

# A Systematic Analysis of Human Disease-Associated Gene Sequences In *Drosophila melanogaster*

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We performed a systematic BLAST analysis of 929 human disease gene entries associated with at least one mutant allele in the Online Mendelian Inheritance in Man (OMIM) database against the recently completed genome sequence of *Drosophila melanogaster*. The results of this search have been formatted as an updateable and searchable on-line database called Homophila. Our analysis identified 714 distinct human disease genes (77% of disease genes searched) matching 548 unique *Drosophila* sequences, which we have summarized by disease category. This breakdown into disease classes creates a picture of disease genes that are amenable to study using *Drosophila* as the model organism. Of the 548 *Drosophila* genes related to human disease genes, 153 are associated with known mutant alleles and 56 more are tagged by P-element insertions in or near the gene. Examples of how to use the database to identify *Drosophila* genes related to human disease genes are presented. We anticipate that cross-genomic analysis of human disease genes using the power of *Drosophila* second-site modifier screens will promote interaction between human and *Drosophila* research groups, accelerating the understanding of the pathogenesis of human genetic disease. The Homophila database is available at <http://homophila.sdsc.edu>.

Studies in the fruit fly *Drosophila melanogaster* have altered our estimate of the evolutionary relationship between vertebrate and invertebrate organisms. Key molecular pathways required for the development of a complex animal, such as patterning of the primary body axes, organogenesis, wiring of a complex nervous system, and control of cell proliferation have been highly conserved since the evolutionary divergence of flies and humans. When these pathways are disrupted in either vertebrates or invertebrates, similar defects are often observed. The utility of *Drosophila* as a model organism for the study of human genetic disease is now well documented. Developmental defects such as the mesenchymal malformations associated with Saethre-Chotzen syndrome (Howard et al. 1997), formation of intracellular inclusions in polyglutamine-tract repeat disorders such as spinocerebellar ataxia and Huntington disease (Fortini and Bonini 2000), and loss of cellular-growth control and malignancy resulting from mutations of tumor suppressor genes (Potter et al. 2000) have been analyzed effectively using *Drosophila* as the model genetic system. The many basic processes that are shared between *Drosophila* and humans, in conjunction with the recent completion of the *Dro-*

*sophila* genomic sequence, provide the necessary ingredients for launching systematic analyses of human disease-causing genes in *Drosophila*. An important question that arises from the combination of this genomic information with the detailed mechanistic understanding of many *Drosophila* genes is, which human disease genes are most appropriate for study in *Drosophila*?

A survey of 289 *Drosophila* genes related to human disease genes has been presented in the context of the *Drosophila* genome sequence release (Rubin et al. 2000) and subsequently by Fortini et al. (2000). Additionally, more focused studies of *Drosophila* ion-channel genes (Littleton and Ganetzky 2000) and cancer-gene related sequences (Potter et al. 2000) have been published. Here, we report on results generated by a cross-genomic analysis of the 929 Locuslink entries of human disease genes known to have at least one mutant allele listed in the current version of the Online Mendelian Inheritance in Man (OMIM) (McKusick 2000) against the complete *Drosophila* genome sequence. We compiled this cross-genomic data into a database called Homophila, which presently contains a set of 714 clear-candidate human disease genes, their *Drosophila* counterparts (548 distinct genes), and any P-elements within 1 kb of these genes. This set of genes was categorized by human disease type and existing mutant alleles of these genes were identified. Analysis

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of this dataset and support material is currently available via the World Wide Web in the form of a searchable cross-genomic database (Homophila, <http://homophila.sdsc.edu>), which has been designed to be automatically updated as the number of disease-associated genes expands.

## RESULTS

### Development of Homophila as a Tool for Cross-Genomic Analysis

As a starting point for our analysis, we wished to determine which human disease genes have clearly related counterparts in *Drosophila*. To this end, we extracted the set of all known human-disease-associated genes from OMIM with Locuslink entries and compared this set of genes to the recently completed *Drosophila* genomic sequence (see Methods). By incorporating information about both the human disease gene and its *Drosophila* counterpart, it is possible to query the search results by key word, disease name, fly gene, and OMIM number. The outline of a typical query to Homophila is illustrated in Figure 1.

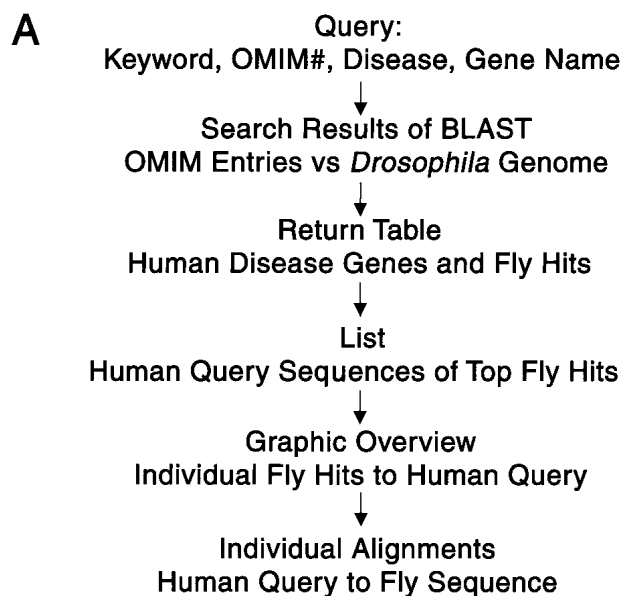
### Identification and Analysis of *Drosophila* Genes Related to Candidate Human Disease Genes

