLeod syndrome**, **Duchenne muscle dystrophy**, **infertility**, and **retinitis pigmentosa** can be inflicted.



Deletion involving *XK* is associated with **reduced expression of Kell blood group antigens** on red blood cells (RBCs) and absence/truncation of the XK protein with **absence of the Kx antigen (McLeod phenotype). As RBCs of healthy individuals carry the XK protein and the Kx antigen, transfusions of Kx+ RBCs could induce alloantibodies against public antigens of the Kx (anti-Kx) and Kell (anti-Km) blood group systems in patients with McLeod phenotype.** Therefore, blood management of Kx- individuals is a challenge, particularly in the context of hematopoietic stem cell transplantation (HSCT)[3].



Case History

- First child of a non-consanguineous family without relevant medical history.
- After uneventful pregnancy, birth at term was complicated by **neonatal asphyxia** requiring **resuscitation** and **intensive care** (Apgar values: 2 after 5 and 10 min.; umbilical artery pH: 7.05). Therapeutic hypothermia for 72 h followed by invasive ventilation for 5 days. Central line **infection with *S. aureus***.
- Readmission at the age of 29 days for suspicion of late onset **sepsis** without detection of a pathogen.
- Readmission at the age of 2.5 months due to **severe pneumonia** with pleural effusions, **muscular hypotonia** and **elevated transaminases** triggering immunological and genetic work-up
- At the age of 4 months immunological diagnosis of **CGD** with **Duchenne muscle dystrophy** and **anemia**.
- Evaluation for **HSCT**

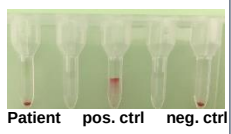
Transfusion Medicine Challenge

→ **Molecular and serologic diagnostics of McLeod phenotype**
→ **Specific blood supply management particularly considering**
  - **Kx- blood donor availability**
  - **Manufacture and limited storage times of irradiated packed (p)RBC products**
  - **Co-ordination with complex patient treatment schedule (HSCT)**

Molecular and serologic diagnostics confirm McLeod phenotype

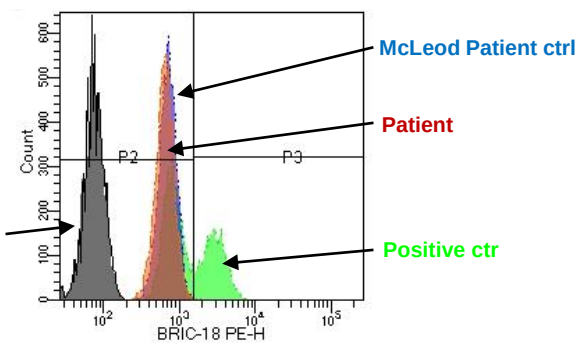
Performed at the Immunohematology Laboratories in Freiburg, Zurich and Baden-Baden.

- **SSP-PCR genotyping** (RBC-Ready Gene KELplus, inno-train Diagnostik GmbH, Kronberg, Germany): **.I *KEL**02 → K-k+, Kp(a-b+), Js(a-b+)**
- **Serology: K-k+**(1+)**, Js(b-), Kp(a-b-), Kx-** → **Kp^b**



