The homo-sapiens solute carrier family 2 member 14 (*SLC2A14*) – further insight into the genomic organization, protein isoforms, substrates and kinetics

By

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Overall Abstract

The *SLC2A14* gene encodes for GLUT14, a novel glucose and dehydroascorbic acid facilitative solute carrier. The literature record on *SLC2A14* and its encoded products are very limited. To date, two different transcripts encoding for two protein isoforms had been described. Genetic variations in *SLC2A14* are associated with chronic diseases such as Alzheimer's disease and Inflammatory Bowel Disease. However, disease mechanism concerning glucose metabolism are undetermined. To investigate disease mechanism, all genomic transcripts and protein isoforms need to be annotated. Our objectives were to describe the *SLC2A14* locus and its encoded products systematically using recent bioinformatics resources and link the gained knowledge to potential disease pathology. Three manuscripts were derived.

In manuscript 1, novel transcripts and individual protein isoforms were identified and annotated. A much more comprehensive list of tissues expressing SLC2A14 was created. All protein isoforms were heterologous expressed in human enterocyte cells to determine subcellular localization to the plasmalemma membrane.

In manuscript 2, the substrate spectrum and kinetics were determined for protein isoform GLUT14A.

In manuscript 3, *SLC2A14's* association to inflammatory bowel diseases was linked to the modulation of innate immune cells based on the expression evidence in intestinal cell types.

The project establishes the core *SLC2A14* biology. This will form the basis to further investigate the disease mechanism associated with *SLC2A14* genetic and genomic variability.

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Overall Introduction and Literature Review

Introduction and literature review

Glucose is a major substrate for energy production in mammalian cells. However, glucose molecules cannot cross the lipid membrane of the cell by simple diffusion due to its polar nature and large size. As Glucose homeostasis is fundamental for human health, so are the solute carriers mediating glucose transmembrane transport. Thus, expanding the knowledge of glucose transporters biochemistry and physiology contributes to a better understanding of whole-body glucose homeostasis under normal and disease conditions.

The significance of glucose transporters is apparent, as they regulate tissue-specific glucose uptake. Most cells express a variety of glucose transporters and the pattern of expression in different tissues is related to specific metabolic requirements (Shah, 2012). Moreover, the roles of glucose transporters had become a focus as therapeutic targets for dietary interventions for various diseases such as diabetes and cancers (Navale, 2016).

Two main types of glucose membrane transporters have been identified: Sodium-dependent glucose cotransporters (SGLTs, encoded by genes of the *SLC5A* family) and facilitated diffusion glucose transporters (GLUTs, encoded by genes of the *SLC2A* family) (Navale,2016). On the basis of sequence similarities, 14 members in this family are divided into three classes. Class I includes GLUT1, GLUT2, GLUT3, GLUT4, and GLUT14. Class II includes GLUT5, GLUT7, GLUT9, and GLUT11. Class III is made up of GLUT6, GLUT8, GLUT10, GLUT12, and GLUT13 (Augustin, 2010).

Although these membrane carrier proteins are called glucose transporters, they are involved in the transport of several different molecules, not just glucose (Augustin, 2010). Substrates other than glucose, include fructose, galactose, mannose, xylose and dehydroascorbate. However, the spectra of substrates for many of the 14 GLUTs are still not characterized (Thorens, 2010).

GLUT14, encoded by the *SLC2A14* gene, was the latest GLUT family member identified. This is owed to the fact that *SLC2A14* is a gene duplication of *SLC2A3* specific to humans and closely

related primates (Wu and Freeze, 2002). This initial report described exclusive expression in testicular tissue and identified two alternatively spliced variants, resulting in two protein isoforms (Wu and Freeze, 2002). The shorter form GLUT14-S is encoded by 10 exons and translates into a 497-amino-acid protein that was described as 94.5% identical to GLUT3. A long isoform GLUT14-L utilized an additional 5' exon and codes for a protein of 520 amino acids that differs from GLUT14-S only at the N-terminus (Wu and Freeze, 2002).

Subsequently, it was demonstrated that both protein isoforms are targeted to the plasmalemma membrane (Amir Shaghaghi, Murph, et al., 2016). Moreover, eight additional exons were identified for the *SLC2A14* locus, but a detailed description of resulting splice variants and protein isoforms are still not published.

Moreover, additional sites of expression were located to eleven tissues which are colon, lung, ovary, blood cells, brain, skeletal muscle, heart, small intestine, kidney, lymph node, and liver (Amir Shaghaghi, Zhouyao, et al., 2016; Wu and Freeze, 2002). However, to date, the tissue expression across human tissues is not completely examined.

Significantly, GLUT14-L and GLUT14-S mediated transmembrane transport of glucose and dehydroascorbic acid (Amir Shaghaghi, Zhouyao, et al., 2017), establishing multi-specificity. However, no additional substrates had been tested. Based on the substrate spectrum of the closely related GLUT3 (Wu and Freeze, 2002), which mediates the transmembrane transport of glucose, galactose, mannose, xylose and dehydroascorbic acid (Deng, 2015; Seatter, 1998), there is a high likelihood that other hexoses or hexose-like molecules are also accepted by GLUT14.

Several reports link *SLC2A14* gene expression to disease states. *SLC2A14* is differential expressed in acute lymphoblastic leukemia (Taylor, Pena-Hernandez, et al., 2007), blastocysts development (Adjaye, Herwig, et al., 2007), drug resistant ovarian cancer cells (Januchowski, Zawierucha, et al., 2013, Januchowski, Zawierucha, et al., 2014), a specific genotype associated with Parkinson's Disease (Infante, Prieto, et al., 2015), specific subtypes of gastric adenocarcinoma (Berlth, Mönig, et al., 2015), poor survival outcomes in papillary thyroid carcinoma (Chai, Yi, et al., 2017), radiation-induced human skin fibrosis (Song, Zhang, et al., 2018), adaption of colon cancer cells to hypoxia (Valli, Morotti, et al., 2019), and Glioblastoma (Sharpe, 2021) (**Figure 1**). However, all but one of the reports on the differential regulation are derived from single studies, and await

future replication, which will be necessary to elucidate on the mechanism linking the disease states to *SLC2A14* expression.

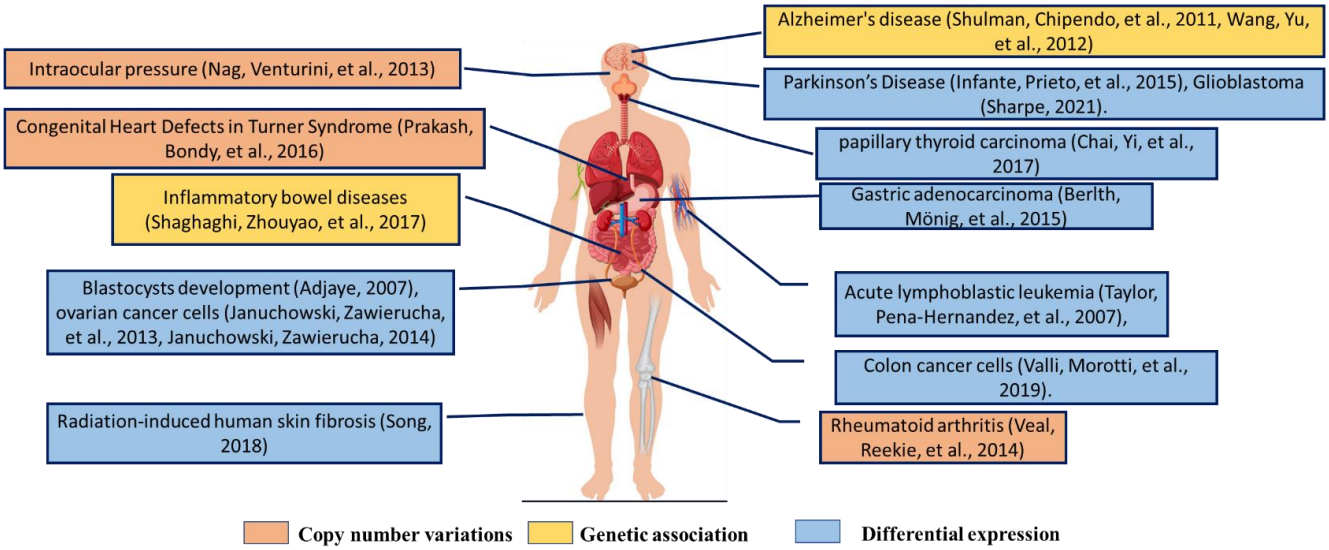


Figure 1: Associations between *SLC2A14* and disease states in different tissues.

Individual *SLC2A14* single nucleotide polymorphisms (SNPs) had been associated with Alzheimer's disease (Shulman, Chipendo, et al., 2011, Wang, Yu, et al., 2012), and inflammatory bowel diseases (Shaghaghi, Zhouyao, et al., 2017).

In addition, copy number variations (CNVs) associated with intraocular pressure (Nag, Venturini, et al., 2013), rheumatoid arthritis (Veal, Reekie, et al., 2014), and left-sided congenital heart lesions in patients with Turner Syndrome (Prakash, Bondy, et al., 2016).

The high similarities between GLUT14 and the paralogous parent GLUT3 warrants attention. GLUT3 was first identified in human in 1988 (Long, 2015). It has one isoform which is a 496 amino acid protein. Although GLUT3 is considered as a neuron-specific glucose transporter specifically in the brain, it is also expressed in tissues with high demand for glucose such as testis, placenta (Augustin, 2010). It is a class I protein with a moderate affinity for glucose of (Km ~1.5 mM) (Thorens, 2010). It can also transport galactose, mannose, maltose, xylose, and dehydroascorbic acid, but not fructose (Deng, 2015; Seatter, 1998). Comparing GLUT3 with other class I transporters, many studies has shown that it has 64.4% and 51.6% identity with GLUT1 and GLUT2, respectively (Long, 2015; Vrhovac, 2014; Simpson, 2008).

Thus far, a few common features of GLUT14 with GLUT3 have emerged. Both mediate the transmembrane transport of glucose, galactose and dehydroascorbic acid and are highly express in testis (Sharpe, 2021; Shaghaghi, Zhouyao, et al., 2017, Wu and Freeze, 2002). However, more detailed analyses are needed on the commonalities and distinctions between GLUT14 with GLUT3 in order to extrapolate on both transporters' contributions to physiology and diseases.

To conclude, the basic information on the *SLC2A14* gene and its encoded products remains inadequate. Moreover, the reports on *SLC2A14* differential expression and genetic associations appear to be spurious. In addition, the similarities and differences between GLUT14 and GLUT3 in the human body are unclear.

Knowledge gaps

The literature and database review indicates the following major knowledge gaps:

1. Exon utilization, splice variants, and encoded protein isoforms are only partially characterized for the *SLC2A14* locus.
2. The full complement of tissue expression is not fully analyzed or published.
3. Additional GLUT14 protein isoforms remain to be described.
4. The subcellular location of the uncharacterized GLUT14 protein isoforms is unknown.
5. The full complement of substrates and kinetics of all GLUT14 protein isoforms are unknown.
6. The similarities and differences between GLUT14 and GLUT3 remain to be described.

Hypotheses

GLUT14/*SLC2A14* encodes more protein isoforms instead of two isoforms mediating glucose transport across the plasmalemma membrane and other substrates.

Objectives

In order to prove this hypothesis, we address the following objectives:

1. Update the knowledge on the genomic locus, specifically the different splice variants translating into distinct protein isoforms.
2. Update the knowledge on the tissue expression.

3. Subclone the individual Open Reading Frames for the transcripts encoding individual protein isoforms.
4. Determine the subcellular localization of the individual protein isoforms.
5. Determine the substrates uptake and kinetics of the individual protein isoforms.
6. The similarities and differences between GLUT14 and GLUT3 remain to be described.

References

Adjaye, J., R. Herwig, T.C. Brink, D. Herrmann, B. Greber, S. Sudheer, et al. 2007. Conserved molecular portraits of bovine and human blastocysts as a consequence of the transition from maternal to embryonic control of gene expression. Physiological Genomics 31: 315-327. doi:10.1152/physiolgenomics.00041.2007.

Amir Shaghaghi, M.A., N. Yurkova, H. Tu, M.A. Levine and P. Eck. 2013. Identification and functional characterization of an alternative 5' exon of the sodium dependent ascorbic acid transporter SLC23A1. Journal of Human Nutrition and Food Sciences 1.

Amir Shaghaghi, M., B. Murphy and P. Eck. 2016. The SLC2A14 gene: Genomic locus, tissue expression, splice variants, and subcellular localization of the protein. Biochemistry and Cell Biology 94: 331-335. doi:10.1139/bcb-2015-0089.

Amir Shaghaghi, M.A., B. Murph and P. Eck. 2016. The SLC2A14 gene: genomic locus, tissue expression, splice variants, and subcellular localization of the protein. Biochemistry and Cell Biology. doi:10.1139/bcb-2015-0165.

Amir Shaghaghi, M., H. Zhouyao, H. Tu, H. El-Gabalawy, G.H. Crow, M. Levine, et al. 2017. The SLC2A14 gene, encoding the novel glucose/dehydroascorbate transporter GLUT14, is associated with inflammatory bowel disease. The American Journal of Clinical Nutrition. doi:10.3945/ajcn.116.147603.

Augustin, R. (2010). The protein family of glucose transport facilitators: It's not only about glucose after all. *IUBMB life*, *62*(5), 315-333.

Berlth, F., S. Mönig, B. Pinther, P. Grimminger, M. Maus, H. Schlösser, et al. 2015. Both GLUT-1 and GLUT-14 are Independent Prognostic Factors in Gastric Adenocarcinoma. Annals of Surgical Oncology 22: 822-831. doi:10.1245/s10434-015-4730-x.

Chai, Y.J., J.W. Yi, S.W. Oh, Y.A. Kim, K.H. Yi, J.H. Kim, et al. 2017. Upregulation of SLC2 (GLUT) family genes is related to poor survival outcomes in papillary thyroid carcinoma: Analysis of data from The Cancer Genome Atlas. Surgery (United States) 161: 188-194. doi:10.1016/j.surg.2016.04.050.

Corpe, C.P., P. Eck, J. Wang, H. Al-Hasani and M. Levine. 2013. Intestinal Dehydroascorbic acid (DHA) transport mediated by the facilitative sugar transporters, GLUT2 and GLUT8. Journal of Biological Chemistry. doi:10.1074/jbc.M112.436790.

Corpe, C.P., J.H. Lee, O. Kwon, P. Eck, J. Narayanan, K.L. Kirk, et al. 2005. 6-Bromo-6-deoxy-L-ascorbic acid: An ascorbate analog specific for Na +-dependent vitamin C transporter but not glucose transporter pathways. Journal of Biological Chemistry 280: 5211-5220.

Corpe, C.P., H. Tu, P. Eck, J. Wang, R. Faulhaber-Walter, J. Schnermann, et al. 2010. Vitamin C transporter Slc23a1 links renal reabsorption, vitamin C tissue accumulation, and perinatal survival in mice. Journal of Clinical Investigation 120: 1069-1083.

Deng, D., Sun, P., Yan, C., Ke, M., Jiang, X., Xiong, L., ... & Yan, N. (2015). Molecular basis of ligand recognition and transport by glucose transporters. Nature, 526(7573), 391-396.

Eck, P., H.C. Erichsen, J.G. Taylor, C. Corpe, S.J. Chanock and M. Levine. 2007. Genomic and functional analysis of the sodium-dependent vitamin C transporter SLC23A1-SVCT1. Genes and Nutrition 2: 143-145.

Eck, P., O. Kwon, S. Chen, O. Mian and M. Levine. 2013. The human sodium-dependent ascorbic acid transporters SLC23A1 and SLC23A2 do not mediate ascorbic acid release in the proximal renal epithelial cell. Physiological Reports 1: e00136. doi:10.1002/phy2.136.

