Cell
Leading Edge
Perspective

# A Call for Systematic Research on Solute Carriers

Adrián César-Razquin,[1,8] Berend Snijder,[1,8] Tristan Frappier-Brinton,[2] Ruth Isserlin,[3] Gergely Gyimesi,[4] Xiaoyun Bai,[5] Reinhart A. Reithmeier,[5] David Hepworth,[6] Matthias A. Hediger,[4,*] Aled M. Edwards,[2,*] and Giulio Superti-Furga[1,7,*]
[1]CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, 1090 Vienna, Austria
[2]Structural Genomics Consortium, University of Toronto, Toronto, Ontario M5G 1L7, Canada
[3]The Donnelly Centre, University of Toronto, Toronto, Ontario, M5S 3E1, Canada
[4]Institute of Biochemistry and Molecular Medicine and Swiss National Center of Competence in Research, NCCR TransCure, University of Bern, 3012 Bern, Switzerland
[5]Department of Biochemistry, University of Toronto, Toronto, Ontario, M5S 1A8 Canada
[6]Worldwide Medicinal Chemistry, Pfizer Worldwide Research and Development, Cambridge, MA 02139, USA
[7]Center for Physiology and Pharmacology, Medical University of Vienna, 1090 Vienna, Austria
[8]Co-first author
*Correspondence: gsuperti@cemm.oeaw.ac.at (G.S.-F.), matthias.hediger@ibmm.unibe.ch (M.A.H.), aled.edwards@utoronto.ca (A.M.E.)
http://dx.doi.org/10.1016/j.cell.2015.07.022

**Solute carrier (SLC) membrane transport proteins control essential physiological functions, including nutrient uptake, ion transport, and waste removal. SLCs interact with several important drugs, and a quarter of the more than 400 SLC genes are associated with human diseases. Yet, compared to other gene families of similar stature, SLCs are relatively understudied. The time is right for a systematic attack on SLC structure, specificity, and function, taking into account kinship and expression, as well as the dependencies that arise from the common metabolic space.**

Individual cells, be they prokaryotic or eukaryotic, must control chemical exchange with their environments, and they use lipid membranes and proteinaceous channels and transporters to this end. The lipid environment of the membrane prevents intrusion or leakage into the *sancta sanctorum* of the inner milieu and buffers the cell against changing and noxious environmental conditions, as well as against attack by phages, viruses, or bacteria (Köberlin et al., 2015; Mulkidjanian et al., 2009). In many respects, the integrity of the membranes represents as critical an element to cellular individuality as does the preservation and transmission of genetic information (Schrum et al., 2010). The protein components of cell membranes import and export most of the chemical matter essential for life, including water, ions, gases, nutrients, vitamins, cofactors, and many drugs (Kell et al., 2011; Kell and Oliver, 2014; Lin et al., 2015). Therefore, regulation of small-molecule transport across membranes is key to a cell's internal physiology and is the gatekeeper to its interface with the environment (Nigam, 2015). Yet, despite their central role in mediating the discussion between chemistry and biology and despite the fact that ~10% of the human genome encodes for transport-related functions (Hediger et al., 2013), transporters, as a class of proteins, do not appear to garner quite the attention that they deserve.

Transporters comprise solute carriers, ion channels, water channels, and ATP-driven pumps, including ABC transporters. Of these, the largest group is formed by the solute carrier proteins (SLCs), which according to the current counting comprises 456 members, distributed in 52 subfamilies that can be further phylogenetically grouped (Hediger et al., 2013, 2004; Schlessinger et al., 2010, 2013b). SLCs are membrane integral proteins localized on the cell surface and in organellar membranes and comprise facilitative transporters, which are equilibrative, and secondary active transporters (symporters and antiporters), which may be concentrative (Hediger et al., 2013). After G-protein-coupled receptors (GPCRs), SLCs are the second-largest family of membrane proteins in the human genome (Hoglund et al., 2011). For detailed information about the individual SLC family members, please refer to www.bioparadigms.org.

## Links to Therapeutics and Human Disease

Much research on SLCs has been spurred by their relevance to pharmacology and drug discovery, either as drug targets themselves or as mediators of drug disposition. Drug targets include SLC6A4 (SERT), the target of the hugely important serotonin uptake inhibitor drug class. Mediators of drug transport include SLCO1B1, which transports statins and allows for preferential drug distribution into the liver compared to other tissues, such as muscle. This tissue distribution of statins is important in driving their therapeutic index by increasing the lipid lowering over the myopathy-causing activity (Giacomini et al., 2010).

SLC-mediated transport of statins and other drug classes can also render their pharmacokinetics susceptible to drug-drug interactions. For example, naringin from citrus fruits inhibits the enterohepatic transporter SLCO1A2 and thus can reduce the bioavailability of drugs that rely on this transporter, such as fexofenadine (Bailey, 2010). Transport can also be affected by the natural pharmacogenomic variability in SLCs (Giacomini et al., 2013). Other SLCs have been studied for their roles in physiology, like SLC25A7 (UCP1), the mitochondrial uncoupling protein involved in the thermogenesis process of brown adipose tissue.

Newer research has implicated SLCs in the action of chemotherapeutics; YM155, a cancer drug in clinical evaluation, was found to be completely dependent on SLC35F2 for entry into human tumor cells (Winter et al., 2014). Increasingly, SLCs are attracting attention because they mediate drug-drug and nutrient-drug interactions. For instance, the investigational

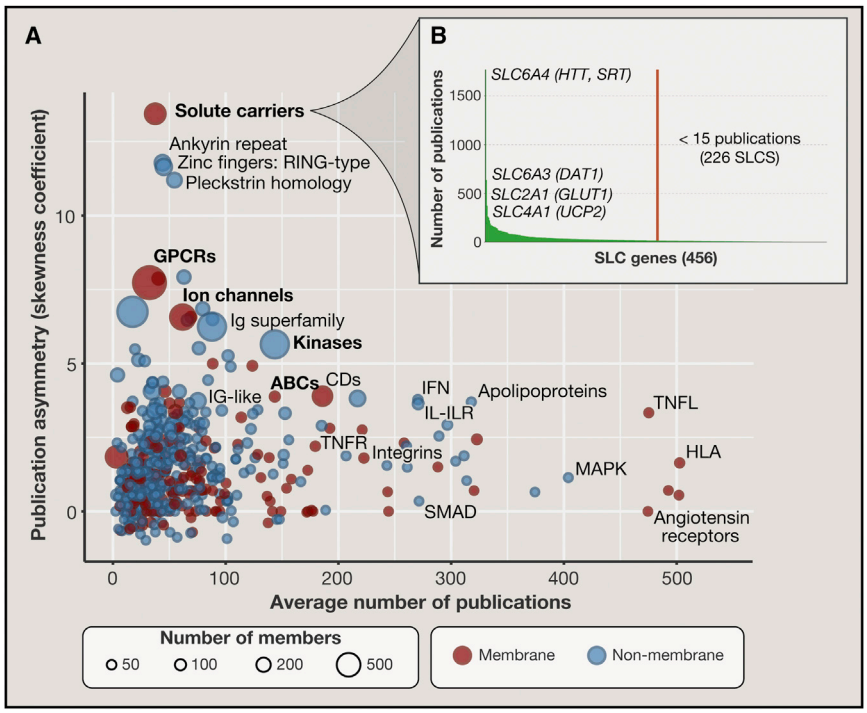


**Figure 1. SLCs Are the Most Neglected Group of Genes in the Human Genome**

(A) Publication asymmetry is plotted against the average number of publications per group of genes. Publication counts per gene were retrieved from the gene2pubmed file provided and curated by NCBI. Gene groups comprise all HGNC gene families and super-families as well as the GO annotations for kinase activity ("kinases") and ion channel activity ("ion channels"). Asymmetry is measured for each group of genes by calculating the skewness (as implemented in R's "moments" package) of the distribution of the number of publications for all genes within the group. A very positive skew thus indicates an uneven distribution where a few genes in the family concentrate a much higher number of publications than the rest. Dot size relates to the number of members in each gene group, and color indicates gene groups where at least 80% of their members are annotated as membrane proteins by GO annotation (see legend). Labels for selected classes are shown.

