Drosophila as a tool for personalized medicine: a primer

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Abstract

The goal of personalized medicine is to treat each patient with the best drug: optimal therapeutic benefit with minimal side effects. The genomic revolution is rapidly identifying the genetic contribution to the diseased state as well as its contribution to drug efficacy and toxicity. The ability to perform genome-wide studies has led to an overwhelming number of candidate genes and/or their associated variants; however, understanding which are of therapeutic importance is becoming the greatest unmet need in the personalized medicine field. A related issue is the need to improve our methods of identifying and characterizing therapeutic drugs in the context of the complex genomic landscape of the intact body. *Drosophila* have proven to be a powerful tool for understanding the basic biological mechanisms of human development. This article will review *Drosophila* as a whole animal tool for gene and drug discovery. We will examine how *Drosophila* can be used to both sort through the myriad of hits coming from human genome-wide scans and to dramatically improve the early steps in pharmaceutical drug development.

Keywords

cancer; diabetes; *Drosophila*; heart disease; multigenic

Hippocrates argued that disease was not a punishment from the gods but was the outcome of environmental factors, diet and living habits; therefore, we should use detailed observation as a basis for directing better health. The belief that observation of the patient would lead to optimal individualized therapy required the use of the best tools at hand. Traditional medicine incorporated patient information such as clinical signs, symptoms and personal history. As medical tools advanced, data was incorporated from imaging of the organs affected by the suspected disease to biochemical assays of easily accessible body fluids such as serum and urine.

In the last 100 years this push towards better diagnosis gradually included genetics, leading to further expansion of the medical toolkit. Individualized healthcare included the taking of family history to the cytogenetic analysis of those suffering from rare Mendelian diseases or cancer. Accurate staging of breast cancer relied upon imaging and staging of lumps. Now ErbB receptor status, microarray data or the prediction of risk by *BRCA1/2* status is used for progressively more accurate patient prognosis. However, only recently have genetics and genomics taken center stage as perhaps the most promising tool for achieving truly

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personalized medicine. Recent advances in genomic sciences, medical genetics and human genetics have enabled a more detailed understanding of the role of genetics in human disease. The advances include the determination of the human genome reference sequence, establishment of multisite collaborations that allow the pooling of thousands of human subjects for association studies and the development of systemic whole-genome analysis through the use of arrays (e.g., RNA, chromatin immunoprecipitation [ChIPS], copy number variation). Now, with the next-generation sequencing technologies, we can more clearly define the level of variation among humans and correlate it to the diseased state as demonstrated by the 1000 Genomes Project, The Cancer Genome Atlas and exomic sequencing of rare Mendelian diseases.

The challenge

Genomic approaches continue to lay the groundwork for identifying genes involved in the human disease process, and expectations are high. However, we are beginning to better appreciate that identifying genetic variants is only the first step. At least three additional steps are required. First, we need to determine which mutations are 'drivers' of a disease and which are 'passengers'. This is difficult as genomic studies often identify dozens or hundreds of variants. Second, we need to determine the biological function of these human disease genes, preferably in the context of the whole animal. Third, we need to identify therapeutics that treat the aberrations, again preferably in the context of the whole body.

These steps need to be addressed cheaply and rapidly. In this article, we explore the use of the fruit fly *Drosophila* as an emerging tool for the next generation 'Genomics 2.0'. In many ways, the lowly fly is a near-perfect match for genomic studies. We explore recent work by *Drosophila* researchers to adapt the remarkable tools available in the *Drosophila* field to the specific needs of genomic studies and personalized medicine. We also consider why the *Drosophila* world has been slow to embrace genomics and personalized medicine, and why this should change.

Why flies?

To understand the advantages and disadvantages of using *Drosophila*, let us first consider their most useful features. Flies have a short lifecycle: approximately 10 days depending on the temperature at which they are raised. After embryogenesis, the developing animal develops through three larval molts; larvae are especially useful for drug studies as they are continuously eating with minimal hesitation or regulation. After pupariating and then completing the pupal stage, the emergent adult has many easily scored structures for genetics (sadly, they emerge as picky eaters). Therefore, a major advantage of flies is that they develop quickly with a life-cycle of approximately 9 days. Because they breed rapidly and are readily kept in vials feeding on inexpensive food, flies are also cheap to maintain and experiment with. Table 1 compares some of the common model systems.