Infante, J., C. Prieto, M. Sierra, P. Sánchez-Juan, I. González-Aramburu, C. Sánchez-Quintana, et al. 2015. Identification of candidate genes for Parkinson's disease through blood transcriptome analysis in LRRK2-G2019S carriers, idiopathic cases, and controls. Neurobiology of Aging 36: 1105-1109. doi:10.1016/j.neurobiolaging.2014.10.039.

Januchowski, R., P. Zawierucha, M. Andrzejewska, M. Ruciński and M. Zabel. 2013. Microarray-based detection and expression analysis of ABC and SLC transporters in drug-resistant ovarian cancer cell lines. Biomedicine and Pharmacotherapy 67: 240-245. doi:10.1016/j.biopha.2012.11.011.

Januchowski, R., P. Zawierucha, M. Ruciński, M. Andrzejewska, K. Wojtowicz, M. Nowicki, et al. 2014. Drug transporter expression profiling in chemoresistant variants of the A2780 ovarian cancer cell line. Biomedicine and Pharmacotherapy 68: 447-453. doi:10.1016/j.biopha.2014.02.002.

Kwon, O., P. Eck, S.L. Chen, C.P. Corpe, J.H. Lee, M. Kruhlak, et al. 2007. Inhibition of the intestinal glucose transporter GLUT2 by flavonoids. Faseb Journal 21: 366-377. doi:10.1096/fj.06-6620com.

Long, W., & Cheeseman, C. I. (2015). Structure of, and functional insight into the GLUT family of membrane transporters. *Cell Health and Cytoskeleton*, *7*, 167.

M, A.S., Z. H, T. H, B. C, E.-G. H, C. G, et al. 2017. The SLC2A14 gene, encoding the novel dehydroascorbic acid/glucose transporter GLUT14, is associated with inflammatory bowel disease. American Journal of Clinical Nutrition AJCN/2016/147603.

Nag, A., C. Venturini, P.G. Hysi, M. Arno, E. Aldecoa-Otalora Astarloa, S. Macgregor, et al. 2013. Copy number variation at chromosome 5q21.2 is associated with intraocular pressure. Investigative ophthalmology & visual science 54: 3607-3612. doi:10.1167/iovs.13-11952.

Navale, A. M., & Paranjape, A. N. (2016). Glucose transporters: physiological and pathological roles. *Biophysical reviews*, *8*(1), 5-9.

Prakash, S.K., C.A. Bondy, C.L. Maslen, M. Silberbach, A.E. Lin, L. Perrone, et al. 2016. Autosomal and X chromosome structural variants are associated with congenital heart defects in Turner syndrome: The NHLBI GenTAC registry. American Journal of Medical Genetics, Part A 170: 3157-3164. doi:10.1002/ajmg.a.37953.

Seatter, M. J., De La Rue, S. A., Porter, L. M., & Gould, G. W. (1998). QLS motif in transmembrane helix VII of the glucose transporter family interacts with the C-1 position of D-glucose and is involved in substrate selection at the exofacial binding site. Biochemistry, 37(5), 1322-1326.

Shaghaghi, M.A., H. Zhouyao, H. Tu, H. El-Gabalawy, G.H. Crow, M. Levine, et al. 2017. The SLC2A14 gene, encoding the novel glucose/dehydroascorbate transporter GLUT14, is

associated with inflammatory bowel disease. American Journal of Clinical Nutrition 106: 1508-1513. doi:10.3945/ajcn.116.147603.

Shah, K., DeSilva, S., & Abbruscato, T. (2012). The role of glucose transporters in brain disease: diabetes and Alzheimer's disease. *International journal of molecular sciences*, *13*(10), 12629-12655.

Sharpe, M. A., Ijare, O. B., Baskin, D. S., Baskin, A. M., Baskin, B. N., & Pichumani, K. (2021). The Leloir Cycle in Glioblastoma: Galactose Scavenging and Metabolic Remodeling. Cancers, 13(8), 1815.

Shulman, J.M., P. Chipendo, L.B. Chibnik, C. Aubin, D. Tran, B.T. Keenan, et al. 2011. Functional screening of alzheimer pathology genome-wide association signals in drosophila. American Journal of Human Genetics 88: 232-238. doi:10.1016/j.ajhg.2011.01.006.

Simpson, I. A., Dwyer, D., Malide, D., Moley, K. H., Travis, A., & Vannucci, S. J. (2008). The facilitative glucose transporter GLUT3: 20 years of distinction. *American Journal of Physiology-Endocrinology and Metabolism*, *295*(2), E242-E253.

Song, J., O. Kwon, S. Chen, R. Daruwala, P. Eck, J.B. Park, et al. 2002. Flavonoid inhibition of sodium-dependent vitamin C transporter 1 (SVCT1) and glucose transporter isoform 2 (GLUT2), intestinal transporters for vitamin C and glucose. Journal of Biological Chemistry 277: 15252-15260.

Song, J., H. Zhang, Z. Wang, W. Xu, L. Zhong, J. Cao, et al. 2018. The Role of FABP5 in Radiation-Induced Human Skin Fibrosis. Radiation Research 189: 177-186. doi:10.1667/RR14901.1.

Taylor, K.H., K.E. Pena-Hernandez, J.W. Davis, G.L. Arthur, D.J. Duff, H. Shi, et al. 2007. Large-scale CpG methylation analysis identifies novel candidate genes and reveals methylation hotspots in acute lymphoblastic leukemia. Cancer Research 67: 2617-2625. doi:10.1158/0008-5472.CAN-06-3993.

Thorens, B., & Mueckler, M. (2010). Glucose transporters in the 21st Century. American Journal of Physiology-Endocrinology and Metabolism, 298(2), E141-E145.

Valli, A., M. Morotti, C.E. Zois, P.K. Albers, T. Soga, K. Feldinger, et al. 2019. Adaptation to HIF1a deletion in hypoxic cancer cells by upregulation of GLUT14 and creatine metabolism. Mol Cancer Res 17: 1531-1544. doi:10.1158/1541-7786.MCR-18-0315.

Veal, C.D., K.E. Reekie, J.C. Lorentzen, P.K. Gregersen, L. Padyukov and A.J. Brookes. 2014. A 129-kb deletion on chromosome 12 confers substantial protection against rheumatoid arthritis, implicating the gene SLC2A3. Hum Mutat 35: 248-256. doi:10.1002/humu.22471.

Vrhovac, I., Breljak, D., & SABOLIĆ, I. (2014). Glucose transporters in the mammalian blood cells. *Periodicum biologorum*, *116*(2), 131-138.

Wang, W., J.T. Yu, W. Zhang, W.Z. Cui, Z.C. Wu, Q. Zhang, et al. 2012. Genetic association of SLC2A14 polymorphism with Alzheimer's disease in a Han Chinese population. Journal of Molecular Neuroscience 47: 481-484. doi:10.1007/s12031-012-9748-y.

Wu, X. and H.H. Freeze. 2003. Erratum: GLUT14, a duplicon of GLUT3, is specifically expressed in testis as alternative splice forms (Genomics (2002) 80 (553-557) PII: S0888754302970102. Genomics 81: 347. doi:10.1016/S0888-7543(03)00033-8.

Wu, X.H. and H.H. Freeze. 2002. GLUT14, a duplicon of GLUT3, is specifically expressed in testis as alternative splice forms. Genomics 80: 553-557. doi:DOI 10.1006/geno.2002.7010.

Xu, H., P.K. Eck, K.E. Baidoo, P.L. Choyke and M.W. Brechbiel. 2009. Toward preparation of antibody-based imaging probe libraries for dual-modality positron emission tomography and fluorescence imaging. Bioorgan Med Chem 17: 5176-5181. doi:DOI 10.1016/j.bmc.2009.05.048.

Transition Statement to Manuscript 1

Up to date, published records have shown incomplete knowledge of *SLC2A14*. In manuscript 1, public databases/resources were re-evaluated to refine the knowledge on the genomic locus, its derived transcripts, protein isoforms, and tissues of expression.

Manuscript 1

Scientific report aimed to be submitted to the Journal Biochemistry and Cell Biology (NRC press).

SLC2A14: The Complement of Transcripts and Protein Isoforms

Abstract

To date, published records have shown incomplete knowledge of the *SLC2A14*. After the reanalysis of publicly available transcripts, six differentially spiced transcripts were identified encoding four protein isoforms. However, we report five protein isoforms, of which one (isoform E, encoded by EST DA225116) is currently not identified in public databases and therefore has no refence protein ID. This record enhances the knowledge of the full complement of *SLC2A14* transcripts, protein isoforms and tissue expression, and the sub-cellular targeting of the protein isoforms by using a mix of bioinformatics and experimental tools.

Introduction

Publications on the *SLC2A14* gene and its disease associations are very limited and appear to be singleton records (Taylor, Pena-Hernandez, et al., 2007; Adjaye, Herwig, et al., 2007; Januchowski, Zawierucha, et al., 2013, Januchowski, Zawierucha, et al., 2014; Infante, Prieto, et al., 2015; Berlth, Mönig, et al., 2015; Chai, Yi, et al., 2017; Song, Zhang, et al., 2018; Valli, Morotti, et al., 2019; Sharpe, 2021; Shulman, Chipendo, et al., 2011; Wang, Yu, et al., 2012; Amir Shaghaghi, Zhouyao, et al., 2017; Nag, Venturini, et al., 2013; Veal, Reekie, et al., 2014; Prakash, Bondy, et al., 2016). It is imperative to possess the complete knowledge of the *SLC2A14* gene to evaluate the biology behind such associations. Unfortunately, published records on the *SLC2A14* gene are incomplete and can be improved through the exploration of data deposited in genetic and genomic databases.

There are discrepancies when comparing the published evidence on the *SLC2A14* genomic locus, its differentially spliced transcripts and encoded protein isoforms with the major genome browsers (NCBI, ENSEMBL and USCS). The most complete record described the *SLC2A14* genomic locus as covering 103,477 nucleotides on chromosome 12p13.31 and containing 20 exons, which is consistent with evidence depicted in the genome browsers (Amir Shaghaghi, Murphy, et al., 2016). Three major transcripts encoding for two protein isoforms (named A and B, in some records Long and Short) were thus far described to be derived from the locus (Amir Shaghaghi, Murphy, et al., 2016). However, the NCBI *SLC2A14* gene page (gene ID 144195) lists six differentially spliced transcripts encoding for four protein isoforms named A-D (**Table 1**).

It has already been established that protein isoforms A and B locate to the plasmalemma membrane and mediate the transmembrane transport of glucose and dehydroascorbic acid (Amir Shaghaghi,

Murphy, et al., 2016, Amir Shaghaghi, Zhouyao, et al., 2017). For protein isoform C and D no literature record exists. Using a mix of bioinformatics and experimental tool, this report aims to describe the full complement of differentially spliced transcripts and protein isoforms derived from the *SLC2A14* genomic locus.

Table 1: The differentially spliced transcripts and encoded protein isoforms listed to be derived form the *SLC2A14* gene in the NCBI database (gene ID 144195, https://www.ncbi.nlm.nih.gov/gene/144195, accessed on 12-23-2021)

GLUT14 Protein Isoform	Number of Transcript	Transcript ID → Protein Name
A (520)	2	NM_001286233 → NP_001273162 NM_153449 → NP_703150.1
B (497)	2	NM_001286234 → NP_001273163 NM_001286235 → NP_001273164
C (411)	1	NM_001286236 → NP_001273165
D (535)	1	NM_001286237 → NP_001273166

Highest *SLC2A14* expression was reported for testis; additional sites are reported for colon, small intestine, lung, ovaries, blood cells, brain, skeletal muscle, heart, kidney, placenta, and liver (Amir Shaghaghi, Murph, et al., 2016). Although only a limited number of tissues seem to express *SLC2A14* the complement of tissues had not been described in full. Utilizing recently deposited next generation sequencing data of human tissues the complement of tissues expressing *SLC2A14* can now be updated.

To conclude, this record aims to describe the full complement of *SLC2A14* transcripts, protein isoforms and tissue expression, as well as the sub-cellular targeting of the protein isoforms.

Material and Methods

Assemblies of the genomic region and identification of alternatively spliced transcripts

The genomic locus was annotated using bioinformatics as we have applied before. Briefly, the gene structure and differentially spliced transcripts were determined with Sequencher^{TM} software, using assemblies of all available transcripts from the NCBI full transcripts and expressed sequence tags databases (Amir Shaghaghi, Murph, et al., 2016, Amir Shaghaghi, Yurkova, et al., 2013).

Protein isoforms

Sequences for GLUT14 isoforms A-D were obtained from the NCBI database (**Table 1**). The sequence of an additional isoform was identified after the genome annotation and its sequence was translated in the Sequencher™ software. Protein isoforms were assembled using Clustal-Alignment (https://www.ebi.ac.uk/Tools/msa/clustalo/).

Gene expression

Thirty-six publicly available sources (mostly depositories or databases containing next generation sequencing data) were interrogated for *SLC2A14* tissue expression (**Supplemental Information Table 1**).

The research criterion was to obtain reliable sources for *SLC2A14* tissue expression in human. Each project was reanalyzed separately by using Microsoft Excel or Microsoft Word to determine the number of *SLC2A14* tissue expression. The obtained results were filtered so that the duplicate tissues were deleted.

Subcloning of coding regions of GLUT14 protein isoforms

Individual coding regions were subcloned after PCR amplification with Phusion® High-Fidelity DNA Polymerase (New England Biolabs, Ipswich, MA) as previously described (Amir Shaghaghi, Murph, et al., 2016, Amir Shaghaghi, Yurkova, et al., 2013). Briefly, amplification conditions and primers can be found in **Supplemental Information Table 2 and 3.** Amplicons were tagged with the Gateway flanking sites to allow subcloning using the Gateway (Thermo Fisher Scientific, Waltham, Massachusetts) system. Ultimately, individual coding regions were tagged or transferred into expression plasmids optimized for fluorescent imaging, using existing lab protocols (Amir Shaghaghi, Murph, et al., 2016, Amir Shaghaghi, Yurkova, et al., 2013, Corpe, Eck, et al., 2013, M, H, et al., 2017). The list of destination vectors used for the individual coding regions can be found in **Supplemental Information Table 4.**

Molecular imaging

Transgenic constructs encoding fluorescent tagged proteins were transfected into CaCo2 cells using PolyJet™ In Vitro DNA Transfection Reagent (SignaGen, Rockville, Maryland). Upon detection of fluorescence life cells were imaged using a Zeiss AxioVert 5 (Carl Zeiss, Jena, Germany) in the 24 well cell culture plate, as described before (Amir Shaghaghi, Yurkova, et al., 2013). Images were processed using Zeiss Axiovert Software (Carl Zeiss, Jena, Germany).