(B) Number of publications per SLC gene is displayed in descending order. The four SLCs with the most publications are annotated. The red line indicates the border at which genes have fewer than 15 publications.

JAK2 inhibitor fedratinib, which was recently terminated from development due to incidence of Wernicke's encephalopathy during trials, has been shown to inhibit thiamine uptake mediated by SLC19A2 (hTHTR2), possibly contributing to the offside effects (Zhang et al., 2014). It would not be surprising if further unplanned SLC-drug interactions were uncovered in the future.

There is also growing interest in SLCs because of their clear genetic link to human diseases; about 190 different SLCs have been found mutated in human disease and through genome-wide association studies (Williams et al., 2012, 2014).

## Are SLCs Getting the Attention They Deserve?

Our sense was that the SLC protein family, despite its clear relevance to health and disease, was comparatively less well studied than other gene families. In an attempt to quantify "SLC knowledge" versus other gene families, we surveyed the literature and analyzed the distribution of publications as reported by NCBI for each gene family annotated by HGNC in an automated, unbiased fashion (Bruford et al., 2008). We then visualized the publication asymmetry, defined by the coefficient of skewness, versus the average number of publications for each family (Figure 1A). SLCs show by far the greatest publication asymmetry of all gene families, i.e., the most uneven distribution of papers over the group members. This does not seem to be simply due to a bias against membrane proteins in general, as ABC proteins, ion channels, and GPCRs appear not so unevenly distributed. Further, SLCs have an average number of publications per member of around 35, which is half of what is observed on average over all families (66 publications). At the other end of the spectrum, one finds, among others, that the small TNF superfamily of ligands are all equally and very well studied.

We then analyzed the asymmetry within the SLC knowledge domain. We performed an automated search for publications per each of the 456 SLC genes (including 65 pseudogenes), which indeed displayed a highly skewed SLC knowledge distribution curve (Figure 1B). A manually annotated search revealed the same general pattern (Figure S1B). Both analyses reveal that some gene members are extremely well studied, whereas most have very few publications. In a phenomenon that appears to be general to all human protein families, the most well-studied SLCs in the last 2 years are almost identical to those that were the most well studied a decade ago (Edwards et al., 2011). Prior to 2003, 20 of the ~400 SLC family members accrued 29% of the publications for the entire family, and those exact same family members garnered 32% of all SLC publications over the period 2012–2014 (Figure S1A).

Rankings of the SLC family members do not seem to be indicative of biological relevance. Some of the most well-studied SLCs appear to have become objects of investigation simply due to their abundance and tissue-specific expression in easily isolated cell types, which greatly facilitated their study in the era before molecular biology. Examples of this type include the so-called "band 3 of erythrocytes" protein (SLC4A1) and the erythrocyte glucose transporter GLUT1 (SLC2A1).

An important factor that contributes to the elevated publication rate of particular transporters has been expression cloning. In the case of the intestinal $Na^+$-glucose transporter SGLT1 (SLC5A1), due to its hydrophobic nature and difficulty in purifying, functional expression in *Xenopus laevis* oocytes finally opened the door to successful cloning and molecular characterization (Hediger et al., 1987). This progress led to a substantial increase in SLC study, ultimately leading to structural

**Table 1. SLCs Specifically Targeted by FDA-Approved Drugs or Drugs in Active Development**

| Drug Status | SLC | Common Protein Name | Examples |
|---|---|---|---|
| **Approved** | SLC5A2 | SGLT2 | canagliflozin; dapagliflozin |
| | SLC6A1 | GAT1 | tiagabine |
| | SLC6A2 | NET | atomoxetine |
| | SLC6A3 | DAT | methylphenidate |
| | SLC6A4 | SERT | fluoxetine; sertraline; citalopram (SSRIs) |
| | SLC12A1/2 | NKCC1/2 | furosemide (loop diuretics) |
| | SLC12A3 | NCC | hydrochlorothiazide (thiazide diuretics) |
| | SLC18A1/2 | VMAT1/2 | reserpine |
| | SLC18A2 | VMAT2 | tetrabenazine |
| | SLC22 family | OATs | probenecid |
| | SLC25A4/5/6 | ANT1/2/3 | clodronate |
| | SLC29A1 | ENT1 | dipyridamole |
| **Phase II+ Clinical Trial** | SLC5A1 (and SLC5A2) | SGLT1 (and SGLT2) | sotagliflozin |
| | SLC6A9 | GlyT1 | bitopertin |
| | SLC9A3 | NHE3 | tenapanor |
| | SLC10A2 | IBAT | elobixibat |
| | SLC22A12 | URAT1 | lesinurad |
| | SLC40A1 | Ferroportin-1 | LY2928057 |

determination (Faham et al., 2008) and development of an antidiabetic drug class (Abdul-Ghani and DeFronzo, 2014) that acts on its renal homolog SGLT2 (SLC5A2).

Other SLCs became highly studied because they were discovered as targets of existing drugs, with VMAT2 (SLC18A2) representing a specific example of this. Reserpine is a drug that was first marketed in the 1950s as a tranquilizer. The actual mode of action of reserpine was only uncovered 40 years later by scoring for cDNAs conferring the ability to sequester the neurotoxin 1-methyl-4-phenylpyridinium (MPP+) in CHO cells, leading to the discovery of the vesicular amine transporter family SLC18 (Liu et al., 1992). As an example of how the availability of research tools has influenced SLC research, there were no publications at all on SLC30A8 until its first cloning and expression in 2004 (Chimienti et al., 2004). Following this publication and a series of papers genetically linking mutations in this protein with diabetes, in recent years SLC30A8 has become one of the most highly studied SLCs (Rutter and Chimienti, 2015). This spike of activity is clearly displayed in Figure S1A. Even more recently, some SLCs that were previously barely studied have been identified to play key roles in physiology. SLC38A9, an SLC recently found to contribute to amino-acid sensing of mTOR, was ranked 288$^{th}$ in the automated ranking of all time SLC publications (Rebsamen et al., 2015; Wang et al., 2015). With the importance of this SLC now clear and tools available to allow its study, one can anticipate an increase in publication rate for this transporter. As for the bottom-ranked 15% of SLC family members, there are more publications in a PubMed search for "star wars" (72 citations) than on these 70 SLCs combined.

