Expression of the ZIP/SLC39A transporters in β-cells: a systematic review and integration of multiple datasets

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Abstract

Background: Pancreatic β-cells require a constant supply of zinc to maintain normal insulin secretory function. Following co-exocytosis with insulin, zinc is replenished via the Zrt- and Irt-like (ZIP; SLC39A) family of transporters. However the ZIP paralogues of particular importance for zinc uptake, and associations with β-cell function and Type 2 Diabetes remain largely unexplored. We retrieved and statistically analysed publically available microarray and RNA-seq datasets to perform a systematic review on the expression of β-cell SLC39A paralogues. We complemented results with experimental data on expression profiling of human islets and mouse β-cell derived MIN6 cells, and compared transcriptomic and proteomic sequence conservation between human, mouse and rat.

Results: The 14 ZIP paralogues have 73–98% amino sequence conservation between human and rodents. We identified 18 datasets for β-cell SLC39A analysis, which compared relative expression to non-β-cells, and expression in response to PDX-1 activity, cytokines, glucose and type 2 diabetic status. Published expression data demonstrate enrichment of transcripts for ZIP7 and ZIP9 transporters within rodent β-cells and of ZIP6, ZIP7 and ZIP14 within human β-cells, with ZIP1 most differentially expressed in response to cytokines and PDX-1 within rodent, and ZIP6 in response to diabetic status in human and glucose in rat. Our qPCR expression profiling data indicate that SLC39A6, −9, −13, and −14 are the highest expressed paralogues in human β-cells and Slc39a6 and −7 in MIN6 cells.

Conclusions: Our systematic review, expression profiling and sequence alignment reveal similarities and potentially important differences in ZIP complements between human and rodent β-cells. We identify ZIP6, ZIP7, ZIP9, ZIP13 and ZIP14 in human and rodent and ZIP1 in rodent as potentially biologically important for β-cell zinc trafficking. We propose ZIP6 and ZIP7 are key functional orthologues in human and rodent β-cells and highlight these zinc importers as important targets for exploring associations between zinc status and normal physiology of β-cells and their decline in Type 2 Diabetes.

Keywords: Type 2 diabetes, Zinc, ZIP, SLC39A, Systematic review, Expression data, Microarray, RNA-seq

Background

Pancreatic β-cells require a constant supply of zinc for normal function in maintaining glycaemic control [1, 2]. Zinc acts at multiple stages within the insulin secretory pathway [3, 4]. Zinc ions (Zn²⁺) are loaded into insulin granules via the predominantly β-cell specific zinc transporter 8 (ZnT8) [5], where two ions co-crystallise with insulin hexamers [6], important for proper insulin processing, protection of insulin from proteolytic degradation [7] and for maintaining granule osmotic stability [8]. Zinc is subsequently co-released with mature insulin upon exocytosis where it is proposed to fulfil additional roles in glycaemic control [9–11].

Significant amounts of Zn²⁺ are lost from β-cells during insulin secretion and coordinated replenishment is required. The Zrt- and Irt-like (ZIP; SLC39A) family of zinc importer proteins, of which 14 paralogues are present within both humans and rodents [12, 13], tightly control cellular Zn²⁺ influx into the cytosol and are thought responsible for restoring β-cell zinc content [14]. ZIP paralogues exhibit differing Zn²⁺ affinities (K₀.₅) and transporting efficiencies, and show cell- and type-specific expression.
condition-dependent expression [12, 15], thus it is expected that the β-cell ZIP profile closely reflects the unique cellular demand for Zn^{2+} and ability to adapt to stresses such as hyperglycaemia and inflammatory cytokines. Since both hyperzincentmic and hypozincemic Zn^{2+} statues are observed in diabetic patients [16–18] and animal models of diabetes [19, 20], one can hypothesise that altered ZIP expression profiles are associated with disease state. However exploration of the β-cell SLC39A transcriptome, and therefore the liable transporters, has been limited to a few studies [4, 14, 21–23], where an importance of ZIP4 [23], ZIP6 [21, 22], ZIP7 [14, 21, 22], ZIP8 [22], and ZIP14 [14, 24] has been suggested.

Type 2 Diabetes is rapidly evolving into a major public health crisis. The disease pathogenesis generally results from an increasingly inadequate insulin response due to enhanced insulin resistance and a compensatory demand on insulin production that eventually leads to β-cell failure. Multiple studies have associated diabetes with hypozincemia, likely caused by hyperzincuria, and a negative correlation between the glycated haemoglobin percentage and plasma zinc [16–18]. Accordingly, there is a positive effect of adequate plasma zinc levels on glycemic control [18], suggesting a compromised zinc status in diabetes [25].

Since zinc plays an integral role within β-cells, understanding its regulation may prove central for targeting loss of secretory function during Type 2 Diabetes. Much of our understanding of β-cell physiology has derived from studies on rodents due to very limited accessibility of human islets [26]. However, differences in physiology between humans and rodents remain often unacknowledged when interpreting rodent studies. We hypothesised that the ZIP transporters most important to β-cells should be robustly expressed and show enrichment relative to other cell types [27], with changes in expression influenced by cellular stresses associated with compromised insulin secretion. We thereby aimed to identify and evaluate the complement of ZIP transporters most important within human and rodent (mouse and rat) β-cells for regulating zinc influx and accumulation.