RESULTS

The SLC2A14 locus and alternatively spliced transcripts and protein isoforms

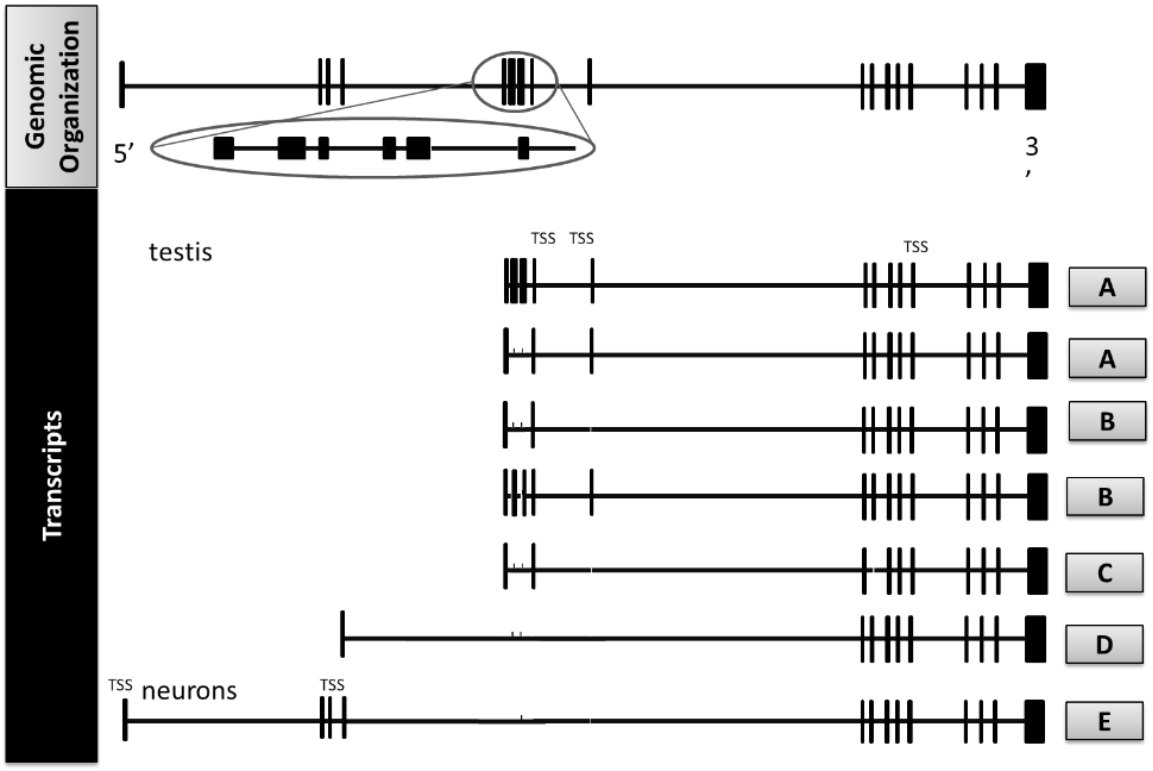
The reanalysis of publicly available transcripts confirms the information previously reported for the size of the *SCL2A14* locus, containing 20 exons (**Figure 1A**). However, seven differentially spiced transcripts were identified (**Figure 1B**). All but one is currently recognized and assigned a reference transcript ID. The EST DA225116 represents a differentially spliced full length transcript not identified as a reference transcript.

A total of five protein isoforms are encoded, of which one (isoform E, encoded by EST DA225116) is currently not identified in public databases and therefore has no refence protein ID (**Figure 1)**.

Protein isoforms

Clustal Alignments of the five GLUT14 protein isoforms illustrates variability of the N-terminal regions, but no discrepancies in all lateral sequences (**Figure 2**). GLUT14 C misses at least 100 amino acids compared to other isoforms, which show only slight distinctions of the N-terminus.

Panel A



Panel B

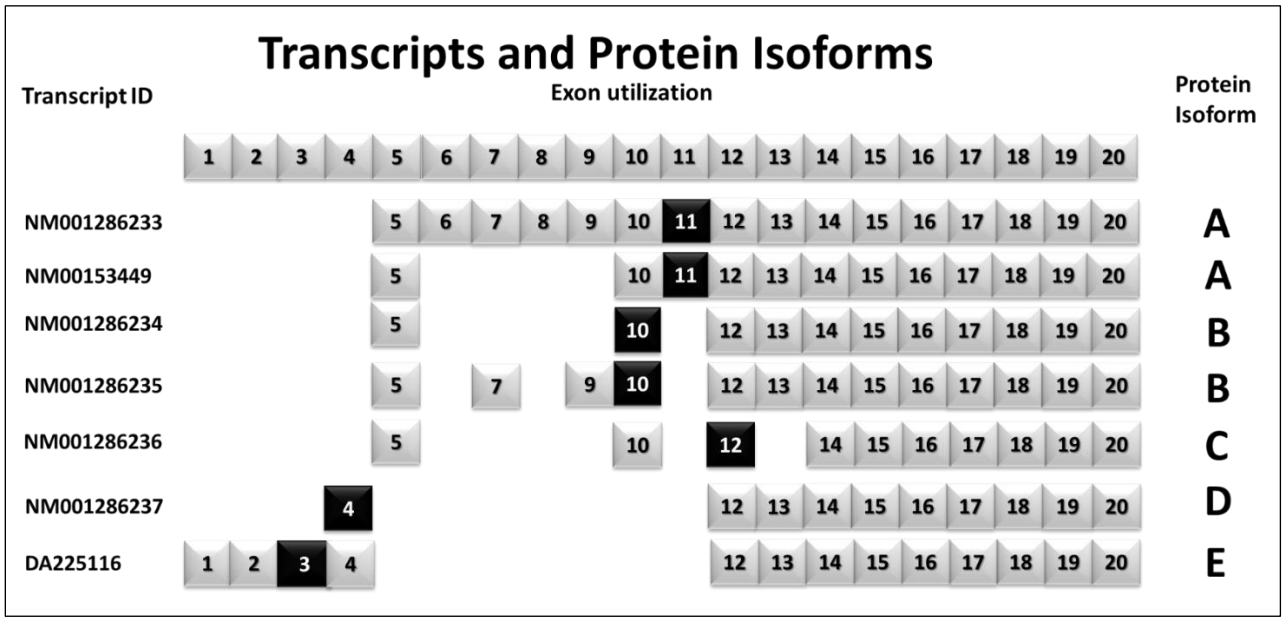


Figure 1A and B: *SLC2A14* genomic organization and the complement of differentially spliced transcripts. Panel A depict the architecture of the 20 exons utilized. Panel B illustrates the utilization of individual exons; exons containing the translational start site are marked in black.

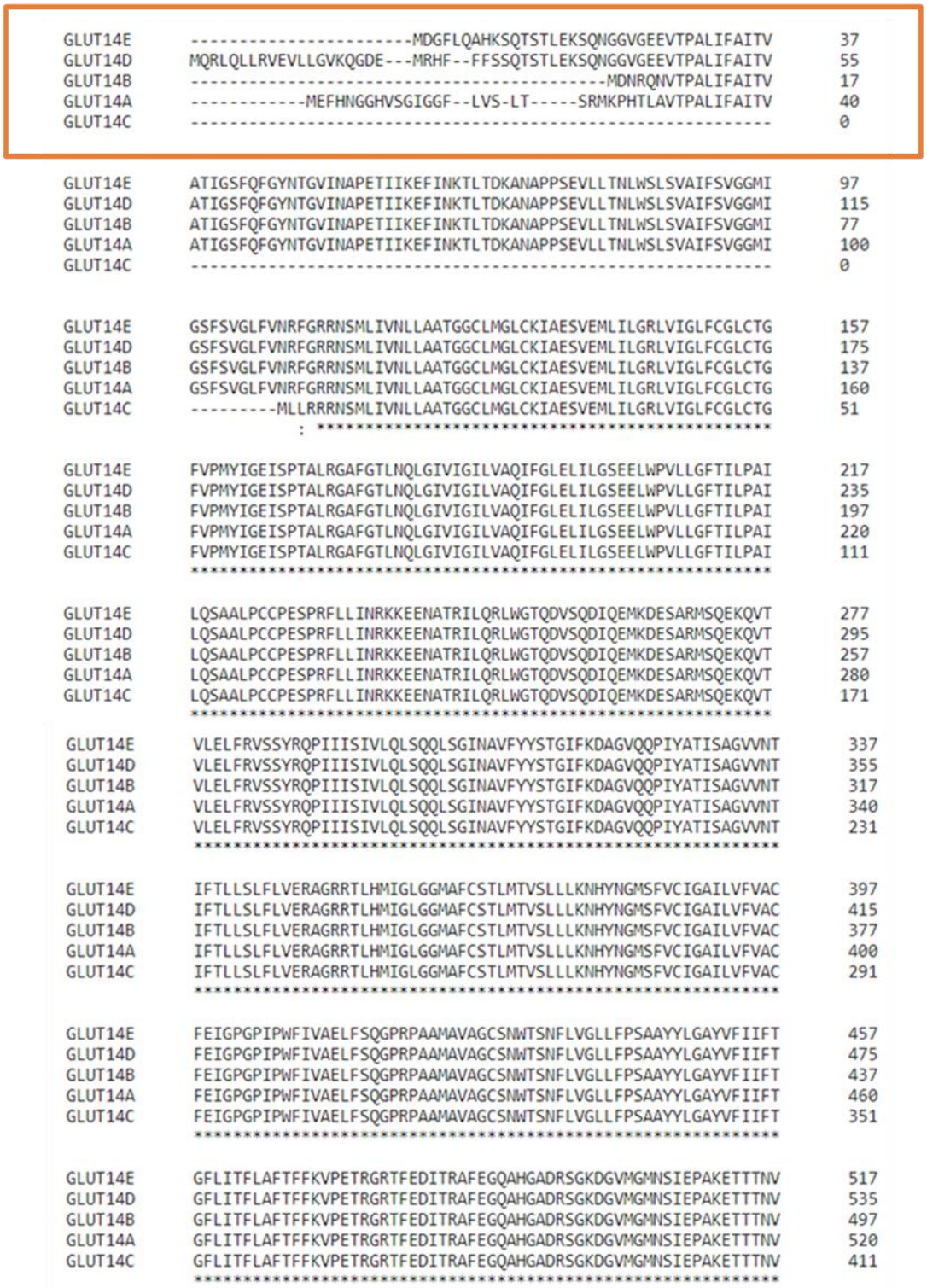


Figure 2: Clustal Alignments of the five GLUT14 protein isoforms indicates variations in the N-terminal region (https://www.ebi.ac.uk/Tools/msa/clustalo/)

Tissues expression

A total of 61 tissues were identified expressing various levels of *SLC2A14* (Table 1). Testis still stands as the tissue with the highest abundance, while skin, skeletal muscle, ovary, colon, and bone marrow also showed sizeable expression. Thirty-three tissues do not express *SLC2A14* while five tissues within the interrogated databases were not tested (**Supplemental Information Table 5**).

Table 1: List of tissues expressing *SLC2A14*.

Expression (61 tissues)	**No Expression (33 Tissues)**	**Untested Experiment (5 Tissues)**
Testis- Spinal cord- Adrenal gland- Ovary- Pons- Choroid plexus- Medulla oblongata- Bone marrow- Cerebellum- Blood- Kidney- Pancreas- Gall bladder- Bladder- Tibial Nerve-Spleen - Small intestine- superior temporal gyrus- entorhinal cortex- pituitary gland- B cell - CD4-positive T cell- Placenta- appendix- liver- lymph node- brain- lung- esophagus- duodenum- heart - prostate- thyroid gland- endometrium- colon- adipose tissue- stomach- skin - salivary gland - breast- eye- rectum- skeletal muscle tissue- Uterus- vagina- fallopian tube- - artery- Basal ganglia- hypothalamus- White matter- amygdala- retina- gallbladder- epididymis- seminal- smooth muscle- tonsil- thymus- sputum_ trachea_ white blood cells	hippocampus-- dorsal thalamus- dura mater- globus pallidus- locus ceruleus- middle frontal gyrus- middle temporal gyrus- mitral valve- olfactory apparatus- parietal lobe- parotid gland- penis- pineal body- pulmonary valve- submandibular gland- tongue- tricuspid valve - vas deferens- basal ganglion - frontal lobe- caudate nucleus- putamen- substantia nigra- large intestine- prostate gland- aorta- atrium auricular region- cerebellar hemisphere- esophagogastric junction - greater omentum- hippocampus proper - saliva- secreting gland- Parathyroid gland	visual cortex - CD8-positive T cell- monocyte- natural killer cell- platelet

Subcellular localization

Targeting to the plasmalemma membrane could be confirmed for GLUT14 isoforms A and B, as previously reported (Amir Shaghaghi, Murph, et al., 2016) (**Figure 3)**. Similar imaging was obtained for isoforms C, D, E, confirming targeting to the plasmalemma membrane (**Figure 3**).

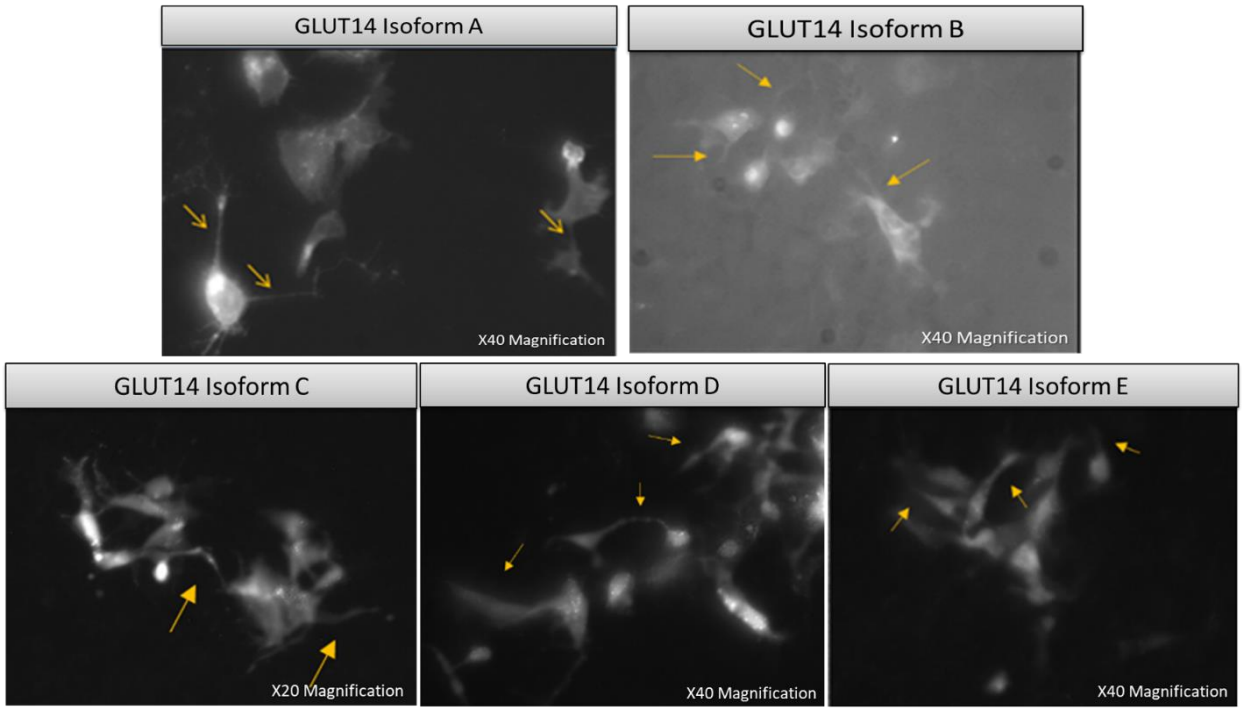


Figure 3: Fluorescent imaging of GLUT 14 isoforms in CaCo2 cells. Arrows indicate membrane extension positive for GLUT14 signals.

Discussion:

Revisiting the *SLC2A14* genomic locus led to the identification of three differentially spliced transcripts of which no published record existed (compare to: Wu and Freeze, 2002, Amir Shaghaghi, Murphy, et al., 2016). Two of these are currently listed as reference transcripts in gene bank (NCBI #: NM001286236 and NM001286237), one is completely novel (encoded by EST# DA225116, NCBI). The fact that the genome browsers failed to identify exons 1-3, covered by EST# DA225116 is likely owed to a very low abundancy/transcript coverage; only a total of three ESTs cover these exons (DA225116, DB466301, DB469509). However, DA225116 is a full-length transcript covering the entirety of the *SLC2A14* locus. Transcript abundance greatly increases upon the utilization of exon 4, which represents the transcriptional start site of 31 transcripts, represented by reference-RNA NM001286237. Transcript abundance further increases from exon 5 onwards, which is covered by 58 transcripts and indicates a strong transcriptional start point; it is represented by ref-RNA 153449. The last alternative transcriptional start site in exon 10 is represented by 30 transcripts. Wu and Freeze (2002) proposed that *SLC2A14* is a simple copy

of the *SLC2A3* gene; however, the emerging complexity in *SLC2A14's* exon utilization indicates a more intricate evolution, which warrants further cross comparison.

