Update on SLC6A14 in lung and gastrointestinal physiology and physiopathology: focus on cystic fibrosis

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Abstract
The solute carrier family 6 member 14 (SLC6A14) protein imports and concentrates all neutral amino acids as well as the two cationic acids lysine and arginine into the cytoplasm of different cell types. Primarily described as involved in several cancer and colonic diseases physiopathological mechanisms, the SLC6A14 gene has been more recently identified as a genetic modifier of cystic fibrosis (CF) disease severity. It was indeed shown to have a pleiotropic effect, modulating meconium ileus occurrence, lung disease severity, and precocity of P. aeruginosa airway infection. The biological mechanisms explaining the impact of SLC6A14 on intestinal and lung phenotypes of CF patients are starting to be elucidated. This review focuses on SLC6A14 in lung and gastrointestinal physiology and physiopathology, especially its involvement in the pathophysiology of CF disease.

Keywords ATB+ · Amino acid transporter · CFTR · Nitric oxide · Pulmonary · Intestine

Introduction
Cystic fibrosis (CF), the most common lethal autosomal recessive genetic disease in Caucasians, is caused by variants in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride channel expressed ubiquitously within epithelia [1–3].

Symptoms can occur as early as birth with meconium ileus (MI), a severe neonatal intestinal obstruction affecting around 15% of CF neonates. This is followed by manifestations of the disease in other organs such as the liver, the pancreas, and the intestine, with lung complications as the main cause of morbidity and mortality in CF patients. In the lungs, absence or dysfunction of CFTR proteins results in altered salt and water transport through the airway epithelium leading to an altered mucociliary clearance, progressive colonization with different pathogens, exacerbation of inflammation, and lung tissue damage. Among the multitude of pathogens colonizing the CF lungs, Pseudomonas aeruginosa is the most common and life-threatening pathogen. Indeed, P. aeruginosa chronic lung colonization has been associated with a more severe lung disease and reduced survival [4].

Although CF is a monogenic disease, considerable phenotypic diversity is observed in patients carrying identical CFTR variants [5–7]. In addition to environmental factors, twins and siblings’ studies have revealed that genetic modifiers outside the CFTR locus are involved in this interindividual variability [5]. It is expected that these modifier genes account for 50% of the lung function variation. The current

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challenge is to identify these variants and determine how they contribute to the severity of the disease by performing in vitro/in vivo functional studies. Among the several modifier genes identified thus far, the solute carrier family 6 member 14 (SLC6A14, also known as ATB0+) has been shown to have pleiotropic effect in CF [8–14]. It was first identified as a modifier of MI occurrence [14], and then associated with lung disease and age at first P. aeruginosa infection [8, 10]. SLC6A14, the protein encoded by this gene, belongs to the solute carrier family 6 and uses the energy provided by Na+ and Cl− gradients to import and concentrate all neutral amino acids as well as the two cationic acids lysine and arginine into the cytoplasm of different cell types. Besides the genotype/phenotype associations, the biological mechanisms explaining the impact of SLC6A14 on intestinal and lung phenotypes of CF patients are beginning to be elucidated as evidenced by recent studies [15–17]. This review focuses on SLC6A14 in the context of CF, especially its involvement in the pathophysiology of CF lung and gastrointestinal disease.

SLC6A14 expression and regulation in the lung and gastrointestinal tract

SLC6A14 gene is located on chromosome X and was cloned in 1999 from a mammary gland cDNA library [18]. This gene produces two transcripts (ENST00000598581.3 and ENST00000463626.1) but only one codes for a protein (ENSG00000087916.7) which is comprised of 642 amino acids with an expected molecular weight of 72 kDa. SLC6A14 is a plasma membrane protein belonging to the solute carrier 6 (SLC6) family which contains 21 human proteins based on the similarity in their amino acid sequences [19]. Although no structural studies have been conducted on SLC6A14 specifically, crystal structure and structural studies on others members of the SLC6 family, as well as topological domain analysis (https://uniprot.org/uniprot/Q9UN76) suggest that SLC6A14 N- and C-terminal domains are cytoplasmic and that the sequence includes 12 putative transmembrane domains and 1 large extracellular domain between transmembrane domains 3 and 4 [19]. In the endoplasmic reticulum, the quality control of SLC6A14 folding involves interactions with the heat shock proteins HSP70 and HSP90 [20]. SLC6A14 trafficking from the endoplasmic reticulum to the Golgi apparatus depends on its interaction with the cargo-recognition protein SEC24 isoform C and the coatomer II (COPII) complex [21]. Further studies are needed to fully understand the mechanisms allowing SLC6A14 trafficking to the plasma membrane.