Here we show through systematic review of micro-array and RNA-seq studies [28, 29] that transcripts for multiple ZIP paralogues are enriched in β-cells and/or show transcriptional regulation in response to cytokines, hyperglycaemia, Type 2 Diabetes status, and pancreatic and duodenal homeobox 1 (PDX-1) activity, the major transcription factor for β-cells. We used quantitative PCR (qPCR) to verify the relative expression of these paralogues within human islets and/or murine MIN6 β-cells. Furthermore, we computationally aligned human, mouse and rat SLC39A mRNA and protein sequences to demonstrate high cross-species conservation of the paralogues identified as key for β-cell zinc homeostasis within our systematic review. We highlight ZIP6, ZIP7, ZIP9, ZIP13 and ZIP14 in human and rodent, and ZIP1 in rodent as biologically important candidates for mediating β-cell Zn^{2+} influx and zinc-signalling processes, such as cell proliferation. In addition to normal physiology, we suggest ZIP6, ZIP7 and ZIP14 downregulation is associated with diabetic status; however the relationship to zinc content in the β-cells/pancreas remains unknown. Critically, our review highlights potentially important differences between human islets and rodent cells in their complements of zinc importers, again demonstrating the limitations of rodent models for human diabetes.

Methods
Systematic review
Identification of eligible expression datasets
This systematic review was conducted in accordance with the guidelines provided in the PRISMA statement. Microarray and RNA-seq expression profiling studies were identified through searching the NCBI PubMed database and the Gene Expression Omnibus (GEO) database [30] to April 2016, using combinations of the following key terms: “β-cell, islet” and “diabetes, gene expression, microarray, RNA-seq”, and compiled studies screened for duplicates. Eligibility was independently assessed through first screening by title and abstract, and then by the full text, based on the following inclusion criteria: original research article published in English, RNA-seq or microarray platform, expression profiling of mature β-cells, islets and/or β-cell line, and human or rodent genome. The eligibility was finally confirmed through verifying the presence of accessible expression data for ZIP transporter transcripts (SLC39A/Slc39a). Included datasets explored: (a) expression within β-cells compared to non-β-cells, (b) expression in response to extracellular cytokines, (c) expression in response to PDX-1 activity, (d) expression in response to extracellular glucose, and (e) expression within human diabetic islets. From each identified dataset, the accession number (if appropriate), platform, species, sample types and sizes, and gene expression data were extracted. This pipeline is depicted in Fig. 1.

Data pre-processing
The heterogeneity of different platforms, gene nomenclature and control samples can cause difficulties when comparing datasets from different sources. Normalisation is therefore critical to reduce the chance of skewing the results and enhances credibility of individual expression changes. To minimise inconsistency, a standardised normalisation method was performed within datasets [31] using Qlucore Omics Explorer (version 3.2; Qlucore
AB, Lund, Sweden). Raw data was log (base = 2; log2) transformed and normalised through applying a standard score (Z-score) transformation, which calculates normalised expression intensities ($y_i$) of each probe as follows:

$$y_i = \frac{x_i - m}{\delta}$$

Where $x_i$ represents raw intensity values ($x_i$, $i = 0...N-1$) for each gene, $m$ represents average gene intensity for the experiment, and $\delta$ represents the standard deviation of all measured intensities.

### Statistical analysis

Statistical analysis was undertaken using the Qlucore Omics Explorer (version 3.2; Qlucore AB, Lund, Sweden) bioinformatics software. Fold differences (FD) in expression between relevant conditions were derived, and significance calculated on the global transcriptomic data set through unpaired t-tests, adjusted using the Benjamini-Hochberg False Discovery Rate (FDR) procedure [32]. Genes were considered differentially expressed in comparisons at an FDR of 15%. Arbitrary FD cut offs of ≥1.5-fold on significantly regulated genes were chosen to indicate biologically relevant differential expression. Full results from analysis are provided in Additional file 1.

### Datasets with inaccessible raw data

Where the full raw datasets were not available for download, normalised data were extracted from supplementary data tables and log2 transformed FD (Log2FD) values and significance extracted/calculated using Excel as appropriate. Data analysed in this way are annotated.

### Experimental analysis

#### Human islet cDNA

Human islet cDNA originating from healthy cadaver donors was obtained via the Human Islet Isolation Unit at King’s College Hospital.

#### Cell line and RNA extraction

The adherent insulinoma β-cell line MIN6 (*Mus musculus*) was maintained within Dulbecco’s Modified Eagle’s Medium supplemented with 15% fetal bovine serum, 4 mM L-glutamine, 50 μM β-mercaptoethanol, 100 μg/ml streptomycin and 100 units/ml penicillin (both Sigma-Aldrich), at 37 °C in a humidified atmosphere of 95% air
and 5% CO₂. Total RNA was extracted using TRIzol Reagent (ThermoFisher), reverse transcribed to cDNA using the high capacity RNA-to-cDNA kit (ThermoFisher), and diluted ≥1:10 prior to experimentation.

