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Understanding Kidney Disease: Toward the Integration of Regulatory Networks Across Species

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Abstract

Animal models have long been useful in investigating both normal and abnormal human physiology. Systems biology provides a relatively new set of approaches to identify similarities and differences between animal models and humans that may lead to a more comprehensive understanding of human kidney pathophysiology. In this review, we briefly describe how genome-wide analyses of mouse models have helped elucidate features of human kidney diseases, discuss strategies to achieve effective network integration, and summarize currently available web-based tools that may facilitate integration of data across species. The rapid progress in systems biology and orthology, as well as the advent of web-based tools to facilitate these processes, now make it possible to take advantage of knowledge from distant animal species in targeted identification of regulatory networks that may have clinical relevance for human kidney diseases.

Keywords

chronic kidney disease; metabolomics; molecular marker; network integration; systems biology; transcriptomics

The use of animal models for understanding human physiology and pathophysiology has a long and illustrious history. From Harvey's measurement of blood pressure in the horse in 1616 to the complete genetic mapping and sequencing of multiple model organisms in the last decade, the analysis of similarities and differences between humans and animal models has often driven physiologic and medical advances. Investigation of model organisms by systems biology approaches can lead to new concepts of kidney pathophysiology, especially when these approaches are combined with analysis of data from humans with kidney disease. These methods include genomics, transcriptomics, proteomics and metabolomics. While some of these methods have been relatively little used in the investigation of kidney disease in humans and animal models, transcriptomic analyses have been performed on model organisms as well as humans with and without kidney diseases.

In this review, we will concentrate on how such genome-wide analyses of mouse models have helped elucidate features of human disease. However, it must be noted that several

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nonmammalian model organisms have been useful in examining kidney development and diseases. Because there are fewer such analyses, these will be briefly considered in the Outlook section at the end of the review. We will also review some currently available web-based tools that may facilitate cross-species integration.

Systems approaches to rodent models of kidney pathophysiology

Because of its size, ease of handling and relative susceptibility to genetic manipulation, the laboratory mouse has become perhaps the major mammalian model of kidney disease over the past few years. The mouse is also genetically and physiologically similar to humans, and the mouse genome has been sequenced and annotated to a high standard, second only to that of human^{1,2}. Systems approaches have been applied to the study of kidney diseases in the mouse in several disease models. The biggest challenge in cross-species transcriptome comparisons is that information has been derived from various technical platforms and data analytical tools for different species. This issue was clearly addressed in a study published by He et al.³, who reported limited overlap between 5 different transcription-profiling approaches to identify glomerulus-enriched genes³, including mouse kidney EST library comparison⁴, mouse “GlomChip” cDNA microarray profiling⁵, mouse Affymetrix Genome 430 2.0 Array profiling³, human SAGE profiling⁶, and human Stanford cDNA microarray profiling⁷. The limited overlap between these different data sets indicates that a simple comparison between several gene lists is insufficient to identify common pathways. Thus, there is a need for more sophisticated tools to explore preserved molecular pathways and networks across different species. In the following sections, we will discuss approaches from several groups that help to overcome this potential disadvantage of cross-species comparisons.

Identification of molecular markers of progressive chronic kidney disease (CKD) progression using a mouse-human comparative genomics approach

Genomics and proteomics techniques are now being intensively applied to identify molecular signatures of kidney diseases and to develop diagnostic and prognostic markers from human and mouse samples. The European Renal cDNA Biopsy Bank (ERCB), established by Kretzler and co-workers, has linked high-quality genome-wide expression data from human kidney glomerular and tubulointerstitial tissues with clinical information and medical treatments of over 2500 patients with various kidney diseases^{8–10}. In parallel, global gene expression profiles obtained from mouse models have identified potential candidate biomarkers for disease initiation, progression and remission, as well as signaling pathways and regulatory networks involved in renal pathophysiology^{11–16}. However, a systematic strategy that takes advantage of mouse-human comparative transcriptomics has not yet been reported for kidney diseases.

