The Human Kell Blood Group Gene Maps to Chromosome 7q33 and its Expression Is Restricted to Erythroid Cells

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The Kell blood group is one of the major antigenic systems in human red blood cells. To determine the location of the Kell gene on human chromosomes, panels containing genomic DNA of human-hamster somatic cell hybrids were hybridized with radiolabeled cDNA probe specific for the Kell locus. Only the samples containing DNA from chromosome 7 gave positive hybridization signals. In situ hybridization analysis, using genomic clones isolated with the cDNA, localized the KEL gene to 7q33. Northern blot analysis of poly(A)^+ RNA from human brain, kidney, lung, fetal and adult liver, and bone marrow showed that Kell transcripts were only present in fetal liver and bone marrow. This indicates that the Kell protein, which carries the Kell antigens, may only be expressed in erythroid tissues.

KELL IS ONE of the major human red blood cell (RBC) groups. It is an important system in clinical medicine, for Kell antibodies can cause severe hemolytic reactions if incompatible blood is transfused. Furthermore, active maternal-fetal Kell group incompatibility may cause severe hemolytic disease in a newborn infant. The Kell blood group system is characterized by its complexity; it is composed of at least 23 different antigens. No example of recombination within the Kell locus has been recorded and it has been assumed that the observed complexity reflects a single gene with many sites at which mutations can occur.1,2

The Kell antigens reside on a 93-kD RBC trans-membrane glycoprotein that is surface-exposed and probably attached to the underlying cytoskeleton.3,4 Molecular cloning of the Kell gene has shown that it is a 731 amino acid protein that spans the membrane once and has a 46 amino acid N-terminal domain within the RBC and a large C-terminal extracellular segment.5

Until recently, Kell was the only major blood group system whose locus had not been mapped. Zelinski et al6 have shown that the Kell blood group locus is linked to the prolactin-inducible protein (PIP) locus, which has been localized to 7q32-36. This assignment was confirmed by linkage analysis between KEL and 3 DNA markers that are linked to the cystic fibrosis marker.7 We have now used genomic clones as probes to localize the KEL gene in a more defined area and we also show that expression of the KEL gene is restricted to erythroid tissues.

MATERIALS AND METHODS

Approval was obtained from the Institutional Review Board of the New York Blood Center for these studies. Persons were informed that samples were obtained for research purposes and that their privacy would be protected.

Northern blot analysis. Human bone marrow was stored at -70°C in 5 vol (wt/vol) of 6 mol/L guanidine thiocyanate, 12.5

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Fig 1. Northern blot analyses of RNA from several tissues. Lanes 1 to 5 contain 10 μg each of poly(A)^+ RNA from human bone marrow, brain, kidney, fetal liver, and adult liver. Lane 6 contains 24 μg of human bone marrow poly(A)^+ RNA. Lane 7 has 10 μg of human lung poly (A)^+ RNA that was analyzed in a separate experiment. Northern blot hybridizations were performed with ^32P-labeled Kell cDNA (upper panel) or with cDNA to human β-actin (lower panel). Molecular weight markers are indicated on the sides of the autoradiograms.
mmol/L sodium citrate, pH 7.0, and 5% 2-mercaptoethanol. RNA was isolated by the combined methods of CsCl-gradient centrifugation followed by acid guanidinium thiocyanate-phenol-chloroform extraction. Poly(A)+ RNA was isolated using an mRNA isolation kit (Fast-Track) from Invitrogen (San Diego, CA). Other poly(A)+ RNAs were purchased from Clonetech Laboratories (Palo Alto, CA).

Poly(A)+ RNAs were separated on 1.2% agarose gels containing formaldehyde. Random-labeled 0.5- and 1.9-kb cDNA fragments from Kell clone no. 191, which contain the entire open reading frame for Kell protein, were used as probes. The analysis was performed as described by Sambrook et al.

Chromosome panels. A human placental DNA genomic library constructed in EMBL-3 (Clonetech) was screened using labeled 0.5- and 1.9-kb cDNA fragments from Kell clone no. 191. Fourteen genomic clones were obtained and three genomic clones (2, 8, and 11) were purchased from Bios Corp (New Haven, CT).

Southern blot hybridizations were performed with random-labeled (Random Primed DNA labeling kit; US Biochemical, Cleveland, OH) 0.5- and 1.9-kb cDNA fragments from Kell clone no. 191 as described by Sambrook et al. Genomic clones. A human placental DNA genomic library constructed in EMBL-3 (Clonetech) was screened using labeled 0.5- and 1.9-kb cDNA fragments from Kell clone no. 191. Fourteen genomic clones were obtained and three genomic clones (2, 8, and 11) were purchased from Bios Corp (New Haven, CT).