**Quantitative PCR**

Quantitative PCR (qPCR) assays were designed using the online Universal Probe Library (UPL) assay design tool (Roche). Assay designs are provided within Additional files 2 and 3: Tables S1 and S2. Primer Blast [33] was used to predict the binding of our primers to mouse and human RNA. The mouse primers bind all respective ZIP transporter isoforms. The human primers bind all isoforms for ZIP2, ZIP4, ZIP5, ZIP6, ZIP8, ZIP10, ZIP11, ZIP12 and ZIP13. Primers for ZIP1, ZIP3, ZIP7, ZIP9 and ZIP14 covered either all the isoforms expressed within human islets, or those predominantly expressed (Additional files 2 and 3: Tables S1 and S2, Additional file 4). PCR plates were loaded using the Biomek FX liquid handling robot (Beckman Coulter) and reactions [20-40 µg cDNA, 0.1 µM UPL probe, 0.2 µM forward primer, 0.2 µM reverse primer and 1X TaqMan Fast Advanced Mastermix (Applied Biosystems)] amplified using the Prism7900HT sequence detection system, Applied Biosystems, and analysed with SDS (sequence detection systems) 2.4 software. All gene expression values were normalised to the housekeeping gene ubiquitin C (UBC), and relative expression calculated using the ΔΔCT method. The efficiencies for primers targeting genes with appreciable expression (mouse: ZIP1, ZIP2, ZIP3, ZIP4, ZIP6, ZIP7, ZIP8, ZIP9, ZIP10, ZIP11, ZIP13, ZIP14, UBC, GADPH; human: ZIP1, ZIP3, ZIP4, ZIP5, ZIP6, ZIP7, ZIP8, ZIP9, ZIP10, ZIP13, ZIP14, UBC, GADPH) ranged from 88 to 111% for mouse and 75–106% for human. Data show an average of two biological repeats for human islets and three biological repeats for MIN6 cells.

**Exploration of zinc transporter heterogeneity**

Human and mouse ZIP orthologues were aligned to assess transcriptomic and proteomic similarities using MUSCLE (3.8) [34, 35] and percent similarity values recorded.

**Results**

**Overview of included datasets**

A systematic review allows integrated analysis of multiple high throughput gene expression datasets. Following the pre-defined criteria, 18 appropriate β-cell/islet expression profiling studies were identified. These studies are summarised in Table 1. Seven studies compared expression within β-cells to non-β-cells, four investigated expression in response to extracellular cytokines, three studied expression in response to PDX-1 activity, one explored expression in response to extracellular glucose, and three measured expression within human diabetic islets.

**Specificity of ZIP transporter expression within β-cells**

Multiple microarray and RNA-seq studies have sought to assess β-cell gene expression relative to other pancreatic cells and additional tissues. Since ZIP paralogues exhibit cell-specific profiles reflecting function [12, 15], β-cell enrichment may indicate important cell-specific roles. Analysis of human islet cell transcriptomics datasets uncovered SLC39A13 and SLC39A14 as enriched within β-cells compared to α-cells [2- to 3-fold], and SLC39A1, SLC39A10 and SLC39A11 as ≥1.5-fold depleted [36, 37]. However, when β-cell expression was compared to sorted pancreatic exocrine cell populations (human duct and acinar cells), enrichment of SLC39A7 and SLC39A9 was observed (1.7- and 1.6-fold respectively) alongside relative depletion of SLC39A5 (11-fold), SLC39A8 (4.3-fold), SLC39A10 (1.8-fold) and SLC39A11 (1.5-fold) (data calculated from supplementary tables). Similarly RNA-seq data from Nica et al. uncovered depletion of SLC39A5 and SLC39A10 within sorted human β-cells over both total islets (2- and 6.8-fold, respectively) and non-β-cells [islet cell populations considered depleted of β-cells (2.8- and 4-fold, respectively)], accompanied by depletion of SLC39A2 (2-fold over total islets and 4-fold over non-β-cells) and SLC39A3 (1.7-fold over both total islets and non-β-cells), with enrichment of SLC39A1 (2.4-fold over total islets and 2.1-fold over non-β-cells), and of SLC39A14 (1.9-fold, only over non-β-cell preparations) [38].

Analysis of microarray datasets of human β-cell-enriched pancreatic samples and 15 other tissues [β-cell-enriched pancreas, pancreatic duct cells, cerebrum, colon, foetal brain, kidney, liver, lung, myocardial, skeletal muscle, prostate, small intestine, spleen, stomach, testis and thymus (dataset GSE30803)] revealed ≥1.5-fold enrichment of SLC39A1, SLC39A6, SLC39A7 and SLC39A14, however without statistical significance [39]. Further investigation of probe-specific expression revealed that relative enrichment was biased by elevated expression of specific paralogues within other tissues (ZIP6 within the brain [40] and prostate [41], ZIP7 within the colon [42] and ZIP14 within the liver [43]). Omitting these tissues indicated ≥3-fold enrichment of SLC39A6 (q < 0.1) and SLC39A14 (q < 0.15), and 1.6-fold SLC39A7 (q < 0.05) enrichment within β-cells compared to the remaining tissues analysed (Fig. 2a).