SLC6A14 mRNA was initially shown to be expressed mainly in the lung, fetal lung, trachea, and salivary gland [18]. Microarray and RNA-sequencing data obtained from Expression Atlas public resource confirm that SLC6A14 is predominantly expressed in human and mouse lung tissue (Table 1). However, SLC6A14 is also detected albeit in extremely low levels in gastrointestinal tissues including intestine and colon.

SLC6A14 expression and regulation in the lung

Before SLC6A14 mRNA and protein were identified in the human lung, Galetta et al. demonstrated the presence of a Na+-dependent amino acid transport at the apical membrane of bronchial epithelial cells isolated from CF or non-CF subjects [32]. Upon showing that SLC6A14 mRNA was strongly expressed in the human lung and trachea compared to other organs [18], Sloan et al. also showed that SLC6A14 protein was expressed in membrane fractions of human airway and distal lung samples from normal, emphysema, and CF patients [33]. Interestingly, the authors pointed out differences in the molecular mass of SLC6A14 protein detected either in the airways or in the distal lung, which was due to alternative splicing or posttranslational modification. Several studies have also shown expression of SLC6A14 in various cell lines of either airway or distal epithelial cell origin, as well as in primary bronchial epithelial cells (Table 2). In the alveolar A549 cell line, SLC6A14 protein was either detected or absent according to the study considered [34, 35]. Di Paola et al. observed that primary cells from individuals with CF or healthy donors showed a similar mRNA level for SLC6A14 [17], suggesting that CFTR pathogenic variants had no impact on SLC6A14 mRNA expression. Interestingly, a recent study using single RNA sequencing revealed that SLC6A14 expression was reduced in alveolar type II cells from idiopathic pulmonary fibrosis (IPF) patients compared to controls [36]. Via single-cell analysis, the authors also showed that SLC6A14 was expressed in basal, club cells, and alveolar type 2 cells.

In vitro, Gorrieri et al. observed that SLC6A14 transcripts were enhanced in bronchial epithelial cells exposed to interleukin-4 [39]. In the human glandular bronchial epithelial cell line Calu-3 and in primary human bronchial epithelial cells, flagellin or lipopolysaccharide from P. aeruginosa exposures increase the expression of SLC6A14 mRNA [17]. Finally, in relation to CF, a study using HEK-293 cells overexpressing SLC6A14 showed that SLC6A14 protein expression was reduced by suprapharmacological concentrations of Vx-770, a CFTR potentiator [40].

SLC6A14 expression and regulation in the gastrointestinal tract

In the human gastrointestinal tract, SLC6A14 appears to be heterogeneously expressed (Table 3). Sloan et al. first detected SLC6A14 mRNA in the stomach, although its levels were significantly lower than those observed in lung samples.
Table 1  SLC6A14 transcript expression in human (A) and mouse (B) (Modified from: Expression Atlas; https://www.ebi.ac.uk)

**A. Human**

| Study                                                                 | Lung | Salivary glands | Stomach | Vermiform appendix | Breast | Lower leg skin | Prostate gland | Urinary bladder | Vagina | Skin | Gall bladder | Duodenum | Esophagus | Esophagus succus | Jejunum | Colon | Rectum | Large intestine | Fallopian tube | Uterus | Sex | Age | Associated references |
|---------------------------------------------------------------------|------|----------------|---------|-------------------|--------|----------------|----------------|----------------|--------|-----|-------------|----------|-----------|-------------------|---------|-------|--------|---------------------|----------------|--------|-----|----|----------------------|
| RNA-seq of coding RNA of 19 human tissues from fetuses with congenital defects (NIH Roadmap Epigenomics Mapping Consortium) | 63   | 28             | 11      | 4                 | 6      | 4              | 3             | 2              | 2      | 1   | 1           | 1        | 1         | 1                 | N/A     | N/A   | N/A    | N/A                 | N/A            | N/A    |     |    | [23]                  |
| Strand-specific RNA-seq of 13 human tissues (Michael Snyder’s lab for the ENCODE project)                              | 42   | 0              | 0       | 0                 | 0      | 0              | 0             | 0              | 0      | 0   | 0           | 0        | 0         | 0                 | N/A     | N/A   | N/A    | N/A                 | N/A            | N/A    |     |    | [24]                  |
| Illumina Body Map                                                   | 23   | 6              | 2       | 0                 | 0      | 0              | 0             | 0              | 0      | 0   | 0           | 0        | 0         | 0                 | N/A     | N/A   | N/A    | N/A                 | N/A            | N/A    |     |    | [25-27]                |
| RNA-seq from 53 human tissue samples (Genotype-Tissue Expression (GTEx) Project)                                     | 14   | 30             | 0       | 1                 | 6      | 1              | 0             | 5              | 5      | 2   | 2           | 0        | 0         | 0                 | mixed   | N/A   | N/A    | N/A                 | N/A            | N/A    |     |    | [28]                  |