Fig 2. Composition of human-hamster somatic cell hybrid panels. The DNA composition of two panels is shown. On the left side is panel I and on the right panel II. Each panel contains 22 lanes. As controls, lanes 1 and 12 of each panel contain human DNA and lanes 11 and 22 contain hamster DNA. Lanes 2 through 10 and 13 through 21 contain chromosomal DNA from the human-hamster somatic cell lines listed. X means that more than 75% of the cells contain the noted human chromosome. The numbers are the percentages of the cell population containing the noted chromosome. D is a deletion at p15.1-15.2 and Dq indicates multiple deletions in 5q. These panels are from Bios Corp, as revised on September 19, 1990.
B) were chosen as probes for in situ hybridization. These three clones, which range in size from approximately 11 to 18 kb, are overlapping and span the entire KEL gene (Lee et al, unpublished).

**Fluorescent in situ hybridization (FISH).** Chromosome mapping was performed by the FISH method of Lichter et al.17 Equal amounts of DNA from genomic clones 2, 8, and B were combined and nick-translated with digoxigenin-11-dUTP. The labeled probes were combined with sheared human DNA and hybridized in 50% formamide, 2× SSC, and 10% dextran to normal metaphase chromosomes. Hybridization was detected with antidigoxigenin-fluorescein isothiocyanate (FITC). The chromosomes were counterstained with propidium iodide. To confirm the identity of the labeled chromosome a similarly labeled probe, specific for the centromere of chromosome 7, was mixed with the three KEL genomic probes and used to cohybridize the chromosomes. The location of the KEL gene on the chromosome was determined by measuring the distance from the centromere to the telomere of the long arm of chromosome 7 and calculating the percent distance of the Kell-specific signal. These procedures were performed by Bios Corp.

**DNA isolation and restriction enzyme analysis.** DNA was isolated from leukocytes present in the buffy coat of blood from a person with common Kell phenotype, as previously described,16 and digested with EcoRI or HindIII. Digested DNA (10 μg) was separated on 0.8% agarose gels and transferred to Hybond-N+ membranes. The filters were hybridized with 32P random-labeled 0.5- and 1.9-kb cDNA fragments of Kell clone no. 1917 as described by Sambrook et al.11

**RESULTS**

**KEL expression is restricted to erythroid tissues.** Northern blot analyses of Poly(A)+ RNA from human bone marrow, brain, kidney, fetal liver, adult liver, and lung were performed with radiolabeled cDNA fragments covering the entire Kell open reading frame. Hybridization signals were only obtained with RNA from bone marrow and fetal liver. The major transcript in both fetal liver and bone marrow was 2.5 kb. Larger transcripts of 6.6, 11.5, and 13.2 kb were also noted in both fetal liver and bone marrow, but the 11.5- and 13.2-kb transcripts were difficult to detect in bone marrow (Fig 1).

The hybridization signal was severalfold greater in fetal liver than in bone marrow. Two different concentrations of Poly(A)+ RNA (10 μg and 24 μg) were used for bone marrow, and in each instance the signal was much less than that noted for fetal liver. Hybridization with 32P-labeled β-actin cDNA was used as a control to evaluate the quantity of RNA from the various tissues (Fig 1).

These data show that Kell transcripts are not detected in brain, kidney, adult liver, and lung, but are expressed in bone marrow and fetal liver, indicating that expression is likely to be restricted to erythroid tissues.

**Chromosome location of KEL by Southern blots of somatic cell hybrids.** Kell cDNA fragments were used to screen two panels containing chromosomal DNA of human-hamster somatic cell hybrids. Each panel also contained human and hamster DNA as controls. The composition of the panels is shown in Fig 2. Positive hybridization signals were obtained in the lanes containing human DNA (lane 1, panels I and II, Fig 3) and in 3 of 36 hybrid cell lines that contained DNA from human chromosome 7 (lane 8, panel I, and lanes 7 and 8, panel II, Fig 3). In these samples, 3 radioactive bands of approximately 11.5, 10.5, and 6.5 kb were noted. The 11.5- and 10.5-kb bands were not well separated and appear as a single signal. Two of the three lanes that gave positive signals (lane 8, panel I, and lane 7, panel II) are derived from cell line no. 1006 and had DNA from human chromosomes 4, 5, 7, 8, 13, 15, 19, and 21. The other cell line (no. 756) that gave a positive signal (lane 8, panel II) had DNA from human chromosomes 5, 6, 7, 12, 13, 14, 19, 20, 21, and Y (Fig 2). All of the other cell lines were negative. They only had weak signals similar to those obtained with hamster DNA (lanes 11 and 22, panels I and II) and different than that seen with human DNA. Absence of a positive signal from these other lanes eliminated human chromosomes 5, 13, 19, and 21.
which, together with chromosome 7, are shared by cell lines 1006 and 756. This combination of positive signals in the presence of DNA from chromosome 7 and absence of signals in cell lines with other chromosomes localizes the KEL gene on chromosome 7.