Analysis of a mouse RNA-seq dataset [44] suggested Slc39a4, Slc39a5 and Slc39a8 are ≥4-fold depleted within sorted β-cells over islets (Fig. 2b). Further investigation of non-β-cell depleted paralogues compared to total islets and six other cell types [brain, liver, lung fibroblasts, neural progenitor cells (NPC), skeletal muscle,
total islet (Fig. 2c) revealed Slc39a7 and Slc39a9 as the most β-cell enriched Zip paralogues in mouse with 2.3- and 3-fold elevated expression, respectively. Analysis of a further rat dataset comparing expression of β-cells over α-cells (dataset GSE13381) displayed ≥1.5-fold enrichment of Slc39a3 and Slc39a6, and ≥2-fold enrichment of
Slc39a6, but without statistical significance. However, there was no differential Slc39a expression between murine islets and five other tissues (adipose, gastrocnemius muscle, hypothalamus, liver, and soleus muscle) from 10-week-old lean and obese C57BL/6 and BTBR mice (dataset GSE10785).

**Cytokine stimulation and ZIP transporter expression**

Pro-inflammatory cytokines profoundly affect cellular metabolism and utilization of nutrients such as metal ions [45]. Chronic exposure of islets to the inflammatory cytokines interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) is associated with β-cell destruction and decreased secretory parameters in both Type 1 and Type 2 Diabetes [46]. Cytokine-dependent expression may indicate ZIP paralogues important for maintaining normal β-cell parameters when adapting to extracellular cytokine stress. RNA-seq dataset analysis of human islets exposed to IL-1β and IFN-γ for two days (dataset GSE35296) revealed 1.4- and 2.0-fold upregulation of SLC39A8 and SLC39A14 transcripts, respectively, and 2-fold downregulation of SLC39A10 [47]. However, an additional microarray study using human islets [48] did not show any ZIP transporter transcripts differentially expressed following 48 h incubation with IL-1β and IFN-γ.

Within independent studies, both fluorescence assisted cell sorting (FACS) purified rat β-cells [49] and the rat INS-1E β-cell line [50] were cultured with IL-1β and IFN-γ before microarray analysis (data from both studies calculated from supplementary data). Within rat β-cells [49] differential regulation of Slc39a1 (2.5-fold) was observed at 2 h, and of Slc39a1 and Slc39a10 at both 12 h (2.0- and −3.5-fold, respectively) and 24 h (1.5-fold and −1.6-fold, respectively). Similarly, INS-1E cells [50] displayed upregulation of SLC39A1 at both 6 and 24 h (2.6- and 2.1-fold, respectively) and of Slc39a6 at 24 h (1.7-fold), alongside downregulation of Slc39a13 at 6 h (−1.5-fold). INS-1E cells were additionally analysed after 6 and 24 h incubation with IFN-γ and TNF-α to show Slc39a1 upregulation (2.8- and 2.4-fold, respectively) and Slc39a14 downregulation (2.8- and 2.4-fold, respectively).

**PDX-1 and ZIP transporter expression**

PDX-1 is the key transcription factor mediating β-cell-specific gene expression within developing and mature β-cells [51]. Changes in ZIP expression as a consequence of PDX-1 activity may indicate roles of respective transporters in maintaining normal β-cell parameters. Critically, constitutive overexpression of PDX-1 sensitizes β-cells to cytokine-induced apoptosis [52, 53]. Overexpression of PDX-1 within rat INS-1αβ cells (dataset GSE40642) resulted in downregulation of Slc39a6 and Slc39a14 (Fig. 3). Stimulation of PDX-1 overexpressing cells with cytokine IL-1β further exacerbated these effects and also upregulated Slc39a1 ≥2.5-fold [54] (Fig. 3). Whereas within rat islets (dataset GSE49786), PDX-1 overexpression upregulated Slc39a8 (2.1-fold, P < 0.01) [55]; however Slc39a5 and Slc39a8 were up- and downregulated 1.5- and −2.6-fold, respectively, in mouse MIN6 cells (dataset E-MTAB-127) [56]. Data analysis from E-MTAB-127 additionally showed 2.8-fold downregulation of Slc39a5 in PDX+/− mouse islets compared to PDX+/+ control mouse islets.