Recently, we combined the molecular analysis of both a murine model of renal disease and human kidney biopsy data to identify and characterize molecular markers as predictors of estimated glomerular filtration rate (eGFR) and clinical stages of CKD in human cohorts¹⁷. This strategy not only allowed us to bypass limitations due to restricted availability of human biopsy tissues, but also provided an experimental system to prospectively test our hypotheses that the molecular signature of glomerular epithelial cell (podocyte) apoptosis at an early stage would predict future disease progression in the same model. Using genome-wide screening, we identified 43 genes differentially expressed at the initiation of disease in 2 subgroups of TGF- β 1 transgenic mice based on glomerular cell apoptosis rate. The candidate genes were tested for their predictive power for disease progression in a longitudinal study by correlating mRNA expression levels of those genes in surgically removed left kidneys at disease initiation with semi-quantitative histopathological score of the right kidneys 2 weeks later when advanced renal damage became apparent in some transgenic mice. Expression levels of 19 genes were found to predict development of progressive kidney damage. To explore the relevance of our findings in human kidney disease, expression values of human orthologs of

these genes in a cohort of ERCB patients^{8,9} were shown to be highly significantly correlated to eGFR in patients with hypertensive nephrosclerosis, IgA nephropathy, minimal change disease, and thin basement membrane disease.

The results of our comparative analysis were remarkably consistent despite the fact that the classification of progressive CKD in mouse and human was based on distinct endpoints and methods (advanced histopathological scores in mouse versus estimated GFR in human cohorts). Nine of the 19 murine genes that were significantly correlated with advanced histopathology scores in the TGF- β 1 transgenic mice were present in the 16 human genes that were significantly correlated with eGFR in humans. This strongly supports the validity and robustness of this mouse-human comparative genomics approach. Because the 19 genes carry predictive power in the mouse longitudinal study it will be of great interest to determine if the common human/mouse markers also predict progression of disease in prospective studies.

Identification of potential pathways of disease progression by a cross-species gene expression approach based on discordance of murine and human responses

Another strategy for using mouse models to help identify gene expression patterns in humans that may be critical for progression of nephropathy is based on the observation that in many cases, murine models do not develop progressive chronic kidney disease leading to kidney failure, and therefore do not mimic the worst forms of human CKD. This principle is certainly true for murine models of diabetic nephropathy and has been the basis for the establishment of the Animal Models of Diabetic Complications Consortium which has recently noted that “no current murine model of diabetic nephropathy displays a consistent progressive decline in kidney function” nor meets all of the other criteria necessary to be a complete model of diabetic nephropathy¹⁸.

This apparent disadvantage can be turned into an advantage by identification of pathways in human progressive diabetic nephropathy that are not observed in murine models. Such human-specific pathways may be related to progressive nephropathy. In one example of this approach, review of transcriptomic data from humans with early and progressive diabetic nephropathy revealed a major increase in expression of members of the JAK/STAT signaling families in both glomeruli in early nephropathy, and in the tubulointerstitium in progressive disease¹⁹. JAK-1, 2 and 3 as well as STAT-1 and STAT-3 were expressed at higher levels in patients with diabetic nephropathy than in controls. The estimated glomerular filtration rate significantly correlated with tubulointerstitial JAK-1, 2, 3 and STAT-1 expression ($R^2=0.30-0.44$) and immunohistochemistry found strong JAK-2 staining in glomerular and tubulointerstitial compartments in DN compared to controls. In contrast, there was little or no increase in expression of JAK/STAT genes in two common mouse models of diabetic nephropathy, the db/db C57BLKS or diabetic DBA/2J mice¹⁹. These two models develop early diabetic nephropathy but not progressive tubulointerstitial fibrosis and progressive CKD^{20, 21}. Although these findings do not confirm the role of enhanced JAK/STAT expression in the tubulointerstitial pathology and progression of diabetic nephropathy, they do strongly suggest that the expression alterations may be important and have pointed to new pathways to dissect in understanding this disease process.