## Exploring SLCs as Drug Targets

Regarding SLCs as drug targets, a recent publication suggests 26 different SLCs being the targets of known drugs, or drugs in development (Lin et al., 2015; Rask-Andersen et al., 2013). A closer inspection using more stringent criteria (FDA-approved drugs whose *primary mode of action* is considered to be through action on an SLC) revealed just 12 drug classes. Only 8 of these drug classes are believed to act through selective action at a single SLC, while 4 classes are believed to act non-selectively via two or more SLCs. Only 6 further SLCs are targeted by drugs in active development in phase II clinical trials or beyond (Table 1). Several drugs interact with SLCs in addition to their purported primary target, e.g., amiloride (SLC9A1, NHE1) or sulfasalazine (SLC7A11, xCT), but in such examples, it has not been clearly established that these effects contribute to their clinical pharmacology. The GPCR family, in contrast, is a well-established drug target class that has been the subject of systematic drug discovery efforts for half a century. Even when considering the possibility that GPCRs may be intrinsically more relevant as drug targets, the difference between a few SLC targets and ~100 GPCR targets is likely to reflect a historical bias. Clearly the SLC family is underexplored from the standpoint of drug discovery. Druggability of SLCs appears not to be the main or only barrier here, as the majority of the well-studied SLCs have reported small-molecule inhibitors.

Is it reasonable to expect more SLC-targeting drugs? Around 75% of SLCs are predicted to carry small organic molecules. It has been proposed that proteins that have evolved to bind such species are, on average, privileged with respect to small-molecule druggability (Fauman et al., 2011). Experiences thus far appear to support this prediction, with molecules of high ligand efficiency (an indicator or protein druggability) (Hopkins et al., 2014) being identified in the cases where medicinal chemistry efforts have been attempted against SLCs. Even SLCs that carry only inorganic species have been shown to be druggable, including, for example, the SLC12 family targets of the loop and thiazide diuretics. Thus, SLCs appear to offer the rare potential of an underexplored gene family with high disease relevance and general small-molecule druggability.

## SLC Genes and Human Disease

Current thinking in biomedicine and drug discovery contends that human genomics will provide the clues to those genes and proteins of particular relevance to disease and therapy. Accordingly, we looked at all SLC genes that are associated with human disease and counted the number of compounds reported for each ($IC_{50}$ < 10 μM), using OpenPHACTS, a platform that provides a single access to disease, chemical, and target databases (Ratnam et al., 2014; Williams et al., 2012). 76% of SLCs (145 out of 190) with an already identified disease link have no compound associated with them (Figure S2). It is notable how few SLC targets have more than 100 active compounds against them in the database, likely to represent another measure indicative of how

few drug discovery programs have been run against the family. In contrast, the most popular targets of monoamine uptake inhibitors (SLC6A2,3,4) have more than a thousand compounds associated with each, with likely thousands more such compounds in pharmaceutical company collections as a result of extensive drug discovery campaigns against these targets.

Of course, it could be argued that involvement of SLC genes in monogenic disorders is a poor reason to call for drug discovery efforts in the corresponding disease areas, as it appears counterintuitive. Yet such arguments need not be always valid, as there is a fundamental difference between life-long genetic loss of function (LOF) and the titrated, reversible pharmacological blockade of a protein. For instance, LOF mutations in the dopamine transporter SLC6A3 lead to early stage Parkinsonism disease (Kurian et al., 2009), but SLC6A3 is also a principal target of methlyphenidate and in the treatment of psychiatric disorders. Further, LOF mutations in SLC12A3 have been found associated with Gitelman's syndrome, characterized by low blood pressure, and SLC12A3 could be mechanistically linked to the action of thiazides that treat hypertension (Brinkman et al., 2006). Even if we take a more stringent connection to disease by counting only the genetic mutations in the OMIM database (103 different SLCs) (Amberger et al., 2015), it is clear that the "disease" zones of the SLC network are not covered nearly enough by chemical agents.

## Why So Little Research Attention Then?

What might have contributed to this apparent anomaly in the distribution of research attention for the SLC gene family, where some members are well studied and so many members not studied at all? First, a unifying nomenclature has been adopted only recently (Hediger et al., 2013, 2004), and as a consequence, common principles and features may have been overlooked. Second, there are a number of technical barriers that may have impeded research in this area. In particular, acquiring competent biological reagents for SLC study can be highly challenging. These are complex integral membrane proteins that are difficult to express and purify and are often poorly detected by typical protocols for mass spectrometry. Accordingly, biochemical, biophysical, and structural biology characterization of SLCs has also been challenging. Indeed, there are so far only three reported human SLC structures (Deng et al., 2014; Gruswitz et al., 2010; Schlessinger et al., 2013a; Deng et al., 2015) (Table S1). Cell-based systems for studying SLC function can likewise be challenging to obtain, as overexpression can cause toxicity (presumably as a result of metabolic perturbation), and loss- or gain-of-function studies can be confounded by endogenous SLCs with overlapping specificities or by compensatory transport or metabolic effects. Even when cell systems with functionally competent SLCs can be obtained, defining their relevant endogenous substrates is not trivial, and establishing screening assays can be difficult. Third, high-quality antibodies are available for only a few SLCs, with the human protein atlas reporting just 45 SLCs for which they have raised reliable antibodies (Uhlen et al., 2015). As a consequence, the current understanding of the subcellular localization of SLCs, crucial for the interpretation of their function, is indeed partial at best. Finally, the transport assays are often challenging, even for those SLCs with known substrates. Artificial lipid vesicles or microinjected frog oocytes, two other useful assay systems, do not necessarily allow for testing function in the context of the regulatory intricacies, and the latter is not always robust enough for large-scale compound screening. In short, despite the post-genomic era, ample evidence for their important physiological role and their druggability, the systematic and parallel structural and functional interrogation of human SLC proteins has not yet been carried out.

## Delving into the "Sparse Zones" of Our Knowledge

Here, we argue that an energetic and detailed exploration of the human "SLCome" is warranted because the family comprises one of the largest "sparse zones" of human biology. Indeed, the concept of the rational filling of sparse zones of knowledge is starting to guide strategies in other collaborative efforts (Rolland et al., 2014; Snijder et al., 2014). Furthermore, we argue that the problem should be tackled systematically to capture the efficiencies that come with economies of scale and the learnings that derive from studying related proteins. Finally, we believe that the initial objective of this effort should focus on generating high-quality, enabling reagents (antibodies, purified proteins, cell-based assays, chemical probes, CRISPR-cell lines) and data sets (protein interaction, tissue and sub-cellular distribution).

Such a concerted effort is not only called for but is also timely due to recent technological developments, listed and referenced in Table 2. Such developments cover protein expression, metabolomics, structure determination, gene knockout technologies, and mass spectrometry, as well as assay development and medicinal chemistry, to deliver high-quality chemical tools into the public domain. We listed possible project aims of a concerted campaign, fully aware that such lists are not comprehensive and are meant to spur additional thoughts. There are several examples of successful de-orphanization of SLCs using recently developed technologies (Abplanalp et al., 2013; Caulfield et al., 2008; Iharada et al., 2010; Rebsamen et al., 2015; Wang et al., 2015; Wikoff et al., 2009; Winter et al., 2014).

In broad terms, the strategy to study proteins by family, where experimental methods on one family member may facilitate analysis of the next (Hoglund et al., 2011; Schlessinger et al., 2010), has been highly successful for tackling the structure and chemical tractability of other gene families such as kinases, GPCRs, and proteins involved in the regulation of the epigenome (Barr et al., 2009; Edwards et al., 2009). Importantly, although one would expect similar success applying this approach to the SLC family, there is an additional opportunity that functional inter-relationships among SLCs, on top of phylogeny, may greatly aid in the design of the experimental strategy.

