CONCISE REPORT

Gene Deletion in a Patient With Chronic Granulomatous Disease and McLeod Syndrome: Fine Mapping of the Xk Gene Locus

By Daniel Frey, Marco Mächler, Reinhard Seger, Werner Schmid, and Stuart H. Orkin

In a patient suffering from X-linked chronic granulomatous disease (X-CGD)—a disorder of phagocytesuperoxide generation—and McLeod syndrome, characterized by the absence of the red cell Kell antigen, we identified a deletion of the entire X-CGD gene by means of DNA hybridization with a cDNA probe. Our findings suggest that the X-CGD and McLeod loci are physically close in the p21 region of the X chromosome proximal to the Duchenne muscular dystrophy locus.

MATERIALS AND METHODS

Clinical Summary

The patient (OM) was born in 1975 as the third child of a healthy mother. During the first months of life he suffered from recurrent bacterial and fungal infections that included several perianal and lymphonodular abscesses, otitis, urinary tract infections, and candida pneumonia. By 3 years of age hepatosplenomegaly was evident. At 5 years of age a microcytic, hypochromic anemia was noted and an anacjthycytosis was first noticed at 7 years of age. At 9 years his height and weight were around the third percentile. Granulomas were found in the stomach and urinary bladder and multiple liver abscesses had to be aspirated. Psychomotor development is normal. No clinical sign of myopathy has appeared.

Hematologic Findings

Granulocytes. Cytochemical nitroblue tetrazolium reduction (NBT test) was 0% (normal values 95% to 100%); O2 production: 0 (normal control 5.5 nmol/10⁶ granulocytes/min), cytochrome b-245 content of granulocytes 0 (control 0.8 nmol per granulocyte).

Red cells. Acanthocytosis, reduced osmotic resistance. Erythrocyte-enzymes were normal (G-6-PD, glutathion reductase, and peroxidase).

Kell blood group antigens. Weak expression of antigen k, Kp", Js", and Ku with no expression of the other Kell antigens, including Kx. This is typical for the McLeod phenotype.

Other Laboratory Findings of Interest

Immunoglobulins were normal, blood creatine kinase level was not elevated. Karyotype: 46,XY; no indication of a deletion in band Xp21. The patient's mother and sister had heterozygous values for NBT, cytochrome b-245, O2⁻, and the Kell antigens.

Analysis With a CGD-cDNA

The CGD/McLeod patient and his family. Equal amounts of high molecular weight DNA, extracted from whole blood from the patient and his family, were digested to completion with various restriction endonucleases (MspI, EcoRI, HindIII, BamHI, BglII, PvuII, HaeIII, and PstI) using conditions recommended by the manufacturer (Boehringer Mannheim). Southern blotting, using 0.8% agarose gels and nitrocellulose membranes, followed established procedures. The probe used for hybridization was a random-primed PstI/KpnI fragment of 3.5 kb containing the majority of the X-CGD cDNA.

Fifteen patients with X-linked CGD without McLeod syndrome. In addition to the CGD/McLeod patient, we examined 15 patients with X-CGD and normal Kell antigenicity. X-linked inheri-
Gene deletion in a CGD-McLeod patient

The deletion signal intensity of the mother and sister, who are both carriers for CGD and McLeod phenotype, was equal to that of a healthy uncle but only 50% of a healthy female control and the two healthy sisters of the patient’s mother. This finding indicates that they are hemizygous for the CGD locus (Fig 2A).

DNA of 15 unrelated CGD patients without McLeod syndrome, digested with MspI, yielded the same nonpolymorphic fragment pattern upon hybridization with the CGD probe; six of these patients are shown in Fig 2B; there was no indication of deletion or gene rearrangement.

