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Localization of the McLeod Locus (XK) Within XP21 by Deletion Analysis

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Summary

The McLeod phenotype is an X-linked, recessive disorder in which the red blood cells demonstrate acanthocytic morphology and weakened antigenicity in the Kell blood group system. The phenotype is associated with a reduction of in vivo red cell survival, but the permanent hemolytic state is usually compensated by erythropoietic hyperplasia. The McLeod phenotype is accompanied by either a subclinical myopathy and elevated creatine kinase (CK) or X-linked chronic granulomatous disease (CGD). Seven males with the McLeod red-blood-cell phenotype and associated myopathy but not CGD, one male with the McLeod phenotype associated with CGD, and two males known to possess large deletions of the Duchenne muscular dystrophy (DMD) locus were studied. DNA isolated from each patient was screened for the presence or absence of various cloned sequences located in the Xp21 region of the human X chromosome. Two of the seven males who have only the McLeod phenotype and are cousins exhibit deletions for four Xp21 cloned fragments but are not deleted for any portion of either the CGD or the DMD loci. Comparison of the cloned segments absent from these two McLeod cousins with those absent from the two DMD boys and the CGD/McLeod patient leads to the submapping of various cloned DNA segments within the Xp21 region. The results place the locus for the McLeod phenotype within a 500-kb interval distal from the CGD locus toward the DMD locus.

Introduction

The McLeod red-blood-cell phenotype (Allen et al. 1961) is a rare disorder in which the cells have acanthocytic morphology, reduced in vivo survival, and weakened antigens in the Kell blood group system (Wimer et al. 1977). Inheritance of the McLeod phenotype is X linked, while the remaining genes involved with intragroup variation in the Kell system have an autosomal dominant mode of inheritance. Kell antigens in red cells of common Kell phenotype are present on a 93-kD glycoprotein (Redman et al. 1984). A 93-kD protein having antigen activity can also be isolated from McLeod red blood cells, but in markedly reduced amounts (Redman et al. 1988). A salient feature of the McLeod red blood cell is the absence of the otherwise ubiquitous Kx antigen (Marsh and Redman 1987). Immunoprecipitation experiments with anti-Kx have established that Kx is a marker on a 37-kD protein (Redman et al. 1988). The McLeod phenotype must be distinguished from the K null (Ko) phenotype. In the latter the cells produce no antigens made by the Kell autosomal gene, lack the Kell 93-kD glycoprotein, and have enhanced Kx antigen activity with an increased amount of immunoreactive 37-kD protein (Redman et al. 1986, 1988). Thus, anti-Kell and anti-Kx antibodies recognize distinct biochemical entities on the red-blood-cell membrane. Abnormal morphology and compromised in vivo survival occur with the McLeod red blood cell but not with the Ko cell, and the McLeod

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cell appears to be associated with the absence of the Kx 37-kD protein.

Among the clinical manifestations of the McLeod syndrome in the absence of CGD are a late-onset myopathy, cardiomyopathy, high levels of muscle-specific serum creatine kinase (CK), and a neurological defect with areflexia and choreiform movements (Marsh et al. 1981; Marsh and Redman 1987). Muscle biopsies of phenotypically similar patients have verified the presence of an active myopathy (Swash et al. 1983).

Several genetic disorders have been characterized without prior knowledge of their aberrant gene products (Friend et al. 1986; Monaco et al. 1986; Royer-Pokora et al. 1986). This characterization was facilitated by the knowledge of where these loci were located within the human genome. One of the means by which their location was determined was the identification of various structural chromosomal abnormalities that were presumed to be the cause of the disorders. A male patient (BB) has been described (Francke et al. 1985) who had the rare McLeod phenotype associated with three X-linked disorders (chronic granulomatous disease [CGD], Duchenne muscular dystrophy [DMD], and retinitis pigmentosa [RP]). This patient was demonstrated to bear an interstitial deletion of the X chromosome short arm (Francke et al. 1985). DNA of BB was used in a competition reassociation and cloning strategy to isolate cloned DNA fragments that were absent from the patient DNA sample (Kunkel et al. 1985). Two of these absent clones, pERT379 and pERT87, were instrumental in the eventual cloning of the loci being altered in CGD and DMD, respectively (Monaco et al. 1986; Royer-Pokora et al. 1986). The remaining absent clones were each potential candidates to approach the loci of the other two disorders that affected the deletion patient.

In this study, 15 cloned DNA segments within and surrounding Xp21 were used to search for alterations in the DNA of seven McLeod patients with mild myopathy but not CGD. Two of these males were found to exhibit deletions for the same four cloned DNA segments. The two men are first cousins and are assumed to bear identical deletions. In addition, one male with the McLeod phenotype associated with CGD and two DMD males known to bear large deletions were tested for the presence of the cloned DNA segments bordering and including the deleted region of the two McLeod cousins.

Material and Methods

Clinical Summaries

Seven males, from six unrelated kindreds, with the McLeod syndrome have been studied (cases 1–7). None has been demonstrated to have CGD by the cytochemical nitroblue tetrazolium test (NBT).