**B. Mouse**

| Study                                                                 | Lung | Testis | Vomeronasal organ | Skin | Olfactory epithelium | Kidney | Thymus | Strain | Sex | Age | Associated references |
|---------------------------------------------------------------------|------|--------|-------------------|------|----------------------|--------|--------|--------|-----|-----|----------------------|
| RNA-seq of the olfactory system of newborn mice                     | 35   | 32     | 35                | 32   |                      |        |        | C57BL/6J | mixed | newborn | N/A                 |
| RNA-seq of mouse DBA/2J x C57BL/6J                                   | 124  | 0,5    | 0                 | 0,5  |                      |        |        | DBA/2J  | N/A  | adult | [29]                |
| Mammalian Kaessmann                                                | 3    | 6      | 21                | 0    |                      |        |        | C57BL/6 | mixed | adult  | [30]                |
| RNA-seq of 9 tissues from an adult male C57BL/6 mouse              | 81   | 6      | 21                | 0    | 0                    |        |        | C57BL/6 | male  | adult  | [31]                |
| Transcription profiling by high throughput sequencing of mouse brain, liver and the whole testis (Soumillon et al.) | 3    | 3      | 3                 | 3    |                      |        |        | C57BL/6 | male  | adult  | [32]                |
| Strand-specific RNA-seq of nine mouse tissues                      | 87   | 4      | 0                 | 0    |                      |        |        | DBA/2J  | N/A  | N/A    | N/A                 |
| Strand-specific RNA-seq of nine mouse tissues                      | 77   | 3      | 0                 | 0    |                      |        |        | CD1     | N/A  | N/A    | N/A                 |
| Strand-specific RNA-seq of nine mouse tissues                      | 58   | 3      | 0                 | 0    |                      |        |        | C57BL/6 | N/A  | N/A    | N/A                 |

Numbers refer as transcripts per million, the gradation of blue illustrates the abundance of the transcript SLC6A14.
Two studies then detected SLC6A14 transcripts in mucosal biopsies from duodenum and rectum, respectively [41, 42]. Finally, Anderson et al. compared the expression of SLC6A14 mRNA throughout the gastrointestinal tract and showed that stomach, duodenum, and descending colon expressed high levels of SLC6A14 transcripts, while low levels were found in jejunum, ileum, ascending colon, and transverse colon [43]. Conflicting results have been obtained on the expression of SLC6A14 in the human colon epithelial cell line Caco-2, reporting either some or no SLC6A14 transcripts or protein [16, 42–44]. Interestingly, in mice, SLC6A14 mRNA expression is negligible in the ileum of control animals, but is strongly induced in epithelial ileal cells of CF mice [45].

In vitro, it has been showed that SLC6A14 expression may be modulated by several factors including toxins, bacterial constituents, and proinflammatory cytokines. Indeed, Flach et al. showed that SLC6A14 mRNA levels are significantly increased after 18 h of stimulation with cholera toxin in Caco-2 cells [42]. Other molecules have also been shown to regulate SLC6A14 expression. For example, in porcine intestinal cells, Wang et al. showed that SLC6A14 mRNA was increased by L-tryptophan [47]. Ikpa et al. also showed that antibiotic treatment of CF mice induces an important reduction of SLC6A14 transcripts in ileal epithelial cells [45].