**Expression in response to glucose stimulation**

Hyperglycaemia is universal within all prediabetic and diabetic cases, and glucose-responsive expression may indicate genes and pathways important for adapting to an enhanced demand for insulin secretion. Examination of microarray datasets of isolated rat islets cultured with 2 mM, 5 mM, 10 mM and 30 mM glucose (dataset GSE12817) uncovered ≥1.5-fold upregulation of Slc39a2, Slc39a4 and Slc39a6 and ≥1.5-fold downregulation of Slc39a3 and Slc39a5 when glucose increases [57].
Expression within islets from type 2 diabetic patients

Transcriptomic datasets of islets derived from normoglycaemic and Type 2 diabetic patients were next analysed to explore relevance to the human disease. Three paralogues (SLC39A2, SLC39A5 and SLC39A8) showed ≥1.5-fold upregulation and four paralogues (SLC39A6, SLC39A7, SLC39A8 and SLC39A14) ≥1.5-fold downregulation in diabetic compared to non-diabetic individuals [results combined from studies GSE25724 [58] and GSE20966 [59], Fig. 4]. However in a cohort of Nordic patients (dataset GSE38642), no ZIP paralogues were found differentially (≥1.5-fold) expressed between diabetic and non-diabetic islets [60].

SLC39A Paralogues identified within our systematic review are experimentally verified to show high β-cell/islet expression and sequence conservation

Enhanced relative expression may suggest a biological relevance of respective putative ZIP orthologues in maintaining intracellular Zn²⁺ homeostasis in β-cells/islets. To verify the biological relevance of the ZIP orthologues we identified in our systematic review in terms of β-cell function, we performed qPCR expression profiling of human and mouse SLC39A mRNA transcripts. There was notably a wider range of mRNA for ZIP transporters expressed in human islets, compared with murine MIN6 cells. We observed highest expression of SLC39A6, SLC39A9, SLC39A13 and SLC39A14 in human islets and of SLC39A6 and SLC39A7 in mouse MIN6 cells (Fig. 5).

We next assessed cross-species homologies between human and putative rodent ZIP orthologues. Enhanced similarity increases the likelihood of inferring molecular function [61], and increases confidence when applying results encompassing data derived from multiple species. We calculated the transcriptomic and proteomic homologies between human and mouse ZIP orthologues, and between mouse and rat ZIP orthologues through bioinformatics. We showed all respective orthologues have high similarities, with lowest protein homology between human and mouse observed for ZIP4 (73%) and highest for ZIP1 (94%). All rat and mouse orthologues showed protein similarities of ≥90% aside from ZIP4 (89.8%) and ZIP14 (89.5%) (Table 2). The high sequence similarities observed indicate that all ZIP paralogues identified as important in our systematic review have the potential to substitute functionally in human, mouse and rat β-cells.

Discussion

Associations between Zn²⁺ status and β-cell function have been extensively described in independent studies [1, 2, 62]. ZnT8 expression is positively correlated with granule Zn²⁺ release and glucose tolerance in mice [63], and high glucose stimulation increases free Zn²⁺ content within mouse islets [22] and hamster HIT-T15 cells [64]. Intracellular Zn²⁺ exhibits roles in protection against oxidative stress-induced apoptosis [65] whereas chronic elevation contributes to β-cell dysfunction [22]. The ZIP importer paralogues responsible for maintaining β-cell Zn²⁺ homeostasis remain largely unexplored and are important to investigate for understanding β-cell function in health and diabetic disease.

A systematic review allows integrated analysis of relative consistencies in differential expression from high throughput gene expression techniques, despite heterogeneities between studies involving experimental design.
and platform used. It has the capacity to identify consistent but modest variations, important for genes involved in processes where small expression changes can have amplified effects. Through this systematic review we reanalysed raw microarray and RNA-seq data in parallel with unannotated high-throughput datasets to compare and contrast β-cell ZIP complements in human, mouse and rat β-cells/islets. We show enrichment of mRNA for ZIP7 and ZIP9 within rodent and ZIP6, ZIP7 and ZIP14 within human, with mRNA for ZIP1, ZIP6 and ZIP14 differentially expressed in response to cytokines and PDX-1 within rodent, and ZIP6 in response to diabetic status in human and glucose in rat. To query the biological relevance of our data, we carried out experimental expression profiling of human islet and MIN6 β-cell cDNA, and computationally aligned human, mouse and rat mRNA and protein sequences. Highest expression was observed for mRNA corresponding to ZIP6, ZIP9, ZIP13 and ZIP14 in human islets and ZIP6 in mouse MIN6 cells, which is in agreement with previous observations [21]. The mRNA profile for ZIPs generated through our qPCR analyses also corresponds well to expression data on specific isoforms in human islets as produced by RNA-seq (Additional file 4). All ZIP