## Working Groups of SLCs

It is highly likely that the transport activity of one SLC may affect the activity of others, acting in parallel or in sequential order, in redundant or interdependent function, integrating with the cellular metabolism in various ways (Nigam, 2015; Thiele et al., 2013). If this is the case, there may be several ways to uncover such functionally linked groups, for example, by analyzing co-expression patterns (Huynen et al., 2003; Jordan et al., 2004; Stuart et al., 2003). Proteins acting together are more likely to be co-expressed across tissues and conditions than if they are

**Table 2. Approaches to Enable SLC De-orphanization**

| Objective | Enabling New Technology | References |
|---|---|---|
| Expression map of SLCs across the human body, at single-cell and sub-cellular resolution | large-scale RNA-seq; single-cell RNA-seq; expression proteomics and antibody mapping efforts; MALDI imaging mass spectrometry; CyTOF | (Bendall et al., 2011; Clemencon et al., 2015; Cornett et al., 2007; Kim et al., 2014; Mele et al., 2015; Uhlen et al., 2015; Wilhelm et al., 2014) |
| Human cell lines mutated in individual SLC genes | CRISPR technology; insertional mutagenesis in haploid cells | (Burckstummer et al., 2013; Carette et al., 2009; Doudna and Charpentier, 2014) |
| Cell lines with multiple SLC gene deletions; cells with minimal SLC repertoire | CRISPR-mediated genomic engineering | (Doudna and Charpentier, 2014; Hsu et al., 2014) |
| SLC genetic interaction landscape | SLCome- and genome-wide CRISPR inactivation and gain of function libraries; k.o. cells | (Cong et al., 2013; Qi et al., 2013) |
| Chemical genomics | high-throughput phenotypic screening | (Carette et al., 2009; Reiling et al., 2011; Winter et al., 2014) |
| SLC interactome | label-free high-throughput AP-MS; BirA-mediated BioID; membrane interaction mapping | (Cox and Mann, 2011; Lambert et al., 2015; Petschnigg et al., 2014; Varjosalo et al., 2013) |
| Metabolomic data and SLC genetic polymorphisms | genetic association studies; population-wide whole-genome sequencing; rare disease genome sequencing coupled with deep metabolomics | (Shin et al., 2014) |
| Metabolome-wide transport assays, in dependence of individual SLC gene alteration | high-throughput accurate LC/ GC-mass spectrometry and databases; libraries of metabolites; k.o. cells | (Kell, 2004) |
| Transport assays using recombinant proteins | proteoliposomes; liposome microarrays; pure solutes, complex body fluids | (Krumpochova et al., 2012; Saliba et al., 2014; Scalise et al., 2013) |
| High-throughput determination of 3D structure | single-particle cryo-EM; high-throughput crystallization protocols; serial femtosecond crystallography | (Bai et al., 2015; Bartesaghi et al., 2015; Chapman et al., 2011; Moraes et al., 2014; Zeev-Ben-Mordehai et al., 2014; Zhou et al., 2015) |
| Potent and selective chemical probes for each SLC | better libraries; more accurate screening technologies; assays to assess target engagement and specificity in cells and tissues | (Edwards et al., 2009; Frye, 2010) |

functionally independent. SLCs that are consistently identified to be co-expressed and thus represent such putative working groups or functional modules may, in turn, help to shed light on the role of the individual family members. Perhaps these connections can be used to distinguish an underlying overall architecture, which might be suggestive of dependencies and vulnerabilities of the system.

To explore this concept more fully, we analyzed different gene expression data sets of human tissues (Fantom5, Illumina body map, and the "32 tissues"; Forrest et al., 2014; Parkinson et al., 2011; Uhlen et al., 2015) and derived a global and high-confidence survey of patterns of co-expression across SLC genes. These patterns for co-expression were analyzed to identify SLCs that are frequently and consistently co-expressed. Co-expression relationships were ranked based on the combined p values of the correlations in the three independent data sets used. For visualization convenience, we chose to display only the top 2,500 co-expressed SLCs observed in at least two data sets. We found at least five major clusters and several smaller ones, perhaps representing fundamental functional relationships (Figure 2A). The edges were colored according to the tissue in which two connected SLCs are most highly, but not necessarily exclusively, expressed. We found that the clusters correspond to individual tissues (kidney, liver, brain, testis, leukocytes). Interestingly, kidney and liver seem to share the highest number of co-expressed SLCs despite their different germ-layer origin. A more fine-grained tissue annotation shows that the kidney/liver intersection harbors the SLCs whose co-expression is highest in intestinal tissue (Figure S3A). This similarity between kidney and liver co-expression is specific for SLCs, as a recently published genome-wide tissue expression comparison revealed a considerably larger "distance" between these organs (Mele et al., 2015).

The network displayed does not automatically reveal all textbook cases of co-expression. For example, expression of SLC26A4 (iodide transporter, pendrin) and SLC5A5 (sodium iodide co-transporter) is well known to be coordinately expressed in thyroid tissue. While a significant level of co-expression is observed in the thyroid, several tissues either express one or the other, suggesting that they may not always be obligatory partners and may have independent functions. Accordingly, the correlation, although significant, did not reach the top