### Genetic association studies in CF

Given the diversity of phenotypic severity in CF patients with the same causal CFTR variants, several genetic studies have been conducted to identify CF modifier genes. Among the identified loci, one locus on chromosome X, near the
The initial evidence showing that SLC6A14 may be a modifier gene in CF has been described by Sun et al. in a “genome wide association study” (GWAS) involving 6135 CF patients [14]. This study identified a significant association between susceptibility to MI and three SLC6A14 genetic variants (rs12839137, rs5905283, and rs3788766).

Table 4 Genetic associations tested between SLC6A14 variants and digestive and pulmonary manifestations in CF patients

| rs ID (Alleles) | MAF  | Variant localization          | Association with                                | Number of CF patients | Cohort/patients characteristics                                                                 | Ref   |
|----------------|------|------------------------------|-------------------------------------------------|-----------------------|--------------------------------------------------------------------------------------------------|-------|
| rs7879546 (T/C) | 0.41 | Intergenic                   | Lung disease severity                            | 6365                  | Mean age: 19.5 years; PI (99.8%); F508del homozygotes (65%)                                      | [8]   |
| rs5905376 (C/A) | 0.23 | Intergenic                   | Lung disease severity                            | 6135                  | Two independent cohorts (patients with two severe CFTR mutations): 3,763 North American (F508del homozygotes 71.4%) and 2,372 French (> 6 years old) and American patients | [14]  |
| rs5952223 (C/T) | 0.23 | Intergenic                   | Lung disease severity                            | 815                   | Mean age of lung function measurements: 12.63 years; F508del homozygotes: 62.3%                   | [10]  |
| rs12839137 (G/A) | 0.12 | Intergenic                   | Presence of meconium ileus                      | 730                   | Median age at first detection of positive P. aeruginosa culture: 5.55 years; F508del homozygotes: 61% |       |
| rs905283 (A/C)  | 0.47 | Intergenic (2 KB Upstream Variant) | Presence of meconium ileus                      | 126                   | Median age of the first available IRT measurement: 0.36 years; F508del homozygotes: 60.3%        | [11]  |
| rs3788766 (G/A) | 0.36 | Regulatory region            | Presence of meconium ileus                      | 111                   | Patients from Colorado, median age at IRT measurement: 2 days; F508del homozygotes: 56%          |       |
| rs12710568 (G/C) | 0.31 | Regulatory region            | Presence of meconium ileus                      | 83                    | Patients with two severe CFTR mutations: 3,763 North American (F508del homozygotes 71.4%) and 2,372 French (> 6 years old) and American patients | [9]   |
| rs5905177 (C/T) | 0.35 | SLC6A14 intron                | Presence of meconium ileus                      | 79                    | Brazilian patients                                                                                | [12]  |

Chromosomic position (forward strand); minor allele in the European population, Minor allele Frequency (MAF), data were collected from Ensembl, 1000 Genomes, European population. IRT immunoreactive trypsinogen, PI pancreatic insufficiency.
In a study involving more than 6700 CF patients from the International CF Gene Modifier Consortium, Gong et al. recently replicated the association between susceptibility to MI and rs3788766 [9]. This study also identified an association between MI susceptibility and two new variants (rs12710568 and rs5905177) located within the SLC6A14 regulatory region and SLC6A14 intron, respectively. Several groups performed sex-specific association analysis based on the fact that SLC6A14 gene is located within the region associated with random X-inactivation [9, 14]. Interestingly, they found higher odd ratios in male than in female only for genetic variants associated with susceptibility to MI.

Li et al. further assessed the association of MI risk alleles of SLC6A14 with other CF co-morbidities, such as the lung disease severity and age at first P. aeruginosa infection [10]. Their study involved 815 CF Canadian pediatric patients who were genotyped for the following SLC6A14 variants: rs12839137, rs5905283, and rs3788766. Among the variants studied, rs5905283 and rs3788766 risk alleles were associated with pediatric lung disease severity; whilst rs3788766 variant was associated with age at first P. aeruginosa infection, as confirmed later in a smaller cohort [12]. In 2015, a GWAS involving 6,365 patients confirmed that SLC6A14 modifies the severity of the lung disease in CF [8]. Indeed, the authors found a significant association between genotypes of three SLC6A14 intergenic variants (rs7879546, rs5905376, and rs5952223) and the lung disease severity.