Cases 1 and 2 are first cousins. They are 44 years old and 39 years old, respectively. Both have acanthocytic red cells with biochemical and hematological evidence of a compensated hemolytic state. Both have areflexia, cardiomegaly, and high levels of CK (1,250 and 680 IU/liter for cases 1 and 2, respectively). Case 2 now exhibits somewhat severe skeletal muscle wasting and has recently been treated for congestive cardiac failure. The large kindred, which has been reported elsewhere (Symmans et al. 1979), includes both three other males with the serological and clinical features of the McLeod syndrome and six female carriers of the McLeod gene, as demonstrated by their red-blood-cell mosaicism.

Case 3 is the original patient in whom the phenotype was first observed (Allen et al. 1961). He is 52 years old, has red cell acanthocytosis, slight reticulocytosis, and splenomegaly. He also has areflexia and a high level of CK (650 IU/liter). His brother is of common Kell type. His mother and two daughters have mosaicism for both Kell blood antigens and acanthocytosis.

Case 4 is 38 years old and has acanthocytic red blood cells with hematological and biochemical evidence of increased in vivo red cell destruction. He has areflexia and elevated CK (280 IU/liter). In a previous publication this individual and a brother, who also has the serological and clinical manifestations of the McLeod syndrome, were reported to have different Kell phenotypes; thus, they had inherited different Kell autosomal genes (Marsh et al. 1983). These data indicate that the Kell autosomal gene itself is unlikely to play a direct role in the development of the McLeod syndrome.

Case 5 is a 38-year-old male and a regular blood donor without any major clinical symptoms, except acanthocytosis, a compensated hemolytic state, and elevated CK (260 IU/liter). No CGD symptoms were found in this patient.

Case 6 is a 57-year-old male with mild skeletal muscle wasting and congestive cardiac failure. Laboratory analysis showed acanthocytes and elevated CK (360 IU/liter). Signs of neuropathy with muscular de-

nervation were found without CGD symptoms. The patient died recently, and two male family members available for study showed red-blood-cell antigens characteristic of McLeod syndrome.

Case 7 is a 41-year-old man who has mild skeletal muscle wasting, acanthocytosis, and a compensated hemolytic state. He has moderate cardiomegaly, electrocardiogram abnormalities, and elevated CK (350 IU/liter) but no CGD symptoms.

Case 8 (also identified as OM) is a 12-year-old boy with both CGD and McLeod syndrome. Clinical and biochemical evidence of X-linked CGD is demonstrated by negative NBT and absence of the spectrum of cytochrome b-245 in the patient's granulocytes. The McLeod phenotype is demonstrated by red cell acanthocytosis and absence of the Kx antigen from red cells.

Case 9 (also identified as WP7) is a DMD patient whose DNA was sent to the Kunkel laboratory as part of a large collaborative study of DMD deletion patients. The patient, as described elsewhere, presents no evidence of any clinical disorder other than DMD (Greenberg et al. 1987).

Case 10 (also identified as SS) is a DMD patient with severe mental retardation (Wilcox et al. 1986) whose DNA was sent to the Kunkel laboratory as part of a large collaborative study of DMD deletion patients. He was tested extensively for evidence of several other X-linked disorders often associated with DMD—e.g., RP, CGD, the McLeod phenotype (XK), and glycerol kinase (GK) deficiency. No evidence was found to indicate that he presented any disease phenotype other than DMD (Wilcox et al. 1986).

DNA Isolation, Restriction-Enzyme Cleavage, and Southern Blotting

Total genomic DNA was isolated from peripheral white blood cells by the following procedure: red blood cells were lysed in 0.85% ammonium chloride solution at room temperature, and the pelleted red cell ghost and white cells were resuspended in a 10-v solution containing 10 mM Tris-HCl, pH 7.8, 1 mM EDTA, 4 M urea, and 1% Sarkosyl. The viscous solution was digested with 200 μg proteinase K/ml at 37 C overnight. DNA was extracted with phenol containing 0.1% of 8-hydroxyquinoline, followed by extraction with chloroform:isoamylalcohol 24:1 (v/v). Following ethanol precipitation, the DNA was digested to completion with both *Hin*dIII and *Pst*I, separated by electrophoresis, and blotted according to methods described elsewhere (Monaco et al. 1985; Bertelson et al. 1986). The cloned DNA fragments used in the present study were each radiolabeled (O'Farrell 1982) and hybridized to nitrocellulose filters bearing the patient DNA samples.

Cloned DNA Segments Used as Hybridization Probes

The cloned DNA fragments used in this study span the region of Xp21.1–Xp21.3. The majority of the characteristics of these cloned segments have been described. The ordering of a number of these cloned segments has been established by mapping them relative to various translocation breakpoints of X chromosomes segregated in somatic cell hybrids (Aldridge et al. 1984; de Martinville et al. 1985; Kunkel et al. 1985), by the analysis of deletion patients (de Martinville et al. 1985; Francke et al. 1985, 1987; Bertelson et al. 1986; Monaco et al. 1987), and by genetic linkage analysis in families (Goodfellow et al. 1985).