Using Homophila, we found that 714 of the 929 (77%) OMIM human disease gene entries have highly similar ( $E \leq 10^{-10}$ ) cognates in *Drosophila* (Fig. 2), which we refer to as “related genes” hereafter. An  $E$  value of  $\leq 10^{-10}$  indicates that the odds are  $< 1$  in  $10^{10}$  that such a match would happen by chance alone given the sizes of the two compared databases (e.g., OMIM Locuslink entries and Flybase). We are aware that these *Drosophila* cognates may not be functional orthologs to the human disease genes and are using the less-stringent term “related genes” to describe these similar sequences. It is notable, however, that even at a higher  $E$ -value cut-off, a significant fraction of human disease genes have matches in *Drosophila* (Fig. 2, e.g.,  $> 54\%$  with  $E \leq 10^{-40}$  and  $29\%$  with  $E \leq 10^{-100}$ ). A list of disease phenotypes resulting from mutations in genes that are highly related to *Drosophila* genes ( $E < 10^{-10}$ ) is available as a separate table on the Homophila Web site (Reiter et al. 2000) as the clear-hit list, and has been categorized into various subclasses based on clinical phenotype (Table 1). Because some of the 714 distinct human disease genes match the same *Drosophila*-related sequences, the total number of different *Drosophila* counterparts of human clear-hit genes is 548 distinct *Drosophila* genes. We found a large number of human disease genes involved in nonmyelin-associated neurological disorders (74), cancer (79), skeletal disorders (26), and other developmental defects (35), as noted in previous studies. We also found

a large number of metabolic and storage disorders (160), which were not highly sampled categories of genes in prior surveys. Consistent with the prevalence of disorders affecting metabolism and other general cellular functions, 409 of the clear-hit human genes (e.g., 57%) also have cognates in yeast (e.g.,  $E \leq 10^{-10}$ ). An interesting feature of this inclusive data set, which also was not evident from the earlier analyses of more selective sets of diseases, is the high-number of human genes affecting the visual (43), cardiovascular (26), auditory (13), skeletal (26), and endocrine (50) systems with *Drosophila* counterparts.

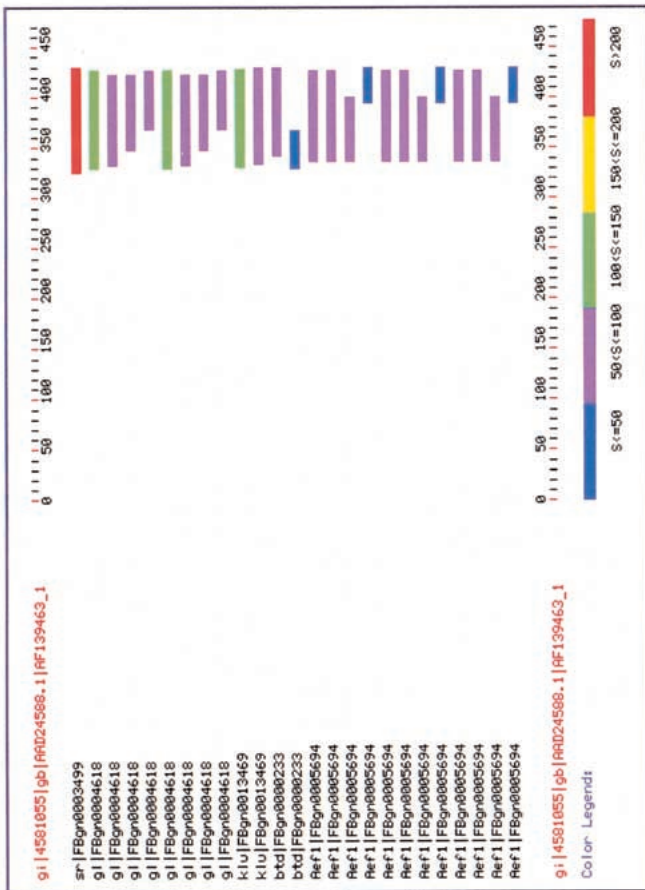
To determine what fraction of *Drosophila* clear-hit genes already have been analyzed by loss-of-function genetics, we examined each entry in the list of 548 cognates of human disease genes in the clear-hit list and searched for alleles of each of these genes systematically using allele and gene tables available from Flybase (Flybase 1999) (see Methods). In this manner, 153 mutant alleles were identified (e.g., 28% of *Drosophila* clear-hit genes). These alleles and the human disease-related sequences can be found on our Web site in tabular form (Reiter et al. 2000).

A notable result of this allele analysis is that the great majority of *Drosophila* genes related to human disease genes (e.g., 395 of 548) have not yet been analyzed by loss-of-function genetics, which is consistent with the finding that only 14% of the genes identified by the *Drosophila* genome project had been identified previously by individual researchers working on specific hypothesis-driven projects (Rubin et al. 2000). We then determined what fraction of the 395 predicted *Drosophila* transcription units without known mutant



**Figure 1** (continues on following page)





```
>sr|FBgn0003499|CT23724|FBan0007847 "transcription factor"
mol_weight=128311 located on: 3R 90E1-90E2;
Length = 1186
```

```
Score = 206 bits (518), Expect = 2e-53
Identities = 89/103 (86%), Positives = 96/103 (92%)
```

```
Query: 325 KVPNRPSTFPVHERYPVCPAEGCDRFRSRDELTRHRIHTGKPFQCRICMRFSRSDH 384
KVPNRPSTFPVHERYPYCP ECDRFRSRDELTRHRIHTG KPFQCRICMR+FSRSDH
Sbjct: 1021 KVPNRPSTFPVHERYACPVENCDFRFRSRDELTRHRIHTGKPFQCRICMRFSRSDH 1080
```

```
Query: 385 LTTHTHTGKPFACDYCGRKFARSDERKRHTYKHLRQKERK 427
LTTHTHTGKPF+CD CGRKFARSD+KRH K+HL+Q+ +K
Sbjct: 1081 LTTHTHTGKPFSCDICGRKFARSDERKRHAKVHLRQRIKK 1123
```

**Figure 1** How to query the Homophila database. (A) Schematic of a Homophila query. The user enters the text query in the form of human disease name, Online Mendelian Inheritance in Man (OMIM) number, fly gene name, or keyword search through the human disease entry box. The database then opens a window with information on the disease name, and human and fly genes that match the key word query. The user then can examine the details of an individual human to *Drosophila* BLAST comparison to get more information on the specific BLAST score, alignment, and other hits to this gene. In addition, *P*-element information is found at this level. (B) Example Homophila query using the keyword "neuropathy". The user enters the key word and the database will return any human entry or *Drosophila* gene description that contains the key word. In this case, there are several human neuropathies listed, including a gene for peripheral neuropathy, which is a transcription factor (*Krox20*). By clicking the "details" button in this first window, one can examine the particular BLAST comparisons of the human genes as well as the *P*-element information. By scrolling down in this window, one can look at particular alignments between the query sequence and its *Drosophila* matches. In this case, the human *Krox20* matches *Drosophila stripe* gene most strongly in the DNA-binding domains, but also retains some overall sequence similarity in other domains as can clearly be seen on the color graphical alignment of similar sequences.