Gong et al. recently integrated GWAS and tissue-specific gene expression data to determine whether modifier loci on chromosome X (encompassing SLC6A14) influence SLC6A14 mRNA expression levels in different tissues [9]. This kind of analysis indicates whether eQTL (expression quantitative trait loci) colocalize with loci associated with CF phenotypes that may indicate the existence of a genetic regulator. Their results showed that SLC6A14 mRNA expression in CF nasal epithelia and in the pancreas colocalize with the lung disease and MI-associated variants, respectively, suggesting that each locus impacts SLC6A14 expression with tissue specificity. Besides, neither association between SLC6A14 genetic variants and early exocrine pancreatic phenotype nor immunoreactive trypsin levels at birth have been found [10, 11].
Putative biological roles of SLC6A14 in CF

Several studies showed that SLC6A14 plays a primary role as an amino acid transporter in various epithelial cells and models [32, 48–52]. Taken together, SLC6A14 expression data in human and genetic studies suggest that SLC6A14 may have an important role in the lung and intestinal pathophysiology of CF patients (see Parts 1 and 2).

SLC6A14 function in the lung

In the lung, it was first suggested that the apical transport of amino acid in the airway epithelial cells may play an important role in infection resolution [32] as pathogens need amino acids to proliferate into the airways. The authors suggested that amino acid transporter at the apical membrane may be activated following infection to rapidly decrease the amino acid concentration of the airway surface liquid (ASL) (Fig. 2). This phenomenon could then help to fight against infections. This hypothesis is supported by the unique characteristics of SLC6A14 allowing it to strongly concentrate all essential amino acids into the cytoplasm of epithelial cells.

Ahmadi et al. recently reported that arginine transport through SLC6A14 increased F508del-CFTR Cl− efflux in CF airway epithelial cells stimulated with or without a CFTR corrector, lumacaftor [15]. They also observed that this increase in CFTR function induced an increase in the ASL height and that the potentiation of F508del-CFTR channel function in CF cells induced by SLC6A14 arginine uptake occurred via the nitric oxide (NO) signaling pathway (Fig. 3). Finally, they suggested that SLC6A14 activation may be considered as a complement therapy to CFTR correction and potentiation in CF patients.

SLC6A14 function in the gastrointestinal tract

Ahmadi et al. also conducted another study to determine the biological function of SLC6A14 in the murine gastrointestinal tract [16]. They first demonstrated that SLC6A14 is a major apical amino acid transporter in the murine colon. Indeed, Slc6a14(−/−) mice exhibited almost 75% reduction in apical arginine transport compared to WT mice. In CF mice, they observed that Slc6a14 disruption induced a reduction in weight gain and BMI post-weaning and worsen the intestinal phenotype, i.e., decreased F508del-CFTR-mediated fluid secretion. They also highlighted that SLC6A14 does not seem to affect...
the processing or stability of F508del-CFTR neither co-
immunoprecipitated with F508del-CFTR in an F508del-
CFTR BHK over-expression system, which led them to
investigate intracellular signaling such as NO synthesis.
They observed that SLC6A14 inhibition impaired argi-
nine uptake by intestinal epithelial cells inducing both
a decrease in NO production and cGMP regulation of
F508del-CFTR. These results suggest that an increase
in SLC6A14 activity may enhance NO production and
F508del-CFTR residual activity in CF tissues. However,
it is not known whether these mechanisms are conserved
in humans.

It has to be emphasized that in the lung or the intes-
tine, functional studies were conducted with either over-
expression (plasmid) or inhibition experiments (siRNA) of
the whole gene. The role of the specific genetic variants
identified in genetic studies (see Part entitled “Genetic
association studies in CF”) is not yet known and further
elucidation is warranted.

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The diagram shows the relationships between SLC6A14, F508del-CFTR, and the nitric oxide signaling pathway.