Identification of potential markers of disease progression by murine urine metabolomic screening

The preceding murine-human comparisons used elements of transcriptomic analyses to parse out markers or pathways relevant to progressive kidney disease. Similar approaches are obviously possible with other systems biology techniques, such as metabolomics, though there is a scarcity of published reports. A recent publication used a mass spectrometry based metabolomic approach to assess all the metabolites in the urine of DBA/2 diabetic and control

mice, some of which had been treated with the thiazolidinedione, rosiglitazone. This diabetic model is one of the more robust models currently available, and though it does not show a progressive decline in glomerular filtration rate, develops extensive diabetic glomerular disease^{20,21}. Treatment with rosiglitazone prevented or ameliorated all the changes of diabetic nephropathy²².

In order to determine if the urinary metabolite profile was altered in this diabetic model and whether rosiglitazone therapy reversed these alterations, the urine was analyzed using a liquid chromatography electrospray ionization time of flight mass spectrometer. Urine samples were diluted and normalized to creatinine content. There were a total of 1988 molecular features (potential metabolites) that were identified in all the samples. Of these, 1023 features were present in all diabetic and/or diabetic + rosiglitazone samples and 668 features were present in all nondiabetic and/or nondiabetic + rosiglitazone samples. A total of 56 features showed up- or down-regulation by >2-fold in diabetic animals (Fig. 1). Of these, 21 features were returned to normal by rosiglitazone therapy²². Preliminary identification of these molecular features was performed using available exact mass databases. Of these, 9 compounds that returned back to baseline with rosiglitazone therapy were identified, raising the possibility that they could be potential biomarkers for resolution of DN. These included ubiquinone, indoxyl sulfate, n-methylfromamide, n-formyl-L-methionine, imidazoleacetic acid riboside, 6-ketoestriol, thiamine monophosphate, and 2'-deoxyinosine-5'-diphosphate. Urine from patients with diabetic nephropathy is now being examined to determine whether a similar pattern of metabolite changes occurs.

Comprehensive strategy of network integration: TALE for effective integration of knowledge from cross-species studies

In addition to the strategies described above, a more sophisticated use of cross-species gene expression studies compares complex regulatory networks across species for conserved network structures. To best define the shared transcriptional networks between humans and mouse model of human nephropathies, an index-based method, TALE²³ (a Tool for Approximate Subgraph Matching of Large Queries Efficiently), can be applied. Species-specific transcriptional networks are built using available primary literature-based network mapping tools like Bibliosphere (Genomatix Software GmbH, Munich, Germany), MiMI (Michigan Molecular Interaction, NCIBI, University of Michigan, Ann Arbor) or other similar networking mapping tools. Together with orthologous mapping of the significant genes from human and mouse, these two networks can be used for network merging. In the merging process, TALE distinguishes the genes by their importance (number of connections with other genes) in the individual network structure, which may contain hundreds to thousands of genes and interactions. The most connected genes are first matched between the two networks, defining the key network nodes (=hubs) of the shared network. The neighborhood of the key conserved nodes is then scanned to identify interactions that might or might not be present in the two species' original lists (Fig. 2A). TALE thus has the advantage of identifying interactions present in both species along with some interactions that are present in only one species but within a regulatory context that is conserved in both species (Fig. 2B). In this way, the integration is not at the single gene level, but at a regulatory network/pathway level, which is more likely to be conserved across species. This tool has been used to generate unique regulatory gene expression networks that are shared by several diabetic mouse models and humans with type II diabetes (unpublished data, J. Hodgin, M. Kretzler and F. Brosius).

Web-based tools for effective integration of knowledge from cross-species studies

Large sets of genomic and EST-based expression data in various species are more and more accessible to the public, though often these data are stored in heterogeneous formats and scattered over a multitude of species-specific databases (ZFIN for zebrafish^{24,25}, BDGP²⁶ and Flybase^{27–30} for *Drosophila*, GXD^{31–33}, EMAGE^{34–36} and GUDMAP³⁷ for mouse, Nephromine³⁸ for human kidney related disease) that are largely non-interoperable. Fortunately, investigators are currently in the process of establishing a central platform which will allow comparison of gene expression and networks across many different species^{39,40, 41}. To achieve this goal, orthologous gene libraries and orthology predictions are critically important. Here we briefly summarize some of these resources, most of which are publicly available.

