An Overview of Synergistic Data Tools for Biological Scrutiny


1 Introduction

A main focus of our research is the use of genomics and bioinformatics tools to obtain novel biological insights. These approaches involve the application of computational strategies to biological information management and analysis. We develop data compendia that assist in obtaining a better understanding of how genes and their variations underlie different human phenotypes. We focus on several parallel threads, including human olfaction, Mendelian and complex diseases, embryonic development and stem cell research. In the spirit of systems biology, we help generate genome-wide views to facilitate gene-based inquiry. One of our long-time fortes is a gene-centric view of the human genome universe, as embodied in GeneCards. More recently, we generated in parallel the human disease compendium MalaCards. Both are richly annotated from numerous web resources and provide focused information to external data structures (Figure 1). A special liaison is between the Weizmann data structure and the LifeMap Discovery database, which provides a unique angle on embryonic development, stem cell research and regenerative medicine, which links to both GeneCards and MalaCards.

2 GeneCards

The individual scientist, seeking research knowledge about a gene of interest, can be overwhelmed by the deluge of data from worldwide genome projects. The laborious task of sifting through hundreds of thousands of records can be reduced by the use of integrated flexible and user-friendly databases. For over 15 years, we have developed and expanded GeneCards (www.genecards.org), a comprehensive, authoritative compendium of annotative information about human genes, accessed yearly by more than two million unique users. Its gene-centric content is automatically mined and integrated from over 100 electronic sources, including HGNC, NCBI, Ensembl, UniProt, UCSC, REACTOME, and BioGPS, resulting in web-based cards with sections encompassing a variety of topics (e.g., genomic location, gene function, transcription, disorder, literature, and pathways) for each of more than 122,000 human gene entries. [1] GeneCards also fea-

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Marilyn Safran is the head of development for the multi-disciplinary GeneCards/MalaCards team in the Lancet lab at the Weizmann Institute of Science in Israel. Prior to joining in 1997, she did software development and management at Ubique Inc., the CS department at Weizmann, Bell Laboratories, MIT’s Lincoln Laboratory, and the Memorial Sloan Kettering Cancer Center, in the fields of bioinformatics, web applications, compilers, databases, and medicine. Marilyn received a BA in Math with Honors from Queens College of CUNY in 1974, and an MS in Computer Science from Boston University in 1978.

Prof. Lancet, head of the Weizmann Institute’s Crown Genome Center, has a PhD from Weizmann and postdoctoral training from Harvard and Yale. He pioneered research on the biochemistry, genetics and evolution of olfaction, and investigates human rare disease genes. Lancet and his team developed GeneCards, a world-renowned web compendium of human genes, and more recently initiated a companion database MalaCards, a comprehensive web tool for human diseases. Lancet was awarded the Takasago and Wright Awards in the USA and the Landau Prize in Israel. He is a member of EMBO and (until 2012) of the HUGO Council.

Non-coding RNA (ncRNA) genes in the human genome have been increasingly studied in the past few years, and new ncRNA classes have been discovered.[4] However, until recently, no comprehensive non-redundant database for all ncRNA human genes was available. We have recently completed a major unification effort of ncRNA gene entries within GeneCards, integrated from 15 different data sources by mining four secondary sources[5] and several additional primary sources. An integration algorithm based on mapping to genomic coordinates employed, among others, another GeneCards suite member, GeneLoc[6] (Section 2.3). This was used to cluster overlapping entries at unified locations, thereby merging parallel versions of the same gene, as well as precursors with their mature transcripts.[ii] A total of ~64,000 new human ncRNA genes were added to GeneCards, resulting in a total of almost 80,000 entries belonging to 14 RNA classes, including 22,000 piwi-interacting RNAs (piRNAs) and 17,000 long non-coding RNAs (LncRNAs). GeneCards V3.09 now contains ~122,500 gene entries, with a greater than fivefold enhancement of the ncRNA count, compared to the pre-unification version 3.07 (Figure 2A). Among the annotations provided is a quality score, reflecting the degree of confidence that the entry is indeed a bona fide gene, based on functional annotations as well as expression. Current work in progress includes a probabilistic categorization, indicating source-related ambiguities in gene classification (Figure 2B).