Table 2 Percentage similarity between human, mouse and rat ZIP/SLC39A protein and mRNA sequences

| Gene     | Refseq transcript ID Human | Refseq transcript ID Mouse | Refseq transcript ID Rat | Entrez protein ID Human | Entrez protein ID Mouse | Entrez protein ID Rat | Percentage similarities (human:mouse) Transcript | Percentage similarities (human:mouse) Protein | Percentage similarities (mouse:rat) Transcript | Percentage similarities (mouse:rat) Protein |
|----------|----------------------------|----------------------------|--------------------------|-------------------------|-------------------------|------------------------|-------------------------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|
| SLC39A1  | NM_014437.4                | NM_013901.2                | NM_001134577.1           | Q9NY26                  | Q9QZ03                  | BSDEF5                 | 80.77                                           | 93.83                                       | 91.60                                       | 98.46                                       |
| SLC39A2  | NM_014579.3                | NM_001039676.2             | NM_001107260.1           | Q9NP94                  | Q2HIZ9                  | D3ZIN1                 | 78.51                                           | 77.99                                       | 91.87                                       | 94.82                                       |
| SLC39A3  | NM_144564.4                | NM_134143.1                | NM_00108356.1            | Q6BRY0                  | Q9K24                   | Q5U1X7                 | 80.99                                           | 84.04                                       | 91.23                                       | 97.16                                       |
| SLC39A4  | NM_017767.2                | NM_028064.2                | NM_001107266.1           | Q6F5W5                  | Q78IQ7                  | A0JPN2                 | 74.55                                           | 73.17                                       | 91.98                                       | 89.79                                       |
| SLC39A5  | NM_173596.2                | NM_028051.3                | NM_001108731.1           | Q6ZMH5                  | Q9O5S6                  | D3ZSF7                 | 83.42                                           | 84.30                                       | 92.24                                       | 94.00                                       |
| SLC39A6  | NM_012319.3                | NM_139143.3                | NM_001024745.1           | Q13433                  | Q8C145                  | QSV887                 | 80.04                                           | 88.20                                       | 92.01                                       | 95.28                                       |
| SLC39A7  | NM_006979.2                | NM_08082.2                 | NM_001164744.1           | Q92504                  | Q31125                  | Q6MGB4                 | 81.72                                           | 85.90                                       | 90.62                                       | 93.72                                       |
| SLC39A8  | NM_022154.5                | NM_000113510.1             | NM_00110116.1            | Q9C0K1                  | Q9W10                   | QSVFQ0                 | 76.15                                           | 89.35                                       | 90.74                                       | 96.10                                       |
| SLC39A9  | NM_018375.4                | NM_026244.2                | NM_001034929.1           | Q9NUM3                  | Q8BFU1                  | Q3KR82                 | 78.04                                           | 93.69                                       | 93.35                                       | 92.33                                       |
| SLC39A10 | NM_001127257.1             | NM_172653.2                | NM_001108739.6           | Q9ULF5                  | Q6F5W6                  | D4A517                 | 84.50                                           | 88.36                                       | 92.25                                       | 96.16                                       |
| SLC39A11 | NM_001159770.1             | NM_000166503.1             | NM_001101304.1           | Q8N155                  | Q8BWY7                  | Q6P6S2                 | 77.62                                           | 90.32                                       | 91.32                                       | 95.22                                       |
| SLC39A12 | NM_001145195.1             | NM_001012305.2             | XM_006254285.3           | Q504Y0                  | Q5F6W7                  | D4A8R5                 | 78.31                                           | 90.15                                       | 91.15                                       | 90.41                                       |
| SLC39A13 | NM_001128252.2             | NM_001290765.1             | NM_001039196.1           | Q9KH72                  | Q8BH2                  | Q2MK4                 | 81.54                                           | 90.58                                       | 93.42                                       | 93.84                                       |
| SLC39A14 | NM_000112843.2             | NM_001135151.1             | NM_001107275.1           | Q15043                  | Q8S7N3                  | D3ZM4M                 | 75.84                                           | 86.91                                       | 90.11                                       | 89.53                                       |

Comparisons were generated using Clustal multiple sequence alignment by MUSCLE (3.8) [34, 35].

Fig. 5 Expression profiles of SLC39A mRNA transcripts in human islets and murine MIN6 cells. a The human SLC39A transcriptome. N = 2 and error bars show ± range. b The MIN6 β-cell SLC39a transcriptome. N = 3 and error bars show ±SEM.
orthologues displayed high sequence conservation between species. Surprisingly ZIP4, which is essential for intestinal zinc uptake in both mouse and human [27, 66], shows the lowest homology (73%) between these two species and it also does not appear to play a major role in β-cells. Based on their expression levels, relative enrichment in β-cells/islets (compared with other cells/tissues), and regulation in response to conditions relevant to diabetes, we propose that ZIP6, ZIP7 and ZIP14 in human, and ZIP6 and ZIP7 in rodent may be of particular importance for β-cell Zn\(^{2+}\) uptake and/or homeostasis. This conclusion is similar to that of Liu et al. [21], who highlighted the roles of ZIP6 and ZIP7 in β-cell zinc transport and viability. Our study also identifies ZIP1, ZIP9 and ZIP13 as being of potential additional significance for β-cell function.