The cloned fragment C7 (DXS28) (Dorkins et al. 1985) was provided by J. L. Mandel. Its localization was based mainly on its hybridization characteristics with regard to DNA samples isolated from patients with GK deficiency and DNA deletions (de Martinville et al. 1985; Francke et al. 1985, 1987; Bertelson et al. 1986). Probe L1 maps in a manner similar to that seen with C7 (de Martinville et al. 1985) but is separable and more centromeric in the DNA samples isolated from GK-deficient patients (Francke et al. 1987; L. M. Kunkel, unpublished data). J66 and JBir represent the terminal breakpoint junction clones of two different DMD patients whose deletions extend terminal from the pERT87 (DXS164) locus (Monaco et al. 1987); both are present in the DNA of BB. p87 (DXS164) is represented by a number of genomic clones spanning a region of 220 kb of contiguous DNA. This locus is absent from the DNA of patient BB and has been mapped distal to two Xp21 translocation breakpoints (Monaco et al. 1987). XJ1.1 (DXS206) is a genomic clone that was obtained from the breakpoint of the t(X;21) translocation female (Ray et al. 1985). p84 (DXS142) is a genomic probe that contains the 5′-most exon of the DMD cDNA (Koenig et al. 1987). J66, JBir, p87, XJ1.1, and p84 all represent portions of the DMD gene locus (Burghes et al. 1987; Koenig et al. 1987). 754 (DXS84) was provided by P. Pearson and has been mapped as residing ≤800 kb centromeric from p84

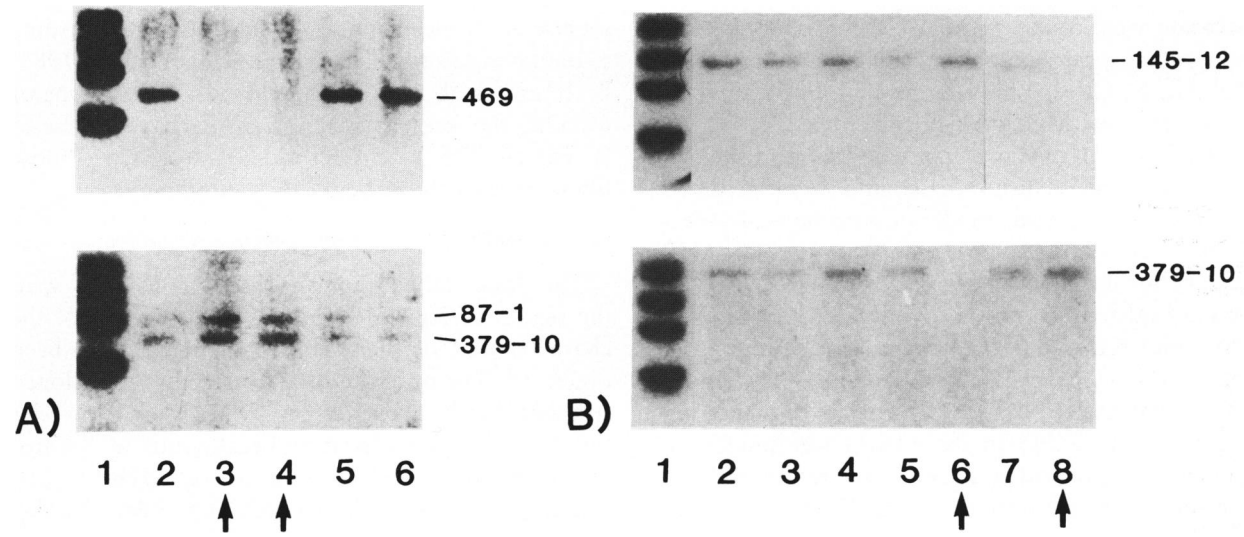

Figure 1 Hybridization of Xp21 cloned DNA fragments to various patient DNA samples. *A*, DNA was isolated from four males with the McLeod phenotype and associated myopathy and from a normal control male and was cleaved, separated by gel electrophoresis, transferred to nitrocellulose, and hybridized as described in Material and Methods. An autoradiograph of the hybridization for the cloned DNA segments p379-10 (3′ CGD cDNA), p87-1 (DXS164), and p469-1 is given. DNA samples were loaded in the following manner: lane 1, *Hin*dIII-cleaved lambda DNA as a molecular-weight standard; lane 2, a normal male; lanes 3–6, cases 1–4, respectively. The autoradiograph is a composite of the results obtained from three separate experiments. The arrows are used to highlight the lane in which absence of a cloned fragment is observed. *B*, Autoradiograph of the hybridization of the cloned fragments p145-12 (DXS141) and p379-10 (3′ CGD cDNA) to the following DNA samples: lane 1, *Hin*dIII-cleaved lambda DNA as a molecular-weight standard; lane 2, a normal male; lanes 3–8, cases 5–10, respectively. The arrows are used as noted above.