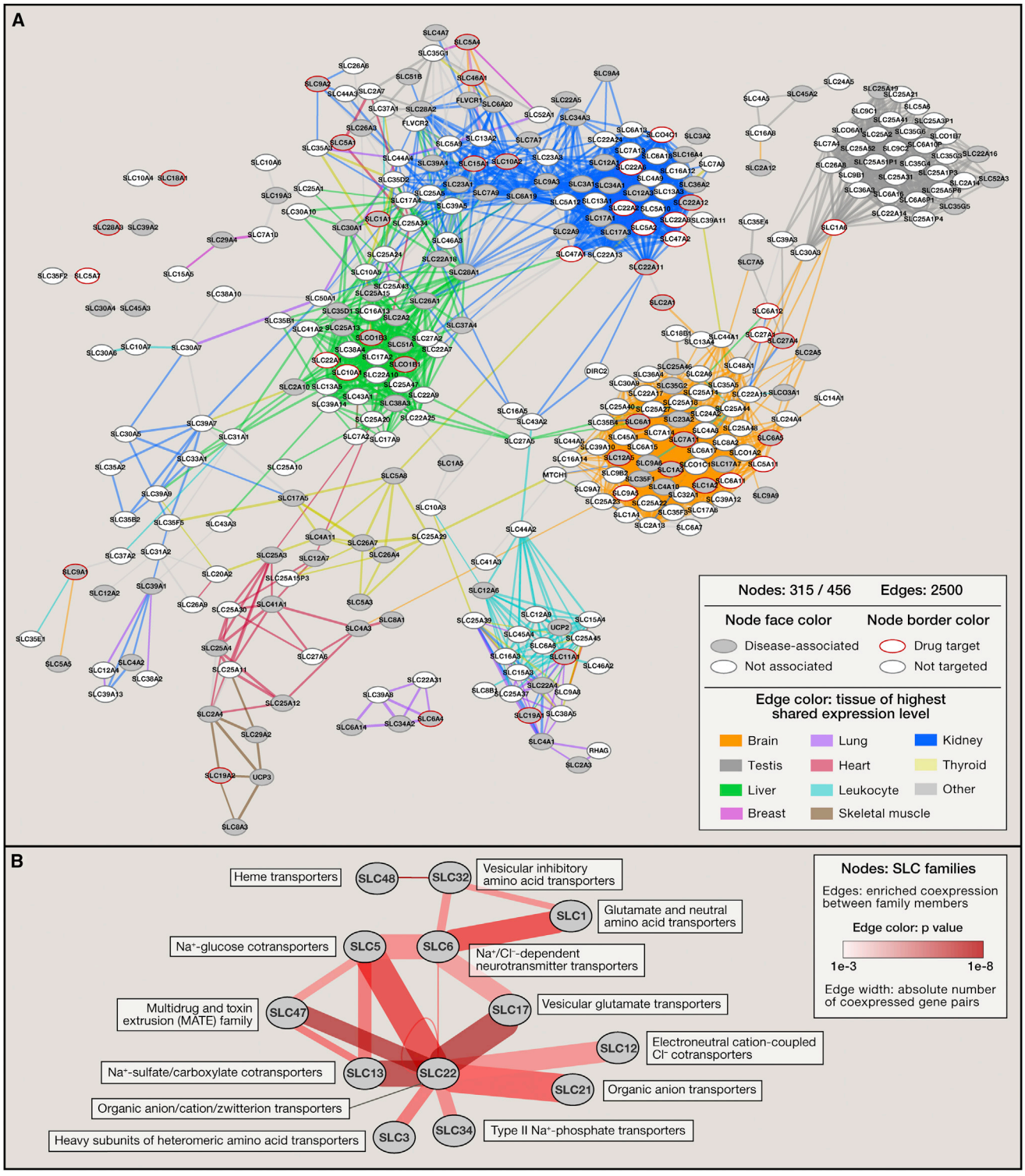


**Figure 2. SLCs Are Expressed in Robust Tissue-Dependent Modules**

(A) Network visualization of SLC co-expression. Nodes in the network represent SLCs. Edges between nodes correspond to significant correlations consistently retrieved in three independent expression data sets from healthy human tissues. Only the top 2,500 most significant edges are shown, based on combined p values of the three independent correlations. Gray nodes indicate SLCs with at least one disease association, and red node outlines indicate the presence of at

*(legend continued on next page)*

2,500. To allow the interrogation of the full data set beyond what can be reasonably visualized in a single network, we include an extended list of SLC co-expression pairs across all tissues (Table S2).

Not all SLCs are represented in the network because their expression does not correlate strongly enough with any given other to be among the top 2,500 that we chose to represent for visualization. Of these SLCs, some 48 appear to be expressed in all tissues and may thus represent a "core" of housekeeping functions (Table S3). Membership to this group may make some of them attractive to study for pharmacokinetic considerations.

We then looked at the distribution of disease-associated SLCs across the network (dark gray filled nodes, Figure 2A). All clusters (except the testis cluster) contain several positive SLCs, confirming that SLC gene functions bear important pathophysiological implications across many tissues and processes. The SLCs for which high-affinity chemical agents have been developed are marked; their distribution was considerably less even (red halos, Figure 2A). At least three cluster regions seem relatively sparse in terms of drugs: heart and skeletal muscle, leukocytes, and the intersection of liver and kidney. Perhaps these regions merit more attention in the future.

The SLC families do not appear to group in clusters or tissues (i.e., most SLC families appear distributed over the different tissues), but there is a non-random pairing of co-expression between different SLC families whose pattern likely reflects metabolic/biochemical dependencies (Figure 2B). For instance, strong interaction between the SLC13 and SLC22 families is likely to reflect an integration of energy and homeostatic regulation of intermediate metabolism, particularly the Krebs cycle. Enrichment in the interactions between families SLC5 (glucose reuptake), SLC13 (citrate/dicarboxylate reuptake), and SLC47 (toxin/xenobiotic secretion) might be also explained by the role of some of their members in kidney, where a coordinated transport of their cargos is required. Furthermore, the sodium and chloride symporter family SLC6, which transports monoamine neurotransmitters and amino acid neurotransmitters, is heavily linked with glutamate/neutral amino acid transporters of the SLC1 family. This link suggests a connection at both a metabolic and physiological level, especially important in brain tissue.

The robustness of SLC co-expression patterns across different large-scale data sets was very high and clearly exceeded, for example, that of protein kinases (Figure S3B). Possibly, SLC function has a particularly high degree of interdependence reflective of the integrative nature of metabolism required for homeostatic stability. The resulting co-expression networks are likely to be reinforced by the integration with environmental parameters.

The patterns of SLC co-expression may reflect normal cell function; when we compared co-expression in different cancer cell lines, we observed massive changes, corresponding to a general loss of structural organization in the network. The network of SLC co-expression in cancer is not robust, i.e., it is not as reproducible across data sets, and it shows considerably less clustering (Figure S3C). The degree by which cancer perturbs the SLC co-expression pattern is much higher than the differences between normal tissues and cancer cell lines observed with protein kinases.

Could loss of these "healthy" SLC co-expression patterns be a good marker for the loss of homeostasis in certain diseases? This analysis may suggest that there is a SLC regulatory circuitry that may be crucial to medical and pharmacological considerations and that might assist strategic choices in the effort to fill the SLC knowledge gap. Armed with this knowledge, redundancy and dependency are not annoying impediments of the large SLC group but, rather, exploitable features.

## Conclusions

In summary, SLCs are particularly understudied and fascinating proteins, vital for correct cellular function by controlling the correct import and export of the molecules of life across membranes. They are important in disease and in the action and transport of drugs. A broad attack on their structure, expression, regulation, chemical structure-activity relationships, and functional characterization in terms of transport and signaling is warranted. The study of their regulation and interdependencies should be particularly fascinating, as the functional target may be not only a single protein but the vulnerability within the functional network, perhaps involving ATP-dependent efflux transporters of the ABC family as well. A full-force effort to study the "SLCome" would open the doors to the interface between human health and metabolism, nutrition, and the environment. The large and important family of SLCs should be neglected no longer.

## SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures and three tables and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2015.07.022.

## ACKNOWLEDGMENTS

We thank all of the participants of the SLC workshop in Vienna, November 6 and 7, 2014 at CeMM and various members of the Superti-Furga laboratory for discussions and H.E. Susan le Jeune d'Allegeershecque and the British Embassy for graceful hosting. The work was funded by the Austrian Academy of Sciences (G.S.-F., A.C.R., B.S.), ERC grant agreement number 250179 (G.S.-F), the Swiss National Science Foundation (project number P300P3_147897 to B.S.), the Marie Curie Actions International Fellowship Program (IFP) TransCure (G.G.), and the Swiss National Center of Competence in Research (NCCR TransCure). D.H. is an employee and shareholder of Pfizer, Inc. G.S.-F. is a founder and shareholder of Haplogen, GmbH. The SGC is a registered charity (number 1097737) that receives funds from AbbVie, Bayer Pharma AG, Boehringer Ingelheim, Eshelman Institute for Innovation, Genome Canada, Janssen, Merck & Co., Novartis Pharma AG, the Ontario Ministry of Economic Development and Innovation, Pfizer, São Paulo Research Foundation-FAPESP, Takeda, and the Wellcome Trust.

least one interacting small compound with an $IC_{50}$ below 10 μM (OpenPHACTS). Edges are colored according to the tissue in which the two connected SLCs share the highest expression (highest mean rank; Illumina Body Map data set), as indicated in the legend.

(B) SLC family co-expression enrichment network. Nodes in the network represent SLC families. Edges correspond to statistically enriched co-expression between members of the connected SLC families, as calculated by a hypergeometric test. Edge color relates to the significance of the enrichment and edge width is proportional to the number of co-expressed SLC pairs (see legend).

## REFERENCES


Abdul-Ghani, M.A., and DeFronzo, R.A. (2014). Lowering plasma glucose concentration by inhibiting renal sodium-glucose cotransport. J. Intern. Med. *276*, 352–363.