In addition to the two previously described GLUT14 protein isoforms (named A and B in NCBI gene bank, or correspondent long and short [compare: Wu and Freeze, 2002, Amir Shaghaghi, Murphy, et al., 2016]). Three additional isoforms are described here; NCBI gene bank lists isoforms C (NP_001273165.1) and D (NP_001273166.1), however, does not recognize isoform E as a reference protein, yet. The protein isoforms show no discrepancies beyond the N-terminal amino acids and therefore are likely performing the same biological functions. All GLUT14 protein isoforms locate to the plasmalemma membrane, and therefore have the potential to function as glucose and dehydroascorbic acid transporters, as described for isoforms A and B (Amir Shaghaghi, Zhouyao, et al., 2017). However, GLUT14 C is the most distinct isoform, lacking a minimum of 100 N-terminal amino acids compared to the other. The elimination of such a significant portion of the protein might render it non-functional.

SLC2A14 expression is more widespread than previously reported, with 61 tissues showing specific RNA signals. However, it is still a spatially restricted gene expression profile, specifically when considering that the abundance in most tissues is low or very low.

Conclusions

This report significantly enhances the literature record on *SLC2A14's* core biology, annotating five additional splice variants, three additional protein isoforms locating to the plasma membrane, and defining the very confined spatial gene expression. It serves as a refence to determine the biology of disease associations.

References

Adjaye, J., R. Herwig, T.C. Brink, D. Herrmann, B. Greber, S. Sudheer, et al. 2007. Conserved molecular portraits of bovine and human blastocysts as a consequence of the transition from maternal to embryonic control of gene expression. Physiological Genomics 31: 315-327. doi:10.1152/physiolgenomics.00041.2007.

Amir Shaghaghi, M., B. Murphy and P. Eck. 2016. The *SLC2A14* gene: Genomic locus, tissue expression, splice variants, and subcellular localization of the protein. Biochemistry and Cell Biology 94: 331-335. doi:10.1139/bcb-2015-0089.

Amir Shaghaghi, M.A., B. Murph and P. Eck. 2016. The *SLC2A14* gene: genomic locus, tissue expression, splice variants, and subcellular localization of the protein. Biochemistry and Cell Biology. doi:10.1139/bcb-2015-0165.

Amir Shaghaghi, M.A., N. Yurkova, H. Tu, M.A. Levine and P. Eck. 2013. Identification and functional characterization of an alternative 5' exon of the sodium dependent ascorbic acid transporter SLC23A1. Journal of Human Nutrition and Food Sciences 1.

Amir Shaghaghi, M., H. Zhouyao, H. Tu, H. El-Gabalawy, G.H. Crow, M. Levine, et al. 2017. The *SLC2A14* gene, encoding the novel glucose/dehydroascorbate transporter GLUT14, is associated with inflammatory bowel disease. The American Journal of Clinical Nutrition. doi:10.3945/ajcn.116.147603.

Berlth, F., S. Mönig, B. Pinther, P. Grimminger, M. Maus, H. Schlösser, et al. 2015. Both GLUT-1 and GLUT-14 are Independent Prognostic Factors in Gastric Adenocarcinoma. Annals of Surgical Oncology 22: 822-831. doi:10.1245/s10434-015-4730-x.

Chai, Y.J., J.W. Yi, S.W. Oh, Y.A. Kim, K.H. Yi, J.H. Kim, et al. 2017. Upregulation of SLC2 (GLUT) family genes is related to poor survival outcomes in papillary thyroid carcinoma: Analysis of data from The Cancer Genome Atlas. Surgery (United States) 161: 188-194. doi:10.1016/j.surg.2016.04.050.

Corpe, C.P., P. Eck, J. Wang, H. Al-Hasani and M. Levine. 2013. Intestinal Dehydroascorbic acid (DHA) transport mediated by the facilitative sugar transporters, GLUT2 and GLUT8. Journal of Biological Chemistry. doi:10.1074/jbc.M112.436790.

Infante, J., C. Prieto, M. Sierra, P. Sánchez-Juan, I. González-Aramburu, C. Sánchez-Quintana, et al. 2015. Identification of candidate genes for Parkinson's disease through blood transcriptome analysis in LRRK2-G2019S carriers, idiopathic cases, and controls. Neurobiology of Aging 36: 1105-1109. doi:10.1016/j.neurobiolaging.2014.10.039.

Januchowski, R., P. Zawierucha, M. Andrzejewska, M. Ruciński and M. Zabel. 2013. Microarray-based detection and expression analysis of ABC and SLC transporters in drug-resistant ovarian cancer cell lines. Biomedicine and Pharmacotherapy 67: 240-245. doi:10.1016/j.biopha.2012.11.011.

Januchowski, R., P. Zawierucha, M. Ruciński, M. Andrzejewska, K. Wojtowicz, M. Nowicki, et al. 2014. Drug transporter expression profiling in chemoresistant variants of the A2780 ovarian cancer cell line. Biomedicine and Pharmacotherapy 68: 447-453. doi:10.1016/j.biopha.2014.02.002.

Nag, A., C. Venturini, P.G. Hysi, M. Arno, E. Aldecoa-Otalora Astarloa, S. Macgregor, et al. 2013. Copy number variation at chromosome 5q21.2 is associated with intraocular pressure. Investigative ophthalmology & visual science 54: 3607-3612. doi:10.1167/iovs.13-11952.

Prakash, S.K., C.A. Bondy, C.L. Maslen, M. Silberbach, A.E. Lin, L. Perrone, et al. 2016. Autosomal and X chromosome structural variants are associated with congenital heart

defects in Turner syndrome: The NHLBI GenTAC registry. American Journal of Medical Genetics, Part A 170: 3157-3164. doi:10.1002/ajmg.a.37953.
Sharpe, M. A., Ijare, O. B., Baskin, D. S., Baskin, A. M., Baskin, B. N., & Pichumani, K. (2021). The Leloir Cycle in Glioblastoma: Galactose Scavenging and Metabolic Remodeling. Cancers, 13(8), 1815.
Shulman, J.M., P. Chipendo, L.B. Chibnik, C. Aubin, D. Tran, B.T. Keenan, et al. 2011. Functional screening of alzheimer pathology genome-wide association signals in drosophila. American Journal of Human Genetics 88: 232-238. doi:10.1016/j.ajhg.2011.01.006.
Song, J., H. Zhang, Z. Wang, W. Xu, L. Zhong, J. Cao, et al. 2018. The Role of FABP5 in Radiation-Induced Human Skin Fibrosis. Radiation Research 189: 177-186. doi:10.1667/RR14901.1.
Taylor, K.H., K.E. Pena-Hernandez, J.W. Davis, G.L. Arthur, D.J. Duff, H. Shi, et al. 2007. Large-scale CpG methylation analysis identifies novel candidate genes and reveals methylation hotspots in acute lymphoblastic leukemia. Cancer Research 67: 2617-2625. doi:10.1158/0008-5472.CAN-06-3993.
Valli, A., M. Morotti, C.E. Zois, P.K. Albers, T. Soga, K. Feldinger, et al. 2019. Adaptation to HIF1a deletion in hypoxic cancer cells by upregulation of GLUT14 and creatine metabolism. Mol Cancer Res 17: 1531-1544. doi:10.1158/1541-7786.MCR-18-0315.
Veal, C.D., K.E. Reekie, J.C. Lorentzen, P.K. Gregersen, L. Padyukov and A.J. Brookes. 2014. A 129-kb deletion on chromosome 12 confers substantial protection against rheumatoid arthritis, implicating the gene SLC2A3. Hum Mutat 35: 248-256. doi:10.1002/humu.22471.
Wang, W., J.T. Yu, W. Zhang, W.Z. Cui, Z.C. Wu, Q. Zhang, et al. 2012. Genetic association of *SLC2A14* polymorphism with Alzheimer's disease in a Han Chinese population. Journal of Molecular Neuroscience 47: 481-484. doi:10.1007/s12031-012-9748-y.

Supplemental Information

Supplemental Information Table 1: list of depositories or databases utilised as sources to determine the complement of *SLC2A14* expression in tissues.

GLUT14 (36 Projects)

Project	Source	Tissues No.	Expression Tissues No.	Unit
20 transcriptomics Projects	_ Ensembl: http://uswest.ensembl.org/Homo_sapiens/Gene/ExpressionAtlas?g=ENSG00000173262;r=12:7812512-7891148 _ EMBL-EBI https://www.ebi.ac.uk/gxa/genes/ENSG00000173262?bs=%7B%22homo%20sapiens%22%3A%5B%22ORGANISM_PART%22%5D%7D&ds=%7B%22kingdom%22%3A%5B%22animals%22%5D%7D#baseline	146	26	TPM
5 Proteomics Projects	_ Ensembl http://uswest.ensembl.org/Homo_sapiens/Gene/ExpressionAtlas?g=ENSG00000173262;r=12:7812512-7891148 _ EMBL-EBI https://www.ebi.ac.uk/gxa/genes/ENSG00000173262?bs=%7B%22homo%20sapiens%22%3A%5B%22ORGANISM_PART%22%5D%7D&ds=%7B%22kingdom%22%3A%5B%22animals%22%5D%7D#baseline	146	10	TPM
GTEX	_ GTEx Portal https://www.gtexportal.org/home/gene/SLC2A14	54	54	TPM
The consensus dataset (GTEX + HPA)	_ THE HUMAN PROTEIN ATLAS https://www.proteinatlas.org/ENSG00000173262-SLC2A14/tissue	55	53	nTPM
HPA	_ THE HUMAN PROTEIN ATLAS https://www.proteinatlas.org/ENSG00000173262-SLC2A14/tissue	52	50	nTPM
GTEX	_ THE HUMAN PROTEIN ATLAS https://www.proteinatlas.org/ENSG00000173262-SLC2A14/tissue	27	26	nTPM
FANTOM	_ THE HUMAN PROTEIN ATLAS https://www.proteinatlas.org/ENSG00000173262-SLC2A14/tissue	46	15	Scaled Tags Per Million
SINGLE CELL TYPES (THE HUMAN PROTEIN ATLAS)	_ THE HUMAN PROTEIN ATLAS https://www.proteinatlas.org/ENSG00000173262-SLC2A14/single+cell+type	25	20	nTPM
HPA RNA-seq normal tissues	_ NCBI https://www.ncbi.nlm.nih.gov/gene/144195	27	27	RPKM
RNA sequencing of total RNA from 20 human tissues	_ NCBI https://www.ncbi.nlm.nih.gov/gene/144195	20	20	RPKM
Illumina bodyMap2 transcriptome	_ NCBI https://www.ncbi.nlm.nih.gov/gene/144195	16	16	RPKM
Genevisible	_ Genevisible https://genevisible.com/tissues/HS/Gene%20Symbol/SLC2A14	10	10	NA
Analysis of the Human Tissue-specific Expression by Genome-wide Integration of Transcriptomics and Antibody-based Proteomics	_ Mol Cell Proteomics Analysis of the Human Tissue-specific Expression by Genome-wide Integration of Transcriptomics and Antibody-based Proteomics* - Molecular & Cellular Proteomics (mcponline.org)	27	27	FPKM

Sources:

_(http://uswest.ensembl.org/Homo_sapiens/Gene/ExpressionAtlas?g=ENSG00000173262;r=12:7812512-7891148)

_(https://www.ebi.ac.uk/gxa/genes/ENSG00000173262?bs=%7B%22homo%20sapiens%22%3A%5B%22ORGANISM_PART%22%5D%7D&ds=%7B%22kingdom%22%3A%5B%22animals%22%5D%7D#baseline)

GLUT14 (20 Transcriptomics Projects)		
HDBR developing brain - Carnegie Stage 13	HDBR developing brain - Carnegie Stage 16	19 NIH Epigenomics Roadmap
GTEx	ENCODE (M. Snyder lab)	Hallstrom et al., 2014 - Organism part
32 Uhlen's Lab	HDBR developing brain - 14 post conception weeks	68 FANTOM5 project - adult
Zhu et al., 2018 - Organism part	HDBR developing brain - 11 post conception weeks	HDBR developing brain - 16 post conception weeks
Illumina Body Map	HDBR developing brain - 9 post conception weeks	HDBR developing brain - Carnegie Stage 14
HDBR developing brain - 13 post conception weeks	HDBR developing brain - 10 post conception weeks	Mammalian Kaessmann
HDBR developing brain - Carnegie Stage 17	HDBR developing brain - Late 8 post conception weeks	
GLUT14 (5 Proteomics Projects)		
McKetney J et.al., 2019 - Individual - 1c	McKetney J et.al., 2019 - Individual - 1f	McKetney J et.al., 2019 - Individual - 1h
Wang et al. 2019	Human Proteome Map – adult	

Supplemental Information Table 2: Amplification conditions for Phusion™

COMPONENT	50 µl REACTION	FINAL CONCENTRATION
Nuclease-free water	to 50 µl	
5X Phusion GC Buffer	10 µl	1X
10 mM dNTPs	1 µl	200 µM
10 µM Forward Primer	2.5 µl	0.5 µM
10 µM Reverse Primer	2.5 µl	0.5 µM
Template DNA	variable	< 250 ng
DMSO (optional)	(1.5 µl)	3%
Phusion Hot Start Flex DNA Polymerase	0.5 µl	1.0 units/50 µl PCR

Thermocycling conditions for a routine PCR at 65°C for all GLUT14 isoforms

STEP	TEMP	TIME
Initial Denaturation	98°C	30 Seconds
25-35 Cycles	98°C 45-72°C 72°C	5-10 Seconds 10-30 Seconds 15-30 Seconds/kb
Final extension	72°C	5-10 minutes
Hold	4-10°C	

Supplemental Information Table 3: Primers utilised for the amplification of the transcripts encoding the GLUT14 isoforms coding regions:

Primer name	Sequence
***SLC2A14* isfA F1 GTW**	GGGGACAAGTTTGTACAAAAAAGCAGGCTACCATGGAGTTTC ACAATGGTGGCC
***SLC2A14* isfB F1 GTW**	GGGGACAAGTTTGTACAAAAAAGCAGGCTACCATGGACAACA GACAGAATGTC
***SLC2A14* isfC F1 GTW**	GGGGACAAGTTTGTACAAAAAAGCAGGCTACCATGCTCCTGA GACGGCGCAATT
***SLC2A14* isfD F1 GTW**	GGGGACAAGTTTGTACAAAAAAGCAGGCTACCATGCAAAGAC TCCAACTGTTG
***SLC2A14* isfE F1 GTW**	GGGGACAAGTTTGTACAAAAAAGCAGGCTACCATGGATGGTT TTCTTCAGGCCC

***SLC2A14* isf R1 GTW**	GGGGACCACTTTGTACAAGAAAGCTGGGTCTTAGACATTGGTG GTGGTCTCC

Supplemental Information Table 4: Gateway destination vectors utilised for GLUT 14 isoforms.

