Biochemistry and Biophysics Reports 10 (2017) 215–223

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Structure, organization and tissue expression of the pig *SLC13A1* and *SLC13A4* sulfate transporter genes

Samuel K. Barnes[a], Yvonne A. Eiby[b], Soohyun Lee[a], Barbara E. Lingwood[b], Paul A. Dawson[a,*]

[a] Mater Research Institute, The University of Queensland, Woolloongabba, Queensland, Australia
[b] UQ Centre for Clinical Research, The University of Queensland, Brisbane, Queensland, Australia

ARTICLE INFO

Keywords:
Sulfate
Sus scrofa
Pig
Gene
Transporter
Placenta
Kidney

ABSTRACT

Sulfate is an obligate nutrient for fetal growth and development. In mice, the renal Slc13a1 sulfate transporter maintains high maternal circulating levels of sulfate in pregnancy, and the placental Slc13a4 sulfate transporter mediates sulfate supply to the fetus. Both of these genes have been linked to severe embryonal defects and fetal loss in mice. However, the clinical significance of *SLC13A1* and *SLC13A4* in human gestation is unknown. One approach towards understanding the potential involvement of these genes in human fetal pathologies is to use an animal model, such as the pig, which mimics the developmental trajectory of the human fetus more closely than the previously studied mouse models. In this study, we determined the tissue distribution of pig *SLC13A1* and *SLC13A4* mRNA, and compared the gene, cDNA and protein sequences of the pig, human and mouse homologues. Pig *SLC13A1* mRNA was expressed in the ileum and kidney, whereas pig *SLC13A4* mRNA was expressed in the placenta, choroid plexus and eye, which is similar to the tissue distribution in human and mouse. The pig *SLC13A1* gene contains 15 exons spread over 76 kb on chromosome 8, and encodes a protein of 594 amino acids that shares 90% and 85% identity with the human and mouse homologues, respectively. The pig *SLC13A4* gene is located approximately 11 Mb from *SLC13A1* on chromosome 8, and contains 16 exons spanning approximately 70 kb. The pig SLC13A4 protein contains 626 amino acids that share 91% and 90% identity with human and mouse homologues, respectively. The 5'-flanking region of *SLC13A1* contains several putative transcription factor binding sites, including GATA-1, GATA-3, Oct1 and TATA-box consensus sequences, which are conserved in the homologous human and mouse sequences. The 5'-flanking sequence of *SLC13A4* contains multiple putative transcription factor consensus sites, including GATA-1, TATA-box and Vitamin D responsive elements. This is the first report to define the tissue distribution of pig *SLC13A1* and *SLC13A4* mRNAs, and compare the gene, cDNA, 5'-flanking region and protein sequences to human and mouse.

1. Introduction

Sulfate is an important nutrient for numerous cellular and metabolic processes in human physiology [1]. Sulfate conjugation (sulfonation) to steroids and thyroid hormone leads to their inactivation by preventing their binding to receptors [2]. Sulfonation also plays an important role in the detoxification and urinary elimination of xenobiotics and some pharmacological agents [3,4]. In addition, the sulfate content of proteoglycans, such as heparan sulfate and chondroitin sulfate, is important for sequestering growth factors (e.g. VEGF) which contributes to regulating tissue growth and development [5,6]. Importantly, sufficient circulating levels of sulfate need to be maintained for sulfonation reactions to function effectively and to achieve the required biological balance of sulfonated to unconjugated substrates [7].

In humans and mice, inorganic sulfate is absorbed in the ileum and then maintained at abundant levels in circulation (human 0.3 mmol/L, mice 1.0 mmol/L) by the kidneys [8]. The solute linked carrier 13A1 (SLC13A1) sulfate transporter is expressed in the ileum and kidney where it mediates sulfate absorption and reabsorption, respectively [9]. During mouse pregnancy, increased *Slc13a1* mRNA expression in the kidney leads to increased maternal plasma sulfate levels that peak (2-fold increase) in third trimester when fetal growth and sulfate demands are high [10]. In human gestation, maternal plasma sulfate level also increases approximately 2-fold, suggesting that the physiological requirement for high circulating sulfate level in pregnancy is conserved across species [11]. A related sulfate transporter, SLC13A4, is expressed in the placenta where it mediates sulfate transfer to the developing fetus [10,12]. The physiological importance of maintaining high maternal plasma sulfate levels via SLC13A1, as well as placental sulfate transfer via SLC13A4, has been highlighted with findings of late-