Enrichment analysis of high-throughput data is a key method used to glean new biological insights from experimental results.[7] GeneDecks, a GeneCards suite member (www.genecards.org/GeneDecks), is an annotation-based enrichment analysis tool for human genes and gene sets. It constitutes a systems biology facilitator, leveraging GeneCards’ unique wealth of combinatorial annotations. In its Set Distiller mode, GeneDecks exposes commonalities within a list of genes, shedding functional light upon the constituent individual genes (Figure 3A). Descriptors that are enriched in the query gene set are sorted first by their statistical significance and then by their shared gene count. GeneDecks’ strength lies in the broad range of attributes available for use in its analyses, coming from eight categories, such as disorders, compounds, pathways, Gene Ontology (GO) terms and expression. It produces
superior results compared to parallel analysis systems, such as DAVID.\[8\] The new disease-centric database MalaCards (Section 4.1) strongly relies on GeneDecks for many of its annotations, by harnessing Set Distiller to find shared descriptors for the genes associated with a disease.

In its Partner Hunter mode, one can “GeneDecks” a given gene with respect to a selected combinatorial annotation in order to obtain a set of similar genes, i.e., potential functional paralogs. GeneDecks portrays functional partners in descending order of similarity (Figure 3B),

Figure 1. Data integration and information flow. One hundred varied external sources provide detailed information to the GeneCards integrated database of human genes, along with its suite members, GeneLoc exon-based genomic map and GeneDecks sets analysis. GeneCards has recently greatly enhanced its ncRNA gene coverage. GeneCards empowers specific databases as shown, covering tools for olfactory genes as well as disease-specific genes and biomarkers. Two novel medically focused databases, MalaCards and LifeMap Discovery, together with GeneCards, form a powerful trio of mutually enriching bioinformatics tools. Double-lined arrows depict data sharing, single-lined arrows depict links, and the number of links between each pair of sources is also indicated.
often discovering relations that might be overlooked based on sequence similarity alone.\textsuperscript{[8b]}\textsuperscript{[8c]}

2.3 GeneLoc

The GeneLoc algorithm (genecards.weizmann.ac.il/gene loc/) creates an integrated location map of genes and markers within the entire human genome.\textsuperscript{[6]} It eliminates redundancies and assigns each gene a meaningful chromosomal megabase location. This is used, in turn, to create a gene identifier, which serves as a GeneCards ID. GeneLoc currently uses gene sets from NCBI, Ensembl, and newly added ncRNA sources. It compares these collections, deciding which entries should be consolidated and which are to remain discrete. The resulting GeneLoc “gene territory” reflects the range of the unified genomic coordinates of a given gene, taking into account every exon. Additionally, DNA segments classified by categories (such as EST clusters) are presented, alongside the genes, on a single megabase-scale map, with further information and links to relevant databases. GeneLoc’s important advantage is in generating a simple tabulated list of genes and markers in a genomic interval of choice, or flanking a gene of interest, with ready links to GeneCards and other databases such as Ensembl, Genethon and NCBI Gene (Figure 4). In this way, it complements graphic maps such as provided by the UCSC.
3 Olfactory Databases

Olfaction, the sense of smell, is a molecularly complex sensory processing system, capable of producing accurate odor perception. Olfactory receptors (ORs), which detect and recognize a multitude of odorants, constitute the largest multigene family in the mammalian genome. They serve as an example of paralog and interindividual diversity, and reveal a very high count of pseudogenes, with the unusual phenomenon that intact and inactive genes may segregate in the population. Downstream from the ORs are auxiliary genes that mediate sensory transduction.

