PERSPECTIVE

Accelerating SLC Transporter Research: Streamlining Knowledge and Validated Tools

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A modern, rational approach to a large class of proteins broadly relevant for pharmacology is enabling both from a postgenomic perspective and for reasons of economy of scale. The international public–private consortia RESOLUTE (https://re-solute.eu/) and REsolution (https://re-solute.eu/resolution) are generating a systematic functional assignment of each of the 451 different human solute carrier (SLC) proteins and, at the same time, trying to catalog and interpret the associated main genetic variants.

SLCs are a superfamily of facilitative and secondary active transporters, subclassified by sequence homology, and represent an understudied target class relevant both as source of drug targets (e.g., glifozins, selective serotonin reuptake inhibitors) and for drug disposition (e.g., organic anion transporters).[1,2] What if we were to take such a target class and wanted to rapidly "unlock" it for the scientific community to explore more broadly and easily? How would one go about it in a rational, postgenomic way? This is what RESOLUTE and REsolution, two public–private partnership projects funded by the Innovative Medicine Initiative (IMI, https://www.ihi.europa.eu/) of the European Union and the European Federation of Pharmaceutical Industries and Associations (EFPIA), are well underway doing. Here we report on the status of the two programs, describe access to new public resources of data and reagents, and briefly describe what may be applicable for similar target class-wide efforts in the future.

RESOLUTE'S AND RESOLUTION'S CULTIVATION STRATEGIES

RESOLUTE is formed by six academic partners (CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Leiden University, Max Planck Institute for Medical Research, University of Liverpool, University of Oxford, and the University of Vienna), one medium-sized enterprise (Axxam SpA), and six EFPIA partners (Pfizer, Bayer, Boehringer Ingelheim, Novartis, Sanofi, and Vifor Pharma). The project started in 2018 and its mission is to empower the scientific community with biological tools for SLC research, with packages of reliable knowledge for deorphanization and functionalization of the majority of SLCs, as well as through development of robust functional assays.[3] To expand the scope of RESOLUTE, the REsolution spin-off project started in 2021. REsolution is formed by the same six academic partners and medium-sized enterprise as well as EFPIA partners Pfizer and Bayer. Taking advantage of the increasing availability of human genomics data[4,5] and responding to the need to understand genetic variation, REsolution builds on RESOLUTE's toolkit to extend its scope towards creating medically relevant impact. To that end, REsolution compiles publicly available evidence on genetic variation in SLC genes and their association with diseases and combines this information with functional data. The consequence of genetic variants is investigated experimentally in functional assays for selected SLCs, either with a limited set of relevant variants or by deep mutational scanning. Respective results are combined with structural, stability, and evolutionary conservation analyses to train and improve variant effect predictors for membrane proteins or SLCs.

THE FIRST FRUITS

Through the open access repository Addgene, RESOLUTE made available a collection of 892 plasmids. This collection consists of codon-optimized ORF sequences for 446 human SLC genes with or without a stop codon. Within only one-and-a-half years we count more than 1,000 requests and expect increasing interest in the future. As a second set of tools, two collections of genetically modified cell lines were released by the consortium in 2021.

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One of these consists of more than 500 different HEK293 Jump-In T-REx cell lines with inducible overexpression of 429 individual human SLC proteins fused with Twin-Strep-Tag and HA epitopes at the C or the N terminal of the protein, which are available upon request (**Table 1**). Cell lines were mycoplasma tested and characterized by RNA sequencing, Western blotting, and immunofluorescence. The second collection consists of 399 different HCT-116 human colon cancer cell lines with specific CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats–CRISPR-associated protein 9)–mediated ablation of 202 individual SLC genes, which were validated by Sanger and RNA sequencing (**Table 1**). All reagents are available to any organization for research use only. As part of the quality control and characterization of HEK293 Jump-In T-REx cell lines, RNA sequencing and high-content imaging results were released in 2021. Researchers can interactively explore the differential transcriptomic profiles between overexpressed and basal conditions of each SLC on the RESOLUTE web portal (**Table 1**). Full RNA sequencing data sets are available through the repository European Nucleotide Archive (ENA). Immunofluorescence images of each HA-tagged SLC in combination with a panel of organelle markers are accessible for interactive exploration on the RESOLUTE web portal (**Table 1**).