(DXS142) (van Ommen et al. 1986). In the present study the remaining cloned segments are mapped relative to each other and represent either the original 200-bp pERT clone (Kunkel et al. 1985) or subclones of phage recovered from a genomic library by hybridization with the original pERT clone. p469-1 is a subcloned DNA fragment that comes from a human genomic phage containing 13.2 kb of contiguous DNA. It has been mapped relative to deletion and translocation breakpoints (van Ommen et al. 1986; L. M. Kunkel, unpublished data). p634 is an original 200-bp pERT clone that can be separated from p469-1. The p469-1 clone is absent from a rodent/human hybrid cell line with X-chromosome representation from Xp11.3-Xqter (Wieacker et al. 1984), while the remaining clones, including p634, are present. p378-40-1 is a cloned DNA fragment that comes from a human genomic phage containing 27.2 kb of contiguous DNA. p145-12 is a cloned DNA fragment that comes from a human genomic phage containing 10.2 kb of contiguous DNA. The linear order between p378-40-1 and 145-12 with reference to the centromere has yet to be established. p379-10 is a genomic subclone that contains the 3′ exon of the CGD cDNA. The 5′ CGD cDNA was provided by S. Orkin. p55-5 is a cloned DNA fragment that has been placed on the centromeric side of CGD. Its positioning was based on the most likely interpretation of both the results presented here and those presented elsewhere (Baehner et al. 1986), but a terminal location relative to CGD cannot be excluded.

Results

Based on the assumption that BB had the McLeod phenotype as a consequence of a large interstitial deletion, a likely conclusion would be that one or more of the cloned DNA fragments absent from BB might be close to the XK locus. To test this possibility, 15 X chromosome–specific cloned DNA segments within and surrounding Xp21 were hybridized to panels of *Pst*I and *Hin*dIII digests of isolated DNA from patients with the McLeod phenotype only, with CGD and the McLeod phenotype, or with DMD only. As shown in figure 1*A*, two of the males with the McLeod red cell phenotype could be demonstrated to have Xp21 deletions. The cloned DNA fragments p379-10 and p87-1 are both present in the DNA of

Table 1

Hybridization Results of Patient Samples with Various Xp Cloned DNA Segments

Probe	DNA Sample										
	0	1	2	3	4	5	6	7	8	9	10
JBir	+	+	+	+	+	NT	NT	NT	NT	−	−
p87-30	+	+	+	+	+	NT	NT	NT	NT	−	−
p87-15	+	+	+	+	+	NT	NT	NT	NT	−	−
p87-8	+	+	+	+	+	NT	NT	NT	NT	−	−
p87-1	+	+	+	+	+	NT	NT	NT	NT	−	−
JMD	+	+	+	+	+	NT	NT	NT	NT	−	−
p84-10	+	+	+	+	+	NT	NT	NT	NT	−	−
754	+	+	+	+	+	+	+	+	+	−	−
p469-1	+	−	−	+	+	+	+	+	+	−	−
p634	+	−	−	+	+	+	+	+	+	−	−
p145-12	+	−	−	+	+	+	+	+	+	+	−
p378-40-1	+	−	−	+	+	+	+	+	+	+	−
p379-10	+	+	+	+	+	+	+	+	−	+	+
5′ CGD cDNA	+	+	+	+	+	+	+	+	−	+	+
p55-5	+	+	+	+	+	+	+	+	−	+	+

NOTE.—Hybridization results for each of the patients described are presented. The DNA samples are numbered (0–10) across the top, and the cloned DNA segments are indicated at the left, proceeding from the terminal side of the DMD locus toward the centromere. The probes defining the loci are as follows: JBir, p87 (DXS164), JMD, p84 (DXS142), 754 (DXS84), p469-1, p634, p145-12 (DXS141), p378-40-1, p379-10 (3′ CGD cDNA), 5′ CGD cDNA, and p55-5 (DXS140). The DNA samples include 0, (a normal male) and cases 1–10, respectively. + = Present; − = absent; and NT = not tested.

the control male (fig. 1*A*, lane 2), as well as in the four males with McLeod phenotype (fig. 1*A*, lanes 3–6). In contrast, the cloned DNA segment p469-1 is completely absent from the DNA of the two cousins (fig. 1*A*, lanes 3, 4) but is present in the DNA of the control male (fig. 1*A*, lane 2) and in the two McLeod males (fig. 1*A*, lanes 5, 6).

Owing to the frequent association of the McLeod phenotype with either myopathy or CGD, the subclones of the DMD (Koenig et al. 1987) and CGD loci (Royer-Pokora et al. 1986) were carefully tested in the patients' DNA samples. The hybridization results are partly demonstrated in figure 1*A* and 1*B* and are summarized in table 1. p379-10 is present in all the McLeod-only DNA samples tested, as is the 5′ portion of the CGD cDNA (table 1). Results found for p87-1, p84-10, and J-Bir indicate that these cloned segments are present in the DNA of all the McLeod patients tested. For both the CGD and DMD loci, no disruptions were found for any part of either locus in the McLeod patients studied. Hybridization results for all 15 cloned segments are summarized in table 1. Together with p469-1, three additional Xp21 clones—p378-40-1, p634, and p145-12—are also absent from these same two cousins with the McLeod phenotype. These cloned fragments are representative of ~50 kb of DNA derived from genomic phage clones (see Material and Methods). Since these phage clones do not overlap, the deletion in these two cousins therefore cannot be <50 kb.