Many aspects of these developmental stages have been remarkably well characterized, making *Drosophila* perhaps the best-understood developing animal in terms of the mechanisms and pathways that direct tissue assembly and maturation. Several basic signaling pathways were first discovered in flies, a point reflected by their unusual names: Hedgehog (larvae cuticle resembles that of a hedgehog), Notch (notched wings) and Wnt (a contraction that includes wingless). The success of *Drosophila* in identifying key molecular pathways active in development was recognized by the awarding of the Nobel Prize in Medicine in 1995 to Eric Wieschaus, Christiane Nusslein-Volhard, and Edward Lewis. Wieschaus and Nusslein-Volhard used fly genetics to identify every zygotic gene required to assemble the young embryo, a saturation approach that should prove particularly useful as members of the community move towards studying disease mechanisms.

Tools

A key strength of *Drosophila* is the powerful tools available to manipulate genes. Simply put, the activity of almost any gene in *Drosophila* can be increased or decreased within nearly any cell or cell type and at any stage of development. This ability to manipulate genes at will represents one of the two most important *Drosophila* approaches relevant to personalized medicine. The detailed techniques – including the FRT/FLP recombination approach to generate discrete patches of mutant tissue in a wild-type background, the GAL4/ upstream activation sequence (UAS) system for mis-expression studies, and the use of inverted repeats to generate RNAi-mediated gene knockdown – have been well reviewed (e.g., [1–3]) and we will introduce the most disease-relevant techniques briefly below. *Drosophila* has a long history of tool building and with each passing year the ability to generate transgenic *Drosophila* genes, manipulate gene expression and direct site-specific changes becomes easier, quicker and more powerful.

The genetic modifier screen

Perhaps no tool has proven more powerful in *Drosophila* than the genetic-modifier screen. If you are wondering how *Drosophila* can aid your own work, this remarkably powerful technique should likely be your first consideration.

The basis of the genetic modifier screen is that functionally related loci will show a dominant genetic interaction. For example, the EGF receptor (EGFR) acts, in part, through the small GTPase Ras to promote tumorigenesis [4–6]. To model aspects of this process, activated EGFR isoforms were targeted to the *Drosophila* eye; the result was over-proliferation of the eye epithelium and a 'rough eye' in the adult [Levine B, Cagan R, Unpublished Data] [7]. Removing a single genomic copy of *Drosophila* Ras by itself has no effect on eye development (removing both genomic copies – flies are diploid – is cell and embryonic lethal). But removing a genomic copy of Ras in the presence of activated EGFR suppressed the overproliferation phenotype, yielding a less rough eye. That is, the genomic copy number of Ras is rate-limiting for EGFR activity. Conversely, removing a genomic copy of the pathway inhibitor RasGAP enhanced activated EGFR. In the fly community we refer to Ras and RasGAP as 'suppressor' and 'enhancer' loci of activated EGFR, respectively, and this data is considered strong evidence for a functional link between these loci. Owing to the availability of off-the-shelf fly stocks containing single mutations that, together, account for most of the predicted loci in the fly genome, screens for functionally linked loci can be readily accomplished. The payoff is identification of truly novel loci that act in your pathway of interest.

Enhancer and suppressor loci are conceptually similar to the modifier/susceptibility loci commonly searched for in personalized medicine studies. Later we will provide disease-related examples to illustrate how genetic modifier screens were used to address specific questions in human therapeutics.

Targeted expression, knockdown

In addition to using genetic tools to test functional links between proteins, advances have made targeted expression or knockdown of genes both easier and more precise. Two major techniques are commonly used to achieve this in stable transgenics, and we briefly outline how they are used.

Targeted transgene expression—Many diseases are due to the expression of disease-specific isoforms of specific proteins. for example, many solid tumors express the oncogenic Ras isoform Ras^{G12V}, leading to activation of the Ras signal transduction pathway and

tumorigenesis. Expression of one or more disease genes is readily accomplished in flies: researchers typically use either direct fusion of a useful promoter to the gene of interest or, more commonly, by use of the GAL4/UAS mis-expression system [8]. Figure 1 shows the basic approach, which combines GAL4 fused to a tissue-specific promoter with the transgene of interest (e.g., ras^{G12V}) fused to the GAL4 target sequence *UAS*. For example, crossing *eye-GAL4* flies to *uas-ras^{G12V}* flies targets oncogenic Ras to the eye; progeny are ready to score in less than 2 weeks. As we discuss below, this approach can provide a useful assay for both genetic screens and for drug discovery/validation.