GLUT14 Protein Isoform	Transcript ID	Donor Vector	Destination Vector	Fluorescent Protein
A (520)	NM_001286233 & NM_153449	pDONR221	pcDNA 3.2/V5 DEST	mCherry-N terminal tag
B (497)	NM_001286234 & NM_001286235	pDONR221	pcDNA 3.2/V5 DEST	tGFP-N terminal tag
C (411)	NM_001286236	pDONR221	362 pCS Cherry DEST	mCherry-N terminal tag
D (535)	NM_001286237	pDONR221	362 pCS Cherry DEST	mCherry-N terminal tag
E (517)	DA225116	pDONR221	362 pCS Cherry DEST	mCherry-N terminal tag

Supplemental Information Table 5: tissues expressing *SLC2A14*

Expression (61 tissues)	No Expression (33 Tissues)	NA Experiment (5 Tissues)
Testis- Spinal cord- Adrenal gland- Ovary- Pons- Choroid plexus- Medulla oblongata- Bone marrow- Cerebellum- Blood- Kidney- Pancreas- Gall bladder- Bladder- Tibial Nerve-Spleen - Small intestine- superior temporal gyrus- entorhinal cortex- pituitary gland- B cell - CD4-positive T cell- Placenta- appendix- liver- lymph node- brain- lung- esophagus- duodenum- heart - prostate- thyroid gland- endometrium- colon- adipose tissue- stomach- skin - salivary gland - breast- eye- rectum- skeletal muscle tissue- Uterus- vagina- fallopian tube- - artery- Basal ganglia- hypothalamus- White matter- amygdala- retina- gallbladder- epididymis- seminal- smooth muscle- tonsil- thymus- sputum_ trachea_ white blood cells	hippocampus-- dorsal thalamus- dura mater- globus pallidus- locus ceruleus- middle frontal gyrus- middle temporal gyrus- mitral valve- olfactory apparatus- parietal lobe- parotid gland- penis- pineal body- pulmonary valve- submandibular gland- tongue- tricuspid valve - vas deferens- basal ganglion - frontal lobe- caudate nucleus- putamen- substantia nigra- large intestine- prostate gland- aorta- atrium auricular region- cerebellar hemisphere- esophagogastric junction - greater omentum- hippocampus proper - saliva-secreting gland- Parathyroid gland	visual cortex - CD8-positive T cell- monocyte- natural killer cell- platelet

Transition Statement to Manuscript 2

Manuscript 2 adresses the fact that by sequence similarities to other members of the GLUT family GLUT14 is predicted to be a multispecific solute carrier. For GLUT14 isoform A, a spectrum of substrates is tested through cmpetitive inhibition. The substrate kinetics for the transport of 2-deoxy-D-glucose is determined for GLUT14 isoform A.

CHAPTER 3

Manuscript 2

Designed as a short report to one of the NRC journals

SLC2A14/GLUT14 isoform A: Spectrum of substrates and glucose uptake kinetics

Abstract

GLUT3 meditates the facilitated transmembrane diffusion of the hexose/hexose like substrates D-glucose, D-galactose, D-mannose, D-xylose and D-fucose, L-arabinose and D-lyxose. Based on the high homology between GLUT3 and GLUT14, it can be extrapolated that GLUT14 will also be a multi-specific solute carrier. The substrate specificity of GLUT14 was determined by competitive inhibition of hexose sugars while GLUT14 kinetics for 2-deoxyglucose uptake were determined in *Xenopus laevis* oocytes. In this recored,we indicate a function of GLUT14 isoform A as multi-specific solute carrier as well as its kenitics. We also show that both GLUT3 and GLUT14 isoform A are not only share 94.5% amino acid similarity, but also have similar biochemical properties.

Introduction:

SLC2A14 encoding for GLUT14 is a novel member of the family of facilitated glucose transporter with very recent evolutionary origins (Wu and Freeze, 2002). Two protein isoforms are described in the literature, denominated A and B, both confirmed to mediate glucose and dehydroascorbic acid transmembrane transport (Amir Shaghaghi et al., 2017, Amir Shaghaghi et al., 2016). It has also been suggested that GLUT14 mediates galactose transport; however, these studies in glioblastoma only extrapolated on *SLC2A14* expression and did not distinguish between GLUT14 and other GLUTs contributions (Sharpe, 2021).

The GLUT14 protein isoform A has a 94.5% amino acid identity with GLUT3, which is encoded by the *SLC2A3* gene and is the evolutionary parent of the *SLC2A14* gene (Wu and Freeze, 2002). Based on the high similarities between the GLUT14 isoform A and GLUT3, it could be expected that both have an identical or highly similar biochemistry. In contrast to *SLC2A14*/GLUT14, there is a well-established record on *SLC2A3*/GLUT3 locus, its biology and disease associations.

GLUT3 was first described in humans in 1988 (Long, 2015) and had been associated with Huntington's disease (Covarrubias-Pinto, 2015), congenital syndromic heart defects (Prakash et al. 2016), gastric cancer (Schlößer, 2017), and esophageal adenocarcinoma (Fonteyne, 2009).

GLUT3 meditates the facilitated transmembrane diffusion of the hexose/hexose like substrates D-glucose, D-galactose, D-mannose, D-xylose and D-fucose, L-arabinose and D-lyxose (Deng, 2015). It also recognizes various disaccharides, such as maltose and cellobiose (Deng, 2015); however, these are likely to bind to the active site but not be translocated.

In this manuscript we report the spectrum of substrates and some putative inhibitors for GLUT14 isoform A.

Material and Methods

SLC2A14/GLUT14 isoform-A subcloning was conducted as previously described (Amir Shaghaghi et al., 2017, Amir Shaghaghi et al., 2016).

Radiolabeled [^{14}C]Dehydroascorbic acid was prepared from crystalline [^{14}C]Ascorbic acid (6.6 mCi/mmol, PerkinElmer Life Sciences) (Rumsey, 1997). Radiolabeled Deoxy-D-glucose, 2-[1,2-3H(N)] (25-50Ci/mmol, PerkinElmer Life Sciences) was adjusted to required concentrations in transport buffer. Previously described *Xenopus laevis* oocyte isolation and injection techniques were used to express the GLUT14 isoform-A (Soreq, 1992).

Transport of radiolabeled substrates was determined using groups of 10–20 oocytes in OR-2 buffer at 21°C. Individual oocytes were dissolved in 500 µl of 10% SDS, and internalized radioactivity was determined using scintillation spectrometry. Transport was analyzed and plotted using Microsoft Excel, Student's *t* test is used to determined statistical differences. Data are expressed as the arithmetic mean ± S.D. of 10–20 oocytes analyzed at each data point.

All data shown are means ± SEM with *N*=number of oocytes, *N*=number of replicates. Excel, or Graphpad Prism 8 (version 8.4.2, Graphpad Software Inc, CA, USA) was used to determine statistic differences through one-way ANOVA analyses.

Results

Determination of GLUT14 Substrates

The substrate specificity of GLUT14 was determined by competitive inhibition of hexose sugars in heavy access (100 mM) on radiolabeled 2-deoxy-glucose uptake in Xenopus laevis oocytes. The uptake of radiolabeled traced 2-deoxy-glucose was competitively abolished by the presence of glucose, galactose, mannose, xylose and arabinose (**Figure 1**). Glucosamine inhibited but did not abolish the uptake, while fructose and uric acid did not compete with 2-deoxy-glucose uptake (**Figure 1**). In accordance with earlier reports, dehydroascorbic acid was transported by GLUT14 (**Figure 2**) (Amir Shaghaghi et al., 2017).

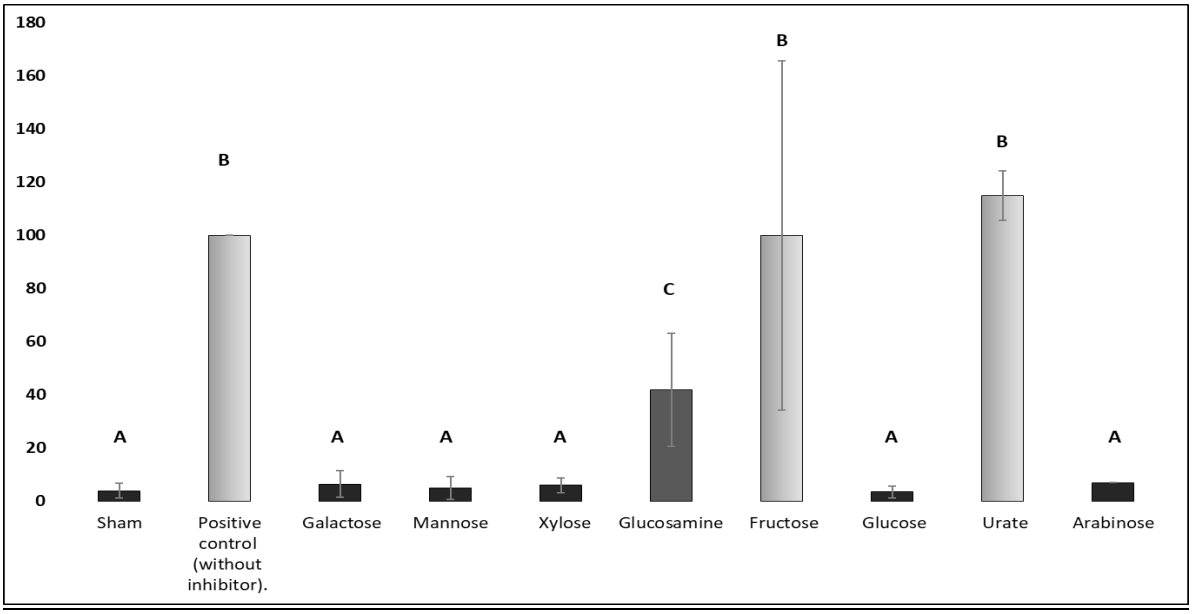


Figure 1: Competitive inhibition of the uptake of radiolabeled traced 2-deoxy-glucose by monosaccharides or other putative substrates. Relative inhibition in % of the positive control (without inhibitor) is depicted as the averages from three independent experiments.

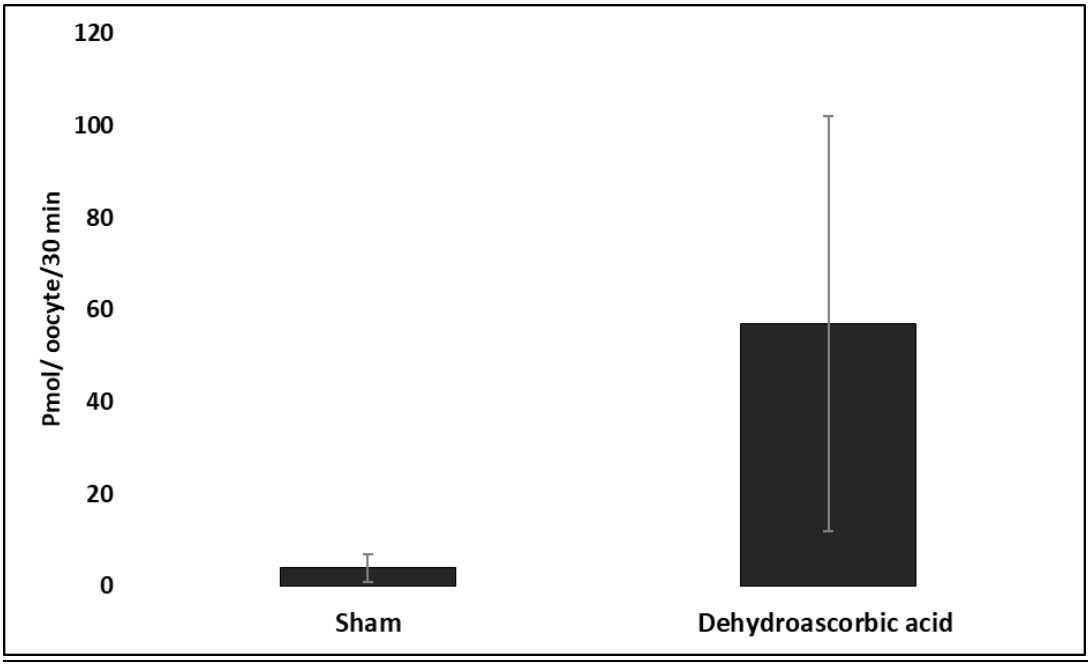


Figure 2: Uptake of radiolabeled dehydroascorbic acid mediated by GLUT14 in Xenopus laevis oocytes.

Determination of GLUT14 disaccharides recognition

Amongst disaccharides that might be recognized/bound to GLUT14 substrate binding site, but not translocated, maltose showed strong inhibition, while lactose and sucrose moderately inhibited radiolabeled traced 2-deoxy-glucose uptake into Xenopus laevis oocytes, while raffinose did not inhibit (**Figure 3**).

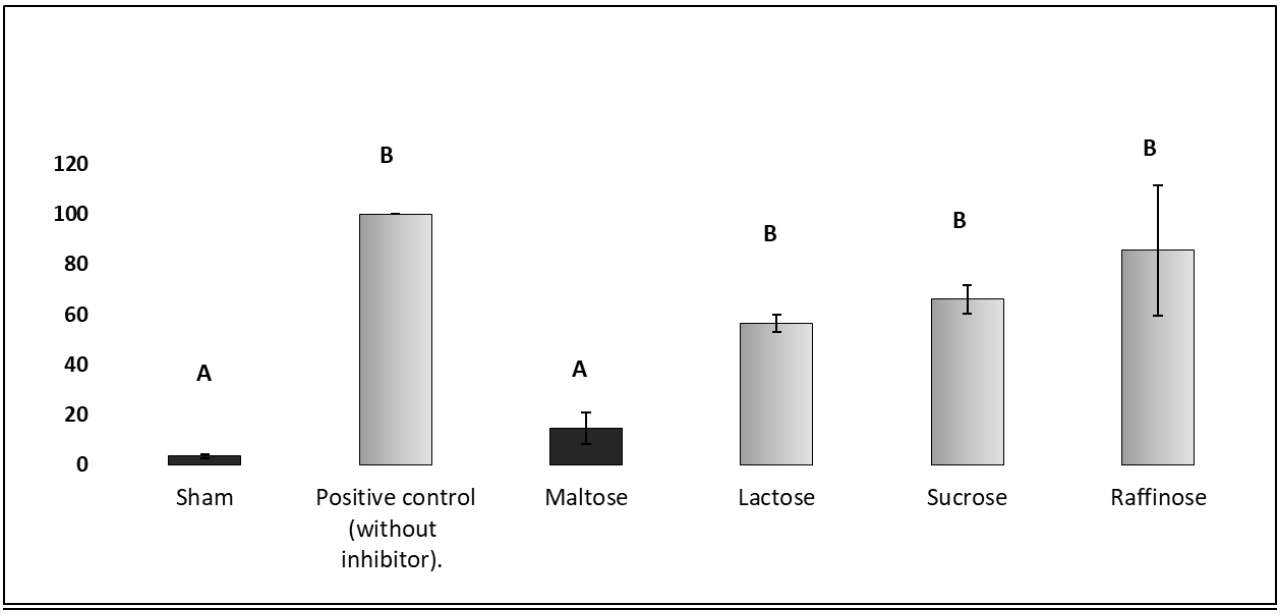


Figure 3: Competitive inhibition of the uptake of radiolabeled traced 2-deoxy-glucose by disaccharides. Relative inhibition in % of the positive control (without inhibitor) is depicted as the averages from three independent experiments.

Determination of GLUT14 isoform A kinetics

GLUT14 kinetics for 2-deoxyglucose (2-DG) uptake were determined in *Xenopus laevis* oocytes (**Figure 4**). Utilizing Michaelis–Menten analysis for substrate saturation a K_m value of 2.5 ± 0.2 mM and Vmax = 2,523 ± 83 pmol/oocyte/30 min.