A CGD/McLeod patient was known to exhibit a deletion of a portion of the CGD locus (D. Frey, unpublished data). The extent of this deletion was examined to determine whether it overlapped with the deletion detected in the McLeod cousins. The DNA of this patient is deleted for the 5′ and 3′ cloned segments of the CGD gene (one of which is demonstrated in fig. 1*B*, lane 6) and for the p55-5 clone (table 1). The deletion did not encompass any of the four absent clones deleted from the McLeod cousins.

Two males have been described (Wilcox et al. 1986; Greenberg et al. 1987) who bear large deletions that extend from the DMD locus centromeric to the locus represented by the 754 probe (Wilcox et al. 1986; Greenberg et al. 1987; Monaco et al. 1987). One male (fig. 1*B*, lane 7; summarized in table 1) exhibits a deletion for the cloned DNA segments 754, p469-1, and p634. This DMD male's deletion partially overlaps with the deletion of the two McLeod cousins. The second DMD patient demonstrates a deletion of both 754 and all four cloned

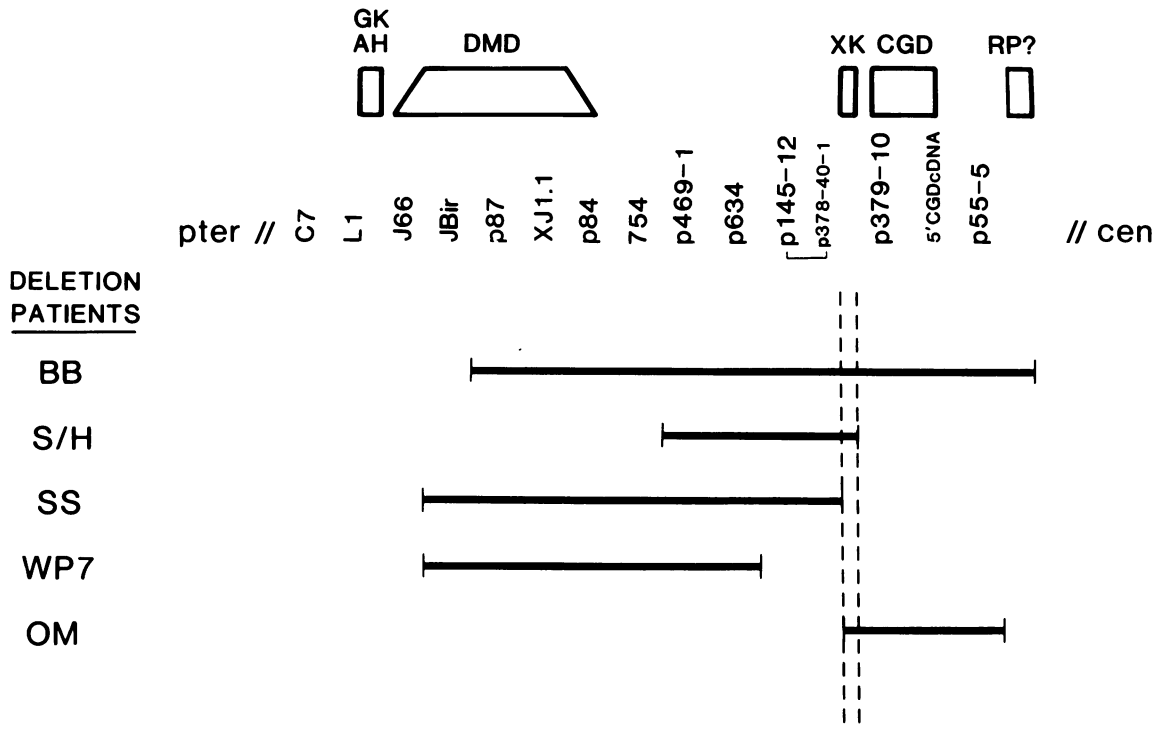

Figure 2 Schematic representation of Xp21 disorders and cloned loci. The following disorders are boxed at the top of the figure: GK, adrenal hypoplasia (AH), DMD, XK, CGD, and RP. The cloned DNA segments are aligned below and are spaced equidistant along the chromosome in the figure. The cloned DNA segments are described in the Material and Methods and include the following DNA segments: C7 (DXS28), LI (DXS68), J66, JBir, p87 (DXS164), XJ1.1 (DXS206), p84 (DXS142), 754 (DXS84), p469-1, p634, p145-12 (DXS141), p378-40-1, 379-10 (3′ CGD cDNA), 5′ CGD cDNA, and p55-5 (DXS140). Below the loci are shown the extent of the deletions: patient BB (Francke et al. 1985); S/H, cases 1 and 2; SS (Wilcox et al. 1986), case 10; WP7 (Greenberg et al. 1987), case 9; and OM, case 8. The location of the McLeod locus is indicated by the dotted vertical lines. GK, AH, and DMD were positioned with reference to these cloned DNA segments on the basis of previously published information (Bertelson et al. 1986; van Ommen et al. 1986; Francke et al. 1987).