The National Center for Biotechnology Information (NCBI) Homologene (<http://www.ncbi.nlm.nih.gov/homologene>) is a commonly used gene homology tool to identify putative orthologs based on nucleotide sequences between pairs of organisms. Curated orthologs are incorporated from a variety of sources: Online Mendelian Inheritance in Man (OMIM), Mouse Genome Informatics (MGI), Zebrafish Information Network (ZFIN) Saccharomyces Genome Database (SGD), Clusters of Orthologous Groups (COG), and FlyBase. Other orthologous gene resources with open access though limited species includes: ProbeMatchDB from BRAINARRAY (<http://brainarray.mbni.med.umich.edu/Brainarray>, University of Michigan), and Orthogene library from ArrayTrack (National Center for Toxicological Research) (<http://edkb.fda.gov/webstart/arraytrack>). The Genomatix Portal also offers user friendly tools for orthologous gene mapping. In addition, OrthoMCL DB⁴² (<http://www.orthomcl.org/cgi-bin/OrthoMclWeb.cgi>) and Inparanoid⁴³ (<http://inparanoid.sbc.su.se/cgi-bin/index.cgi>) have been shown to display very good overall balance between sensitivity and specificity in orthology prediction⁴¹. Altogether, there are many different programs available (for a detailed review see the paper by Kuzniar, et al.⁴⁴), although their accuracy varies. Therefore, we recommend combining ortholog information from several different resources and evaluating the output cautiously.

Several groups have begun to establish a central platform for gene and network cross-species comparisons. Haudry and co-workers have integrated expression patterns for zebrafish, drosophila, medaka and mouse into a public repository called 4DXpress (<http://4dx.embl.de/4DXpress/>; expression database in four dimensions – interpreted either as: 3 spatial dimensions and time, or as species, gene, developmental stage, and anatomical structure).⁴⁰ Users of 4DXpress can query anatomy ontology-based expression annotations across species and obtain relative information for orthologs in other species⁴⁰. An expression similarity measure is used to find genes with similar expression patterns and links to all original data sources are provided. While 4DXpress does not include human expression information, COMPARE (<http://compare.ibdml.univ-mrs.fr>) is a multi-organism web-based resource system that does include human data and is designed to easily retrieve, correlate and interpret data across species⁴¹. The COMPARE interface provides access to genomic structure, expression data, annotations, pathways and literature links for human and three animal models (zebrafish, *Drosophila* and mouse). The ortholog-finding pipeline enables accurate comparison of data across species. This allows the researcher to take information from well studied species to apply to research of more poorly annotated species.

Outlook

While we have concentrated our review on mouse models of kidney disease, non-rodent models such as the zebrafish, *Danio rerio*, the fruitfly, *Drosophila melanogaster*, and the toad, *Xenopus*

laevis, should also be considered for systems biology approaches as they have some advantages over rodent models, including smaller size, shorter generation time, high repopulation rate and greater ease of genetic manipulation. Kidney systems biology researchers have not yet taken full advantage of these non-mammalian models. One reason may be the difficulty of comparing their evolutionarily distant genomes to that of humans. Newer systems approaches may help overcome this barrier as shown in several cross-species studies^{45–50} which have demonstrated that novel functions for conserved molecules and conserved signaling pathways in human kidney development and disease can be extracted from data mining of large scale networks in *Drosophila* or other small animal models. The rapid advancement in orthology and web-based network integration tools (like TALE) has made it possible to integrate the regulatory networks from distant species to provide a strategy for rapid and targeted identification of genes and pathways that may have clinical relevance for human kidney diseases. Nonetheless, cross-species integration requires cautious study design and data interpretation, including careful selection of an animal model, determination of how to compare similarities and differences between species, and choice of the proper bioinformatics tools. Usually pathway or network comparisons will yield more reliable analyses than comparisons of individual gene expression. In addition, functional validation should be included as part of any comparative study.

One critical step in integration across species is to develop robust bioinformatics structures to integrate systems biology data from various animal models derived from high throughput technologies with similar data from patient oriented studies in order to achieve a more comprehensive understanding of genes/pathways/networks that underlie the pathophysiology of human kidney disease. With such tools being developed, we are hopeful that findings in model organisms will be rapidly translated into knowledge that can directly contribute to patient care.