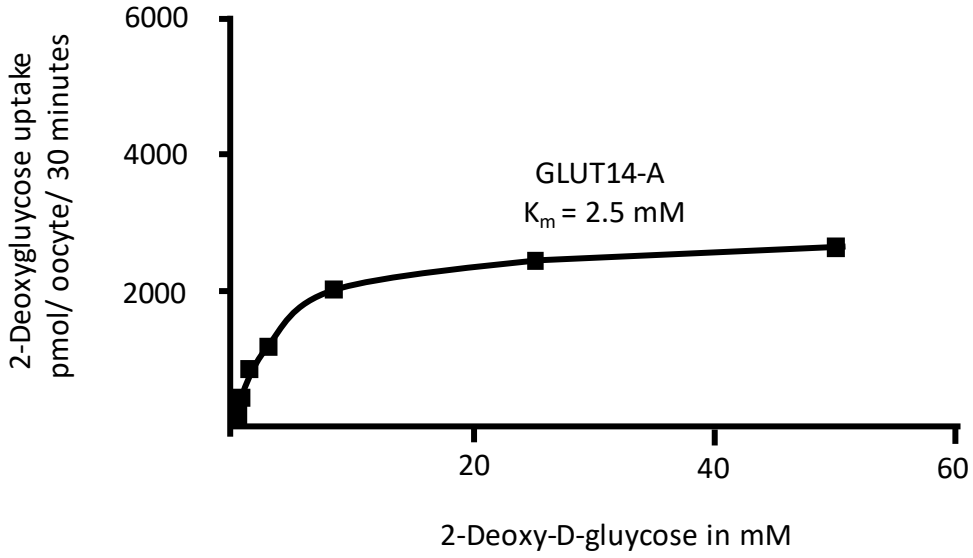


Figure 4: Kinetic parameters for 2-DG transport mediated by GLUT14 isoform A in Xenopus laevis oocytes. The data were fitted using the Michaelis–Menten non-linear fit, yielding a K_m = 2.5 ± 0.2 mM and V_{max} = 2,523 ± 83 pmol/oocyte/30 min.

Discussion:

This report substantially expands on the substrate range and kinetics reported for GLUT14 isoform A. GLUT14 isoform A substrates include galactose, mannose, xylose, arabinose, and glucosamine, in addition to the previously reported glucose and dehydroascorbic acid (Amir Shaghaghi et al., 2017). This multi-specificity confirms a substrate range like the one reported for GLUT3, the closest paralogue (Custódio, 2021).

The disaccharide maltose, composed of two glucose molecules, was also recognized as a GLUT14 isoform A inhibitor. Maltose binding to GLUT3 had been demonstrated and classified as exofacial inhibition, where maltose is recognized by the substrate binding site but cannot be translocated through the membrane transporter protein (Deng, 2015). Our data indicate identical process for GLUT14 isoform A. Moreover, lactose and sucrose inhibit a similar effect to a much lesser extend, which is likely related to exofacial inhibition as well.

GLUT14 isoform A kinetics data mirror the high sequence identity with GLUT3, as both proteins show very similar kinetics. The K_m of 2.5 mM and the V_{max} of 2.5 µmol/oocyte/30 min characterize GLUT14 isoform A in the higher affinity range compared to the other GLUT family members, indicated that it could have a significant role in basal hexose transport. This means that disease pathologies can now be related to GLUT14 expressions, and individual substrates and how they may contribute to the disease outcome can be interrogated.

Conclusion

GLUT14 isoform-A has a multi-specific substrate range for hexoses and hexose like substrates, similar to GLUT3. Specifically, glucose, galactose, mannose, xylose and arabinose are preferred substrates. GLUT14 isoform-A has a high affinity to glucose and may therefore contribute to the basal transport in cell types where it is adequately expressed.

References

Amir Shaghaghi, M., B. Murphy and P. Eck. 2016. The SLC2A14 gene: Genomic locus, tissue expression, splice variants, and subcellular localization of the protein. Biochemistry and Cell Biology 94: 331-335. doi:10.1139/bcb-2015-0089.

Augustin, R. (2010). The protein family of glucose transport facilitators: It's not only about glucose after all. IUBMB life, 62(5), 315-333.

Corpe, C.P., P. Eck, J. Wang, H. Al-Hasani and M. Levine. 2013. Intestinal Dehydroascorbic acid (DHA) transport mediated by the facilitative sugar transporters, GLUT2 and GLUT8. Journal of Biological Chemistry. doi:10.1074/jbc.M112.436790.

Corpe, C.P., J.H. Lee, O. Kwon, P. Eck, J. Narayanan, K.L. Kirk, et al. 2005. 6-Bromo-6-deoxy-L-ascorbic acid: An ascorbate analog specific for Na +-dependent vitamin C transporter but not glucose transporter pathways. Journal of Biological Chemistry 280: 5211-5220.

Corpe, C.P., H. Tu, P. Eck, J. Wang, R. Faulhaber-Walter, J. Schnermann, et al. 2010. Vitamin C transporter Slc23a1 links renal reabsorption, vitamin C tissue accumulation, and perinatal survival in mice. Journal of Clinical Investigation 120: 1069-1083.

Covarrubias-Pinto, A., Moll, P., Solís-Maldonado, M., Acuña, A. I., Riveros, A., Miró, M. P., ... & Castro, M. A. (2015). Beyond the redox imbalance: oxidative stress contributes to an impaired GLUT3 modulation in Huntington's disease. Free Radical Biology and Medicine, 89, 1085-1096.

Custódio, T. F., Paulsen, P. A., Frain, K. M., & Pedersen, B. P. (2021). Structural comparison of GLUT1 to GLUT3 reveal transport regulation mechanism in sugar porter family. Life science alliance, 4(4).

Deng, D., Sun, P., Yan, C., Ke, M., Jiang, X., Xiong, L., ... & Yan, N. (2015). Molecular basis of ligand recognition and transport by glucose transporters. Nature, 526(7573), 391-396.

Eck, P., H.C. Erichsen, J.G. Taylor, C. Corpe, S.J. Chanock and M. Levine. 2007. Genomic and functional analysis of the sodium-dependent vitamin C transporter SLC23A1-SVCT1. Genes and Nutrition 2: 143-145.

Fonteyne, P., Casneu, V., Pauwels, P., Van Damme, N., Peeters, M., Dierckx, R., & Van de Wiele, C. (2009). Expression of hexokinases and glucose transporters in treated and untreated oesophageal adenocarcinoma. Histology and histopathology.

Kwon, O., P. Eck, S.L. Chen, C.P. Corpe, J.H. Lee, M. Kruhlak, et al. 2007. Inhibition of the intestinal glucose transporter GLUT2 by flavonoids. Faseb Journal 21: 366-377. doi:10.1096/fj.06-6620com.

Long, W., & Cheeseman, C. I. (2015). Structure of, and functional insight into the GLUT family of membrane transporters. Cell Health and Cytoskeleton, 7, 167.

Prakash, S. K., Bondy, C. A., Maslen, C. L., Silberbach, M., Lin, A. E., Perrone, L., ... & Milewicz, D. M. (2016). Autosomal and X chromosome structural variants are associated with congenital heart defects in Turner syndrome: The NHLBI GenTAC registry. American Journal of Medical Genetics Part A, 170(12), 3157-3164.

Rumsey, S. C., Kwon, O., Xu, G. W., Burant, C. F., Simpson, I., & Levine, M. (1997). Glucose transporter isoforms GLUT1 and GLUT3 transport dehydroascorbic acid. Journal of Biological Chemistry, 272(30), 18982-18989.

Schlößer, H. A., Drebber, U., Urbanski, A., Haase, S., Baltin, C., Berlth, F., ... & Alakus, H. (2017). Glucose transporters 1, 3, 6, and 10 are expressed in gastric cancer and glucose transporter 3 is associated with UICC stage and survival. Gastric Cancer, 20(1), 83-91.

Sharpe, M. A., Ijare, O. B., Baskin, D. S., Baskin, A. M., Baskin, B. N., & Pichumani, K. (2021). The Leloir Cycle in Glioblastoma: Galactose Scavenging and Metabolic Remodeling. Cancers, 13(8), 1815.

Soreq, H., & Seidman, S. (1992). [14] Xenopus oocyte microinjection: From gene to protein. Methods in enzymology, 207, 225-265.

Transition Statement to Manuscript 3

A previous study confirmed that individual SLC2A14 single nucleotide polymorphisms had been associated with inflammatory bowel diseases (Shaghaghi, Zhouyao, et al., 2017). In manuscript 3, publicly available sources were utilized to identify expressing various levels of SLC2A14 and to understand the correlation between intestinal cell types and SLC2A14 in the pathophysiology of inflammatory bowel diseases.

CHAPTER 4

Manuscript 3

Intended as a short report to an immune focused journal.

Single Cell Transcriptomics Narrows *SLC2A14's* Role in Intestinal Immunity

Abstract

A previous study confirmed that individual *SLC2A14* single nucleotide polymorphisms (SNPs) had been associated with inflammatory bowel diseases (Shaghaghi, Zhouyao, et al., 2017). It was speculated that the disease-causing mechanisms were related to the weakening of the intestinal integrity or decreased intestinal immunity. In this report, we determine *SLC2A14* expression in intestinal cell types to investigate its role in the pathophysiology of inflammatory bowel diseases by using the data from The Human Protein Atlas project.

Introduction

The *SLC2A14* gene encodes GLUT14, a putative multi-specific solute carrier. To date, it is confirmed to mediate the facilitative transmembrane diffusion of glucose and dehydroascorbic acid (Amir Shaghaghi, Zhouyao, et al., 2017).

Three alleles in the *SLC2A14* gene had been independently associated with two phenotypes of Inflammatory Bowel Disease (IBD) (Amir Shaghaghi, Zhouyao, et al., 2017). More specifically, the risk for ulcerative colitis (UC) and Crohn's disease (CD) were elevated in carriers of the *SLC2A14* SNP rs2889504-T allele by 2.6- and 3.6-fold, respectively. Independently, the SNP rs10846086-G allele associated with an approximately 2-fold increased risk for ulcerative colitis as well as Crohn's disease. In a third independent association, susceptibility to Crohn's disease was elevated by 1.2-fold for ulcerative colitis and by 0.6-fold for Crohn's disease (Amir Shaghaghi, Zhouyao, et al., 2017). It was speculated that the disease-causing mechanisms were related to the weakening of the intestinal integrity or decreased intestinal immunity (Amir Shaghaghi, Zhouyao, et al., 2017). However, there are no records available which attempted to resolve if the putative mechanisms relate to the integrity of the intestinal mucosa or immunity.

Intestinal integrity is maintained through the strength of the structure of the barrier enterocyte layer and specific intestinal immunity mediated by resident Paneth cells and blood derived immune cells (Vancamelbeke, 2017).

SLC2A14 tissue expression had been reported for all parts of the gastrointestinal tract involved in Inflammatory Bowel Diseases (Amir Shaghaghi, Murphy, et al., 2016); however, it had not been

reported in which cell-types the expression resides. Recent advances in high throughput single cell RNA sequencing technology and the deposition of these data in public databases enable the accurate identification of cell identities and expression of individual genes (Mereu et al. 2020).

For this report, public resources were utilized to determine *SLC2A14* expression in intestinal cell types to extrapolate on its role in the pathophysiology of inflammatory bowel diseases.

Material and Methods

Bioinformatics

We obtained the information from The Human Protein Atlas (https://www.proteinatlas.org/ENSG00000173262-SLC2A14/single+cell+type). Single cell transcriptomics data for 25 tissues and peripheral blood mononuclear cells (PBMCs) were analyzed based on meta-analysis of literature on single cell RNA sequencing and single cell databases that include healthy human tissue. All data was sourced from Genotype-Tissue Expression (GTEx) bulk RNAseq data which was used to profile gene cell type specificity. Our inclusion criteria were *SLC2A14* expression in intestinal cell types and blood derived immune cells. We determined the required information and then transferred the data manually to Microsoft Excel while the figures were modified by Microsoft PowerPoint.

Results

In the small intestine, *SLC2A14* was almost solely expressed in the Paneth cells (6 nTPM), besides an extremely low signal in enterocytes (0.1 nTPM) (**Figure 1**). No expression was detected in undifferentiated cells and goblet cells.

In the colon, a similar pattern emerged, where Paneth cells express approximately 6 nTPM *SLC2A14* compared to approximately 1 nTPM in enterocytes. Significantly, Granulocytes express about 9 nTPM *SLC2A14* (**Figure 2**). Again, no expression was detected in undifferentiated cells and goblet cells. Moreover, B-cells and T-cells showed no expression, as well as enteroendocrine cells.

Expression in the rectum mirrors the other two intestinal segments, with the highest *SLC2A14* expression in the Paneth cells (3 nTPM) (**Figure 3**). Undifferentiated cells (~0.4 nTPM) and

goblet cells (~0.1 nTPM) had only residual signals. However, in contrast to the other two intestinal sections, where expression was extremely low, no signal was detected in enterocytes.

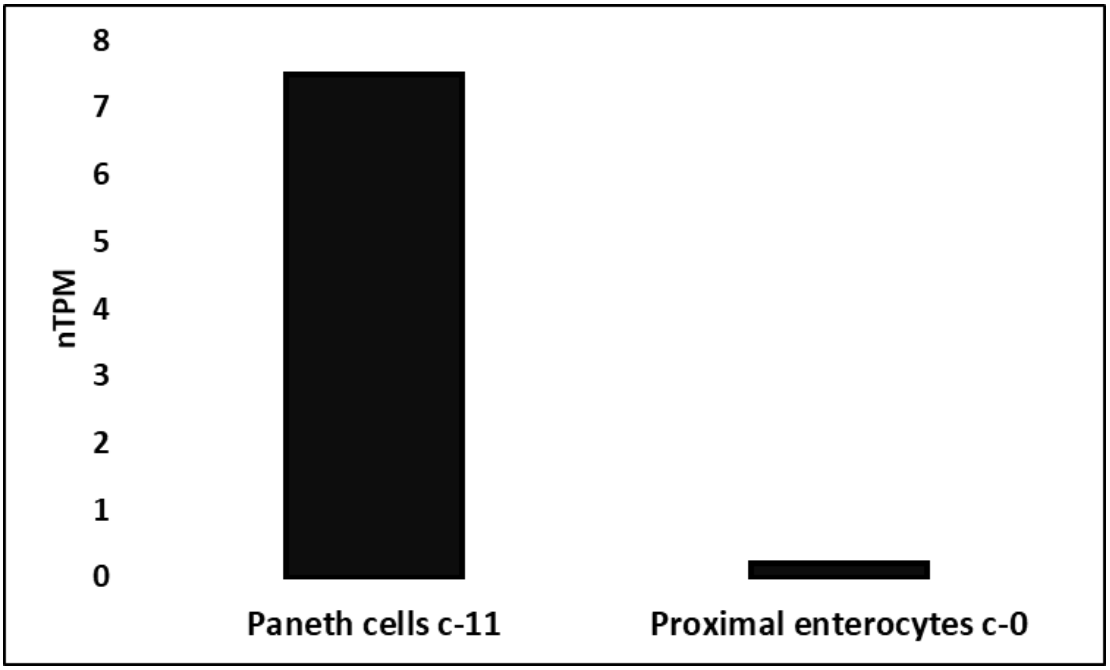


Figure 1: *SLC2A14* expression in cell types of the small intestine.

