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BRAIN RESEARCH

Research Report

# The Kell and XK proteins of the Kell blood group are not co-expressed in the central nervous system

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ARTICLE INFO

*Article history:*
Accepted 15 January 2007
Available online 2 February 2007

*Keywords:*
Metalloproteases of the M13 family
Localization
Kell blood group antigens
McLeod syndrome
Rodent
Human

ABSTRACT

The Kell blood group is constituted by two covalently linked antigens at the surface of red blood cells, Kell and Kx. Whereas Kell is a metalloprotease with demonstrated *in vitro* enzymatic activity, the role of Kx thereon, and/or alone, remains unknown, although its absence is linked to the McLeod syndrome, a neuroacanthocytosis. In the central nervous system, the expression of Kell and XK has been suggested, but their expression patterns remain uncharacterized, as are the post-translational pathogenic mechanisms involved in the development of the McLeod syndrome. The distributions of Kell and XK were thus studied by *in situ* hybridization as well as immunohistochemistry in rodent and human brain. The results reveal an independent localization of the two constituents of the Kell blood group, XK (Kx) being expressed throughout this tissue, whereas Kell expression is restricted to red blood cells in cerebral vessels. The XK protein is shown to be neuronal, located mainly in intracellular compartments, suggesting a cell specific trafficking pattern, possibly associated with specific physiological functions.



## 1. Introduction

The Kell blood group system is formed by two disulfide-linked proteins, Kell and XK (Redman and Marsh, 1993; Redman and Lee, 1995). The Kell protein is a type II membrane glycoprotein sharing sequence homology with members of the M13 family of zinc-dependent metalloproteases including neprilysin (NEP) and the endothelin-converting enzymes (ECE-1 and ECE-2) (Lee et al., 1991). Because these proteases play important homeostatic functions through their involvement in maturation and/or inactivation processes of messenger peptides (Turner et al., 2000, 2001), the metalloprotease nature of Kell has long been sought, and recently brought to light. Thus, Kell was shown to possess enzymatic activity characteristic of the M13 metalloproteases, cleaving the Big-endothelin inactive intermediates, yielding mature endothelins, inactivating tachykinins and binding the inhibitory compounds phosphoramidon and thiorphan with affinities in the nanomolar range (Lee et al., 1999; Claperon et al., 2005). Although Kell was originally thought to be expressed exclusively in erythroid tissues, dot-blot experiments have evidenced its expression in testis, heart and liver and suggested

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*Abbreviations:* ER, endoplasmic reticulum; ET, endothelin; CNS, central nervous system; SSC, standard saline citrate; RT, room temperature; RBC, red blood cell; MLS, McLeod syndrome; ISHH, *in situ* hybridization histochemistry; IHC, immunohistochemistry; GAR, goat anti-rabbit IgG; GAM, goat anti-mouse IgG

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doi:10.1016/j.brainres.2007.01.106

its presence in brain regions. The Kell protein has been observed in muscle fibers, tonsils and Sertoli cells of the testis, but never in the central nervous system (CNS) (Russo et al., 2000; Camara-Clayette et al., 2001; Jung et al., 2001a).

Linkage of Kell to a smaller protein was first suggested by immunoprecipitation experiments using a monoclonal anti-Kell K1 antibody (Redman et al., 1988; Jaber et al., 1989), instrumental in the subsequent isolation by affinity chromatography of the Kell partner, XK, from red blood cells (RBCs) (Khamlichi et al., 1995; Carbonnet et al., 1997). Coded by the XK gene, the XK protein is constituted by 444 amino acids with a polytopic structure predicted to contain 10 membrane-spanning domains, reminiscent of certain transport proteins (Ho et al., 1994). The covalent nature of the association of the Kell and Kx antigens was thereafter demonstrated by site directed mutagenesis revealing that Kell $Cys^{72}$ was linked to XK $Cys^{347}$ (Russo et al., 1998).

Absence of the Kx antigen at the surface of RBCs and weakened Kell antigens define the McLeod phenotype. Although this phenotype is related to Kell, it is the Kx antigen which is either deleted or mutated and no longer able to bind Kell. The phenotype is moreover associated to the McLeod syndrome (MLS), an X-linked (Bertelson et al., 1988) neuroacanthocytosis characterized by misshapen erythrocytes and elevated serum creatine kinase (Marsh et al., 1981). MLS is defined by late-onset muscular dystrophy and a typical triad of neurological symptoms highly reminiscent of those observed in early Huntington's disease (HD), including choreic movement disorder, psychiatric abnormalities and progressive cognitive impairment (Danek et al., 1994, 2001, 2005; Danek and Walker, 2005; Jung et al., 2001b).