Toward meeting its objective to improve assayability of SLCs, RESOLUTE published a comprehensive overview of cell-based assay platforms, which are developed and used by consortium partners.[6] The technologies cover uptake, binding, and functional assays for both plasma membrane and intracellular localized SLCs. We plan a second compilation of assays at the end of the project and anticipate being able to issue valuable recommendations for suitable assays for each SLC family and many individual members. Protocols and results of individual assays and SLCs are available through the RESOLUTE web portal already and several assays with the phenotypic, label-free impedance technology have been published.[7,8]

With the conclusion of its first year, the REsolution consortium released the genetic assessments for the entire SLC family (**Table 1**). The RESOLUTE web portal provides an SLC-focused overview of evidence on SLC genetic links to diseases and molecular phenotypes assembled from a variety of sources. A flexible scoring algorithm was developed to quantify and compare the variants across all SLCs. Users can select a customized set of associations and data sources to adapt the score to their specific investigation, which should assist researchers in a tailored exploration of human SLC genetics.

THE FUTURE HARVEST

Described cell lines are explored by the RESOLUTE consortium in a variety of omics technologies for elucidation of SLC function in a family-wide manner, resulting in the release of further data packages in the next year. Among those are transcriptomics analyses of SLC knock-out cell lines with or without induced corresponding SLC overexpression, as well as targeted metabolomics of genetically modified cell lines for at least 80% of the family. Metabolic profiles include roughly 150 metabolites and are obtained upon doxycycline-induced overexpression of individual SLCs either in wild-type or cognate knock-out cell lines. They provide experimental assessments of the metabolic consequences of SLC expression and often suggest functional relationships between different SLCs. Cellular uptake and release assays in human plasma coupled with untargeted metabolomics studies are carried out for a subset of orphan SLCs with the aim of elucidating their possible substrate. A combinatorial genetic interaction screen is underway, aiming at deciphering the functional redundancies in the HCT-116 cell line and is expected to lead to uncovering SLC functional dependencies useful to the deorphanization campaign as proven in a pioneering study using HAP1 cells.[9] A central piece of the RESOLUTE effort focuses on the SLC interactome. A protein interaction network based on affinity purification coupled with mass-spectroscopy of individual SLCs provides the interaction partners of each SLC under a steady state, unstimulated condition. It is expected that the SLC interactome will help to elucidate the SLC function and to identify obligatory binding partners with SLCs, contextualizing links to the signaling, biosynthetic/proteostatic, metabolic, and transport machinery of the cell. Moreover, each SLC interactome is interpreted in light of its experimentally determined subcellular localization obtained by high-content imaging. Integrative analysis of all data should drive efficient hypothesis generation on the biochemical function for each SLC. For this, we are employing (i) experimentally determined and alphafold predicted structure; (ii) interactome; (iii) metabolome; (iv) transcriptome; (v) localization; (vi) genetic interactions; and (vii) previous functional annotation. This should provide an unbiased, data-based, degree of similarity that, through the guilt-by-association principle, should pinpoint with precision the likelihood of a biochemical function, regulatory principles, and transporter cross talk.

Some of the hypotheses, especially on so-called orphan SLCs, accounting for around 30% of the family, will be tested using the established assay technology platforms or through collaboration with expert laboratories in our extended cooperation network. We are confident that, not unlike a giant puzzle, general features and themes will appear, including principles of transporter regulation and functional coordination.

An important contribution to the community will occur through the release of sequences for high-affinity protein binders. Roughly 40 SLCs are explored in different platforms for the generation of nanobodies, sybodies or other protein scaffolds, which are evaluated for *in vitro* binding affinity and in cellular applications such as immunofluorescence and immunoprecipitation. We expect these protein binders to become valuable tools and assist the structural and cell biological research on SLCs as well as allow new types of functional modulation. Comparison of the different binder-generating platforms for suitability for different SLCs as well as utility for various applications will provide guidance for the future generation of high-affinity protein binder for SLC transporters and perhaps membrane proteins in general.