**Table 1.** Classification of 714 Clear-Hit *Drosophila* Genes According to Human Disease Phenotypes

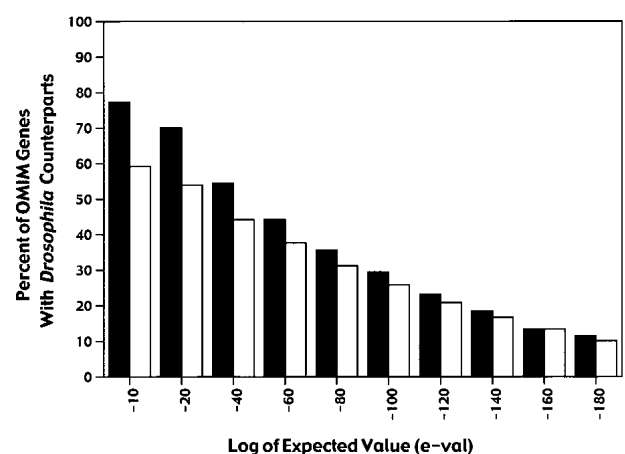
Disorder	No. of genes
<b>Neurological</b>	<b>74</b>
Neuromuscular	20
Neuropsychiatric	9
CNS/Developmental	8
CNS/Ataxia	9
Mental retardation	6
Other	22
<b>Endocrine</b>	<b>50</b>
Diabetes	10
Other	40
<b>Deafness</b>	<b>13</b>
Syndromic	7
Nonsyndromic	6
<b>Cardiovascular</b>	<b>26</b>
Cardiomyopathy	10
Conduction defects	4
Hypertension	7
Atherosclerosis	3
Vascular malformations	2
<b>Ophthalmologic</b>	<b>43</b>
<b>Anterior segment (13)</b>	
Aniridia	1
Rieger syndrome	1
Mesenchymal dysgenesis	2
Iridogoniodysgenesis	2
Corneal dystrophy	2
Cataract	3
Glaucoma	2
<b>Retina (30)</b>	
Retinal dystrophy	1
Choroideremia	1
Color vision defects	4
Cone dystrophy	2
Cone rod dystrophy	1
Night blindness	8
Leber congenital amaurosis	2
Macular dystrophy	4
Retinitis pigmentosa	7
<b>Pulmonary</b>	<b>4</b>
<b>Gastrointestinal</b>	<b>13</b>
<b>Renal</b>	<b>13</b>
<b>Immunological</b>	<b>33</b>
Complement mediated	11
Other	22
<b>Hematologic</b>	<b>42</b>
Erythrocyte, general	29
Porphyrias	7
Platelets	6
<b>Coagulation abnormalities</b>	<b>28</b>
<b>Malignancies</b>	<b>79</b>
Brain	3
Breast	4
Colon	11
Other gastrointestinal	3
Genitourinary	5
Gynecologic	3
Endocrine	3
Dermatologic	3
Xeroderma pigmentosa	6
Other/sarcomas	9
Hematologic malignancies	29
<b>Skeletal development</b>	<b>26</b>
Craniosynostosis	5
Skeletal dysplasia	13
Other	8

**Table 1.** (Continued)

Disorder	No. of genes
<b>Soft tissue</b>	<b>2</b>
<b>Connective tissue</b>	<b>18</b>
<b>Dermatologic</b>	<b>25</b>
<b>Metabolic/mitochondrial</b>	<b>123</b>
<b>Pharmacologic</b>	<b>12</b>
<b>Peroxisomal</b>	<b>9</b>
<b>Storage</b>	<b>37</b>
Glycogen storage	11
Lipid storage	13
Mucopolysaccharidosis	10
Other	3
<b>Pleiotropic developmental</b>	<b>35</b>
Growth, immune, cancer	7
Apoptosis	1
Other	27
<b>Complex other</b>	<b>9</b>
<b>Total</b>	<b>714</b>

Totals for categories of disease are in bold, subcategory totals are in parenthesis, and individual categories are in plain text.

alleles have *P*-elements inserted in or near them (e.g., within 1 kb of the gene-coding region). By aligning the map positions for 3442 known *P*-element insertions listed by the Berkeley genome project with the map positions of the *Drosophila* genes related to human clear-hit genes, we found 190 distinct *P*-element insertions that lie within or near disease-gene-related sequences. When corrected for multiple insertions, these 190 *P*-elements reduce to 102 distinct clear-hit *Drosophila* genes. Further analysis determined that 56 of



**Figure 2** Number of *Drosophila* sequences related to human disease genes as a function of *E*-value. A graph of the percent of human disease genes with similar sequences in *Drosophila* as a function of *E*-value. Black-filled bars indicate the percent of human Locuslink entries (929 total) with matches to *Drosophila* sequences. White-filled bars indicate the percent of unique *Drosophila* sequences that match one or more human disease gene sequences. Note that even at *E*-values of  $\leq 10^{-40}$ , 54% of human disease genes have matches to *Drosophila* sequences.

these *P*-element insertions are the only known alleles of these genes. Using routine genetic methods in *Drosophila*, it should be possible to create null alleles of the 56 *P*-element tagged genes with relatively little difficulty by remobilizing the *P*-elements and screening for imprecise excisions that delete all or parts of the coding regions. Thus, loss-of-function analysis should be straightforward for 209 (153 + 56) of the 548 clear-hit genes, which represents a substantial proportion of these genes (e.g., 38%).