**Restriction enzyme analysis.** To determine the approximate size of the gene, genomic DNA was digested with EcoRI and HindIII and hybridized with probes that encompass the entire open reading frame of Kell protein. The sum of the respective EcoRI or HindIII fragments indicated that Kell gene is approximately 30 kb (data not shown).

**FISH.** To define further the segment of chromosome 7 in which the Kell gene is located, three Kell genomic clones (Kell clones 2, 8, and B) were labeled with the fluorescent tag digoxigenin and in situ hybridization to human chromosome metaphase spreads was performed. Specific hybridizations were seen on the long arm of a C-group chromosome whose morphology indicated that it was chromosome 7. To confirm the identity of the labeled chromosome, a probe (D7Z1) specific for the centromeric region of chromosome 7 was cohybridized with genomic clones no. 2, 8, and B to metaphase chromosomes. The results are shown in Fig 4. All chromosomes that contained a centromeric signal, identifying them as chromosome 7, also contained hybridization signals on the distal long arm. Measurements of hybridized chromosomes indicate that the Kell hybridization signal is

![Fig 4. In situ hybridization showing labeling of chromosome 7. Normal human metaphase (A) and prometaphase chromosomes (B) were cohybridized with fluorescent-labeled Kell-specific genomic DNA and with a chromosome 7-specific centromeric probe.](image-url)
located 82% of the distance from the centromere to the telomere of the long arm of chromosome 7. This distance corresponds to band 7q33. A diagram showing the location where the KEL gene maps is given in Fig 5.

**DISCUSSION**

Many RBC membrane proteins occur, or have similar counterparts, in other tissues. However, the 93-Kd protein that carries the Kell antigens appears to be only expressed in erythroid tissues. Northern blot analyses did not detect transcripts in human brain, kidney, lung, or adult liver, but expression was noted in bone marrow and in fetal liver. Kell protein is not homologous to any known protein, but has most similarity with the common acute lymphoblastic antigen (CALLA) and the enkephalinases, a group of zinc metallo-endoproteinases that are widely distributed in many cell types and have broad substrate specificities. The role of these endopeptidases is to process peptide hormones such as the enkephalins, neurotensin, angiotensin, oxytocin, and bradykinin. In addition, Kell also shares a pentameric consensus sequence with a large family of zinc metalloproteases present in nearly all organisms and having a variety of proteolytic roles. The physiologic role of Kell on RBC membranes has not been elucidated, but because zinc metalloproteases are widely distributed, it may not be necessary for Kell, if it has peptidase activity, to be expressed in nonerythroid tissues. It should be noted that Ko(null) persons, who lack Kell protein on RBC membranes, are healthy and, presumably, there are compensatory mechanisms that take over the role of Kell.

Some of the zinc endopeptidases are membrane-spanning proteins that act as cell-surface antigens. For example, CALLA and BP-1/6C3 antigens are both, like Kell, proteins that span the membrane once, have small cytoplasmic domains, and contain the common zinc-binding endopeptidase active site consensus sequence on the extracellular segment. It has been suggested that the role of these enzymes on the surfaces of several different cell types may be to control growth and differentiation in both hematopoietic and epithelial systems. However, other roles, such as processing of extracellular hormones or matrices, must also be considered.

In bone marrow and fetal liver, the major transcript noted by Northern blot analysis was 2.5 kb. This size is in the range expected to code for an 80-Kd protein, such as deglycosylated Kell. The reasons for the presence of larger transcripts, of approximately 6.6, 11.5, and 13.2 kb, are not clear. Both bone marrow and fetal liver have a 6.6-kb transcript and fetal liver also has larger 11.5- and 13.2-kb sizes. Only a single 93-Kd glycoprotein has been identified as carrying Kell antigens, and thus only a single-size mRNA may be expected. However, mRNAs with large 3′ untranslated regions are not uncommon and these larger transcripts may have such untranslated structures.

The in situ hybridization studies localized the KEL gene to a single chromosome region 7q33. This confirms the genetic linkage studies of Zelinski et al, who have mapped the KEL gene to 7q32-36 based on its linkage with the prolactin-induced protein. Two other blood group loci, Colton (CD) and Cartwright (YT), have also been assigned to chromosome 7. Colton is on the short arm of chromosome 7q21.2 and YT, which has a loose linkage with KEL, is an antigen located on erythrocyte acetylcholinesterase, which maps to 7q22. Spence et al reported the linkage of the classical genetic marker for phenylthiocarbamide-tasting (PTC) to KEL and, thus, PTC has also been assigned to chromosome 7. As noted by Zelinski et al, now only 2 of the 19 recognized blood group systems (Diego and Dombrock) have not yet been assigned a chromosomal location.
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