* Corresponding author.
E-mail address: paul.dawson@mater.uq.edu.au (P.A. Dawson).

http://dx.doi.org/10.1016/j.bbrep.2017.04.005
Received 8 November 2016; Received in revised form 27 February 2017; Accepted 12 April 2017
Available online 13 April 2017

gestational fetal demise in *Slc13a1* and *Slc13a4* knockout mice [13–15].

The lethal consequence of targeted *Slc13a1* and *Slc13a4* disruption on mouse development has potential clinical relevance to fetal development in human gestation. Both genes are highly conserved (> 80% identity) and have similar tissue expression patterns in mice and humans [16,17]. In addition, loss-of-function mutations in human *SLC13A1* lead to renal sulfate wasting and reduced plasma sulfate levels [18,19], as found in the *Slc13a1* null mouse [13]. Over the past decade, several studies on the *Slc13a1* and *Slc13a4* null mice have provided valuable insights into the roles of these genes, particularly their obligate requirement for supplying sulfate from mother to fetus [13–15]. However, the importance of sulfate in human pregnancy is underappreciated and is not routinely measured in clinical settings [9]. In addition, dietary sulfate intake during pregnancy is not usually considered, despite evidence that diet can impact on circulating sulfate levels and sulfonation capacity [8]. Accordingly, further studies are warranted to investigate the potential pathogenetic involvement of *SLC13A1* and *SLC13A4* in human gestation, as well as the consequences of reduced sulfate levels in mother and child.

One approach towards investigating the potential clinical relevance of reduced sulfate availability to the fetus, as a consequence of disrupted *SLC13A1* or *SLC13A4* function, is to use a preclinical animal model such as the pig that mimics certain aspects of human gestation more closely than the mouse models [13,15,20]. For example, the pig fetus has a similar size, organ architecture and tissue developmental trajectory when compared to the human fetus, and its gestational period (115 days) is closer to the length of human gestation (259–280 days) than that of the mouse (19–21 days) [21,22]. However, before we consider any studies to investigate sulfate biology in the pig fetus, we first need to determine whether the tissue expression profiles and gene structures of pig *SLC13A1* and *SLC13A4* are conserved with human and mouse homologues.

In this study, we report the gene, cDNA and protein sequences of pig *SLC13A1* and *SLC13A4*, and compare those to the human and mouse homologues. Our study also reports the tissue distribution of *SLC13A1* and *SLC13A4* mRNA expression in several pig tissues, as well as putative response elements in the 5'-flanking regions of these genes.