Abplanalp, J., Laczko, E., Philp, N.J., Neidhardt, J., Zuercher, J., Braun, P., Schorderet, D.F., Munier, F.L., Verrey, F., Berger, W., et al. (2013). The cataract and glucosuria associated monocarboxylate transporter MCT12 is a new creatine transporter. Hum. Mol. Genet. *22*, 3218–3226.

Amberger, J.S., Bocchini, C.A., Schiettecatte, F., Scott, A.F., and Hamosh, A. (2015). OMIM.org: Online Mendelian Inheritance in Man (OMIM(R)), an online catalog of human genes and genetic disorders. Nucleic Acids Res. *43*, D789–D798.

Bai, X.C., McMullan, G., and Scheres, S.H. (2015). How cryo-EM is revolutionizing structural biology. Trends Biochem. Sci. *40*, 49–57.

Bailey, D.G. (2010). Fruit juice inhibition of uptake transport: a new type of food-drug interaction. Br. J. Clin. Pharmacol. *70*, 645–655.

Barr, A.J., Ugochukwu, E., Lee, W.H., King, O.N., Filippakopoulos, P., Alfano, I., Savitsky, P., Burgess-Brown, N.A., Muller, S., and Knapp, S. (2009). Large-scale structural analysis of the classical human protein tyrosine phosphatome. Cell *136*, 352–363.

Bartesaghi, A., Merk, A., Banerjee, S., Matthies, D., Wu, X., Milne, J.L., and Subramaniam, S. (2015). Electron microscopy. 2.2 Å resolution cryo-EM structure of β-galactosidase in complex with a cell-permeant inhibitor. Science *348*, 1147–1151.

Bendall, S.C., Simonds, E.F., Qiu, P., El-ad, D.A., Krutzik, P.O., Finck, R., Bruggner, R.V., Melamed, R., Trejo, A., and Ornatsky, O.I. (2011). Single-cell mass cytometry of differential immune and drug responses across a human hematopoietic continuum. Science *332*, 687–696.

Brinkman, R.R., Dube, M.P., Rouleau, G.A., Orr, A.C., and Samuels, M.E. (2006). Human monogenic disorders - a source of novel drug targets. Nat. Rev. Genet. *7*, 249–260.

Bruford, E.A., Lush, M.J., Wright, M.W., Sneddon, T.P., Povey, S., and Birney, E. (2008). The HGNC Database in 2008: a resource for the human genome. Nucleic Acids Res. *36*, D445–D448.

Burckstummer, T., Banning, C., Hainzl, P., Schobesberger, R., Kerzendorfer, C., Pauler, F.M., Chen, D., Them, N., Schischlik, F., Rebsamen, M., et al. (2013). A reversible gene trap collection empowers haploid genetics in human cells. Nat. Methods *10*, 965–971.

Carette, J.E., Guimaraes, C.P., Varadarajan, M., Park, A.S., Wuethrich, I., Godarova, A., Kotecki, M., Cochran, B.H., Spooner, E., Ploegh, H.L., et al. (2009). Haploid genetic screens in human cells identify host factors used by pathogens. Science *326*, 1231–1235.

Caulfield, M.J., Munroe, P.B., O'Neill, D., Witkowska, K., Charchar, F.J., Doblado, M., Evans, S., Eyheramendy, S., Onipinla, A., Howard, P., et al. (2008). SLC2A9 is a high-capacity urate transporter in humans. PLoS Med. *5*, e197.

Chapman, H.N., Fromme, P., Barty, A., White, T.A., Kirian, R.A., Aquila, A., Hunter, M.S., Schulz, J., DePonte, D.P., Weierstall, U., et al. (2011). Femtosecond X-ray protein nanocrystallography. Nature *470*, 73–77.

Chimienti, F., Devergnas, S., Favier, A., and Seve, M. (2004). Identification and cloning of a beta-cell-specific zinc transporter, ZnT-8, localized into insulin secretory granules. Diabetes *53*, 2330–2337.

Clemencon, B., Fine, M., Schneider, P., and Hediger, M.A. (2015). Rapid method to express and purify human membrane protein using the Xenopus oocyte system for functional and low-resolution structural analysis. Methods Enzymol. *556*, 241–265.

Cong, L., Ran, F.A., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, P.D., Wu, X., Jiang, W., and Marraffini, L.A. (2013). Multiplex genome engineering using CRISPR/Cas systems. Science *339*, 819–823.

Cornett, D.S., Reyzer, M.L., Chaurand, P., and Caprioli, R.M. (2007). MALDI imaging mass spectrometry: molecular snapshots of biochemical systems. Nat. Methods *4*, 828–833.

Cox, J., and Mann, M. (2011). Quantitative, high-resolution proteomics for data-driven systems biology. Annu. Rev. Biochem. *80*, 273–299.

Deng, D., Xu, C., Sun, P., Wu, J., Yan, C., Hu, M., and Yan, N. (2014). Crystal structure of the human glucose transporter GLUT1. Nature *510*, 121–125.

Deng, D., Sun, P., Yan, C., Ke, M., Jiang, X., Xiong, L., Ren, W., Hirata, K., Yamamoto, M., Fan, S., et al. (2015). Molecular basis of ligand recognition and transport by glucose transporters. Nature. Published online July 15, 2015. http://dx.doi.org/10.1038/nature14655.

Doudna, J.A., and Charpentier, E. (2014). Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science *346*, 1258096.

Edwards, A.M., Bountra, C., Kerr, D.J., and Willson, T.M. (2009). Open access chemical and clinical probes to support drug discovery. Nat. Chem. Biol. *5*, 436–440.

Edwards, A.M., Isserlin, R., Bader, G.D., Frye, S.V., Willson, T.M., and Yu, F.H. (2011). Too many roads not taken. Nature *470*, 163–165.

Faham, S., Watanabe, A., Besserer, G.M., Cascio, D., Specht, A., Hirayama, B.A., Wright, E.M., and Abramson, J. (2008). The crystal structure of a sodium galactose transporter reveals mechanistic insights into Na+/sugar symport. Science *321*, 810–814.

Fauman, E.B., Rai, B.K., and Huang, E.S. (2011). Structure-based druggability assessment–identifying suitable targets for small molecule therapeutics. Curr. Opin. Chem. Biol. *15*, 463–468.

Forrest, A.R., Kawaji, H., Rehli, M., Baillie, J.K., de Hoon, M.J., Haberle, V., Lassman, T., Kulakovskiy, I.V., Lizio, M., Itoh, M., et al. (2014). A promoter-level mammalian expression atlas. Nature *507*, 462–470.

Frye, S.V. (2010). The art of the chemical probe. Nat. Chem. Biol. *6*, 159–161.

Giacomini, K.M., Huang, S.M., Tweedie, D.J., Benet, L.Z., Brouwer, K.L., Chu, X., Dahlin, A., Evers, R., Fischer, V., Hillgren, K.M., et al. (2010). Membrane transporters in drug development. Nat. Rev. Drug Discov. *9*, 215–236.

Giacomini, K.M., Balimane, P.V., Cho, S.K., Eadon, M., Edeki, T., Hillgren, K.M., Huang, S.M., Sugiyama, Y., Weitz, D., Wen, Y., et al. (2013). International Transporter Consortium commentary on clinically important transporter polymorphisms. Clin. Pharmacol. Ther. *94*, 23–26.