Targeted knockdown—The *Drosophila* tool set includes the ability to knock out genes in specific tissues. For disease models, a more common approach has been targeted knockdown of a gene using RNAi; this approach reduces gene activity by destabilizing their transcript. Expression of inverted repeat, dsRNA leads to targeting of homologous sequences for degradation by a Dicer-mediated complex (reviewed in [9]). This approach works particularly well in flies. Currently, three separate collections of flies are available that cover the predicted 20,000 fly transcripts: each of the 20,000 fly lines contains an inverted repeat transgene that targets a single transcript; this transgene is fused with the *UAS* promoter to allow precise GAL4-mediated targeting of a gene for knockdown (see earlier). For example, knocking down the tumor suppressor P53 in the wing simply requires crossing 'wing'-GAL4 flies to UAS-P53 flies. The F1 knockdown adults will emerge in less than 2 weeks. The ability to knockdown any predicted transcript with off-the-shelf reagents simplifies the effort, time and cost required.

RNAi: *in vivo* & cell culture

Many drug and functional screens are more readily accomplished in cell lines. *Drosophila* cell lines have proven especially useful for this approach, and the *Drosophila* Genomics Resource Center makes a large number of well-characterized lines available. Although fly cell lines do not take advantage of the ability to screen in a whole-animal setting, they are quicker, cheaper and more quantitative than whole-fly screens. Moreover fly cell lines have unique advantages that have attracted numerous researchers of mammalian systems. They are robust, thrive at room temperature (flies are ectothermic) and, critically, readily take up RNA added to the media. That is, libraries of dsRNA can be screened rapidly through 'systemic RNAi' screening, in which single or pools of RNAi constructs are added to the media. The Perrimon laboratory has established an automated facility that permits laboratories to screen a validated dsRNA library, typically against a luciferase readout of the pathway of interest [10]. This approach has been hugely successful in identifying regulators of several pathways including Hedgehog, Wnt, receptor tyrosine kinase/Ras, metabolism, and other pathways [11–15].

Disease models

In studies of development, *Drosophila* proved useful for its powerful genetics but also its emphasis on examining mechanisms *in situ*. This whole-animal approach is also invaluable for disease studies. Many of the difficulties translational researchers have had can be traced to the differences between diseased tissue within a whole animal, cell culture models or conceptually related xenograft models. In this section we consider specific examples of how *Drosophila* can be used to rapidly and inexpensively generate disease models that are useful for studies of both mechanism and therapeutics. Our purpose is not to be comprehensive, but to point to a small number of illustrative examples of how *Drosophila* can impact specific issues of disease and personalized medicine.

Cancer

Flies provide a natural tool to study the genomics of cancer, as most oncology-related pathways have a long history of study in *Drosophila*. Indeed, the first tumor suppressor was identified in *Drosophila* through the pioneering work of Peter Bryant and his colleagues [16,17]. However, only recently has the fly community embraced cancer as a specific field of focus. The number of fly workers studying cancer is rapidly rising. We bring an *in situ* sensibility to questions of tissue development and homeostasis, and are comfortable considering impact on the animal as a whole. As such, we anticipate flies will have an increasing impact on our understanding of how mutated genes and gene combinations affect both tumor outcome and even drug response.

Multiple endocrine neoplasia type 2—Several recent examples serve to demonstrate the utility of flies in understanding how mutations in oncogenes and tumor suppressors can lead to progressively more aggressive tumors. At the simple end of the mutation spectrum is the 'one-hit' solid tumor syndrome multiple endocrine neoplasia type 2 (MEN2). This syndrome is characterized by mutations in the Ret protein, a transmembrane receptor tyrosine kinase. Patients with either spontaneous or inherited oncogenic isoforms of Ret invariably develop medullary thyroid carcinoma (MTC), a potentially aggressive overgrowth of the para-follicular C cells that can lead to metastatic disease if not treated early. MTCs have proven resistant to traditional chemotherapies.

Nearly all patients with MEN2, and many with MTC, have activated isoforms of Ret, suggesting that Ret is both necessary and sufficient for development of MTCs. To develop a whole-animal transgenic model, various oncogenic Ret isoforms were targeted to the developing fly eye epithelium. The result was overproliferation, switches in cell fate, compensatory apoptosis and other aspects of tumorigenesis that suggested this fly MEN2 model could prove useful [18]. The eyes displayed a 'rough eye' phenotype that is readily scored with a dissecting microscope, yielding a useful screening tool (Figure 1).