The abundance of ZIP transporters varies substantially between tissues and cells, allowing those with differing Zn\(^{2+}\) affinities, cellular localizations and regulatory mechanisms to tightly maintain the homeostatic balance [67]. We found significant differences in ZIP mRNA abundance between β-cells and non-pancreatic tissues; specifically, enrichment of ZIP7 and ZIP9 within mouse β-cells, and ZIP6, ZIP7 and ZIP14 within human islets. With the exception for ZIP7, which is found in the endoplasmic reticulum (ER) and in some cells in the Golgi apparatus, these zinc channels are operating at the plasma membrane [13, 68]. ZIP6 and ZIP7 enrichment is consistent with a report [21], suggesting that ZIP6 and ZIP7 mediate influx of zinc into the β-cell cytosol in tandem from the plasma membrane and the ER. In addition to their roles in transporting zinc, ZIP6, ZIP7 and ZIP14 strongly stimulate cell proliferation, drastically increasing the number of cells in G\(_2\)/M phase, and their expression changes in cancers [27, 69–73]. Also of potential importance is that ZIP14 mediates import of both zinc and non-heme iron [74, 75] and that ZIP9 has been identified as a plasma membrane androgen receptor [76]. Interestingly, transcripts of ZIP9 and ZIP14, which were both found expressed at comparable abundances to ZIP6 and ZIP7 within human islets by ourselves and others [21], were additionally enriched within β-cells. ZIP9 and ZIP14 both show predicted localization at the plasma membrane (with localization of ZIP9 at the Golgi and trans-Golgi network additionally described) [77, 78], and currently remain unexplored in this context. Our expression profiling further identified ZIP13 as highly expressed in both human islets and MIN6 cells. ZIP13 is phylogenically grouped with ZIP7 [79] and studies have suggested ZIP13 localises at the ER, Golgi [80, 81] and intracellular vesicles [82]. However, to our knowledge ZIP13 has not been studied in β-cells. ZIP9, ZIP13 and ZIP14 may represent novel targets for understanding β-cell zinc uptake and homeostasis.

PDX-1 is the major transcriptional regulator in mature β-cells and mediates expression of key β-cell genes, with homozygous mutations linked to Type 2 Diabetes development [83]. Furthermore, PDX-1 drives β-cell (re)generation from neurogenin-3 positive endocrine precursors and pancreatic α-cells [84, 85], and β-cell-specific recovery of activity within Ins\(^2\)Akita mice (βPdx1; Ins\(^2\)Akita mice) promotes significantly improved glucose tolerance compared to control littermates [86]. Of interest, PDX-1 binds enhancers (cis elements) of the ZnT8 gene SLC30A8 [87], indicating a role of PDX-1 in β-cell zinc homeostasis parallel to its role in insulin gene regulation [88]. Our analysis suggests PDX-1 activity sensitizes the β-cell zinc response to cytokines through ZIP6 and ZIP14 downregulation and ZIP1 upregulation within rat INS-1ab cells. We additionally established ZIP1 to be consistently upregulated following stimulation with IL-1β and IFN-γ, and IFN-γ and TNF-α within rat β-cells [49] and INS-1E cells [50], highlighting ZIP1 as potentially important in the adaptive response to cytokines. Interestingly, ZIP1 and ZIP6 abundances have been negatively correlated with the obesity-associated inflammatory state [89]. In contrast to the data in rodents, our review further identified ZIP8 and ZIP14 upregulation in response to the inflammatory cytokines IL-1β and IFN-γ in human islets [47]. Inflammatory mediators such as lipopolysaccharides (LPS) and TNF-α upregulate SLC39A8 within human lung epithelia [90] and Slc39a14 is upregulated in response to LPS-initiated inflammation within the mouse pancreas and liver [43] and shows an acute-phase gene response to IL-6 [91].

Hyperglycaemia is well recognised as a universal driver in the pathogenesis of Type 2 Diabetes [92]. Our analysis showed high glucose stimulation of rat islets significantly enhanced ZIP6 mRNA expression, consistent with glucose-dependent increases of additional ZIP7 and ZIP8 upregulation [22]. Similarly, analysis of islets from human type 2 diabetic donors displayed ZIP6, ZIP7, ZIP8 and ZIP14 mRNA downregulation compared to normoglycaemic controls [58, 59]. Decreased transcript expression supports a disease relevance of these paralogues for mediating β-cell zinc accumulation, indicating abnormally low zinc uptake may occur within diabetic β-cells as a result of disrupted ZIP6, ZIP7, ZIP8 and/or ZIP14 expression.

At a proteomic level no significant differences in protein abundances were observed for any ZIP parologue within human islets incubated with high or low glucose [93–95]. Though in one of these studies non-significant trends for enrichment of ZIP6 (2.6-fold) and ZIP14 (1.6-fold) in human islets were observed following culture in high compared to low glucose [95]. However, these proteomic studies likely bias towards proteins with higher abundances [96], and accurately evaluating less
abundant species away from central pathways and those in complexes remains challenging, with membrane proteins imposing further challenges [97]. Although it is acknowledged that mRNA abundances often poorly correlate with protein abundances and functional activity [98], transcriptomic analysis remains important for pinpointing expression control and pathways of disruption during disease states.