DNA segments that are absent from the DNA of the two McLeod cousins (including p145-12, shown in fig. 1*B*, lane 8). On the basis of the results presented in table 1, the minimum region of deletion overlap to yield the McLeod phenotype must reside between the loci defined by the cloned segments p145-12/p378-40-1 (the relative order of these two clones is unknown) and the CGD locus.

Discussion

The DNA of the patient, BB, has been instrumental in the cloning of the cDNAs for both CGD and DMD (Monaco et al. 1986; Royer-Pokora et al. 1986). We have used the knowledge of the chromosomal location of these two genes as a basis on which to attempt to define the location of the XK locus that is responsible for the McLeod phenotype. Deletions of a subset of cloned segments from Xp21, detected in two cousins with the McLeod phenotype (table 1), allow mapping of the XK locus between DMD and CGD. The region becomes even more well defined with the addition of a nonoverlapping deletion observed in a patient with CGD and the McLeod phenotype, as well as with the addition of two overlapping deletions observed in non-McLeod/DMD patients. An interpretation of the hybridization results (table 1), together with previously published mapping results (Aldridge et al. 1984; de Martinville et al. 1985; Francke et al. 1985, 1987; Baehner et al. 1986; Bertelson et al. 1986; van Ommen et al. 1986; Burghes et al. 1987; Monaco et al. 1987), allows the construction of a map of the Xp21 region (fig. 2).

The McLeod phenotype is associated with either a mild myopathy with substantial cardiac involvement or with X-linked CGD. Here, two cousins are de-

scribed who have demonstrable deletions in Xp21 that do not involve either the CGD or DMD loci. The clinical absence of the CGD phenotype combined with the fact that the 3′ and 5′ ends of the CGD cDNA were present in the seven McLeod men we tested leads us to conclude that the CGD locus is not altered in these patients. The presence of myopathy in non-CGD/McLeod subjects might indicate a disruption of the DMD locus due to the close proximity of the DMD and XK loci. The deletions detected in the two cousins, however, start substantially centromeric from the 5′-most exon of the DMD locus, making this hypothesis unlikely. Therefore it is unlikely that the myopathy observed in McLeod patients is caused by a disruption of the DMD gene. Placement of the deletion in these subjects between the CGD and DMD loci, without altering either, indicates that the locus responsible for the McLeod phenotype must be an entity separate from CGD and DMD. The XK locus can be further defined by the data presented in table 1 and depicted in figure 2. Given the analysis of DNA samples of patients, we conclude that the McLeod locus must be in a region between clones p145-12/p378-40-1 and the CGD locus and is defined by the overlapping deletions. Presumably the region of deletion overlap that contains XK was not detectable with the cloned segments presented here. If the remaining disease locus, RP, is truly in this region, then, on the basis of deletion analysis presented here and elsewhere (Francke et al. 1985; Baehner et al. 1986), RP must reside on the centromere side of CGD very near the breakpoint of the BB deletion.

The association of myopathy with the McLeod phenotype is therefore difficult to explain because, unlike the course of DMD, the myopathy observed with the McLeod phenotype is a late-onset type of muscular dystrophy and is not rapidly progressive. The CGD/McLeod association is clear, since some of these patients have deletions that must remove portions of the McLeod gene. Much of the understanding of the interaction of the XK locus with its surrounding loci will have to await the cloning of this locus and the characterization of its protein product. One potential protein-product candidate is the 37-kD protein recognized by anti-Kx serum, which is present in red blood cells of all common Kell phenotypes and in Ko cells but is absent from McLeod cells. The deletion patients described here define where the search for this gene should begin. On the basis of current estimates (Francke et al. 1985; Wilcox et al. 1986), BB's deletion probably encompasses 5–10 million bp of DNA. If the eight pERT clones and the locus 754 (DXS84) are distributed evenly along the DNA molecule, then each should be ~500–1000 kb apart. The distances for the most terminal of the pERT clones are reasonably well established from the characterization of the DMD locus (Burmeister and Lehrach 1986; van Ommen et al. 1986; Kenwrick et al. 1987; Koenig et al. 1987). The distal clone, pERT87, is ~300 kb from the BB distal deletion breakpoint, with pERT84 ~500 kb more centromeric. The clone 754 has been estimated to be ≤800 kb centromeric from pERT84. With the relatively random distribution of these three clones, which are indeed separated by ~500 kb, the estimate that the four pERT clones absent both in the McLeod cousins and in the DMD boys are spaced >2,000 kb is not unreasonable. Because there is a region of deletion overlap between the DMD and McLeod patients yet no overlap of phenotype, the XK locus must extend beyond the loci represented by the probes p378-40-1 and p145-12, toward the CGD locus. On the basis of the assumption given above, the distance between these flanking cloned loci and the XK locus can be estimated to be on the order of 500 kb. Chromosome walking (Bender et al. 1983) and long-range restriction-endonuclease mapping associated with HTF island localization (Bird 1986) will be initiated with the centromeric CGD locus and with the more terminal loci defined by the probes p145-12 and p378-40-1. Conservation of nucleotide sequences within this region might ultimately lead to both the cloning of the McLeod locus and a better understanding of how the disruption of various loci within Xp21 generates the different phenotypes observed in affected individuals.