Multigenic cancer models—An important foundation of personalized medicine lies in the ability to recognize and model multigenic disease states. This plays to the strengths of *Drosophila*, which permit rapid and inexpensive manipulation of multiple genes. In the past decade a small number of *Drosophila* laboratories have developed multigenic cancer models. Pioneers include the laboratories of Tian Xu, Helena Richardson, Dirk Bohmann and Maria Dominguez; we have also been active in this area. Work with the potent oncogene Ras has demonstrated that it can direct proliferation on its own but that true transformation requires synergy with second loci, a point increasingly embraced in work with other organisms as well. For example, pairing oncogenic Ras isoforms with mutations in the cell polarity protein Scribble, led to strong proliferation and aggressive metastasislike migration by Ras/Scribble cells. Labeling these cells with GFP highlighted their movement to distant sites. More recent work has shown similar synergy between oncogenic Ras and mutations that activate the cytoplasmic tyrosine kinase Src.

In both *Drosophila* and humans, EGFR and its downstream effectors, Ras and Raf, were also found to synergize with Src kinase activity. The result was both increased proliferation and metastasis-like behavior [19]. This is a particularly clinically relevant pairing: for example, approximately 70% of triple-negative breast tumors show high activity of EGFR plus Src [20–27]. Pairing these not only synergizes with respect to proliferation, but also shows emergent tendencies for transformed cells to undergo metastasis-like migration [28–33]. This migration relied on local interactions between the Ras/Src transformed cells and their neighbors within the epithelium. These increasingly tumor-like phenotypes highlight the potential for flies to screen candidate therapeutics in the context of richer, more complex

whole-animal models, and to target aspects such as metastasis, which relies on interactions between tumor cells and their environment.

Work with *Drosophila* on cancer models has also highlighted another important potential use: the ability to identify candidate modifier loci. An elegant example of this was provided by Dominguez and colleagues [34]. The *Drosophila* community has developed a number of overexpression tools that permit screening of randomly overexpressed loci [35–37]. Hyperactivation of the Notch signaling pathway has been linked to tumorigenesis owing to its ability to promote excess proliferation [38–43]. Ferres-Marco *et al*. used an eye overgrowth model to screen and identify two neighboring loci that, when ectopically coexpressed with the Notch ligand-δ, strongly enhanced the Notch-dependent eye overgrowth phenotype (Figure 2) [34]. Pipsqueak and Lola are transcriptional regulators that act by regulating histone modification [44–46]. The authors provide evidence that these two nuclear factors synergize with active Notch pathway signaling to target cell cycle genes including *Rb*. Similar to work demonstrating synergy between Ras and Scribble or Src, the connection between Notch signaling and epigenetic regulation of the cell cycle points to new targets to explore in cancer.

Glioma

This simple but powerful approach – targeting multiple transgenes with a single driver to establish multigenic cancer models – will be utilized to establish increasingly sophisticated models designed to match the ever-increasingly genomic data. An excellent example of this comes from Read *et al*., who established and explored a 'two-hit' glioma model [47]. Targeting activated isoforms of *Drosophila* EGFR plus the P110 subunit of PI3K to glia yielded aggressive neoplasias that proved invasive into surrounding nervous system tissue (Figure 2). This transformation was specific to glia; neural tissue did not show similar transformation phenotypes, emphasizing that different cell types respond differently to specific oncogene combinations. Detailed histological and genetic evidence bolstered the view that these flies modeled fundamental aspects of human gliomas. Once in hand, these transgenic flies were then used to explore the downstream signals that mediate these effects, implicating orthologs of Rb, Myc, Elk-1, and so on, as critical regulators. This model should prove useful both for continuing to explore mechanisms and potentially as a first-line screen for candidate therapeutics.

Heart disease

Heart disease remains the number one source of mortality in the developed world, and yet we still only partly understand the mechanisms that lead to progressive heart deterioration. *Drosophila* does indeed have a heart; they also have structures similar to other organs including the kidneys, lungs and the liver. The pioneers of *Drosophila* models of heart disease have been Rolf Bodmer and Karen Ocorr, who have taken tools honed in developmental studies to explore specific issues of disease. They have demonstrated progressive heart deterioration in aging adult flies and have shown that this can be reversed by reducing activity of the insulin signaling pathway [48,49]. The structural proteins and channels that regulate human heart function are conserved in *Drosophila* – several were initially discovered in flies – and likely perform similar functions within a fairly simple, nonchambered tube that moves fly blood (hemolymph) through the body (reviewed in [48,50,51]).