Gruswitz, F., Chaudhary, S., Ho, J.D., Schlessinger, A., Pezeshki, B., Ho, C.M., Sali, A., Westhoff, C.M., and Stroud, R.M. (2010). Function of human Rh based on structure of RhCG at 2.1 A. Proc. Natl. Acad. Sci. USA *107*, 9638–9643.

Hediger, M.A., Coady, M.J., Ikeda, T.S., and Wright, E.M. (1987). Expression cloning and cDNA sequencing of the Na+/glucose co-transporter. Nature *330*, 379–381.

Hediger, M.A., Romero, M.F., Peng, J.B., Rolfs, A., Takanaga, H., and Bruford, E.A. (2004). The ABCs of solute carriers: physiological, pathological and therapeutic implications of human membrane transport proteinsIntroduction. Pflugers Arch. *447*, 465–468.

Hediger, M.A., Clemencon, B., Burrier, R.E., and Bruford, E.A. (2013). The ABCs of membrane transporters in health and disease (SLC series): introduction. Mol. Aspects Med. *34*, 95–107.

Hoglund, P.J., Nordstrom, K.J., Schioth, H.B., and Fredriksson, R. (2011). The solute carrier families have a remarkably long evolutionary history with the majority of the human families present before divergence of Bilaterian species. Mol. Biol. Evol. *28*, 1531–1541.

Hopkins, A.L., Keseru, G.M., Leeson, P.D., Rees, D.C., and Reynolds, C.H. (2014). The role of ligand efficiency metrics in drug discovery. Nat. Rev. Drug Discov. *13*, 105–121.

Hsu, P.D., Lander, E.S., and Zhang, F. (2014). Development and applications of CRISPR-Cas9 for genome engineering. Cell *157*, 1262–1278.

Huynen, M.A., Snel, B., von Mering, C., and Bork, P. (2003). Function prediction and protein networks. Curr. Opin. Cell Biol. *15*, 191–198.

Iharada, M., Miyaji, T., Fujimoto, T., Hiasa, M., Anzai, N., Omote, H., and Moriyama, Y. (2010). Type 1 sodium-dependent phosphate transporter (SLC17A1 Protein) is a Cl(-)-dependent urate exporter. J. Biol. Chem. *285*, 26107–26113.

Jordan, I.K., Marino-Ramirez, L., Wolf, Y.I., and Koonin, E.V. (2004). Conservation and coevolution in the scale-free human gene coexpression network. Mol. Biol. Evol. *21*, 2058–2070.

Kell, D.B. (2004). Metabolomics and systems biology: making sense of the soup. Curr. Opin. Microbiol. *7*, 296–307.

Kell, D.B., and Oliver, S.G. (2014). How drugs get into cells: tested and testable predictions to help discriminate between transporter-mediated uptake and lipoidal bilayer diffusion. Front. Pharmacol. *5*, 231.

Kell, D.B., Dobson, P.D., and Oliver, S.G. (2011). Pharmaceutical drug transport: the issues and the implications that it is essentially carrier-mediated only. Drug Discov. Today *16*, 704–714.

Klijn, C., Durinck, S., Stawiski, E.W., Haverty, P.M., Jiang, Z., Liu, H., Degenhardt, J., Mayba, O., Gnad, F., Liu, J., et al. (2015). A comprehensive transcriptional portrait of human cancer cell lines. Nat. Biotechnol. *33*, 306–312.

Kim, M.S., Pinto, S.M., Getnet, D., Nirujogi, R.S., Manda, S.S., Chaerkady, R., Madugundu, A.K., Kelkar, D.S., Isserlin, R., Jain, S., et al. (2014). A draft map of the human proteome. Nature *509*, 575–581.

Koberlin, M.S., Snijder, B., Heinz, L.X., Baumann, C.L., Fauster, A., Vladimer, G.I., Gavin, A.C., and Superti-Furga, G. (2015). A Conserved Circular Network of Coregulated Lipids Modulates Innate Immune Responses. Cell *162*, 170–183.

Krumpochova, P., Sapthu, S., Brouwers, J.F., de Haas, M., de Vos, R., Borst, P., and van de Wetering, K. (2012). Transportomics: screening for substrates of ABC transporters in body fluids using vesicular transport assays. FASEB J. *26*, 738–747.

Kurian, M.A., Zhen, J., Cheng, S.Y., Li, Y., Mordekar, S.R., Jardine, P., Morgan, N.V., Meyer, E., Tee, L., Pasha, S., et al. (2009). Homozygous loss-of-function mutations in the gene encoding the dopamine transporter are associated with infantile parkinsonism-dystonia. J. Clin. Invest. *119*, 1595–1603.

Lambert, J.P., Tucholska, M., Go, C., Knight, J.D., and Gingras, A.C. (2015). Proximity biotinylation and affinity purification are complementary approaches for the interactome mapping of chromatin-associated protein complexes. J. Proteomics *118*, 81–94.

Lin, L., Yee, S.W., Kim, R.B., and Giacomini, K.M. (2015). SLC transporters as therapeutic targets: emerging opportunities. Nat. Rev. Drug Discov. Published online June 26, 2015. http://dx.doi.org/10.1038/nrd4626.

Liu, Y., Peter, D., Roghani, A., Schuldiner, S., Prive, G.G., Eisenberg, D., Brecha, N., and Edwards, R.H. (1992). A cDNA that suppresses MPP+ toxicity encodes a vesicular amine transporter. Cell *70*, 539–551.

Mele, M., Ferreira, P.G., Reverter, F., DeLuca, D.S., Monlong, J., Sammeth, M., Young, T.R., Goldmann, J.M., Pervouchine, D.D., Sullivan, T.J., et al. (2015). Human genomics. The human transcriptome across tissues and individuals. Science *348*, 660–665.

Moraes, I., Evans, G., Sanchez-Weatherby, J., Newstead, S., and Stewart, P.D. (2014). Membrane protein structure determination - the next generation. Biochim. Biophys. Acta *1838*, 78–87.

Mulkidjanian, A.Y., Galperin, M.Y., and Koonin, E.V. (2009). Co-evolution of primordial membranes and membrane proteins. Trends Biochem. Sci. *34*, 206–215.

Nigam, S.K. (2015). What do drug transporters really do? Nat. Rev. Drug Discov. *14*, 29–44.

Parkinson, H., Sarkans, U., Kolesnikov, N., Abeygunawardena, N., Burdett, T., Dylag, M., Emam, I., Farne, A., Hastings, E., Holloway, E., et al. (2011). ArrayExpress update–an archive of microarray and high-throughput sequencing-based functional genomics experiments. Nucleic Acids Res. *39*, D1002–D1004.

Petschnigg, J., Groisman, B., Kotlyar, M., Taipale, M., Zheng, Y., Kurat, C.F., Sayad, A., Sierra, J.R., Mattiazzi Usaj, M., Snider, J., et al. (2014). The mammalian-membrane two-hybrid assay (MaMTH) for probing membrane-protein interactions in human cells. Nat. Methods *11*, 585–592.

Qi, L.S., Larson, M.H., Gilbert, L.A., Doudna, J.A., Weissman, J.S., Arkin, A.P., and Lim, W.A. (2013). Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell *152*, 1173–1183.

Rask-Andersen, M., Masuram, S., Fredriksson, R., and Schioth, H.B. (2013). Solute carriers as drug targets: current use, clinical trials and prospective. Mol. Aspects Med. *34*, 702–710.