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**Table 5** Expression of SLC6A14 in other diseases

| Disease                  | SLC6A14 expression | Methods                          | Ref                      |
|--------------------------|--------------------|----------------------------------|--------------------------|
| Cancer                   | Cervical           | Up-regulated                     | PCR, IF/IH, HIS          | [54]          |
|                          | Colorectal         | Up-regulated                     | PCR, Northern blot, IH   | [55]          |
|                          | Pancreatic         | Up-regulated                     | Microarray, qPCR, WB, IF, IH | [56, 57] | |
|                          | Breast (ER+)       | Up-regulated                     | PCR, IF                  | [58]          |
| Colonic diseases         | Crohn’s disease    | Up-regulated                     | qPCR                     | [46]          |
| Colonic diseases         | Ulcerative colitis | Up-regulated                     | qPCR, microarray         | [41, 59, 60] |
| Colonic diseases         | Long vs. short duration of ulcerative colitis | Down-regulated | Microarray | [61]          |
| Cholera                  | Acute vs. convalescence phase | Up-regulated | qPCR | [42]          |
| IPF                      | IPF                | Down-regulated                   | scRNAseq                 | [36]          |
| IPF                      | IPF vs. NSIP       | Up-regulated                     | Microarray               | [62]          |

ER+ estrogen receptor-positive, IPF idiopathic pulmonary fibrosis, NSIP non-specific interstitial pneumonia. If not specifically mentioned, the expression of SLC6A14 is relative to control patients. IF immunofluorescence, IH immunohistochemistry, HIS hybridization in situ, PCR polymerase chain reaction, WB western blot.
**SLC6A14 in non-CF diseases**

**SLC6A14 expression in other diseases**

SLC6A14 expression has been shown to be differentially up-regulated in several pathological contexts (Table 5), especially in cancer and colonic diseases (reviewed in [53]).

**Cancer**

SLC6A14 is significantly upregulated in tissues from cervical [54], colorectal [55], pancreatic [56, 57], and estrogen receptor-positive (ER+) breast cancer [58]. In ER+ breast cancer, high expression of SLC6A14 mRNA has been correlated with a better survival among patients [63]. Using a mouse model of spontaneous breast cancer, Babu et al. showed that its development and progression was significantly decreased when the mice were crossed with Slc6a14−/− mice [64]. The molecular mechanisms associated with these up- or down-regulations are largely unknown.

SLC6A14 expression was shown to be regulated by estrogen [58] which explains its specific increased expression in ER+ but not in ER- breast cancer. Also, inverse expression patterns of SLC6A14 mRNA and the microRNA (miR)-23a [65, 66] were found suggesting its regulatory effect. However, no functional studies (using miR mimic or inhibitors) confirmed miR-23a involvement in SLC6A14 expression regulation. In contrast, inverse correlation of miR-23b-3p [67] and SLC6A14 expression was recently confirmed. Functional studies have shown that the downregulation of SLC6A14 observed in endocrine therapy (ER+ breast cancer standard of care)-resistant cells is associated with an increase of miR-23b-3p [63].

**Colonic diseases**

SLC6A14 mRNA levels are significantly higher in colonic mucosal specimens obtained from patients with Crohn’s disease compared to controls [46]. SLC6A14 expression was also increased in rectal and colonic biopsies from patients with ulcerative colitis or infectious/ischemic colitis compared to controls, suggesting that SLC6A14 upregulation might be the result of the inflammatory context rather than a specific pathophysiological consequence of the ulcerative colitis [41, 59, 60, 68]. Low et al. further observed that SLC6A14 was down-regulated in colonic biopsies from patients with long-duration of ulcerative colitis compared with patients with short duration [61]. In rats, D’Argenio et al. showed that experimental colitis induced a marked decrease in SLC6A14 transcript expression in the colon [69]. Finally, Kou et al. found that colon cancer cell lines overexpressed SLC6A14 compared to normal colon cells [44].

**Idiopathic pulmonary fibrosis**

SLC6A14 has been found to be downregulated in alveolar-type II cells of idiopathic pulmonary fibrosis (IPF) patients [36], while it is overexpressed in specimens from explanted lungs of patients with non-specific interstitial pneumonia compared to specimens from IPF patients [62].
Infectious diseases

SLC6A14 mRNA levels have been shown to be increased in biopsies of duodenum collected during the acute phase of cholera compared to biopsies collected during convalescence phase [42].

Genetic associations studies in other diseases

Four additional SLC6A14 genetic variants have been associated with phenotypic variability in other diseases than CF (Table 6).