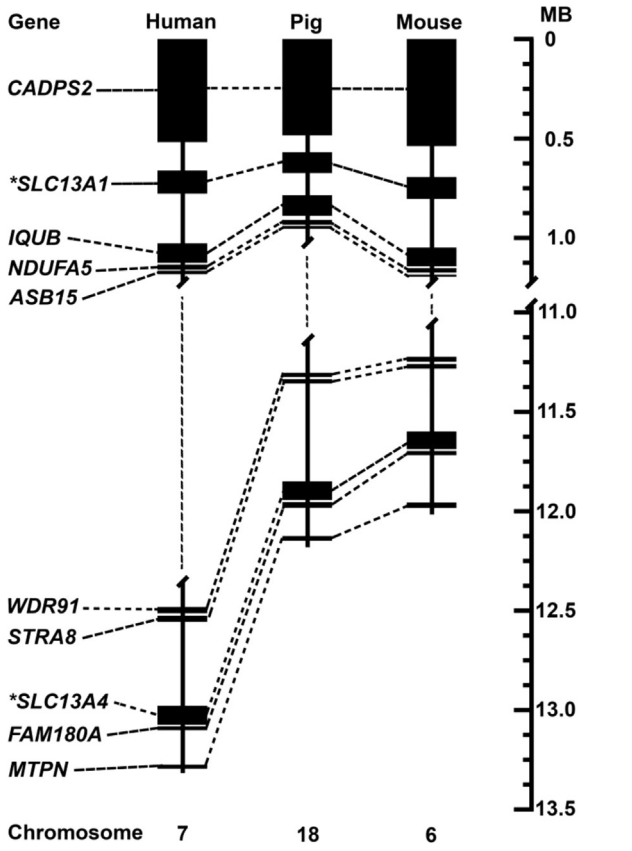

Fig. 1. Pig *SLC13A1* and *SLC13A4* chromosomal localization. *Comparative locations of *SLC13A1* and *SLC13A4* on pig chromosome 18 (assembly Sscrofa10.2, NC_010460.3), human chromosome 7 (assembly GRCh38.p7, NC_000007.14) and mouse chromosome 6 (assembly GRCm38.p4, NC_000072.6). Also shown are those genes surrounding *SLC13A1* (*CADPS2*, *IQUB*, *NDUFA5*, *ASB15*) and *SLC13A4* (*WDR91*, *STRA8*, *FAM180A*, *MTPN*).

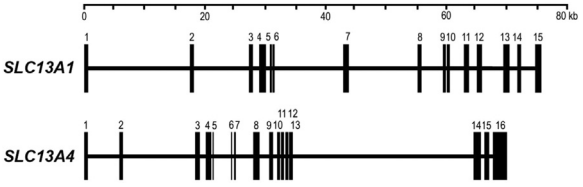

Fig. 2. Pig *SLC13A1* and *SLC13A4* gene structures. Exon-intron organization showing exons (vertical lines) and introns (horizontal lines) spread over approximately 76 kb (*SLC13A1*) and 70 kb (*SLC13A4*).

2. Materials and methods

2.1. Gene, cDNA and protein sequences

We undertook a search of the NCBI Gene, Nucleotide and Protein databases (https://www.ncbi.nlm.nih.gov/) using the terms "*SLC13A1*" or "*SLC13A4*", and "*Sus scrofa*" within the date range of 1 April 2016 to 1 May 2016. To determine the nucleotide sequences of intron/exon junctions, transcription initiation start sites, and the 5'-flanking regions, we aligned the curated pig *SLC13A1* (XM_013985680.1) and *SLC13A4* (XM_003134643.3) mRNA sequences with pig genome sequence (NC_010460.3). Putative transcription factor binding motifs within the first 1000 nucleotides of the 5'-flanking regions of pig *SLC13A1* and *SLC13A4* were identified using MatInspector software [23], and compared to the published *SLC13A1* and *SLC13A4* gene promoter findings for human and mouse [16,24–26]. Amino acid sequences of pig SLC13A1 (XP_013841134.1) and SLC13A4 (XP_003134691.1) were aligned to human and mouse homologue proteins using ClustalW software [27]. Potential transmembrane domains (TMDs), protein kinase A, protein kinase C, casein kinase II and N-glycosylation sites were identified based on conserved amino acid sequences in the homologous proteins of human and mouse [16,24,26,28].

2.2. Animals, tissues and RNA isolation

Large White/Landrace cross sows were fed water ad libitum and standard pig feeds: Riverina Pig Grower for non-pregnant sows, and Riverina Pig Breeder for pregnant sows. The levels of total protein (16.00%) and methionine (RPG 0.22%; RPB 0.20%), as well as the sulfate salts of zinc (120 mg/kg), manganese (45 mg/kg), iron (100 mg/kg) and copper (10 mg/kg) were similar between both diets (Riverina Stock Feeds, Australia). Approximate body masses of non-pregnant and pregnant sows were 80 and 250 kg, respectively. Three non-pregnant sows were selected at 16 weeks of age to be euthanized for collection of kidney, heart, skin, ileum, lung, spleen, muscle, liver, ovary, uterus, eye, and dissected brain regions (frontal lobe and choroid plexus). Two additional pregnant sows at approximately 2 years of age were selected for collection of placental tissue at 98 days gestation (term = 115 days). All procedures were approved by the University of Queensland Animal Ethics Committee. Total RNA was isolated from each tissue using TRIzol® reagent according to the manufacturer's protocol (Invitrogen). First strand cDNA was generated using 2 μg of DNase I treated RNA and a Transcriptor cDNA Synthesis Kit (Roche).