The presence of normal fragment patterns with the three Xp21 probes OTC, 754, and CX5.7 indicates that these loci are not deleted (results not shown). The deletion is thus confined to the CGD locus and the locus Xk encoding the Kell precursor substance Kx. As depicted in Fig 1 the deletion breakpoints lie between the OTC locus and DXS148 (CX5.7) and span a region of probably not more than 1,000 kb.
kb in Xp21.1, according to a large range restriction map of this region.\textsuperscript{23}

DISCUSSION

In recent years, several patients manifesting various associations of different X-linked disorders have been reported: patient KC, a female heterozygous for OTC deficiency, CGD, and probably DMD\textsuperscript{24}, BB, a male patient with DMD, CGD, McLeod syndrome, and retinitis pigmentosa\textsuperscript{25}; NF, a patient male with DMD, and CGD\textsuperscript{2}; various patients with DMD, glycerol kinase deficiency (GKD), and adrenal hypoplasia (AH).\textsuperscript{25} In these patients detection of deletions of varying extents, all confined to Xp21, was greatly facilitated by use of the rapidly developed DNA probes from this region. The deletion analysis has permitted construction of an almost contiguous deletion map localizing the OTC, CGD, DMD, AH, and GKD loci to Xp21.\textsuperscript{22,23}

We report here a male patient manifesting an association of CGD and McLeod syndrome. The patient’s mother and sister both showed mosaicism for NBT-positivity and for acanthocytosis, as well as reduction of the b-cytochrome spectrum. These findings demonstrate heterozygosity for both CGD and the McLeod syndrome. Cytogenetic analysis, which can identify deletions of 3 to 5 megabases, revealed no abnormality in the patients X chromosome. Hybridization with the cDNA probe of the X-CGD gene, however, identified a deletion that removed the CGD gene and we assume the locus for Kx. Given the long-range restriction map of the Xp21 region deleted in patient BB, we estimate that the deletion in our patient spans no more than 500 to 1,000 kb of DNA (Fig 1).

The observation of recombinants between the CGD and McLeod loci\textsuperscript{11} and the demonstration of a lack of Kx on normal and CGD granulocytes,\textsuperscript{26} which conflicts with previous studies,\textsuperscript{27} provide evidence that the CGD and Xk loci are, in fact, different. While it appears that the CGD and McLeod loci are relatively close in Xp21, their precise order remains to be determined. Based on the report of two patients with McLeod syndrome manifesting mild subclinical myopathy with elevated creatine kinase, myopathic EMG, and features of active myopathy in a muscle biopsy,\textsuperscript{28} we might expect that the McLeod locus is distal (relative to the centromere and CGD) rather than on the centromero-proximal region of the CGD locus.

The blood creatine kinase level in our patient was normal. Previously, 11 males with McLeod syndrome reported by Marsh et al\textsuperscript{29} had elevated serum creatine kinase; males with McLeod and CGD have normal or elevated levels of the enzyme.\textsuperscript{29}

The deletion in the patient described here is not de novo, but inherited from the mother. Mosaicism for CGD and acanthocytosis indicates that the deleted chromosome is subject to random rather than preferential inactivation.\textsuperscript{24}

In families such as that reported here, prenatal diagnosis of CGD by DNA analysis can be accomplished readily by detecting or excluding the deletion in a male fetus. In the majority of X-linked CGD families prenatal diagnosis by either functional analysis of fetal neutrophils aspirated by fetoscopy\textsuperscript{30} or analysis with closely linked DNA markers from Xp21 can be made available. Since the X-CGD locus is not sufficiently polymorphic in our experience with more than 60 restriction enzymes (unpublished data SHO), use of CGD gene probes for prenatal diagnosis is limited to those few families in which gene deletion is the molecular basis of the disease; so far, only two of 30 unrelated X-CGD patients showed a deletion of the CGD gene.\textsuperscript{31}

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REFERENCES

13. Marsh WL: Chronic granulomatous disease, the McLeod syndrome and the Kell blood groups. Birth Defects (original article series) 14:9, 1978
27. Marsh WL, Oyen R, Nichols E: Kx antigen, the McLeod phenotype and chronic granulomatous disease: further studies. Vox Sang 31:356, 1976
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D Frey, M Machler, R Seger, W Schmid and SH Orkin

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