SLC6A14 genetic variants have been associated with obesity in different populations (Table 6). In a candidate gene analysis, later replicated in an independent cohort, Suviolahti et al. found significant differences in SLC6A14 rs2011162 genetic variant allele frequencies between obese and non-obese subjects [72]. Another study suggested an association between the rs2011162 and fat oxidation in women which may be, when not adapted to fat intake, responsible to weight gain over time [73]. Finally, in a French family cohort study comprising of 1,267 obese adults and 649 lean control subjects, Durand et al. found a significant association between rs2011162 genetic variant and obesity. They observed that the risk allele was associated with higher body fat and modified perception of hunger and satiety in adult women with moderate obesity and in obese girls [71]. Durand et al. also identified an association between SLC6A14 rs2071877 genetic variant and obesity in a French cohort [71]. This variant has also been associated with sum of triceps and subscapular skinfolds thickness, an objective measure of adiposity, in boys 7–8 of age [70]. Finally, Miranda et al. also found evidences of associations between two other genetic variants, rs2312054 and rs12391221, and several parameters used to assess the food intake in children [70].

SLC6A14 genetic variants have also been associated with male infertility. Indeed, Noveski et al. found that rs2011162 alone and rs2011162(G)/rs2071877(C)/rs2312054(A) haplotype were differently distributed among fertile and infertile groups in their cohort [74]. As rs2011162 is located within the 3‘UTR region of SLC6A14, they investigated the possible consequences of this genetic variation on the RNA secondary structure. They found a significant structural effect of this genetic variant that may result in a differential mRNA expression depending on the allele.

It was recently shown that SLC6A14 expression quantitative trait loci (eQTL) from nasal epithelial cells and pancreas tissues coincide with lung disease and meconium ileus-associated variants, respectively, supporting an important role for SLC6A14 variants in CF [9]. However, whether in CF or other diseases, the functional in vitro/in vivo consequences of identified SLC6A14 genetic variants on SLC6A14 protein expression and/or function have never been studied. This gap in knowledge needs to be addressed to better understand the molecular mechanisms by which SLC6A14 affect phenotypes or diseases. SLC6A14 genetic variants described in this review are located in the non-coding region either intergenic or located in the regulatory region (promoter), introns, or in the 3‘UTR region of SLC6A14, and, subsequently, do not modify the amino acid sequence of SLC6A14 protein. However, these variants may have multiple effects not only on SLC6A14, but also on nearby and/or distant genes. For example, genetic variants located in the promoter may affect transcriptional activity by altering transcription factor binding. Other functional consequences of these SNPs have to be studied including DNA methylation and histone modifications, alternative splicing, conformation and stability of mRNA as well as structure, expression level, and function of proteins. Even if bioinformatic tools may predict functional consequences of genetic variants, downstream in vitro/in vivo experimental studies will also be necessary. Successfully used for several complex traits, genome-editing technologies may also be used to create isogenic cell lines with specific alleles to assess their functionality including chromatin structure, transcription factor binding, gene and protein expression, and specific cellular assays [75]. Furthermore, as previously mentioned, in addition to the impact of genetic variants, SLC6A14 expression can be regulated by environmental factors (inflammatory molecules, pathogens, and pharmacological treatments) and possibly age (fetal vs. adult, Table 1). Thus, the development of different experimental models to identify the causality of SLC6A14 expression and function variability over the course of disease progression will be a major challenge.

Conclusion

SLC6A14 seems to be predominantly expressed in epithelial cells of the human lung and to a lesser extent in the human gastrointestinal epithelium. Importantly, SLC6A14 expression profiles appear to be different between human and mice, suggesting that studies investigating the biological roles of this protein in murine models may not directly correlate with findings obtained in human models. Several groups have observed that SLC6A14 levels are up- or down-regulated in pathological conditions, however, the mechanisms involved in these dysregulations have mostly not been elucidated. Moreover, some studies have demonstrated that inflammatory mediators and pathogen molecules may impact SLC6A14 expression.

Furthermore, genetic studies highlight that SLC6A14 genetic variants modulate the severity of digestive and pulmonary diseases in CF patients. The biological function of this pleiotropic modifier gene is not fully explained and
the biological direct consequences of identified variants in genetic studies remained to be clarified. However, some recent studies suggest that SLC6A14 may play an important role in the response to respiratory infection and fluid secretion related to CFTR. Thus, SLC6A14 may be a potential therapeutic target to improve anti-infective response and CFTR function and/or correction in CF patients in a personalized way.

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