Heart function can be measured in detail and precise M-modes can be generated that demonstrate rhythmicity. With these tools plus high-sensitivity fluorescence imaging [52], the fly heart is improving in its ability to be screened for genes and drugs that compromise or improve function. An excellent example of the utility of flies comes from the study of the

KCNQ potassium channel. In humans, mutations in this potassium channel lead to long QT syndrome (LQT) Type 1, a delay in repolarization of the heart that is associated with arrythmia and sudden death. Flies mutated for *kcnq* survive to adult and, also similar to humans, are susceptible to cardiac failure when the heart is artificially 'paced' [53]. Also promising from a personalized medicine standpoint, *Drosophila* has also conserved the 'human Ether-a-gogo' channel (HERG, named after the fly protein due to mutants' uncontrolled movements). HERG has been linked to drug-induced LQT, a potentially fatal side effect common with many drugs tested in the clinics. Mutation of the fly ortholog led to increased rates of arrythmicity [54], holding the promise that *Drosophila* could provide a rapid whole-animal assay to detect this serious drug contraindication.

Diabetes

Perhaps the fastest growing disease field in *Drosophila* research is its use as a tool to explore metabolism-related diseases. Here, we consider work in diabetes, often referred to as an 'epidemic' that has tripled in incidence in the USA in just the past 30 years. The utility of *Drosophila* as a tool for studying diabetes was highlighted by Rulifson, Kim and Nusse [55,56], who developed a model of Type 1 diabetes. To mimic loss of the insulin-secreting β-cells in human patients, they expressed a pro-apoptosis gene in the *Drosophila* insulin-producing cells (IPCs). Loss of these neuroendocrine cells led to aspects reminiscent of the human disease, including hyperglycemia, hyperinsulinemia, aspects of insulin resistance and altered fat profiles [56]. Further work has demonstrated the presence of cells that function similar to our α-cells; these cells secrete a glucagon-like peptide that is predominant in regulating hemolymph glucose levels [55].

Most diabetes patients suffer from the more common Type 2 diabetes mellitis (T2DM). New *Drosophila* models of T2DM are being developed that rely on simple high-carbohydrate feeding paradigms [Palankar L, Fink J, Cagan R, Baranski T, Unpublished Data]. These lead to a broad array of effects described in human patients and show both similarities and some differences with Type 1 diabetes. A close examination of heart and kidney function in these high-sugar-fed flies revealed many defects observed in human patients [Na J, Baranski T, Ocorr K, Bodmer R, Cagan R, Unpublished Data]. Genetic screens have indicated a remarkable complexity to the defects imposed by a high-sugar diet, and have highlighted both the complexity of the disease and the need for a powerful genetic system such as *Drosophila* to sort out the intricacies.

Genomic mapping

Regarding personalized medicine, perhaps the greatest place for future growth in the *Drosophila* field over the next few years is their potential to provide biological context to the flood of genomic data. *Drosophila* commonly screen hundreds or even thousands of genes in a study, assessing functional links and generating new pathways based on functional criteria. In this section we cite recent examples in which flies have been used with success to address specific issues that emerged in genomic disease studies.

Susceptibility loci

The potential utility of fly's genetic tools for identifying susceptibility (modifier) loci is underappreciated, even by fly biologists. Enhancers identified in genetic screens are often excellent candidates to act as modifiers of a primary genetic insult. To explore the potentially utility of *Drosophila*, we again look to the *Drosophila* MEN2 model described earlier.

Nearly all patients with MEN2 develop MTC, but only a subset develops additional tumors, such as pheochromocytomas [57–59]. This suggests that susceptibility to these adrenal-

based tumors requires differences at secondary loci. A *Drosophila* genetic-modifier screen – described earlier – identified 140 loci that were functionally linked to the oncogenic activity of *Drosophila* Ret. We then crossmatched many of these loci to copy number studies in *MEN2* tumor tissue and eventually identified two candidate susceptibility loci for the development of pheochromocytomas by MEN2 patients: the cytoplasmic kinase TNIK and the chromatin remodeling complex member CHD3 [18]. These loci are at least biomarkers for susceptibility to pheochromocytomas in these patients; whether they play a similar dominant role in promoting these adrenal tumors is not known.

This work highlights the ability of flies to rapidly identify pathway members that promote disease. Linking these functional genetic screens with high-throughput genomic analysis represents a powerful combination for exploring the subtleties of a disease's genomic complexities.