The expression pattern of *XK* has to date been studied using Northern blotting which showed the transcript to be highly expressed in erythroid tissues, skeletal muscle, as well as in heart and brain at more moderate levels (Ho et al., 1994). In the CNS, Kell expression remains unstudied, and the XK expression pattern uncharacterized. Moreover, whether MLS is due to Kell weakened expression and/or to the absence of Kx remains open for debate, particularly since the K0 phenotype (no Kell expression) is not associated with any neurological disorder. As Kell and/or XK may be relevant to the etiology and development of the neurological impairments associated with MLS, expression of their transcripts and of the proteins they encode was investigated using *in situ* hybridization histochemistry (ISHH) and immunohistochemistry (IHC) in rodent and human brain. The cellular and sub-cellular localization of XK was further explored using double immunofluorescent labeling experiments on brain sections and hippocampal cultures.

## 2. Results

### 2.1. *Expression of Kell and XK transcripts in the central nervous system*

The expression of Kell and XK mRNA in the CNS was first investigated by RT-PCR using RNA preparations from mouse total brain as neither Kell nor XK has to date been cloned in rat. Because testis expresses both Kell (Russo et al., 2000; Camara-Clayette et al., 2001) and XK transcripts (Collec et al., 1989), this tissue was also chosen as a positive control of specific amplification. Using cDNA produced from both these tissues, single amplification products of expected sizes were observed after PCR. Their respective authenticity was verified by subcloning and sequencing thus unambiguously confirming their identity. Control actin amplifications were used as a reflection of both RNA integrity and of quantities used for PCR amplifications (Fig. 1).

Results of these RT-PCR experiments, a typical example of which is displayed in Fig. 1, showed that Kell is indeed expressed in testis but not in brain as no mRNA coding for this protein was evidenced in this tissue (Fig. 1), even though permissive, 30-cycle protocols of amplification were performed. Transcripts coding for XK were however expressed both in testis and brain, as reflected by the presence of the XK-specific RT-PCR product, suggesting that the XK protein is expressed alone in rodent CNS (Fig. 1).

While ISHH experiments using the Kell-specific cRNA probe did not reveal the Kell transcript in rodent brain (data not shown) further confirming the above RT-PCR results, using the specific XK cRNA probe, ISHH brought to light a quasi-ubiquitous distribution of this transcript throughout the adult mouse brain (Fig. 2). In the cerebral cortex, cells expressing XK were present in all cortical layers (Figs. 2A, B, 3A), with a strong labeling of the piriform cortex (Fig. 2B). Intense labeling was detected in the pyramidal cells of the CA1, CA2 and CA3 subfields of the hippocampal formation (Figs. 2A, B, C) and in the granular cells of the gyrus dentatus (Figs. 2A, B, C, 3C, D), in the neurons of the hile (Fig. 3C), in the stratum oriens layer (Fig. 3D) as well as in the entorhinal cortex. The XK transcript was also observed in the thalamus, with moderate expression in the habenula, paraventricular and lateral mediodorsal nuclei (Figs. 2A, B) and a more pronounced expression in sensory thalamic nuclei, i.e. in the ventroposterior medial (VPM) and ventroposterior lateral (VPL) or the medial geniculate (MG) nuclei (Figs. 2B, 2C, 3E). The hypothalamus expressed high levels of *XK*, with for instance, strong labeling of the dorsomedial and ventromedial nuclei. In the basal forebrain,

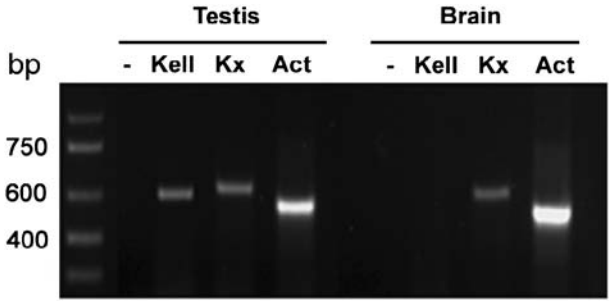


**Fig. 1 – RT-PCR analysis of Kell and XK expression in adult mouse brain and testis. RT-PCR experiments were performed using specific primers designed to amplify fragments of either 600 bp for Kell or 637 bp for XK. Negative control amplifications without cDNA are displayed in lanes 1 and 5 (-), Kell-specific amplifications are in 2 and 6 (Kell) while XK amplicons are observed both in lanes 3 and 7 (Kx). Positive actin control amplifications (Act) were electrophoresed in 4 and 8.**