#### Defects at Multiple Tiers of Conserved Signal-Transduction Pathways Cause Human Disease

To explore the cross-genomic nature of the clear-hit gene dataset further, we subcategorized genes into one of several signal transduction pathways known to play important developmental functions in *Drosophila* and looked for trends in the resulting human phenotypes. Signal transduction pathways typically are activated by one or several ligands binding to one or more transmembrane receptors. Ligand binding activates the receptor and leads to modification of cytoplasmic transducers that enters the nucleus-altering gene expression. A feature common to many signaling pathways is that multiple ligands activate specific receptors, which converge upon one or a few common cytoplasmic transducer(s).

Among the disease genes on the clear-hit list, 56 (corresponding to 38 distinct *Drosophila* genes) encode components acting in well-characterized signaling pathways such as the bone morphogenic protein (BMP), receptor tyrosine kinase/reticular activating system (RTK/RAS), G-coupled receptor, JAK/STAT, Toll, Integrin, and axonal-guidance pathways (Table 2). Signaling components in these pathways have been ordered in Table 2 with respect to their position in known signaling cascades in *Drosophila* (e.g., ligand->receptor->cytoplasmic transducer->transcription-factor effector). A notable trend apparent in these tabulated data is that mutations affecting particular ligands or cell-type-specific receptors generally result in restricted developmental abnormalities in humans, whereas mutations in universally employed receptor subunits or downstream intracellular signal transducers tend to cause more global loss of cellular growth control or cancer in humans. For example, in the case of the BMP signaling pathway (Fig. 3), defects in specific BMP ligands result in human bone malformation (e.g., brachydactyly) and mutations of a selective type I BMP receptor subunit cause venous malformations (e.g., hereditary hemorrhagic telangiectasia). On the other hand, loss of the universal type II BMP receptor subunit, or the core signal transducer (e.g., vertebrate SMAD4 = *Drosophila* Medea) results in cancer in humans. This trend in which mutations in generally used

signaling components often lead to loss of cellular growth control and cancer is consistent with many signaling pathways being directly or indirectly involved regulating cell proliferation.

#### DISCUSSION

Our goal in conducting the analysis described in this study was to define a subset of human disease genes that would benefit most from molecular-genetic analysis in *Drosophila*. To this end we used Homophila, a searchable interactive database, to define a set of candidate human disease genes that have clearly related genes in *Drosophila*.

A strength of our current analysis with respect to previous studies is that it is inclusive and encompasses a much larger nonredundant set of human disease genes listed in OMIM with Locuslink entries. In contrast, previous studies have been more restrictive surveys focusing only on a subset of 289 genes selected a priori, which are known to be causally linked to a human disease (Fortini et al. 2000; Rubin et al. 2000) or those involved with a particular category of disease state (Littleton and Ganetzky 2000; Potter et al. 2000). This is a critical distinction because the current analysis reveals the relative proportions of different disease subclasses available for study in *Drosophila*. For example, in the previous two survey studies, genes affecting hearing and visual systems were relatively rare because of the more restrictive selection criteria used. Additionally, we identified 123 metabolic genes (17% of those analyzed) whereas the previous studies only included 17 metabolic genes in the dataset (6% of those analyzed).

Another problem with any type of cross-genomic analysis is that one must determine which sequence matches are significant enough to be considered similar in evolutionary origin. In addition, one must be able to distinguish domain-specific matches (e.g., a cross-species match of leucine zipper domains) versus matches that span the entire amino-acid sequence. For these reasons, we have provided a graph of the percent of human disease genes with *Drosophila* counterparts at a variety of *E*-values (Fig. 2). We also implemented a graphical interface for each match; this will provide the user with information about annotated domains of both the human and fly proteins (see Fig. 1).

#### Approximately Three-Quarters of the Candidate Human Disease Genes are Clearly Related to Genes in *Drosophila*

Analysis of the set of potential human disease genes related to *Drosophila* genes as defined in this study is informative in several respects. First, we find a high prevalence of neurological and neurodegenerative con-

**Table 2.** *Drosophila* Genes From the Clear-Hit List That are in Known Signaling Pathways and the Human Phenotypes Associated with These Disease Genes