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References

Aldridge, J., L. Kunkel, G. Bruns, U. Tantravahi, M. Lalande, T. Brewster, and E. Moreau. 1984. A strategy to reveal high frequency RFLPs along the human X chromosome. Am. J. Hum. Genet. 36:546–564.

Allen, F. H., Jr., S. M. R. Krabbe, and P. A. Corcoran. 1961. A new phenotype (McLeod) in the Kell blood group system. Vox Sang. 6:555–560.

Baehner, R. L., L. M. Kunkel, A. P. Monaco, J. L. Haines, P. M. Conneally, C. Palmer, N. Heerema, and S. H. Orkin. 1986. DNA linkage analysis of X chromosome–linked chronic granulomatous disease. Proc. Natl. Acad. Sci. USA 83:3398–3401.

Bender, W., M. Arkam, F. Karch, P. A. Beachy, M. Peifer, P. Spierer, E. B. Lewis, and D. S. Hogness. 1983. Molecular genetics in the bithorax complex in *Drosophila melanogaster*. Science 221:23–29.

Bertelson, C. J., J. A. Bartley, A. P. Monaco, C. Colletti-Feener, K. Fischbeck, and L. M. Kunkel. 1986. Localization of Xp21 meiotic exchange points in Duchenne muscular dystrophy families. J. Med. Genet. 23:531–537.

Bird, A. P. 1986. CpG-rich islands and the function of DNA methylation. Nature 321:209–213.

Burghes, A. H. M., C. Logan, X. Hu, B. Belfall, R. G. Worton, and P. N. Ray. 1987. A cDNA clone from the Duchenne/Becker muscular dystrophy gene. Nature 328:434–437.

Burmeister, M., and H. Lehrach. 1986. Long range restriction map around the Duchenne muscular dystrophy gene. Nature 324:582–585.

de Martinville, B., L. M. Kunkel, G. Bruns, F. Morlé, M. Koenig, J. L. Mandel, A. Horwich, S. A. Latt, J. F. Gusella, D. Housman, and U. Francke. 1985. Localization of DNA sequences in region Xp21 of the human X chromosome: search for molecular markers close to the Duchenne muscular dystrophy locus. Am. J. Hum. Genet. 37:235–249.

Dorkins, H., C. Junien, J. L. Mandel, K. Wrogemann, J. P. Moison, M. Martinez, J. M. Old, S. Bundey, M. Schwartz, N. Carpenter, D. Hill, M. Lindlof, A. de la Chapelle, P. L. Pearson, and K. E. Davies. 1985. Segregation analysis of a marker localised Xp21.2-Xp21.3 in Duchenne and Becker muscular dystrophy families. Hum. Genet. 71:103–107.

Francke, U., J. F. Harper, B. T. Darras, J. M. Cowan, E. R. B. McCabe, A. Kohlschütter, W. K. Seltzer, F. Saito, J. Goto, J.-P. Harpey, and J. E. Wise. 1987. Congenital adrenal hypoplasia, myopathy, and glycerol kinase deficiency: molecular genetic evidence for deletions. Am. J. Hum. Genet. 40:212–227.

Francke, U., H. D. Ochs, B. de Martinville, J. Giacalone, V. Lindgren, C. M. Distèche, R. A. Pagon, M. H. Hofker, G.-J. B. van Ommen, P. L. Pearson, and R. J. Wedgwood. 1985. Minor Xp21 chromosome deletion in a male associated with expression of Duchenne muscular dystrophy, chronic granulomatous disease, retinitis pigmentosa, and the McLeod syndrome. Am. J. Hum. Genet. 37:250–267.

Friend, S. H., R. Bernards, S. Rogelj, R. A. Weinberg, J. M. Rapaport, D. M. Albert, and T. P. Dryja. 1986. A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. Nature 323:643–645.

Goodfellow, P. N., K. E. Davies, and H.-H. Ropers. 1985. Report of the Committee on the Genetic Constitution of the X and Y Chromosomes: Human Gene Mapping 8. Cytogenet. Cell Genet. 40:296–352.

Greenberg, C. R., J. L. Hamerton, M. Nigli, and K. Wrogemann. 1987. DNA studies in a family with Duchenne muscular dystrophy and a deletion at Xp21. Am. J. Hum. Genet. 41:128–137.

Kenwrick, S., M. Patterson, A. Speer, K. Fischbeck, and K. Davies. 1987. Molecular analysis of the Duchenne muscular dystrophy region using pulsed field gel electrophoresis. Cell 48:351–357.

Koenig, M., E. P. Hoffman, C. J. Bertelson, A. P. Monaco, C. Feener, and L. M. Kunkel. 1987. Complete cloning of the Duchenne muscular dystrophy (DMD) cDNA and preliminary genomic organization of the DMD gene in normal and affected individuals. Cell 50:509–517.

Kunkel, L. M., A. P. Monaco, W. Middlesworth, H. D. Ochs, and S. A. Latt. 1985. Specific cloning of DNA fragments absent from the DNA of a male patient with an X chromosome deletion. Proc. Natl. Acad. Sci. USA 82:4778–4782.