REsolution, the RESOLUTE sister project focused on genetic variants, will publish target-focused analyses on a subset of therapeutically relevant SLCs and their genetic variants observed in the population from a structure–function perspective. Mutational

Table 1 Overview of available resources from the RESOLUTE and REsolution consortia

		Link	Description
	Resources		
	Web portal	https://re-solute.eu	Web portal with public resources and data for RESOLUTE and REsolution
	Knowledgebase	https://re-solute.eu/knowledgebase	Curated information on individual SLC members and families
	Protocols/reports	https://zenodo.org/communities/resolute/	Protocols and public reports from RESOLUTE
	Reagents		
	Plasmids	https://addgene.org/depositor-collections/re-solute/	RESOLUTE plasmids and plasmid libraries collection
	Cell lines	https://re-solute.eu/resources/reagents	Collection of cell lines with specific SLCs genetically deleted by CRISPR-Cas9 or with an inducible overexpression construct inserted
	Data		
	Public data sets	https://re-solute.eu/resources/datasets	Overview of full data sets and links to public repositories
	SLC genetics and disease associations dashboard	https://re-solute.eu/resources/dashboards	Collection of evidence from a variety of sources with an algorithm to score sources based on individual interest
	Transcriptomics dashboard	https://re-solute.eu/resources/dashboards	RNA sequencing data of HEK293 Jump-In T-REx cell lines with inducible overexpression of individual SLC proteins fused with Twin-Strep-Tag and HA epitopes
	Localization dashboard	https://re-solute.eu/resources/dashboards	High-content imaging data of cellular organelle markers and individual SLCs overexpressed in HEK293 Jump-In T-REx cell lines
	Transport assays	https://re-solute.eu/resources/reagents	Transport assay data released by RESOLUTE

Web links and brief descriptions of different types of resources which are publicly available and created by the RESOLUTE and REsolution consortia.
CRISPR-Cas9, clustered regularly interspaced short palindromic repeats–CRISPR-associated protein 9; SLC, solute carrier.

scanning coupled with the assessment of transport activity of selected SLCs from different families is expected to provide insight into the individual transport mechanisms and, in addition, combined with stability predictions and evolutionary analysis, enable a better understanding of the structure–function relationship of SLCs. Such understanding may allow for improving current variant effect predictors in an SLC-specific manner, also through machine-learning, which in turn may lead to a benefit for patient diagnosis for variants of unknown significance for transporters and other membrane proteins.

Data from both consortia are integrated together with publicly available data into the RESOLUTE knowledgebase (https://re-solute.eu/knowledgebase), which we envision to serve as a compass to navigate the SLC family for basic researchers, drug discoverers, and medical doctors.

FERTILE SOIL FOR FURTHER CULTIVATION

Even though we are still in the middle of both projects and hopefully ahead of many new discoveries and tools, we are convinced that both the scientific output of these consortia and the network of experts created across organizations will have a significant, long-standing impact on transporter research well beyond the projects' duration and scope. On one hand, the tools, the data, and knowledge created by the consortia will enable individual researchers anywhere in the world to use and translate them into pathophysiological context and, specifically, for drug discovery initiatives and bioavailability studies. SLCs are routinely explored in absorption, distribution, metabolism, and elimination studies. The output from the consortia is expected to expand both the number of pharmacokinetically relevant SLCs as well as assays and cell lines for respective studies. On the other hand, it is time to consider what may come after RESOLUTE and REsolution. Opportunities aimed at benefitting from the multidisciplinary and efficient research teams, grown to be expert in an exciting emerging target class, should be considered, both from a public benefit and a biotech/pharma perspective. Now that the end of this large, cumbersome effort on providing the basic knowledge and tools to "unlock" this target class is within sight, future work should aim at leveraging the enhanced knowledge of SLC function and regulation to design effective therapeutics targeting SLCs. As SLCs are eminently druggable, and we have hundreds of assays and validation tools aligned, a plan for the systematic identification of specific chemical probes for each transporter is within reach.[10] Given the integral role of SLC in human physiology and disease, a focus on the top 10–20% of all SLCs in terms of disease target attractivity should represent a priority focus of the drug discovery community to match the rate of successfully exploited top targets known from other target classes as GPCRs, kinases, channels, and proteases. We are confident that the long-term impact of this dedicated research effort on SLCs will ultimately result in more approved drugs targeting SLCs.

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CONFLICT OF INTEREST

G.S.-F. is founder and shareholder of Solgate and Proxygen. G.S.-F. is a shareholder of Exscientia Ltd. C.M.S. is an employee of Pfizer. All other authors declared no competing interests for this work.

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