3.1 HORDE

HORDE, the human olfactory data explorer (genome .weizmann.ac.il/horde/), presents a complete compendium of human OR genes, pseudogenes and segregating pseudogenes, as well as ORs from four other vertebrate species (chimpanzee, dog, opossum and platypus). This database is generated by an automated OR-specialized computational pipeline, which mines OR gene and pseudogene sequences out of complete genomes. The pipeline has the capacity to annotate OR pseudogenes, which are usually not identified by standard whole-genome annotation pipelines, making HORDE a unique resource for all OR loci. Human location information produced by this pipeline is supplied to HGNC and to GeneCards. Importantly, HORDE’s automatic pipeline generates gene symbols based on sequence-similarity classification for each gene. For example, OR1A2 indicates family 1, subfamily A, member 2. This nomenclature system was subsequently applied to dog, opossum, and platypus. Its potential as a tool for the study of mammalian ecological adaptation was recently demonstrated.
In an attempt to provide insights into the evolution, structure and function of the complete human OR repertoire, HORDE integrates diverse bioinformatic analyses and additional resources into the database, and provides links to/from HGNC, GeneCards, ORDB and others. Thus, information is given on the genomic organization of the ORs into clusters, expression data (obtained from ESTs and microarray data),\textsuperscript{[17]} gene model,\textsuperscript{[18]} annotation of putative functional amino acid residues,\textsuperscript{[19]} identification of synthetic clusters,\textsuperscript{[14]} and more (Figure 5A). This information is pertinent to several open questions such as the control of gene expression in the olfactory system, in-

**Figure 5.** An example of a HORDE card. A) Partial view of the information provided for the olfactory receptor gene OR7C2. B) The haplotype section for OR7C2. Only segregating positions are shown, with the frequency of each haplotype in the population (%Freq) and its predicted functionality score (CORP\textsuperscript{[43]}).
cluding epithelial zone–specific expression as well as expression with locus and allele exclusion, ectopic expression in non-olfactory tissues, such as sperm, and biased expression of certain OR genes and pseudogenes (e.g., reference [23]).

Genetic variations in OR genes underlie odorant sensitivity differences, specific anosmia (diminished sensitivity) and specific hyperosmia (enhanced sensitivity). To address the universe of human interindividual variation, we recently integrated a comprehensive catalog of genomic variations in human ORs, collected from eleven sources, including The 1000 Genomes Project. This encompasses single-nucleotide polymorphisms (SNPs) and copy-number variation (CNV), as well as deleterious mutational events. Using this catalog, HORDE now presents all inferred protein variants (haplotypes) for all intact OR genes, potentially related to differences in odorant binding (Figure 5B). The catalog provides a realistic and up-to-date view of the personal OR repertoire, where about two-thirds of the loci segregate between intact and inactivated alleles, and every individual genome contains different OR combinations.

Future plans include the expansion of HORDE to additional OR repertoires of terrestrial vertebrates. The availability of such a collection with the unified standard nomenclature system of HORDE across species will allow researchers to make facile cross-species transitions in the complex OR gene family.