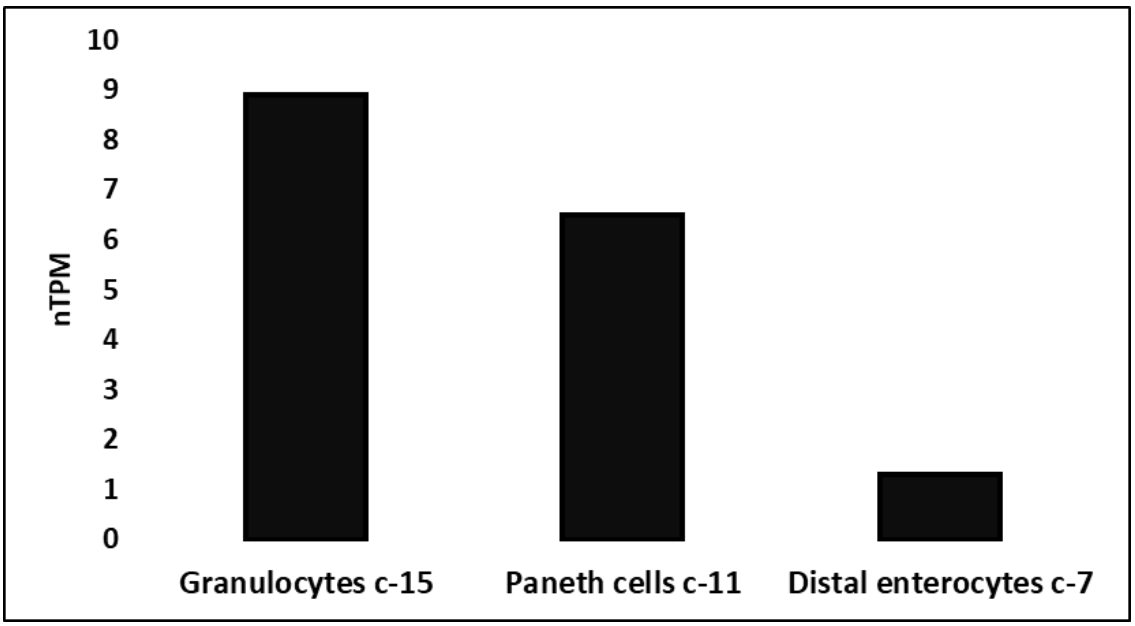


Figure 2: *SLC2A14* expression in cell types of the colon.

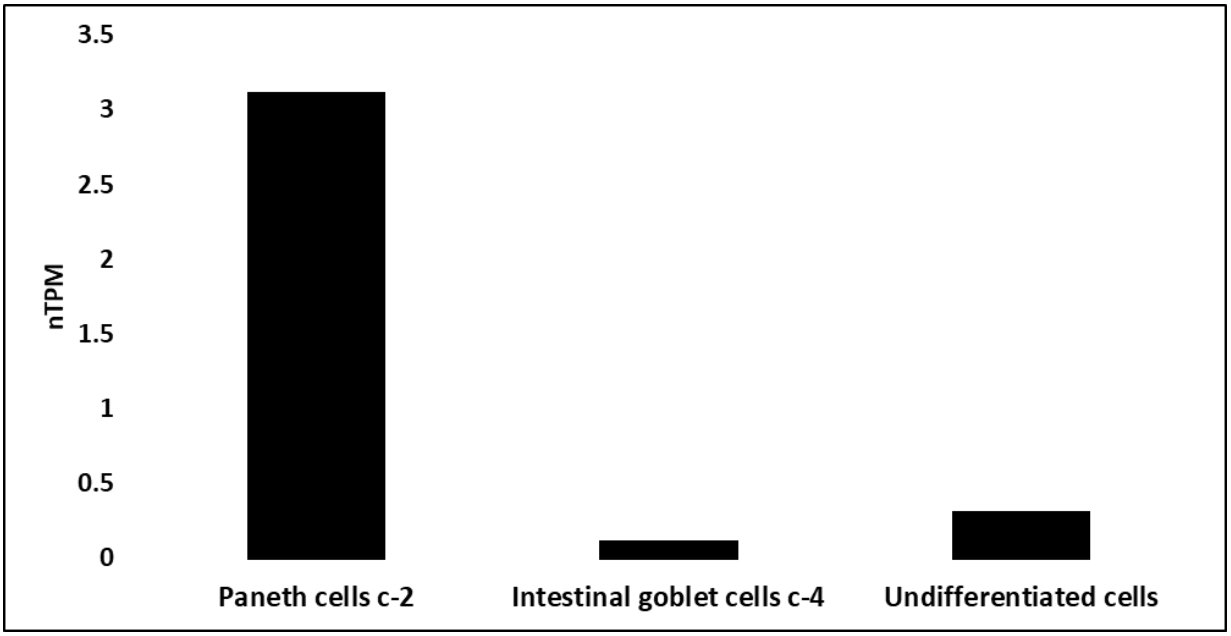


Figure 3: *SLC2A14* expression in cell types of the rectum.

Highest *SLC2A14* expression in colon granulocytes indicate an involvement of blood derived immune cells. The term granulocytes do not define a cell type, but is includes the immune cells neutrophils, eosinophils, and basophils (Todd, 2021). Therefore, info on *SLC2A14* expression in blood derived immune cells was analyzed. Amongst blood derived immune cells, basophiles (3.7 nTPM) and neutrophiles (3.4 nTPM) express highest *SLC2A14* levels, whilst eosinophiles (1 nTPM) and others express less than 1 nTPM (**Figure 4**).

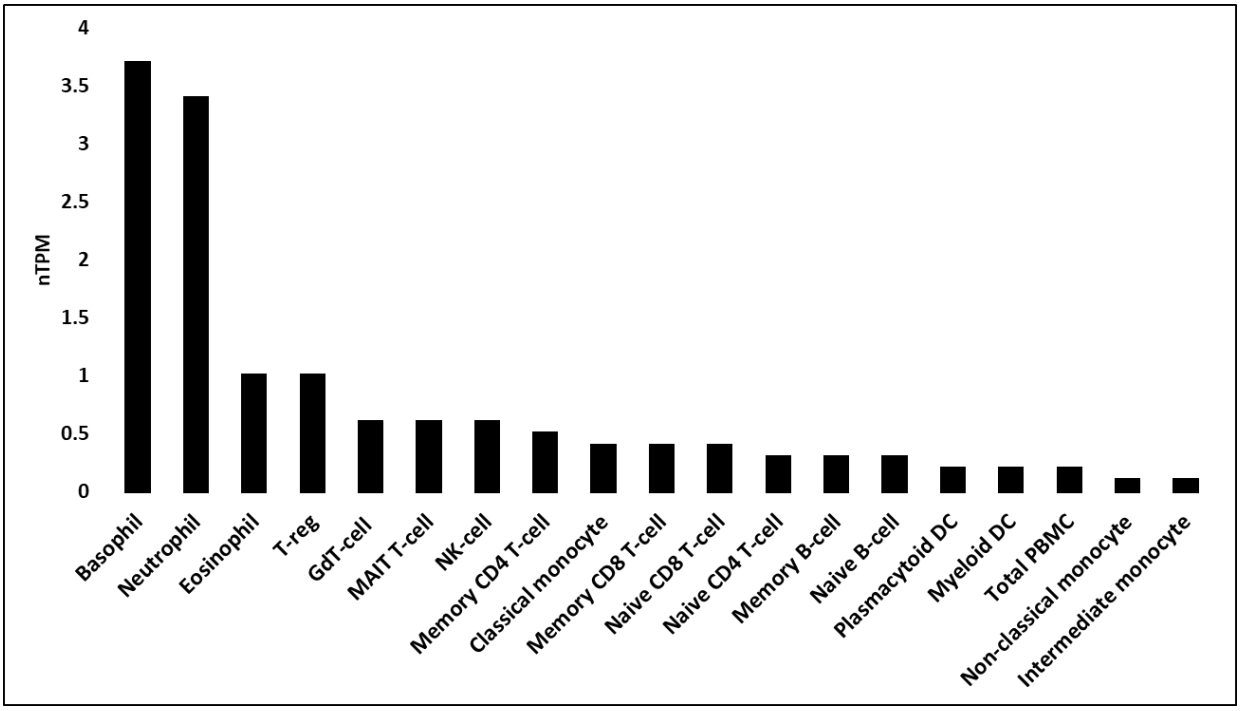


Figure 4: *SLC2A14* expression in blood derived immune cells.

Discussion:

The evidence presented informs on the potential mechanism of *SLC2A14's* genetic associations with inflammatory bowel diseases. The *SLC2A14* gene expression in intestinal tissue is mainly confined to Paneth cells as well as neutrophiles, eosinophiles and basophiles, all mediating an innate immune response. This strongly implies that potential disease mechanism would be mediated through a dysregulated immune response rather than weakened mechanical barrier functions. This adds to the hypothesis that a dysregulated immune response against the intestinal microbiome is now believed to be a key driver of inflammation (Sartor, 2017).

Paneth cells predominantly locate in the small intestine, but can also be induced by inflammation in the colon. They secrete antimicrobial peptides, most prominently the antibacterial α-defensins, human defensin 5 (HD5) (Jones, 1992) and, human defensin 6 (HD6) (Wehkamp, 2006), and lysozyme (Deckx, 1967). Therefore, Paneth cells are the basis of an antibacterial chemical barrier and any defect in Paneth cell function may compromise mucosal integrity (Wehkamp, 2020). Due to the high abundance of Paneth cells in the lateral small intestine some authors coined the term ileal Crohn's disease as "Paneth's disease" (Wehkamp 2010).

The human defensin 5 controls the constitution of the commensal microbiome as a direct antibacterial (Salzman, 2010), demonstrated in “knock-in” mice (Salzman, 2003); therefore, it seems to have the dual role of controlling the commensal microbiome and protecting from invaders.

Impaired Paneth cell function with diminished human defensing 5 and 6 levels were reported from Crohn’s disease patients (Wehkamp, 2004; Wehkamp, 2005). This was attributed to impaired intracellular recognition of bacteria via NOD2, a gene also associated with Crohn’s disease (Hugot, 2001; Ogura, 2001). Environmental factors, such as smoking, antibiotic exposure and vitamin D deficiency also negatively impact Paneth cells functioning (Piovani, 2019) and are known risk factors for Crohn’s disease. Hence, multiple lines of evidence seem to support a role of decrease Paneth cells immune functioning in some forms of inflammatory bowel disease, specifically with ileal involvement.

Neutrophils constitute up to 70% of all white blood cells in humans (Actor, 2011) and are quickly recruited to sites of injury where they are involved in acute inflammation (Rosales, 2018). Like Paneth cells, neutrophils release soluble anti-microbials, amongst them various types of alpha-defensins, specifically human neutrophile peptide 1-4. In addition, they phagocyte pathogens and generate neutrophil extracellular traps to neutralize a microbial challenge (Hickey, 2009), and form reactive oxygen species and other toxic molecules. The primary function of neutrophils in the gut is to kill luminal microbes that translocate across the epithelium and invade the mucosa. Significantly, in chronic granulomatous disease their function is impaired and patients develop bowel inflammation identical to Crohn’s disease (Levine, 2013).

Basophils represent about 0.5% to 1% of circulating white blood cells (Mukai, 2013). They are multifunctional and involved in many stages of inflammatory reactions. Basophils produce the anticoagulant heparin (Guyton, 2006), as well as the vasodilator histamine, and could play a role in the regulation of T-cells (Nakanishi, 2010). Upon activation, they release proteolytic enzymes (Janeway Jr, 2001). Most significantly, basophils are innate effector cells of allergen-induced IgE-dependent allergic diseases (Sarfati, 2015). Basophils accumulate in inflamed masses, both IgE-dependent and IgE-independent, where they augment memory T cells effector responses.

Increased numbers of basophils are observed in blood of patients with chronic inflammatory bowel diseases, both Crohn's disease and ulcerative colitis (Sarfati, 2015; Chapuy, 2014). Concordantly, increased basophils are noticed in the inflamed relative to the non-inflamed colonic mucosa, while none can be detected in in the intestinal mucosa of healthy individuals. This suggests that they contribute to disease pathogenesis via the regulation of T cell responses (Chapuy, 2014).

Eosinophils are multifunctional producing cytotoxic proteins, regulatory cytokines and chemokines. They primarily reside in the gut where they are integral to gut-associated immunity (Loktionov, 2019). In intestinal immunity, they modulate neutrophil influx to the lamina propria followed by transepithelial migration to colorectal mucus. In addition to being an element of the innate immunity, eosinophils modulate complex immune responses at the surface of the gastrointestinal tract (Lee, 2010). Eosinophils cooperate with the colonic epithelium to produce a range of neutrophil chemo-attractants (Dent, 2014).

Increased eosinophiles' abundance in the gut mucosa correlates with disease severity in UC and some CD cases (Loktionov, 2019). During disease remission activated eosinophils persist in the lamina propria of UC, but not CD patients.

IBD pathogenesis is multifactorial, but the dysregulation of the innate immune system is proven to be involved (Kaser, 2010). *SLC2A14* expression is found in the major cell types constituting the intestinal mucosal innate immune system, but not in the enterocyte monolayer forming the physical barrier. This evidence suggests that *SLC2A14* disease association is mediated through immune response modulation, specifically in the innate effector cells.

GLUT14 mediates glucose as well as dehydroascorbate uptake, and both of these substrates play a role in the homeostasis and viability of innate immune cells.

The glucose metabolism is a key component in the metabolic shift that follows the sensing of microbial ligands to initiate the antimicrobial activity of innate immune cells (Tucey, 2018). This upregulation of glycolysis delivers energy to support the antimicrobial action (Freemerman, 2014). Compounds that reduce glucose availability (e.g., 2-deoxyglucose) or inhibit the metabolic shift to high glycolysis (e.g., inhibitors of the target of rapamycin pathway, or the antidiabetic drug metformin) diminish the immune response by reducing the production of

antimicrobial agents (Cheng, 2016; Gleeson, 2016; Lachmandas, 2016; Tannahill, 2013; Wickersham, 2017). Moreover, the upregulation of glycolytic enzymes controls the upregulation of antimicrobial cytokines and peptides (Millet, 2016; Palsson-McDermott, 2015). The specific impact of GLUT14 on these pathways in intestinal innate immune cells had not been studied and remains to be determined in the search for future therapeutic interventions for individuals of specific *SLC2A14* genotypes associated with intestinal inflammation.

Dehydroascorbic metabolism plays a specific role in the so-called oxidative burst of immune cells, mainly exhibited by neutrophiles. During the oxidative burst reactive oxygen species are produced and released or used to directly harm extracellular or invading microbes (Mills, 2016). The importance of the oxidative burst is demonstrated in Chronic Granulomatous Disease which is characterized by abolished oxidative burst in neutrophils resulting in severely delayed clearance of bacterial infections (Fernandez, 2021). During the oxidative burst, extracellular ascorbic acid is oxidized and converted into dehydroascorbic acid, which is a GLUT14 substrate. In a process termed “ascorbic acid recycling” large quantities of dehydroascorbic acid are transported into the neutrophil, which leads to a up to 20 fold increased intracellular ascorbic acid, an important antioxidant (Washko,1993).

The increased antioxidant capacity might contribute to the neutrophil’s protection against its own oxidative burst and prolonged survivability or enhanced functioning (Parker, 2011).

Neutrophils from scorbutic animals had decreased chemotaxis, phagocytosis, oxidant production and microbial killing, compared with controls; normal activity was restored upon ascorbic acid supplementation (Shilotri, 1977; Shilotri, et al., 1977; Goldschmidt, 1991). Moreover, vitamin C decreases the development of Neutrophils Extracellular Traps in septic Gulo knockout mice and in stimulated human neutrophils Vitamin C: a novel regulator of neutrophil extracellular trap formation (Mohammed, 2013). In addition, in stimulated neutrophiles the formation of Neutrophils Extracellular Traps was decreased (Bozonet, 2019). Although neutrophils extracellular traps immobilize pathogens, it had been postulated that they are inflammatory, damaging host tissues and resulting in various pathologies (Czaikoski, 2016).

After vitamin C repletion, enhanced chemotaxis and oxidant production was observed in neutrophiles from vitamin C depleted humans (Bozonet, 2015). However, this was not observed for neutrophiles from humans of adequate vitamin C status (Bozonet, 2019).

In regard to the potential protection for surrounding host tissues from the oxidative burst, in isolated phagocytes ascorbate had a dual role, by enhancing intracellular oxidant generation, but scavenging extracellular oxidants (Anderson, 1987).

Conclusion

Expression data indicate a role of intestinal cells mediating innate immunity in the previously reported association of intestinal inflammation with genetic variations in the *SLC2A14* gene. Such comprehensive insight is essential and gives a new understanding for glucose and dehydroascorbic acid pathophysiology in IBD.