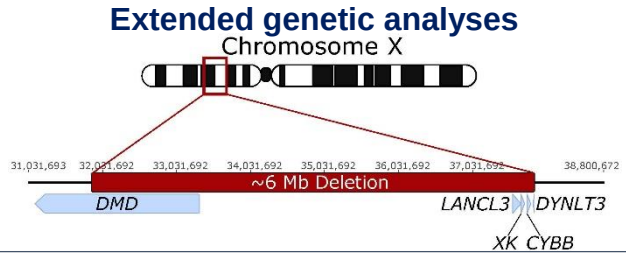
- **Flow cytometry** (BRIC-68)**:**

no expression of **KEL/Kx complex**



Extended genetic analyses

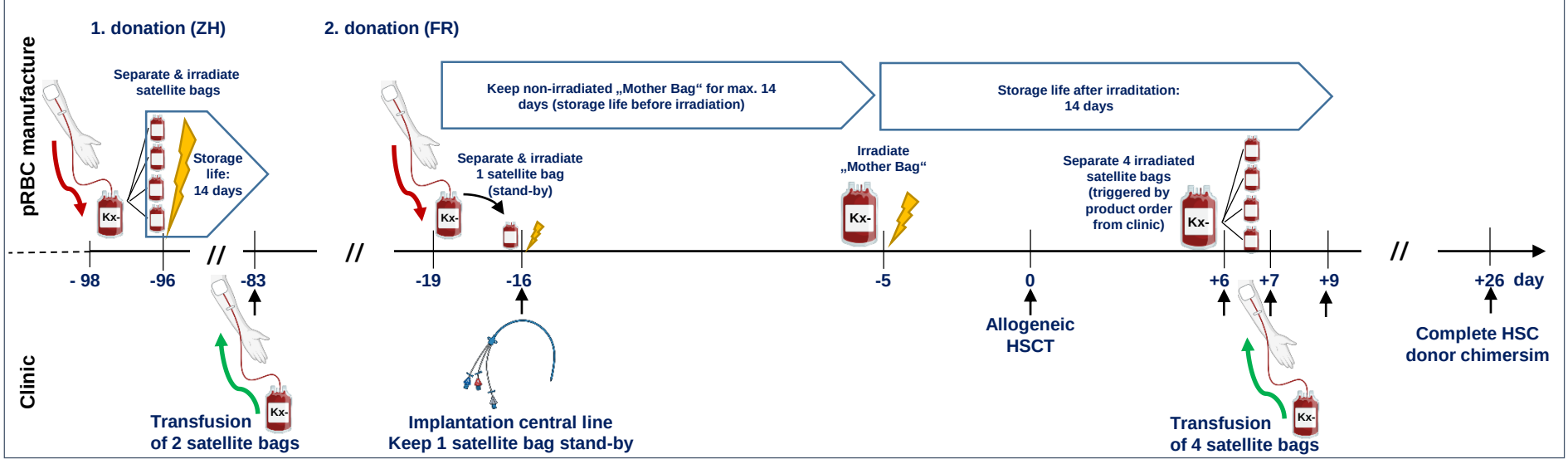
Schematic representation of the **large, previously unknown ~ 6 Mb deletion** identified by stepwise partitioning of the Xp21.1 region.



Genes involved by this deletion and their orientation of transcription are displayed as blue arrows.

Managing the supply with Kx- pRBCs

- **Aim 1: Allocate Kx- pRBCs**
  No Kx- and RhD compatible donors were identified by International Rare Donor Programs. The Blood Transfusion Service Zurich, Switzerland had previously identified individuals with McLeod phenotype without hematologic, neuromuscular, or cerebral involvement[4], and offered to assess their potential eligibility and availability for blood donation. A compatible donor was elected and consented to donate blood, and the legal import of blood product from non-EU jurisdiction to EU jurisdiction was granted by the competent German authority.
- **Aim 2: Elaborate an individual manufacture concept** particularly considering a. the minimal interval between blood donations (55 d), b. the storage life of the pRBCs (14 d before and after irradiation), and c. the production of the satellite pRBC bags.
- **Aim 3: Adjust the manufacture concept** to the availability of the donor and the projected clinical need for Kx- pRBC transfusions.



Clinical follow-up

- The patient was discharged with complete chimerism at day 68, and is well and independent of transfusions at day +250 without signs of GvHD.
- Antibody screen post HSCT was negative for alloantibodies against high frequent antigens of the Kell and Kx blood group systems.

Summary

Mc Leod phenotype poses a substantial challenge for blood management, especially in the context of HSCT, where timing of complex procedures, availability of compatible stem cell and blood donors, as well as logistics and the storage life of irradiated pRBCs must be considered. Close international collaborations with three transfusion medicine institutions and the clinic ensured the successful HSCT treatment of the young patient. Further, we identifed a large, previously unknown, deletion involving *DMD, PRRG1, LANCL3, XK, CYBB* and *DYNLT3.*

References

1. Leiding JW & Holland SM (2016): GeneReviews® Chronic Granulomatous Disease. Ed.: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Mirzaa G & Amemiya A. Seattle (WA).
2. Lhomme F, et al. (2020): Chronic Granulomatous Disease with the McLeod Phenotype: a French National Retrospective Case Series. *Journal of Clinical Immunology* 40 (5), S. 752–762.
3. Hönig M, et al. (2010): Successful hematopoietic stem-cell transplantation in a patient with chronic granulomatous disease and McLeod phenotype sensitized to Kx and K antigens. *Bone Marrow Transplantation* 45(1): 209–211.
4. Jung HH, et al. (2003): McLeod phenotype associated with a *XK* missense mutation without hematologic, neuromuscular, or cerebral Involvement. *Transfusion* 43:928-938.