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References

1. Frese KK, Tuveson DA. Maximizing mouse cancer models. *Nat Rev Cancer* 2007;7(9):645–658. [PubMed: 17687385]
2. Mattison J, van der Weyden L, Hubbard T, Adams DJ. Cancer gene discovery in mouse and man. *Biochim Biophys Acta* 2009;1796(2):140–161. [PubMed: 19285540]
3. He L, Sun Y, Takemoto M, et al. The glomerular transcriptome and a predicted protein-protein interaction network. *J Am Soc Nephrol* 2008;19(2):260–268. [PubMed: 18032794]
4. He L, Sun Y, Patrakka J, et al. Glomerulus-specific mRNA transcripts and proteins identified through kidney expressed sequence tag database analysis. *Kidney Int* 2007;71(9):889–900. [PubMed: 17332733]
5. Takemoto M, He L, Norlin J, et al. Large-scale identification of genes implicated in kidney glomerulus development and function. *EMBO J* 2006;25(5):1160–1174. [PubMed: 16498405]
6. Chabardes-Garonne D, Mejean A, Aude JC, et al. A panoramic view of gene expression in the human kidney. *Proc Natl Acad Sci U S A* 2003;100(23):13710–13715. [PubMed: 14595018]
7. Higgins JP, Wang L, Kambham N, et al. Gene expression in the normal adult human kidney assessed by complementary DNA microarray. *Mol Biol Cell* 2004;15(2):649–656. [PubMed: 14657249]
8. Yasuda Y, Cohen CD, Henger A, Kretzler M. Gene expression profiling analysis in nephrology: towards molecular definition of renal disease. *Clin Exp Nephrol* 2006;10(2):91–98. [PubMed: 16791393]
9. Schmid H, Cohen CD, Henger A, Schlondorff D, Kretzler M. Gene expression analysis in renal biopsies. *Nephrol Dial Transplant* 2004;19(6):1347–1351. [PubMed: 15034163]