3.2 GOSdb

In parallel to the study of odorant sensitivity differences, we also study general olfactory sensitivity (GOS), scantily charted interindividual differences in overall (average) smell sensitivities. An extreme case of the GOS phenotype is isolated congenital general anosmia (CGA), a non-syndromic inborn complete incapacity to perceive odors. Our working hypothesis is that genetic variations in auxiliary olfactory genes, including those mediating transduction and sensory neuronal structure and development, constitute the genetic basis for GOS and CGA. To better study such chemosensory phenotypes, we performed a systematic exploration and created GOSdb, an online resource (genome.weizmann.ac.il/GOSdb) derived from eleven data sources, which integrates auxiliary olfactory genes and their variations. As a primary source, GeneCards was searched for words like “anosmia”. The resulting gene set was fed into GeneALaCart to extract annotations, including aliases, articles, and genomic locations. In parallel, the literature was surveyed, seeking relevant functional in vitro studies, mouse gene knockouts, and human disorders with olfactory phenotypes. Also tackled were published transcriptome and proteome data for genes expressed in olfactory tissues and genes identified in olfactory-related linkage peaks. Finally, we performed in-house next-generation transcriptome sequencing of human olfactory epithelium and mouse olfactory epithelium and bulb, aiming to identify olfactory sensory-enriched transcripts. The information is presented on a web card for each gene, with the main symbol (e.g., FGFR1) linked prominently to GeneCards. Employing a global scoring system based on the attributes of the eleven data sources assembled, we identified 1680 candidate auxiliary olfactory genes. To assess our differential expression data sources’ potential to detect olfactory functional genes, we examined which genes receive high scores. Of the 20 top accumulated scoring genes, we found eleven genes that were previously annotated by other data sources as core olfactory genes, and the rest are candidates for new scrutiny (Figure 6). For a shortlist of the 136 top-scoring genes, we identified genomic variants (probably damaging single-nucleotide polymorphisms, indels and copy-number deletions) gleaned from public repositories. Our GOSdb database of genes and their variants should assist in rationalizing the great interindividual variation in human overall olfactory sensitivity. The database and its variations assist in an ongoing whole-exome sequencing study of 66 Jewish families with CGA. In addition, GOSdb may aid in scrutinizing undeciphered genetic diseases accompanied by olfactory disorders, as exemplified by Kallmann syndrome for which, despite substantial progress, most of the genetic basis remains uncharted. FGFR1 is an example of a GOSdb gene, implicated in anosmia via in vivo and mouse knockout studies, linked to GeneCards, and associated in Malacards with Kallmann Syndrome. Insights into this disease were shared with the MalaCards project, and it was featured as the sample malady on the MalaCards V1.0.1 homepage. Ultimately, GOSdb may illuminate other sensory systems using olfaction as a model, and may contribute to a broader understanding of neurogenetics.

4 Disease Databases

One of the greatest challenges of biomedical research is deciphering the underlying mechanisms of human diseases, which requires accurate classification and annotation. Most human diseases arise due to complex interactions between multiple genetic variants and environmental risk factors. The study of diseases could thus shed light on basic biological mechanisms. In parallel, diagnosis and treatment are facilitated by the huge amount of information coming from genomics and proteomics research, allowing molecular-level support for medical decisions.

The integration of massive existing amounts of information under a single disease nomenclature is an enormous challenge. At present, disease compilation, occurring in more than 60 existing data sources, is incomplete, heterogeneous and often lacking systematic inquiry mechanisms. Each data source focuses on different aspects of
disease annotation, and/or contains a partial specialized list.

Promising attempts to settle the varied disease nomenclature are presented via knowledge representation through standardized vocabularies, to ensure both effective information sharing and interoperability among information systems. There are several vocabularies, ranging from class-specific ones such as the Infectious Disease Ontology (IDO, infectiousdiseaseontology.org/page/Main_Page) to more broadly disposed ones such as the International Classification of Diseases (ICD), the Unified Medical Language System (UMLS), the Systematized Nomenclature of Medicine – Clinical Terms (SNOMED-CT) (16770974), the Medical Subject Headings (MeSH) (www.nlm.nih.gov/mesh/) and the Disease Ontology (DO). Such data structures range from flat lists, such as Online Mendelian Inheritance in Man (OMIM), to hierarchies, as exemplified by the Disease Ontology. However, significant inconsistencies prevail in basic terms pertaining to diseases. Existing vocabularies are only partially cross-connected to each other, and do not define disease concepts uniformly. Moreover, most existing disease databases only partially associate with any ontology, which greatly limits the effectiveness of formalization and definition unification.

4.1 MalaCards

It is clear to us that the realm of disease databasing requires the same “one-stop shop solution” as provided by GeneCards for the gene universe. We have recently introduced MalaCards (www.malacards.org), an integrated database of human maladies and their annotations, modeled on the GeneCards strategy, architecture and information affluence. MalaCards mines and merges 44 data sources to generate a computerized web card for each of 16,919 human disease entries, with disease-specific prioritized annotations, as well as interdisease connections. It leverages the GeneCards relational database, searches, and GeneDecks set analyses (Figure 7A).