Marsh, W. L., N. J. Marsh, A. Moore, W. A. Symmans, C. L. Johnson, and C. M. Redman. 1981. Elevated serum creatine phosphokinase in subjects with McLeod syndrome. Vox Sang. 40:403–411.

Marsh, W. L., and C. M. Redman. 1987. Recent developments in the Kell blood group system. Trans. Med. Rev. 1:4–20.

Marsh, W. L., E. F. Schnipper, C. L. Johnson, K. A. Mueller, and S. A. Schwartz. 1983. An individual with McLeod syndrome and the Kell blood group antigen K (K1). Transfusion 23:336–338.

Monaco, A. P., C. J. Bertelson, C. Colletti-Feener, and L. M. Kunkel. 1987. Localization and cloning of Xp21 deletion breakpoints involved in muscular dystrophy. Hum. Genet. 75:221–227.

Monaco, A. P., C. J. Bertelson, W. Middlesworth, C.-A. Colletti, J. Aldridge, K. H. Fischbeck, R. Bartlett, M. A. Pericak-Vance, A. D. Roses, and L. M. Kunkel. 1985. Detections of deletions spanning the Duchenne muscular dystrophy locus using a tightly linked DNA segment. Nature 316:842–845.

Monaco, A. P., R. L. Neve, C. Colletti-Feener, C. J. Bertelson, D. M. Kurnit, and L. M. Kunkel. 1986. Isolation of candidate cDNAs for portions of the Duchenne muscular dystrophy gene. Nature 323:646–650.

O'Farrell, P. 1982. Replacement synthesis method of labelling DNA fragments. Focus [Bethesda Res. Lab.] 3:1–3.

Ray, P. N., B. Belfall, C. Duff, C. Logan, V. Kean, M. W. Thompson, J. E. Sylvester, J. L. Gorski, R. D. Schmickel, and R. G. Worton. 1985. Cloning of the breakpoint of an X;21 translocation associated with Duchenne muscular dystrophy. Nature 318:672–675.

Redman, C. M., G. Avellino, S. R. Pfeffer, T. K. Mukherjee, M. Nichols, P. Rubinstein, and W. L. Marsh. 1986. Kell blood group antigens are part of a 93,000 dalton red cell membrane protein. J. Biol. Chem. 261:9521–9525.

Redman, C. M., W. L. Marsh, K. A. Mueller, G. P. Avellino, and C. L. Johnson. 1984. Isolation of Kell-active protein from the red cell membrane. Transfusion 24: 176–178.

Redman, C. M., W. L. Marsh, A. Scarborough, C. L. Johnson, B. I. Rabin, and M. Overbeeke. 1988. Biochemical studies on McLeod phenotype red cells and isolation of Kx antigen. Br. J. Haematol. 68:131–136.

Royer-Pokora, B., L. M. Kunkel, A. P. Monaco, S. C. Goff, P. E. Newburger, R. L. Baehner, F. S. Cole, J. T. Curnutte, and S. H. Orkin. 1986. Cloning the gene for an inherited human disorder (chronic granulomatous disease) on the basis of its chromosomal location. Nature 322:32–38.

Swash, M., M. S. Schwartz, N. D. Carter, R. Heath, M. Leak, and K. L. Rogers. 1983. Benign X-linked myopathy with acanthocytes (McLeod syndrome): its relationship to X-linked muscular dystrophy. Brain 106:717–733.

Symmans, W. A., C. S. Shepherd, W. L. Marsh, R. Oyen, S. B. Shohet, and B. J. Linehan. 1979. Hereditary acanthocytosis associated with the McLeod phenotype of the Kell blood group system. Br. J. Haematol. 42:575–583.

van Ommen, G.-J. B., J. M. H. Verkerk, M. H. Hofker, A. P. Monaco, L. M. Kunkel, P. Ray, R. Worton, B. Wieringa, E. Bakker, and P. L. Pearson. 1986. A physical map of 4 million bp around the Duchenne muscular dystrophy gene on the human X chromosome. Cell 47: 499–504.

Wieacker, P., K. E. Davies, H. J. Cooke, P. L. Pearson, R. Williamson, S. Bhattacharya, J. Zimmer, and H.-H. Ropers. 1984. Toward a complete linkage map of the human X chromosome: regional assignment of 16 cloned single-copy DNA sequences employing a panel of somatic cell hybrids. Am. J. Hum. Genet. 36:265–276.

Wilcox, D. E., A. Cooke, J. Colgan, E. Boyd, D. A. Aitken, L. Sinclair, L. Glasgow, J. B. P. Stephenson, and M. A. Ferguson-Smith. 1986. Duchenne muscular dystrophy due to familial Xp21 deletion detectable by DNA analysis and flow cytometry. Hum. Genet. 73:175–180.

Wimer, B. M., W. L. Marsh, H. F. Taswell, and W. R. Galey. 1977. Haematological changes associated with the McLeod phenotype of the Kell blood group system. Br. J. Haematol. 36:219–224.