10. Henger A, Schmid H, Kretzler M. Gene expression analysis of human renal biopsies: recent developments towards molecular diagnosis of kidney disease. *Curr Opin Nephrol Hypertens* 2004;13(3):313–318. [PubMed: 15073490]
11. Kim JH, Ha IS, Hwang CI, et al. Gene expression profiling of anti-GBM glomerulonephritis model: the role of NF-kappaB in immune complex kidney disease. *Kidney Int* 2004;66(5):1826–1837. [PubMed: 15496153]
12. Susztak K, Bottinger E, Novetsky A, et al. Molecular profiling of diabetic mouse kidney reveals novel genes linked to glomerular disease. *Diabetes* 2004;53(3):784–794. [PubMed: 14988265]
13. Schanstra JP, Bachvarova M, Neau E, Bascands JL, Bachvarov D. Gene expression profiling in the remnant kidney model of wild type and kinin B1 and B2 receptor knockout mice. *Kidney Int* 2007;72(4):442–454. [PubMed: 17579666]
14. Monrad SU, Killen PD, Anderson MR, Bradke A, Kaplan MJ. The role of aldosterone blockade in murine lupus nephritis. *Arthritis Res Ther* 2008;10(1):R5. [PubMed: 18197980]
15. Moreno S, Ibraghimov-Beskrovnaya O, Bukanov NO. Serum and urinary biomarker signatures for rapid preclinical in vivo assessment of CDK inhibition as a therapeutic approach for PKD. *Cell Cycle* 2008;7(12):1856–1864. [PubMed: 18583937]
16. Liu K, Li QZ, Delgado-Vega AM, et al. Kallikrein genes are associated with lupus and glomerular basement membrane-specific antibody-induced nephritis in mice and humans. *J Clin Invest* 2009;119(4):911–923. [PubMed: 19307730]
17. Ju W, Eichinger F, Bitzer M, et al. Renal gene and protein expression signatures for prediction of kidney disease progression. *Am J Pathol* 2009;174(6):2073–2085. [PubMed: 19465643]
18. Brosius FC 3rd, Alpers CE, Bottinger EP, et al. Mouse Models of Diabetic Nephropathy. *J Am Soc Nephrol*. 2009
19. Berthier CC, Zhang H, Schin M, et al. Enhanced expression of Janus kinase-signal transducer and activator of transcription pathway members in human diabetic nephropathy. *Diabetes* 2009;58(2):469–477. [PubMed: 19017763]
20. Qi Z, Fujita H, Jin J, et al. Characterization of susceptibility of inbred mouse strains to diabetic nephropathy. *Diabetes* 2005;54(9):2628–2637. [PubMed: 16123351]
21. Gurley SB, Clare SE, Snow KP, et al. Impact of genetic background on nephropathy in diabetic mice. *American journal of physiology* 2006;290(1):F214–222. [PubMed: 16118394]
22. Zhang H, Saha J, Byun J, et al. Rosiglitazone reduces renal and plasma markers of oxidative injury and reverses urinary metabolite abnormalities in the amelioration of diabetic nephropathy. *American journal of physiology* 2008;295(4):F1071–1081. [PubMed: 18667486]
23. Tian, T.; Patel, JM. TALE: A Tool for Approximate Large Graph Matching. ICDE, International Conference on Data Engineering; Cancún, México. 2008.
24. Sprague J, Bayraktaroglu L, Bradford Y, et al. The Zebrafish Information Network: the zebrafish model organism database provides expanded support for genotypes and phenotypes. *Nucleic Acids Res* 2008;36(Database issue):D768–772. [PubMed: 17991680]
25. Sprague J, Bayraktaroglu L, Clements D, et al. The Zebrafish Information Network: the zebrafish model organism database. *Nucleic Acids Res* 2006;34(Database issue):D581–585. [PubMed: 16381936]
26. Tomancak P, Berman BP, Beaton A, et al. Global analysis of patterns of gene expression during *Drosophila* embryogenesis. *Genome Biol* 2007;8(7):R145. [PubMed: 17645804]
27. Drysdale R. FlyBase : a database for the *Drosophila* research community. *Methods Mol Biol* 2008;420:45–59. [PubMed: 18641940]
28. Wilson RJ, Goodman JL, Strelets VB. FlyBase: integration and improvements to query tools. *Nucleic Acids Res* 2008;36(Database issue):D588–593. [PubMed: 18160408]
29. Crosby MA, Goodman JL, Strelets VB, Zhang P, Gelbart WM. FlyBase: genomes by the dozen. *Nucleic Acids Res* 2007;35(Database issue):D486–491. [PubMed: 17099233]
30. Grumbling G, Strelets V. FlyBase: anatomical data, images and queries. *Nucleic Acids Res* 2006;34(Database issue):D484–488. [PubMed: 16381917]
31. Smith CM, Finger JH, Hayamizu TF, et al. The mouse Gene Expression Database (GXD): 2007 update. *Nucleic Acids Res* 2007;35(Database issue):D618–623. [PubMed: 17130151]