The MalaCards disease list is built from 15 ranked sources, using disease name unification heuristics. Four schemes populate MalaCards sections: (i) direct interrogation of disease resources, to establish integrated disease names and synonyms, as well as additional annotations such as summaries, drugs/therapeutics, clinical features, genetic tests, and anatomical context; (ii) searches of GeneCards for related publications and for associated genes, with corresponding relevance scores; (iii) analyses of disease-associated gene sets using GeneDecks to calculate statistically significant descriptors enriched in this set (e.g., in the “Anaplastic Ependymoma” MalaCard, “tumorigenesis” is entered into the phenotypes section, while “tumor metastasis” is entered into the path-
ways section. This process also assigns a relevance score for every hit, and is employed to populate the related diseases, phenotypes, pathways, compounds and GO terms sections, also providing relevant GeneCards deep links.; (iv) searches in MalaCards itself, e.g., for additional related diseases. The latter form the basis for the construction of a disease network, based on shared MalaCards annotations (Figure 7B). Such networks embody associations based on etiology, clinical features and clinical conditions. This broadly disposed network has a power-law degree.
distribution, as previously indicated for smaller disease networks, implying that some inherent properties are represented within such networks. Our current work in MalaCards includes a more profound classification of diseases, and disease set analyses, hoping to make MalaCards an improved tool for biological as well as medical studies.

4.2 GeneKid

A powerful way to integrate heterogeneous omics data is by using genes as their common denominator, using the genes’ annotation network. The expected increase in patient chronic kidney disease (CKD) afflictions resulting in end-stage renal failure, has inspired SysKid (www.syskid.eu), an EU consortium involving 25 research groups from 16 countries. The aim of the consortium is to associate genes with CKD, so as to assist in the development of new diagnosis and treatment methods. The crucial step in establishing a unified omics network is the connection of each datum, such as RNA expression and metabolites, to a unique approved human gene symbol, a step termed “symbolization”. The robustness of GeneCards’ gene annotation is the basis for overcoming the diversity of gene identifiers supplied by consortium experimentalists. This challenge is significant for microarray probe set and SNP identifiers, and is even more accentuated by the need to associate metabolites to genes; to this end, compound-gene associations were fortified using both The Human Metabolome Database (HMDB) and DrugBank cheminformatics sources that link drugs and compounds to their gene target.

GeneKid is a resource for data storage and management, such as those generated by SysKid. Its database consists of 18 tables storing omics data as the main entity, along with study and sample information. Insertion of data into GeneKid occurs after symbolization. Quantitative measurements detected for each experimental feature are stored in GeneKid, and a combinatorial/compound score is produced for each of them, thus prioritizing their importance in overall SysKid results (Figure 8). The GeneKid user interface enables basic lookup services, allowing collaborating groups to access intermediate results. When testing potential biomarkers, it is highly beneficial to use GeneCards’ posted research reagents. As an example, siRNAs for seven specific genes of interest were extracted from GeneCards; these are already being tested on candidate CKD genes. This knowledge could be the basis for the development of proprietary diagnostic tools.

4.3 Xome

Current advances in next-generation sequencing, such as whole-genome and whole-exome sequencing, have dramatically increased the identification probability of causal variants for genetic disorders. Indeed, during the last two years, more than 100 genes have been identified as participating in rare Mendelian disorders, including our own discovery of deleterious mutations in five diseases, such as the TECPR2 mutation that causes an autosomal recessive form of hereditary spastic paraplegia. However, the tremendous and increasing volume of sequencing data generated by these technologies provides a great bioinformatics challenge in terms of data processing, storage, management and interpretation.

While typical whole-exome sequencing results in ~25,000 variants per individual genome, identification of the causal variant involves the application of various filtering strategies. Commonly adopted procedures include filtering of common variants, type of allele variants, predictions of pathogenicity, and selection of an inheritance mode. A key component in these analyses is a comparison to an appropriate set of control variants, incorporating exome-sequencing data of individuals from the same population and arising from the same sequencing platform. This reduces the number of false positive variants and filters out population-specific variants. In this realm, we developed Xome, a database for whole-exome sequencing data management. The database is currently populated with data from 105 Israeli individuals. These give rise to 474,781 variants. The access to Xome is currently password protected, because the database contains detailed patient information, which is difficult to disguise for this small population.
Figure 9. An example card for an in vivo cell in the LifeMap Discovery website. A) Quick view of the cell anatomical location (kidney → metanephric mesenchyme (compartment) → cell) and summary, notes and development time. B) List of available data on this cell, including gene expression, high-throughput data, signals, related diseases and more. C) An interactive graphical view of the cell’s development tree. Cells lower down are descendants, and cells can have one or more ancestors. D) Insert of the Gene Expression section, with lists of genes curated from literature, in situ and microarrays.
5 LifeMap Discovery