Ratnam, J., Zdrazil, B., Digles, D., Cuadrado-Rodriguez, E., Neefs, J.M., Tipney, H., Siebes, R., Waagmeester, A., Bradley, G., Chau, C.H., et al. (2014). The application of the open pharmacological concepts triple store (open PHACTS) to support drug discovery research. PLoS ONE *9*, e115460.

Rebsamen, M., Pochini, L., Stasyk, T., de Araujo, M.E., Galluccio, M., Kandasamy, R.K., Snijder, B., Fauster, A., Rudashevskaya, E.L., Bruckner, M., et al. (2015). SLC38A9 is a component of the lysosomal amino acid sensing machinery that controls mTORC1. Nature *519*, 477–481.

Reiling, J.H., Clish, C.B., Carette, J.E., Varadarajan, M., Brummelkamp, T.R., and Sabatini, D.M. (2011). A haploid genetic screen identifies the major facilitator domain containing 2A (MFSD2A) transporter as a key mediator in the response to tunicamycin. Proc. Natl. Acad. Sci. USA *108*, 11756–11765.

Rolland, T., Tasan, M., Charloteaux, B., Pevzner, S.J., Zhong, Q., Sahni, N., Yi, S., Lemmens, I., Fontanillo, C., Mosca, R., et al. (2014). A proteome-scale map of the human interactome network. Cell *159*, 1212–1226.

Rutter, G.A., and Chimienti, F. (2015). SLC30A8 mutations in type 2 diabetes. Diabetologia *58*, 31–36.

Saliba, A.E., Vonkova, I., Ceschia, S., Findlay, G.M., Maeda, K., Tischer, C., Deghou, S., van Noort, V., Bork, P., Pawson, T., et al. (2014). A quantitative liposome microarray to systematically characterize protein-lipid interactions. Nat. Methods *11*, 47–50.

Scalise, M., Pochini, L., Giangregorio, N., Tonazzi, A., and Indiveri, C. (2013). Proteoliposomes as tool for assaying membrane transporter functions and interactions with xenobiotics. Pharmaceutics *5*, 472–497.

Schlessinger, A., Matsson, P., Shima, J.E., Pieper, U., Yee, S.W., Kelly, L., Apeltsin, L., Stroud, R.M., Ferrin, T.E., Giacomini, K.M., et al. (2010). Comparison of human solute carriers. Protein Sci. *19*, 412–428.

Schlessinger, A., Khuri, N., Giacomini, K.M., and Sali, A. (2013a). Molecular modeling and ligand docking for solute carrier (SLC) transporters. Curr. Top. Med. Chem. *13*, 843–856.

Schlessinger, A., Yee, S.W., Sali, A., and Giacomini, K.M. (2013b). SLC classification: an update. Clin. Pharmacol. Ther. *94*, 19–23.

Schrum, J.P., Zhu, T.F., and Szostak, J.W. (2010). The origins of cellular life. Cold Spring Harb. Perspect. Biol. *2*, a002212.

Shin, S.Y., Fauman, E.B., Petersen, A.K., Krumsiek, J., Santos, R., Huang, J., Arnold, M., Erte, I., Forgetta, V., Yang, T.P., et al. (2014). An atlas of genetic influences on human blood metabolites. Nat. Genet. *46*, 543–550.

Snijder, B., Kandasamy, R.K., and Superti-Furga, G. (2014). Toward effective sharing of high-dimensional immunology data. Nat. Biotechnol. *32*, 755–759.

Stuart, J.M., Segal, E., Koller, D., and Kim, S.K. (2003). A gene-coexpression network for global discovery of conserved genetic modules. Science *302*, 249–255.

Thiele, I., Swainston, N., Fleming, R.M., Hoppe, A., Sahoo, S., Aurich, M.K., Haraldsdottir, H., Mo, M.L., Rolfsson, O., Stobbe, M.D., et al. (2013). A community-driven global reconstruction of human metabolism. Nat. Biotechnol. *31*, 419–425.

Uhlen, M., Fagerberg, L., Hallstrom, B.M., Lindskog, C., Oksvold, P., Mardinoglu, A., Sivertsson, A., Kampf, C., Sjostedt, E., Asplund, A., et al. (2015). Proteomics. Tissue-based map of the human proteome. Science *347*, 1260419.

Varjosalo, M., Sacco, R., Stukalov, A., van Drogen, A., Planyavsky, M., Hauri, S., Aebersold, R., Bennett, K.L., Colinge, J., Gstaiger, M., et al. (2013). Interlaboratory reproducibility of large-scale human protein-complex analysis by standardized AP-MS. Nat. Methods *10*, 307–314.

Wang, S., Tsun, Z.Y., Wolfson, R.L., Shen, K., Wyant, G.A., Plovanich, M.E., Yuan, E.D., Jones, T.D., Chantranupong, L., Comb, W., et al. (2015). Metabolism. Lysosomal amino acid transporter SLC38A9 signals arginine sufficiency to mTORC1. Science *347*, 188–194.

Wikoff, W.R., Anfora, A.T., Liu, J., Schultz, P.G., Lesley, S.A., Peters, E.C., and Siuzdak, G. (2009). Metabolomics analysis reveals large effects of gut

microflora on mammalian blood metabolites. Proc. Natl. Acad. Sci. USA *106*, 3698–3703.

Wilhelm, M., Schlegl, J., Hahne, H., Moghaddas Gholami, A., Lieberenz, M., Savitski, M.M., Ziegler, E., Butzmann, L., Gessulat, S., Marx, H., et al. (2014). Mass-spectrometry-based draft of the human proteome. Nature *509*, 582–587.

Williams, A.J., Harland, L., Groth, P., Pettifer, S., Chichester, C., Willighagen, E.L., Evelo, C.T., Blomberg, N., Ecker, G., Goble, C., et al. (2012). Open PHACTS: semantic interoperability for drug discovery. Drug Discov. Today *17*, 1188–1198.

Williams, A.L., Jacobs, S.B., Moreno-Macias, H., Huerta-Chagoya, A., Churchhouse, C., Marquez-Luna, C., Garcia-Ortiz, H., Gomez-Vazquez, M.J., Burtt, N.P., Aguilar-Salinas, C.A., et al. (2014). Sequence variants in SLC16A11 are a common risk factor for type 2 diabetes in Mexico. Nature *506*, 97–101.

Winter, G.E., Radic, B., Mayor-Ruiz, C., Blomen, V.A., Trefzer, C., Kandasamy, R.K., Huber, K.V., Gridling, M., Chen, D., Klampfl, T., et al. (2014). The solute carrier SLC35F2 enables YM155-mediated DNA damage toxicity. Nat. Chem. Biol. *10*, 768–773.

Zeev-Ben-Mordehai, T., Vasishtan, D., Siebert, C.A., Whittle, C., and Grunewald, K. (2014). Extracellular vesicles: a platform for the structure determination of membrane proteins by Cryo-EM. Structure *22*, 1687–1692.

Zhang, Q., Zhang, Y., Diamond, S., Boer, J., Harris, J.J., Li, Y., Rupar, M., Behshad, E., Gardiner, C., Collier, P., et al. (2014). The Janus kinase 2 inhibitor fedratinib inhibits thiamine uptake: a putative mechanism for the onset of Wernicke's encephalopathy. Drug Metab. Dispos. *42*, 1656–1662.

Zhou, Q., Huang, X., Sun, S., Li, X., Wang, H.W., and Sui, S.F. (2015). Cryo-EM structure of SNAP-SNARE assembly in 20S particle. Cell Res. *25*, 551–560.