Signaling pathway	Disease	OMIM#	Fly gene	Signaling component
<i>BMP</i>	Fibrodysplasia ossificans progressiva	112262	( <i>dpp</i> )	Ligand
	Brachydactyly, type C	113100	( <i>dpp</i> )	Ligand
	Acromesomelic dysplasia, Hunter-Thompson type	601146	( <i>dpp</i> )	Ligand
	Hereditary hemorrhagic telangiectasia-2	601284	( <i>sax</i> )	Specific type I receptor
	Persistent Mullerian duct syndrome, type II	600956	( <i>wit</i> )	Specific type II receptor
	Colorectal cancer, familial nonpolyposis, type 6	190182	( <i>put</i> )	General type II receptor
	Polyposis, juvenile intestinal	174900	( <i>med</i> )	Cytoplasmic transducer
<i>Hedgehog</i>	Pancreatic cancer	600993	( <i>med</i> )	Cytoplasmic transducer
	Holoprosencephaly-3	600725	( <i>hh</i> )	Ligand
	Basal cell nevus syndrome	109400	( <i>ptc</i> )	Co-receptor
<i>Wnt</i>	Basal cell carcinoma, sporadic	601309	( <i>ptc</i> )	Co-receptor
	Greig cephalopolysyndactyly syndrome	165240	( <i>ci</i> )	Transcription factor
	Joubert syndrome	213300	( <i>wg</i> )	Ligand
<i>Notch</i>	Simpson dysmorphia syndrome	300037	( <i>dally</i> )	Proteoglycan (co-receptor?)
	Colorectal cancer	116806	( <i>arm</i> )	Cytoplasmic transducer
	Alagille syndrome	601920	( <i>Ser</i> )	Ligand
<i>RTK</i>	Cerebral arteriopathy with subcortical infarcts and leukoencephalopathy	600276	( <i>N</i> )	Receptor
	Obesity with impaired prohormone processing	162150	( <i>Fur1</i> )	Protease: Ligand activation?
	Achondroplasia; Craniosynostosis; Crouzon syndrome	134934	( <i>htl</i> )	Receptor
	Pfeiffer syndrome	136350	( <i>htl</i> )	Receptor
	Venous malformations, multiple cutaneous and mucosal	600221	( <i>htl</i> )	Receptor
	Apert syndrome; Beare-Stevenson cutis gurate	176943	( <i>htl</i> )	Receptor
	Mast cell leukemia; Mastocytosis; Piebaldism	164920	( <i>htl</i> )	Receptor
	Diabetes mellitus, insulin-resistant; Leprechaunism; Rabson-Mendenhall syndrome	147670	( <i>InR</i> )	Receptor
	Renal cell carcinoma	164860	Receptor kinase-like gene	Receptor?
	Predisposition to myeloid malignancy	164770	Putative growth factor receptor	Receptor?
	Bladder cancer	190020	( <i>Ras85D</i> )	Cytoplasmic transducer
	Colorectal adenoma	190070	( <i>Ras85D</i> )	Cytoplasmic transducer
	Colorectal cancer	164790	( <i>Ras85D</i> )	Cytoplasmic transducer
	Colon cancer	600679	<i>Tyrosine phosphatase 99A</i>	Phosphatase
Ehlers-Danlos syndrome, type X	135600	<i>Tyrosine phosphatase 10D</i>	Phosphatase	
<i>Serpentine</i>	Elliptocytosis-1	130500	( <i>cora</i> )	Cytoskeletal scaffolding?
	Hypertension, salt-resistant	108962	<i>guanylate cyclase receptor</i>	Receptor
	Night blindness, rhodopsin-related; Retinitis pigmentosa	180380	( <i>ninaE</i> )	Receptor (Rhodopsin 1)
	Colorblindness, deutan	303800	( <i>ninaE</i> )	Receptor
	Retinitis pigmentosa 4, included; rp4	180380	( <i>ninaE</i> )	Receptor
	Night blindness, congenital stationary, rhodopsin-related	190900	( <i>ninaE</i> )	Receptor
	Autonomic nervous system dysfunction	126452	Dopamine receptor-like gene	Receptor
	Susceptibility to Schizophrenia?	126451	( <i>DopR2</i> )	Receptor
	Night blindness, congenital stationary, type 3	180072	cGMP phosphodiesterase	Phosphodiesterase
	Retinitis pigmentosa, autosomal recessive	180071	cGMP phosphodiesterase	Phosphodiesterase
	Susceptibility to essential hypertension	139130	( <i>Gbeta13F</i> )	Cytoplasmic transducer
	Bleeding diathesis due to GNAQ deficiency	600998	( <i>Alpha49B</i> )	Cytoplasmic transducer
	<i>JAK/STAT</i>	SCID, autosomal recessive, T-negative/B-positive type	600173	( <i>hop</i> )

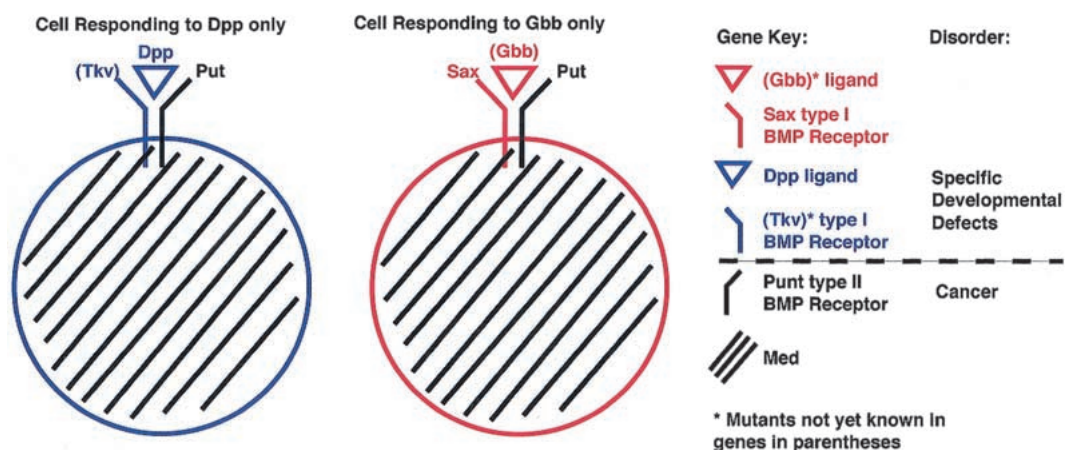
**Table 2.** (Continued)

Signaling pathway	Disease	OMIM#	Fly gene	Signaling component
<i>Toll/NFκB</i>	Leukemia/lymphoma, B-cell	109560	( <i>cact</i> )	Cytoplasmic transducer NFκI-like
<i>Neuronal pathfinding</i>	Propedrin deficiency	312060	Semaphorin family	Repulsive ligand
	Polycystic kidney disease, type I	601313	Slit-like gene	Repulsive ligand
	Antithrombin III deficiency	107300	( <i>sema-5c</i> )	Ligand?
	Transcortin deficiency	122500	( <i>sema-5c</i> )	Ligand?
	Plasmin inhibitor deficiency	262850	( <i>sema-5c</i> )	Ligand?
	Hydrocephalus due to aqueductal stenosis, MASA syndrome, spastic paraplegia	308840	( <i>Nrg</i> )	Adhesion molecule (Neuroglian)
<i>Integrin</i>	Colorectal cancer	120470	( <i>fra</i> )	Receptor
	Glazmann thrombasthenia, type A	273800	( <i>if</i> )	Integrin α-chain
	Epidermolysis bullosa, junctional, with pyloric stenosis	147556	( <i>mew</i> )	Integrin α-chain
	Myopathy, congenital	600536	( <i>mew</i> )	Integrin α-chain
	Glycoprotein Ia deficiency	192974	( <i>mew</i> )	Integrin α-chain

ditions (Table 1). This finding is not entirely unanticipated, because many of the components of neurogenesis (such as factors involving neural induction, guidance cues leading axons to their appropriate targets, the machinery for generating and propagating action potentials, and enzymes and molecular complexes involved in the synthesis and release of neurotransmitters) have been highly conserved during the course of evolution (Salzberg and Bellen 1996; Wu and Bellen 1997). Within the category of neurological diseases, the relatively large number of hearing conditions is noteworthy because these genes represent biologically

analogous systems (e.g., the hairs in the inner ear versus the sensory bristles of *Drosophila*). Without the complete comparisons of the genomes in a database like Homophila, it would not be immediately obvious that genes responsible for human deafness could be functionally analyzed in an organism like *Drosophila*, which has no external auditory specializations analogous to ears. Second, we find that components of signal transduction pathways are frequent targets of human disease. An interesting relationship regarding this category of disease genes is that mutations in different components of various signaling pathways can result