References

Actor, J. K. (2011). Elsevier's Integrated Review Immunology and Microbiology E-Book: with STUDENT CONSULT Online Access.

Amir Shaghaghi, M., H. Zhouyao, H. Tu, H. El-Gabalawy, G.H. Crow, M. Levine, et al. 2017. The *SLC2A14* gene, encoding the novel glucose/dehydroascorbate transporter GLUT14, is associated with inflammatory bowel disease. The American Journal of Clinical Nutrition. doi:10.3945/ajcn.116.147603.

Amir Shaghaghi, M.A., B. Murph and P. Eck. 2016. The *SLC2A14* gene: genomic locus, tissue expression, splice variants, and subcellular localization of the protein. Biochemistry and Cell Biology. doi:10.1139/bcb-2015-0165.

Anderson, R., & Lukey, P. T. (1987). A biological role for ascorbate in the selective neutralization of extracellular phagocyte-derived oxidants. Annals of the New York Academy of Sciences, 498(1), 229-247.

Bozonet, S. M., & Carr, A. C. (2019). The role of physiological vitamin C concentrations on key functions of neutrophils isolated from healthy individuals. Nutrients, 11(6), 1363.

Bozonet, S. M., Carr, A. C., Pullar, J. M., & Vissers, M. (2015). Enhanced human neutrophil vitamin C status, chemotaxis and oxidant generation following dietary supplementation with vitamin C-rich SunGold kiwifruit. Nutrients, 7(4), 2574-2588.

By, R. (2021). Todd Gersten, MD, Hematology/Oncology, Florida Cancer Specialists & Research Institute, Wellington, FL. Review provided by VeriMed Healthcare Network. Also reviewed by David Zieve, MD, MHA, Medical Director, Brenda Conaway, Editorial Director, and the ADAM Editorial team. (https://medlineplus.gov/ency/article/003440.htm)

Chapuy, L., Bsat, M., Mehta, H., Rubio, M., Wakahara, K., Van, V. Q., ... & Sarfati, M. (2014). Basophils increase in Crohn disease and ulcerative colitis and favor mesenteric lymph node memory TH17/TH1 response. Journal of allergy and clinical immunology, 134(4), 978-981.

Cheng, S. C., Scicluna, B. P., Arts, R. J., Gresnigt, M. S., Lachmandas, E., Giamarellos-Bourboulis, E. J., ... & Netea, M. G. (2016). Broad defects in the energy metabolism of leukocytes underlie immunoparalysis in sepsis. *Nature immunology*, *17*(4), 406-413.

Czaikoski, P. G., Mota, J. M. S. C., Nascimento, D. C., Sônego, F., Castanheira, F. V. E. S., Melo, P. H., ... & Cunha, F. Q. (2016). Neutrophil extracellular traps induce organ damage during experimental and clinical sepsis. PloS one, 11(2), e0148142.

Deckx, R. J., Vantrappen, G. R., & Parein, M. M. (1967). Localization of lysozyme activity in a Paneth cell granule fraction. Biochimica et Biophysica Acta (BBA)-Enzymology, 139(1), 204-207.

Dent, G., Loweth, S. C., Hasan, A. M., & Leslie, F. M. (2014). Synergic production of neutrophil chemotactic activity by colonic epithelial cells and eosinophils. Immunobiology, 219(10), 793-797.

Fernandez, J. (2021). Chronic Granulomatous Disease (CGD). MSD Manual Professional Edition. https://www.msdmanuals.com/professional/immunology-allergic-disorders/immunodeficiency-disorders/chronic-granulomatous-disease-cgd

Freemerman, A. J., Johnson, A. R., Sacks, G. N., Milner, J. J., Kirk, E. L., Troester, M. A., ... & Makowski, L. (2014). Metabolic reprogramming of macrophages: glucose transporter 1 (GLUT1)-mediated glucose metabolism drives a proinflammatory phenotype. Journal of Biological Chemistry, 289(11), 7884-7896.

Gleeson, L. E., Sheedy, F. J., Palsson-McDermott, E. M., Triglia, D., O'Leary, S. M., O'Sullivan, M. P., ... & Keane, J. (2016). Cutting edge: Mycobacterium tuberculosis induces aerobic glycolysis in human alveolar macrophages that is required for control of intracellular bacillary replication. The Journal of Immunology, 196(6), 2444-2449.

Goldschmidt, M. C. (1991). Reduced bactericidal activity in neutrophils from scorbutic animals and the effect of ascorbic acid on these target bacteria in vivo and in vitro. The American journal of clinical nutrition, 54(6), 1214S-1220S.

Guyton, A. C., & Hall, J. E. (2006). Textbook of medical physiology: Elsevier Saunders. United States of America, 323.

Hickey, M. J., & Kubes, P. (2009). Intravascular immunity: the host–pathogen encounter in blood vessels. Nature reviews immunology, 9(5), 364-375.

Hugot, J. P., Chamaillard, M., Zouali, H., Lesage, S., Cézard, J. P., Belaiche, J., ... & Thomas, G. (2001). Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature, 411(6837), 599-603.

Janeway Jr, C. A., Travers, P., Walport, M., & Shlomchik, M. J. (2001). The complement system and innate immunity. In Immunobiology: The Immune System in Health and Disease. 5th edition. Garland Science.

Jones, D. E., & Bevins, C. L. (1992). Paneth cells of the human small intestine express an antimicrobial peptide gene. Journal of Biological Chemistry, 267(32), 23216-23225.

Kaser, A., Zeissig, S., & Blumberg, R. S. (2010). Inflammatory bowel disease. Annu. Rev. Immunol, 28, 573–621.

Lachmandas, E., Boutens, L., Ratter, J. M., Hijmans, A., Hooiveld, G. J., Joosten, L. A., ... & Stienstra, R. (2016). Microbial stimulation of different Toll-like receptor signalling pathways induces diverse metabolic programmes in human monocytes. Nature microbiology, 2(3), 1-10.

Lee, J. J., Jacobsen, E. A., McGarry, M. P., Schleimer, R. P., & Lee, N. A. (2010). Eosinophils in health and disease: the LIAR hypothesis. Clinical & Experimental Allergy, 40(4), 563-575.

Lee, K. H., Kronbichler, A., Park, D. D. Y., Park, Y., Moon, H., Kim, H., ... & Shin, J. I. (2017). Neutrophil extracellular traps (NETs) in autoimmune diseases: a comprehensive review. Autoimmunity reviews, 16(11), 1160-1173.

Levine, A. P., & Segal, A. W. (2013). What is wrong with granulocytes in inflammatory bowel diseases?. Digestive diseases, 31(3-4), 321-327.

Loktionov, A. (2019). Eosinophils in the gastrointestinal tract and their role in the pathogenesis of major colorectal disorders. World journal of gastroenterology, 25(27), 3503.

Mereu, E., Lafzi, A., Moutinho, C., Ziegenhain, C., McCarthy, D. J., Álvarez-Varela, A., ... & Heyn, H. (2020). Benchmarking single-cell RNA-sequencing protocols for cell atlas projects. Nature Biotechnology, 38(6), 747-755.

Millet, P., Vachharajani, V., McPhail, L., Yoza, B., & McCall, C. E. (2016). GAPDH binding to TNF-α mRNA contributes to posttranscriptional repression in monocytes: a novel mechanism of communication between inflammation and metabolism. The Journal of Immunology, 196(6), 2541-2551.

Mills, E. L., Kelly, B., Logan, A., Costa, A. S., Varma, M., Bryant, C. E., ... & O'Neill, L. A. (2016). Succinate dehydrogenase supports metabolic repurposing of mitochondria to drive inflammatory macrophages. Cell, 167(2), 457-470.

Mohammed, B. M., Fisher, B. J., Kraskauskas, D., Farkas, D., Brophy, D. F., & Natarajan, R. (2013). Vitamin C: a novel regulator of neutrophil extracellular trap formation. Nutrients, 5(8), 3131-3150.

Mukai, K., & Galli, S. J. (2013). Basophils. eLS.

Nakanishi, K. (2010). Basophils as APC in Th2 response in allergic inflammation and parasite infection. Current opinion in immunology, 22(6), 814-820.

Ogura, Y., Bonen, D. K., Inohara, N., Nicolae, D. L., Chen, F. F., Ramos, R., ... & Cho, J. H. (2001). A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature, 411(6837), 603-606.

Palsson-McDermott, E. M., Palsson-McDermott, E. M., Curtis, A. M., Curtis, A. M., Goel, G., Goel, G., ... & Sheedy, F. J. Pyruvate kinase M2 regulates Hif-1alpha activity and IL-1beta induction and is a critical determinant of the warburg effect in LPS-activated macrophages, Cell Metabol. 21 (2015) 65–80.

Parker, A., Cuddihy, S. L., Son, T. G., Vissers, M. C., & Winterbourn, C. C. (2011). Roles of superoxide and myeloperoxidase in ascorbate oxidation in stimulated neutrophils and H2O2-treated HL60 cells. Free Radical Biology and Medicine, 51(7), 1399-1405.

Piovani, D., Danese, S., Peyrin-Biroulet, L., Nikolopoulos, G. K., Lytras, T., & Bonovas, S. (2019). Environmental risk factors for inflammatory bowel diseases: an umbrella review of meta-analyses. Gastroenterology, 157(3), 647-659.

Rosales, C. (2018). Neutrophil: a cell with many roles in inflammation or several cell types?. Frontiers in physiology, 9, 113.

Salzman, N. H., Ghosh, D., Huttner, K. M., Paterson, Y., & Bevins, C. L. (2003). Protection against enteric salmonellosis in transgenic mice expressing a human intestinal defensin. Nature, 422(6931), 522-526.

Salzman, N. H., Hung, K., Haribhai, D., Chu, H., Karlsson-Sjöberg, J., Amir, E., ... & Bos, N. A. (2010). Enteric defensins are essential regulators of intestinal microbial ecology. Nature immunology, 11(1), 76-82.

Sarfati, M., Wakahara, K., Chapuy, L., & Delespesse, G. (2015). Mutual interaction of basophils and T cells in chronic inflammatory diseases. Frontiers in immunology, 6, 399.

Sartor, R. B., & Wu, G. D. (2017). Roles for intestinal bacteria, viruses, and fungi in pathogenesis of inflammatory bowel diseases and therapeutic approaches. Gastroenterology, 152(2), 327-339.
Shilotri, P. G. (1977). Glycolytic, hexose monophosphate shunt and bactericidal activities of leukocytes in ascorbic acid deficient guinea pigs. The Journal of nutrition, 107(8), 1507-1512.
Shilotri, P. G. (1977). Phagocytosis and leukocyte enzymes in ascorbic acid deficient guinea pigs. The Journal of nutrition, 107(8), 1513-1516.
Tannahill, G. M., Curtis, A. M., Adamik, J., Palsson-McDermott, E. M., McGettrick, A. F., Goel, G., ... & O'Neill, L. A. J. (2013). Succinate is an inflammatory signal that induces IL-1β through HIF-1α. Nature, 496(7444), 238-242.
Tucey, T. M., Verma, J., Harrison, P. F., Snelgrove, S. L., Lo, T. L., Scherer, A. K., ... & Traven, A. (2018). Glucose homeostasis is important for immune cell viability during Candida challenge and host survival of systemic fungal infection. Cell metabolism, 27(5), 988-1006.
Vancamelbeke, M., & Vermeire, S. (2017). The intestinal barrier: a fundamental role in health and disease. Expert review of gastroenterology & hepatology, 11(9), 821-834.
Washko, P. W., Wang, Y. A. O. H. U. I., & Levine, M. (1993). Ascorbic acid recycling in human neutrophils. Journal of Biological Chemistry, 268(21), 15531-15535.
Wehkamp, J., & Stange, E. F. (2020). An update review on the Paneth cell as key to ileal Crohn's disease. Frontiers in immunology, 11, 646.
Wehkamp, J., & Stange, E. F. (2010). Paneth's disease. Journal of Crohn's and Colitis, 4(5), 523-531.
Wehkamp, J., Chu, H., Shen, B., Feathers, R. W., Kays, R. J., Lee, S. K., & Bevins, C. L. (2006). Paneth cell antimicrobial peptides: topographical distribution and quantification in human gastrointestinal tissues. FEBS letters, 580(22), 5344-5350.
Wehkamp, J., Harder, J., Weichenthal, M., Schwab, M., Schäffeler, E., Schlee, M., ... & Stange, E. F. (2004). NOD2 (CARD15) mutations in Crohn's disease are associated with diminished mucosal α-defensin expression. Gut, 53(11), 1658-1664.
Wehkamp, J., Salzman, N. H., Porter, E., Nuding, S., Weichenthal, M., Petras, R. E., ... & Bevins, C. L. (2005). Reduced Paneth cell α-defensins in ileal Crohn's disease. Proceedings of the National Academy of Sciences, 102(50), 18129-18134.
Wickersham, M., Wachtel, S., Lung, T. W. F., Soong, G., Jacquet, R., Richardson, A., ... & Prince, A. (2017). Metabolic stress drives keratinocyte defenses against Staphylococcus aureus infection. Cell reports, 18(11), 2742-2751.

CHAPTER 5

Overall Conclusion

Summary and Implications

The present studies provide novel information about GLUT 14 protein isoforms. To date, all published records identified two alternatively spliced variants, resulting in two protein isoforms. However, here we identify four GLUT14 protein isoforms which are called isoform A, B, C, and D from the NCBI dataset while the fifth one which is isoform E is not assigned in NCBI yet. Moreover, we indicate that all protein isoforms locate in the plasmalemma membrane. After the reanalysis of publicly available transcripts, the information prior reported was confirmed for the size of the *SCL2A14* locus, containing 20 exons.

Although both GLUT3 and GLUT14 isoform A show high similarities in the functional properties, each GLUT has specific functions in the tissues in which they are expressed because of its special physiological and biochemical properties (Simmons, 2017). More comparison is needed about the role of GLUT14 and GLUT3 to be more understandable.

Previous study illustrated the expression of *SLC2A14* is observed in 10 extra-testicular tissues (Amir Shaghaghi, M., 2016). However, we indicate *SLC2A14* expression is more widespread than previously reported with 61 tissues due to the availability of additional RNA-Sequencing data. Mapping out the gene expression is major importance for understanding the functional differences between tissues as well as their development and differentiation (Santos, 2015).

Furthermore, for the first time, we explain the underlying mechanism of association between intestinal cells mediating innate immunity and intestinal inflammation with genetic variations in the *SLC2A14* gene. This increases the possibility that *SLC2A14* could be treated as target in some diseases.

As known, each protein isoform encoded by the same gene can play very different roles in the cell (Andreadis, 1987). Thus, an understanding of their regulatory properties that are directly related to their distinct physiological roles will facilitate an understanding of glucose metabolism and other substrate which are not fully illustrated. Less is still known regarding the biology role of the GLUT14 protein isoforms and their differences in human body.

References

Amir Shaghaghi, M.A., B. Murph and P. Eck. 2016. The *SLC2A14* gene: genomic locus, tissue expression, splice variants, and subcellular localization of the protein. Biochemistry and Cell Biology. doi:10.1139/bcb-2015-0165.

Andreadis, A., Gallego, M. E., & Nadal-Ginard, B. (1987). Generation of protein isoform diversity by alternative splicing: mechanistic and biological implications. Annual review of cell biology, 3(1), 207-242.

Santos, A., Tsafou, K., Stolte, C., Pletscher-Frankild, S., O'Donoghue, S. I., & Jensen, L. J. (2015). Comprehensive comparison of large-scale tissue expression datasets. PeerJ, 3, e1054.

Simmons, R. A. (2017). Cell glucose transport and glucose handling during fetal and neonatal development. In Fetal and neonatal physiology (pp. 428-435). Elsevier.