This systematic review provides an overview of ZIP transcript expression in the context of β-cell specificity, cytokine stimulation, PDX-1 activity, glucose status and Type 2 Diabetes. It has allowed us to collectively analyse ZIP expression within multiple high throughput datasets, complemented by experimental work, providing evidence for differential regulation as a consequence of β-cell stresses associated with decreased insulin secretion. The study’s limitations should nevertheless be acknowledged. Firstly, although all raw datasets (if appropriate) were subjected to the same normalisation process to minimise inconsistencies, the platform and genomic heterogeneities and differing probe hybridization efficiencies could skew global interpretation, and the analysis used may not have been equally suited to all datasets. Secondly, RNA-seq offers unbiased analysis of sequences present however microarray datasets are limited by hybridization efficiencies and the probes present [99], such that in multiple studies probes did not target all ZIP paralogues. Multiple datasets analysed and our qPCR expression data utilised islets incorporating non-β-cells. Relative SLC39A abundances may be impacted by additional cell populations, such as SLC39A14 enrichment in α-cells [100]. Furthermore, although we have shown high conservation of ZIP mRNA and protein sequences between human and mouse, results may not be entirely translatable across species. Finally, several microarray studies identified within the systematic review search criteria were excluded during the final screening due to the absence of available experimental data for download and analysis. Despite these limitations, our systematic review distinguishes specific SLC39A paralogues as important within each human and rodent β-cells. The results are strongly supported by our experimental expression profiling of human islet and MIN6 β-cell cDNA through confirming relative enrichment and a biological relevance.

Conclusions
We have used a systematic approach to identify key ZIP complements in human, mouse and rat β-cells. We have verified a biological importance of these paralogues through proving high relative expression in human islets and/or murine MIN6 β-cells, and have demonstrated their potential to serve as functional orthologues in human and rodent through verifying high sequence similarities. Importantly, our results highlight similarities and potentially biologically relevant differences in zinc regulation between human and rodent ZIP orthologues which may prove critical when evaluating rodent β-cell models of disease. We propose ZIP6 and ZIP7 serve as key functional rodent-human orthologues in β-cells. We further identify ZIP9, ZIP13 and ZIP14 in human and
important for β-cell function (Fig. 6). These paralogues represent interesting targets for future investigation into zinc regulation and homeostasis in β-cell failure and Type 2 Diabetes.

Additional files

- Additional file 1: Analyzed datasets. (XLSX 61 kb)
- Additional file 2: Table S1. Designs for human qPCR assays undertaken. (DOCX 12 kb)
- Additional file 3: Table S2. Designs for mouse qPCR assays undertaken. (DOCX 12 kb)
- Additional file 4: ZIP isoforms in human islets. (DOCX 56 kb)

Abbreviations

BR: Endoplasmic reticulum; FACS: Fluorescence assisted cell sorting; FD: Fold difference; FDR: False Discovery Rate; GEO: Gene Expression Omnibus; IFN-γ: Interferon-gamma; IL-1β: Interleukin-1 beta; Log2: Log2 transformed FD; LPS: Lipopolysaccharides; NPC: Neural progenitor cells; PDX-1: Pancreatic and duodenal homeobox 1; qPCR: quantitative PCR; TNF-α: Tumor necrosis factor-alpha; UPL: Universal Probe Library; ZIP: Zrt- and Irt-like protein; Zn2+: Zinc ions; ZnT8: Zinc transporter 8.

Acknowledgments

Human islet material was obtained from Dr. Pratik Choudhary and Dr. Guo-Cai Wang of the Human Islet Isolation Unit at King's College Hospital. We thank Dr. Matthew Arno (King's College London Genomics Centre) for technical support and Dr. Christine Baldwin (King’s College London) for advice on presenting the systematic review methodology. The authors wish to thank Dr. Theodora Stewart for proofreading the final manuscript.

Funding

RL was supported by the King’s Bioscience Institute and the Guy’s and St Thomas’ Charity Prize PhD Programme in Biomedical and Translational Science. The funding body was not involved in the design of the study, collection, analysis or interpretation of data, or in writing the manuscript.

Availability of data and materials

Datasets analysed during the current study are available in either the GEO repository [https://www.ncbi.nlm.nih.gov/geo/] or the ArrayExpress repository [https://www.ebi.ac.uk/arrayexpress/]. Respective dataset IDs are provided in Table 1.

Authors’ contributions

RL performed the systematic review, all experiments, analysis and drafted the manuscript. CH and WM supervised the study. All the authors reviewed and contributed to the final version of this manuscript.

Ethics approval and consent to participate

The King’s College Hospital Research Ethics Committee has approved human islet isolation and use for research (Protocol number 01-082, Human Islet Isolation and Research), and an assent form is completed by a relative of the cadaver pancreas donor for all islets used for research. The MIN6 cell line was a kind gift from Dr. Jun-ichi Miyazaki [101], distributed to WM under the appropriate materials transfer agreement.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 22 May 2017 Accepted: 5 September 2017
Published online: 11 September 2017

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