### Relation of Position in the BMP Signaling Pathway to Disease Phenotype



**Figure 3** Relationship of a component's position in the bone morphogenetic protein (BMP) pathway to human disease phenotypes. In general, there is a relationship between the position of a component in signaling pathway to the disease phenotype resulting from inactivation of that component. An example of this trend is the BMP pathway. Mutations in components acting at the start of the BMP signal transduction cascade such as a particular BMP ligand (e.g., *Drosophila* Dpp = Human BMP4/BMP2) or a specialized BMP type I receptor (*Drosophila* Saxophone = type I receptor for the Screw and Glass Bottom Boat ligands) result in specific developmental defects (e.g., brachydactyly). Mutations acting on subsequent steps in the BMP pathway, which mediate the effects of several converging upstream inputs such as the universal type II BMP receptor (e.g., *Drosophila* Punt = type II receptor mediating all BMP signaling) or the cytoplasmic/nuclear SMAD transducer (e.g., *Drosophila* Medea = Human SMAD4) result in generalized misregulation of cellular growth control and cancer (e.g., colorectal or pancreatic cancer).



in very different disease phenotypes in humans. Components acting at early stages in a given pathway, such as genes encoding extracellular ligands, tend to have more specific and limited phenotypes, while genes acting in more downstream capacities, such as those encoding ligand receptors and downstream intracellular signaling molecules exhibit broader sets of defects resulting from disruption of several converging upstream signals.

### Loss-of-Function Genetics to Study Human Disease Genes In *Drosophila*

Another striking feature of the list of potential human disease genes with related genes in *Drosophila* (i.e., the clear-hit list) is that only a minority of these genes already have been studied by classical loss-of-function genetics (i.e., 28% of the 548 *Drosophila* genes related to human disease genes on the clear-hit list). This number highlights the substantial number of yet-unstudied *Drosophila* cognates of human disease genes, which could be analyzed using the molecular genetic tools of *Drosophila*. It should be possible to study the majority of these genes using various previously-established methods. For example, wild-type or disease-causing mutant variants of any candidate human disease gene can be misexpressed in *Drosophila* using routine methods and the resulting gain-of-function phenotypes assayed either during development or in the adult. Because developmental pathways have been extensively studied in *Drosophila*, observation of gain-of-function phenotypes often will immediately implicate particular candidate pathways. For example, in the *Drosophila* wing it is possible to distinguish phenotypes resulting from disruption of components in the EGF-Receptor (RTK), Notch, Wingless, Hedgehog, and *Drosophila* decapentaplegic (Dpp) signaling pathways based on wing shape, integrity of the wing border, and the position and number of wing veins. Isolation of a new mutant with phenotypes resembling those of mutants in one of these known pathways would suggest obvious follow-up experiments to verify that the new gene was indeed involved in the suspected pathway. It also is possible to assay neurobehavioral phenotypes in *Drosophila* such as defects in vision, chemosensation, touch, hearing, and rudimentary learning. The few studies of this kind that have been carried out to date are very encouraging in that misexpression of disease alleles of human genes often results in visible morphological defects or behavioral deficits. A particularly promising aspect of several of these studies is that the function of normal versus mutant alleles of the human disease gene can be distinguished. A likely mechanistic basis for the different activity of wild-type versus mutant forms of candidate human-disease genes is that the mutant may act as a dominant negative in *Dro-*

*sophila* as a result of a nonproductive interaction with a conserved component shared between flies and humans.

The function of the endogenous *Drosophila* counterparts of candidate human disease genes also can be analyzed by gain-of-function studies. More critically, however, loss-of-function analyses can be initiated to determine the consequence of removing the activity of these genes in *Drosophila*. Such loss-of-function analysis can be carried out for any of the 56 yet-to-be-analyzed *P*-element tagged genes. Additionally, because it is now practical to make targeted mutants in *Drosophila* (Rong and Golic 2000), it should soon be feasible to generate loss-of-function mutants in any of these genes. If misexpression of a human disease gene (normal or altered) or mutation of its *Drosophila* counterpart leads to scorable phenotypes in flies, second-site modifier screens typically can be designed to identify further genetic components acting in the same pathway as the gene of interest. The use of *Drosophila* to identify second-site modifier loci, which can then be tested for potential contribution to human disease (or modification of disease phenotypes), is likely to emerge as the most valuable application of *Drosophila* as model system for analysis of human disease genes because similar screens cannot be carried out on a significant scale in vertebrate systems.

### Which Candidate Human Disease Genes are Best Suited for Analysis in *Drosophila*?

The motivation for conducting the above analysis was the practical issue of identifying *Drosophila* genes related to candidate human disease genes that are likely to be productively studied in *Drosophila*. It is evident that not all genes on the clear-hit list are necessarily best suited for study in *Drosophila*. For example, the great majority of the clear-hit human disease genes involved in metabolic and mitochondrial disorders (123) also have direct counterparts in yeast. Because many of these genes control similar basic cellular processes in yeast, flies, and humans, they may be more effectively analyzed in yeast rather than in *Drosophila*. It is also the case that some genes common to *Drosophila* and humans may not be performing equivalent functions in these two organisms. For example, it is likely that some of the genes involved in human-blood diseases affecting specific cell types may have other functions in *Drosophila*, which has a relatively simpler hemolymph system compared to the complexity of vertebrate blood.