32. Hill DP, Begley DA, Finger JH, et al. The mouse Gene Expression Database (GXD): updates and enhancements. *Nucleic Acids Res* 2004;32(Database issue):D568–571. [PubMed: 14681482]
33. Ringwald M, Mangan ME, Eppig JT, Kadin JA, Richardson JE. GXD: a gene expression database for the laboratory mouse. The Gene Expression Database Group. *Nucleic Acids Res* 1999;27(1):106–112. [PubMed: 9847152]
34. Richardson L, Venkataraman S, Stevenson P, et al. EMAGE mouse embryo spatial gene expression database: 2010 update. *Nucleic Acids Res*. 2009
35. Venkataraman S, Stevenson P, Yang Y, et al. EMAGE--Edinburgh Mouse Atlas of Gene Expression: 2008 update. *Nucleic Acids Res* 2008;36(Database issue):D860–865. [PubMed: 18077470]
36. Christiansen JH, Yang Y, Venkataraman S, et al. EMAGE: a spatial database of gene expression patterns during mouse embryo development. *Nucleic Acids Res* 2006;34(Database issue):D637–641. [PubMed: 16381949]
37. McMahon AP, Aronow BJ, Davidson DR, et al. GUDMAP: the genitourinary developmental molecular anatomy project. *J Am Soc Nephrol* 2008;19(4):667–671. [PubMed: 18287559]
38. Martini S, Eichinger F, Nair V, Kretzler M. Defining human diabetic nephropathy on the molecular level: integration of transcriptomic profiles with biological knowledge. *Rev Endocr Metab Disord* 2008;9(4):267–274. [PubMed: 18704688]
39. Kuhn A, Luthi-Carter R, Delorenzi M. Cross-species and cross-platform gene expression studies with the Bioconductor-compliant R package 'annotationTools'. *BMC Bioinformatics* 2008;9:26. [PubMed: 18201381]
40. Haudry Y, Berube H, Letunic I, et al. 4DXpress: a database for cross-species expression pattern comparisons. *Nucleic Acids Res* 2008;36(Database issue):D847–853. [PubMed: 17916571]
41. Salgado D, Gimenez G, Coulier F, Marcelle C. COMPARE, a multi-organism system for cross-species data comparison and transfer of information. *Bioinformatics* 2008;24(3):447–449. [PubMed: 18056065]
42. Chen F, Mackey AJ, Stoeckert CJ Jr, Roos DS. OrthoMCL-DB: querying a comprehensive multi-species collection of ortholog groups. *Nucleic Acids Res* 2006;34(Database issue):D363–368. [PubMed: 16381887]
43. O'Brien KP, Remm M, Sonnhammer EL. Inparanoid: a comprehensive database of eukaryotic orthologs. *Nucleic Acids Res* 2005;33(Database issue):D476–480. [PubMed: 15608241]
44. Kuzniar A, van Ham RC, Pongor S, Leunissen JA. The quest for orthologs: finding the corresponding gene across genomes. *Trends Genet* 2008;24(11):539–551. [PubMed: 18819722]
45. Liu J, Ghanim M, Xue L, et al. Analysis of Drosophila segmentation network identifies a JNK pathway factor overexpressed in kidney cancer. *Science* 2009;323(5918):1218–1222. [PubMed: 19164706]
46. Langenau DM, Keefe MD, Storer NY, et al. Effects of RAS on the genesis of embryonal rhabdomyosarcoma. *Genes Dev* 2007;21(11):1382–1395. [PubMed: 17510286]
47. Sreenivasan R, Cai M, Bartfai R, et al. Transcriptomic analyses reveal novel genes with sexually dimorphic expression in the zebrafish gonad and brain. *PLoS One* 2008;3(3):e1791. [PubMed: 18335061]
48. Kobayashi I, Ono H, Moritomo T, et al. Comparative gene expression analysis of zebrafish and mammals identifies common regulators in hematopoietic stem cells. *Blood*. 2009
49. Christensen EI, Raciti D, Reggiani L, Verroust PJ, Brandli AW. Gene expression analysis defines the proximal tubule as the compartment for endocytic receptor-mediated uptake in the *Xenopus* pronephric kidney. *Pflugers Arch* 2008;456(6):1163–1176. [PubMed: 18551302]
50. Raciti D, Reggiani L, Geffers L, et al. Organization of the pronephric kidney revealed by large-scale gene expression mapping. *Genome Biol* 2008;9(5):R84. [PubMed: 18492243]

Figure 1.

Depiction of 56 murine urine metabolite features that were up or down-regulated by >2 fold after normalization to urine creatinine values in urine of diabetic DBA/2 mice with substantial early diabetic nephropathy after 12 weeks of diabetes. These features needed to be present in 5 out of 7 animals tested in each group to be listed. Levels of 35 features in electrospray ionization-positive mode which showed such alterations are depicted.

Figure 2.

A: Overview of the matching algorithm of TALE, adapted from Tian et al.²³ The algorithm first matches the most important nodes conserved between the two networks. Of note, not all of these have to be exact orthologs of those in the other species, but fulfill similar functions (defined as connected to the comparable set of genes, as indicated by the matching of different shades of green, yellow and blue/purple). The algorithm then progressively extends these matches by including genes that are connected to those important genes in either species. B: Species-specific transcriptional networks can then be built based on the identified genes and connections using available primary literature based network mapping tools, which then be used by TALE for network merging. In the merged network, TALE pulls in interactions present in both species (orange lines) along with some interactions that are present in either of the species (purple or green lines).