Understanding how stem and progenitor cells differentiate into mature functional cells during embryonic development is of fundamental interest, and clearly one of the most mystifying areas of multicellular life. Being able to generate functional cells, and grow tissues from these stem and progenitor cells in a dish, is perhaps the greatest challenge for medicine in the coming decades. The LifeMap Discovery database has been created to bridge these separate areas of research, and bring knowledge from the in vivo into the in vitro realm, and from there to clinical utility, facilitating scientific knowledge and future applications of regenerative medicine. The underlying postulate of LifeMap Discovery is that understanding the genes expressed in every developing cell, and the signaling that drives its differentiation, will provide invaluable information for (i) identification and classification of differentiated stem and progenitor cells, and (ii) suggesting mechanisms to derive protocols for differentiating these cells into the desired, more mature cell types.

The LifeMap Discovery database (discovery.lifemap.sc.com) helps users trace the cellular differentiation that occurs during mammalian embryonic development. The annotated cell development tree stems from the zygote, branches to progenitor cells, and terminates at mature cells (Figure 9). The LifeMap Discovery database is based on developmental paths to specific fates, such as blood, endothelium, motor neurons, bone or cartilage. Developmental data are available at cellular and anatomical resolutions. In concert with cellular differentiation, complex organs and tissues are formed; hence each developing cell is also a member of specific anatomical compartments, and associated with tissues composing the developing organs.

LifeMap Discovery is based on systematic gathering and de novo assimilation of various scientific data describing mostly mouse and human development. Due to the inherent difficulties in studying human development, mouse information is far more abundant, and therefore is included in the database. The mouse data serve both as a foundation for further studies and as a model for human development. The resulting annotated cell development ontology tree is thus based on mouse development, but with cross-related human development information wherever available. The information is collected and described for each cell in specific lineages. Examples are the endothelium, blood, muscle, bone, cartilage, heart myocardium, kidney, neuronal cells and more. The anatomy content includes the organ development path supplemented with relevant images, in situ hybridizations, and high-throughput experimental gene-expression data. Cellular information includes the cell development path, qualitative gene expression, signaling that affects the differentiation process, high-throughput gene-expression data, related diseases, and relevant references.

In addition to the in vivo developmental part, the database includes in vitro differentiation protocols, as well as characteristics and cell therapy applications of the various types of human in vitro cultured cells. These include embryonic stem and progenitor cells, adult stem cells, induced pluripotent stem cells, and primary cells. The differentiation protocols are mapped, where possible, to the closest matching in vivo development cells, anatomical compartments and tissues.

The database is divided into the following main parts: (i) in vivo development – the first complete assembly and reconstruction of cell lineages developed in the mammalian body; (ii) stem cell differentiation – cultured cells, their differentiation protocols and cell therapy–related applications; and (iii) regenerative medicine – development of stem and progenitor cells into therapeutic products. These different parts are connected and interlaced by computational and hand-curated methods. Most noteworthy, the in vivo entities are linked to their closest in vitro entities whenever data is available. Matching is mostly based on gene-expression analysis and also on other cell characteristics such as functional and morphological similarity.

The value provided by LifeMap Discovery and its projected effect on stem cell research and therapeutics originate from the combined power of these data, which enables or facilitates identifying, predicting and indicating possible differentiation paths and future regenerative medicine applications. LifeMap Discovery integrates with GeneCards, where the rich information available on each gene is directly linked, and MalaCards, to which it provides the relationships of cells, tissues and organs to the disease potentially targeted by regenerative medicine and cell therapy applications.

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