These general guiding principles should not be adhered to dogmatically, however. For example, the gene for the metabolic disorder acute porphyria (OMIM #176000), a defect in the gene for porphobilinogen deaminase (PBG), has a clearly related gene in *Dro-*

*sophila* ( $E = 10^{-78}$ ). Although there are gene sequences related to this uroporphyrinogen synthetase in many lower organisms, including yeast, the phenotype in humans involves paralysis and seizures as a result of secondary neurotoxicity from the buildup of excess porphyrin precursors. The study of such secondary effects of biochemical defects and the suppressors of these effects is more suited to an organism like *Drosophila*, which has a complex nervous system. As it happens, the fly gene most related to PBG (CG9165) contains a *P*-element insertion EP(3)0419. Thus, this gene seems to be an excellent candidate gene for study in *Drosophila*.

Another limitation of the cross-genomic comparison of human disease genes is that some of the *Drosophila* genes related to human disease genes may not be functionally equivalent (or orthologous) to the human disease gene in question, but rather may be more related by sequence and/or activity to another human gene that has a different function than the human disease gene. It is therefore to be anticipated that the clear-hit list contains matches between human and *Drosophila* genes that are members of a related but functionally diverged gene family. True orthologs may be identified through functional studies of individual genes in *Drosophila*. Thus, an important first step in analyzing any human disease gene in flies will be to demonstrate that the wild-type form of the human disease gene can substitute for (or rescue) loss-of-function mutants in the *Drosophila* gene. It is worth noting in this regard that a significant number of human disease genes have very strong matches to *Drosophila* counterparts (e.g., 274 disease genes = 29% match with  $E \leq 10^{-100}$ ), suggesting that this stringent criterion of functional equivalence will be satisfied in many cases. Our group is in the process of studying several human disease genes using misexpression in *Drosophila*. Our initial findings indicate that at least for the human *CYP2D6* gene, the *Drosophila cyp18* gene is an ortholog and that regulation of the *Drosophila* gene can be disrupted via misexpression of the human gene (L. Reiter, pers. comm.).

With the above considerations and qualifications in mind, we believe that there are broad categories of candidate disease genes that are likely to be particularly amenable to study in *Drosophila*. Thus, among the human disease, clear-hit genes, 74 result in neurological disorders. Given the substantial existing evidence indicating that basic neuronal systems have been conserved between flies and humans, this set of disease genes is likely to be effectively analyzed in *Drosophila*. As mentioned above, analysis of *Drosophila* mutants involved in synaptic transmission and action potential propagation has proven to be directly relevant to these processes in vertebrates (Salzberg and Bellen 1996; Wu and Bellen 1997). Also, the 296 genes that repre-

sent developmental, neurological, cardiovascular, ophthalmologic, and hearing disorders as well as cancers, appear to be good candidates for study using *Drosophila* because there is reason to believe that the underlying molecular mechanisms controlling these organismic processes also are highly similar in flies and humans.

For the individual researcher, we suggest that the best approach to using our dataset is to query Homophila for a particular disease or key word representing a class of disorders. From these results, one can judge the degree of similarity between the human and fly genes as well as the domains that are similar (for example, the *HOX* genes show homology only in the DNA-binding domain). The domain graphic below the best match enables the user to determine if the best match is in a known domain and not across the entire gene. One should be mindful when using this information not to discard hits with only localized domains of homology. For example, in the case of the *HOX* genes, it has been well established that functionally orthologous genes in highly diverged species share high-sequence similarity only within the DNA-binding homeobox domain. Yet this relatively small portion of the molecule seems to carry key developmental information. There are links to both OMIM for the human disease information as well as to Flybase to determine allele and *P*-element information. In addition to determining if the human and fly genes are likely to perform similar functions, reasonable criteria for a good-candidate disease gene for study in *Drosophila* would include the following: (1) There is at least good circumstantial evidence that the gene is involved in the disease condition, (2) the mechanism by which the human gene functions is poorly understood (e.g., has not been placed in the context of a known pathway), and, pragmatically, (3) there is at least one mutant allele in that gene (153 genes) or a *P*-element insertion in or near that gene (56 genes).

#### Future Development of Homophila as an Interactive Tool for Cross-Genomic Analysis

We will continue to develop the Homophila database to bridge the gap between the human disease (OMIM) and *Drosophila* (Flybase) databases, which were not originally designed to facilitate cross-genomic browsing. Given that ~4000 human disease phenotypes may have a genetic basis (Scriver 1995), it seems likely that the number of genes currently listed in OMIM will continue to grow at a rapid pace and that the frequent updates to the Homophila database will provide researchers with state-of-knowledge links to *Drosophila* counterparts of these genes. In addition, we currently are creating software to facilitate discovery of secondary associations among human and fly genes, which is now the focus of our next phase in development of the

database. In particular, we plan to implement a version of the database that will allow for phenotype key word searches in both human and fly databases. This modified alignment technology would create key word strings for each disease-gene entry in OMIM and each fly-gene entry (e.g., by distilling key words from OMIM, Flybase, Interactive Fly, or Medline review abstracts) and then allow researchers to search these unordered strings against one another for statistically significant similarities (e.g., neurological diseases with cell loss in the cerebellum). The idea would be to then examine the fly cognates of genes responsible for similar diseases identified by such key word searches, and ask if these fly genes have some interesting feature or function in common (e.g., they are part of a common signaling pathway or molecular machine of some kind). It also would be possible to do this in reverse (e.g., cluster fly genes and ask if the corresponding human diseases share any common disease phenotypes).

Another addition to Homophila we are planning is software to identify potential candidate disease genes based on predicted phenotypes. This idea is based on the fact that while there are many examples in which several human disease genes belong to a common signaling pathway or functional module, there typically are not known diseases associated with all components in these pathways as defined by studies in *Drosophila* or other systems. In principle, one could guess the types of disease phenotypes that might arise from mutations in human orthologs of these other components (based on the disease phenotypes of mutations in existing components, the phenotypes of mutations of these other components in *Drosophila*, and the expression pattern of these components in mice or other vertebrates). The software we are currently developing will be used to identify human counterparts of fly genes in a systematic fashion and to ask if any diseases matching the predicted phenotypes have been mapped to regions of the human genome containing those genes. We anticipate that with the input of both the human and *Drosophila* genetics communities, Homophila will become a valuable cross-genomics tool in the post-genome-sequence era.

## METHODS

### Identification of *Drosophila* Genes Related to Human Disease Genes

This work reflects version 3.01 of the Homophila database (released Feb. 1, 2001). Our analysis began with the OMIM morbid map, a catalog of genetic diseases and their cytogenetic map locations, which is available electronically at <ftp://ncbi.nlm.nih.gov/repository/OMIM/morbidmap>. It was not possible to simply download the sequences related to each disease in the on-line version of OMIM because the protein

and nucleic sequences associated with each OMIM entry often include unrelated genes mentioned in the text. Thus, a more involved procedure, relying on the NCBI Locuslink database, was required. Beginning with each of the 1792 genetic diseases specified in the OMIM morbid map, each disease was identified in the Locuslink *mim2loc* table, which relates OMIM entries to NCBI locus records. Each locus record then was used to locate the correct protein and nucleic-acid sequence records using the Locuslink *loc2UG*, *loc2acc*, and *loc2ref* tables, which specify entries in the NCBI Unigene, protein, nucleic acid, and RefSeq databases, respectively. This process was simplified by downloading the Locuslink tables (*mim2loc*, *loc2ref*, *loc2acc*, and *loc2UG*) and importing them directly into the Homophila database. The result of this procedure was a list of 4104 protein-sequence entries associated with 929 OMIM disease loci, and 4643 nucleic-acid sequence entries associated with 941 OMIM disease loci. Each of the protein-sequence entries was compared to the complete *Drosophila* genome sequence (Adams et al. 2000) using the BLASTP and TBLASTX programs (Altschul et al. 1997). BLAST comparisons were performed using BLAST v2.09 and the standard BLOSUM 62 and  $E = 10$  settings. Many OMIM disease entries have multiple protein sequences linked to the disease through Locuslink. The BLAST search results for each of the probe sequences are merged, and the most significant hit (smallest  $E$  value) taken to construct the table of clear hits (*Drosophila* cognates of human disease genes, Table 1).

A relational database has been implemented to allow queries on these results and is available on-line (<http://homophila.sdsc.edu>) using the MySQL relational database management system (Dubois 2000). PERL scripts using the DBI package are used to convert queries entered on the Homophila Web pages to SQL queries to the actual RDBMS. A complete list of  $P$ -element locations in the *Drosophila* genomic sequence was kindly provided by FlyBase (Flybase 1999).

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## REFERENCES

- Adams, M.D., Celniker, S.E., Holt, R.A., Evans, C.A., Gocayne, J.D., Amanatides, P.G., Scherer, S.E., Li, P.W., Hoskins, R.A., Galle, R.F., et al. 2000. The genome sequence of *Drosophila melanogaster*. *Science* **287**: 2185–2195.
- Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D.J. 1997. Gapped BLAST and

- PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.* **25**: 3389–3402.
- Dubois, P. 2000. *MySQL*. New Riders Publishing, Indianapolis, IN.
- Flybase. 1999. The FlyBase database of the *Drosophila* Genome Projects and community literature. The FlyBase Consortium. (<http://flybase.bio.indiana.edu/>).
- Fortini, M.E. and Bonini, N.M. 2000. Modeling human neurodegenerative diseases in *Drosophila*: On a wing and a prayer. *Trends Genet.* **16**: 161–167.
- Fortini, M.E., Skupski, M.P., Boguski, M.S., and Hariharan, I.K. 2000. A survey of human disease gene counterparts in the *Drosophila* genome. *J. Cell. Biol.* **150**: F23–30.
- Howard, T.D., Paznekas, W.A., Green, E.D., Chiang, L.C., Ma, N., Ortiz de Luna, R.I., Garcia Delgado, C., Gonzalez-Ramos, M., Kline, A.D., and Jabs, E.W. 1997. Mutations in TWIST, a basic helix-loop-helix transcription factor, in Saethre-Chotzen syndrome. *Nat. Genet.* **15**: 36–41.
- Littleton, J.T. and Ganetzky, B. 2000. Ion channels and synaptic organization: Analysis of the *Drosophila* genome. *Neuron* **26**: 35–43.
- McKusick, V.A. 2000. Online Mendelian Inheritance in Man, OMIM. (<http://www.ncbi.nlm.nih.gov/omim/>).
- Potter, C.J., Turenchalk, G.S., and Xu, T. 2000. *Drosophila* in cancer research: An expanding role. *Trends Genet.* **16**: 33–39.
- Reiter, L., Beir, E., and Gribskov, M. 2000. Homophila. (<http://homophila.sdsc.edu>).
- Rong, Y.S. and Golic, K.G. 2000. Gene targeting by homologous recombination in *Drosophila*. *Science* **288**: 2013–2018.
- Rubin, G.M., Yandell, M.D., Wortman, J.R., Gabor Miklos, G.L., Nelson, C.R., Hariharan, I.K., Fortini, M.E., Li, P.W., Apweiler, R., Fleischmann, W., et al. 2000. Comparative genomics of the eukaryotes. *Science* **287**: 2204–2215.
- Salzberg, A. and Bellen, H.J. 1996. Invertebrate versus vertebrate neurogenesis: Variations on the same theme? *Dev. Genet.* **18**: 1–10.
- Scriver, C.R. 1995. *The metabolic and molecular bases of inherited disease*, 7th ed. McGraw-Hill Health Professions Division, New York, NY.
- Wu, M.N. and Bellen, H.J. 1997. Genetic dissection of synaptic transmission in *Drosophila*. *Curr. Opin. Neurobiol.* **7**: 624–630.

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Lawrence T. Reiter, Lorraine Potocki, Sam Chien, et al.

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