Genome-wide association studies

Another underappreciated use of *Drosophila* genetics is the potential for matching genetic modifiers from *Drosophila* to SNPs or related genome-wide association study mapping in human patients. Often these approaches identify candidate regions but have difficulty determining, first, the exact gene within a region that actually contributes to the disease, or second, testing whether a candidate gene with a subtle genetic alteration is likely to be functionally linked to the disease. For example, recent efforts to match our *Drosophila* T2DM models with genome-wide association studies of T2DM patients has yielded interesting preliminary results [Baranski T, Cagan R, Collins F, Unpublished Data]. *Drosophila* have been able to take several candidate genes within a statistically-defined linkage region and identify a subset that when mutated alter the animal's response to high-sugar feeding. Incumbent on these studies is the need to demonstrate that flies are predictive of *bona fide* human susceptibility loci. With this validation, *Drosophila* may prove a centrally important tool in sorting through the enormous data generated by genomics.

Drug discovery

Drosophila has traditionally been a 'powerhouse' as a genetic system. More recently, *Drosophila* has contributed to the search for compound therapeutics in a small number of other diseases as well. *Drosophila* holds great promise for drug screening: combining sophisticated multigenic models with the ability to be used in a moderately high-throughput drug screen. This approach may identify compounds with better whole-animal efficacy and reduced whole-animal toxicity. Here, we consider early efforts by the fly community to establish whole-animal screening.

MEN2

Earlier we described MEN2, a cancer syndrome typically triggered by activating mutations in the Ret receptor tyrosine kinase. Santoro and colleagues demonstrated that the anilinoquinazoline-type kinase inhibitor ZD6474/Vandetanib was useful for suppressing oncogenic Ret isoforms in cell culture and xenograft studies [60]. To examine vandetanib in a transgenic model, the drug was fed to the oncogenic Ret fly models: the compound proved effective in suppressing most aspects of oncogenic Ret *in situ* (Figure 2). The cell culture and subsequent fly work proved predictive: vandetanib has shown clear efficacy in Phase II clinical trials [61] and is completing Phase III trials [Wells S, Pers. Comm.]. This work provides an important validation of the use of *Drosophila* to predict whole-animal efficacy and toxicity in human patients.

Drosophila still has more to offer MEN2 patients. Most patients that respond to vandetanib show a partial response, and most show significant toxicity at useful doses [61]. The ability

of *Drosophila* to address the full palate of Ret-activated pathways within a whole-animal context promises to find more effective drugs with fewer side effects. Our laboratory has identified new-generation compounds that show increased efficacy with decreased toxicity in fly and human MEN2 cell lines, holding the promise that next-generation drugs will show better therapeutic indices. Furthermore, our increasing knowledge of the genetics of oncogenic Ret will permit a testing of these compounds in the context of multiple genetic backgrounds for more personalized treatment.

Fragile X

We have focused on the use of flies in cancer studies, but the *Drosophila* community is expanding to other diseases as well. Recently, an elegant approach used flies to identify a candidate therapeutic for Fragile X syndrome [62]. A common form of mental retardation, Fragile X is linked to alterations in the Fragile X mental retardation protein (FMRP), which likely leads to excess glutamate production [63–65]. Mutations in the *Drosophila* ortholog FMR1 led to lethality if the food of developing animals was supplemented with glutamate. Using an embryo-sorting machine to screen *fmr1* mutant embryos against a small chemical library, the authors identified nine compounds that rescued glutamate-dependent lethality in *fmr1* flies. Their 'hits' were interesting from the standpoint of mechanism: regulators of both GABAergic and muscarinic cholinergic receptors were identified. The latter was especially surprising and pointed to previous unsuspected complexity of the disease. This work highlights the utility of *Drosophila* to explore neural-based diseases, which can have complex effects through the neural network. Other researchers have reported compound screens against fly models of neuro-degenerative diseases such as triplet-repeat based diseases and Parkinson's [66–84]. Figure 3 shows an example of a factor identified in a genetic screen that successfully rescued degenerating dopaminergic neurons in a fly model of Parkinson's as well as the progressive degeneration observed when disease isoforms of ataxin-1 – linked to the triple repeat-class disease spinocerebellar ataxia type 1 (SCA1) – are expressed in the developing eye. Together, this work further demonstrates the utility of *Drosophila* for whole-animal screening of compounds.

Why have *Drosophila* researchers not contributed more to therapeutics & to personalized medicine?

In this article we have examined the potential for flies as a tool for bringing increased genetic sophistication to issues of therapeutics. This, in turn, is another useful step towards the promise of bringing a similar level of sophistication to therapy in the clinics. Currently, only a few in the *Drosophila* community have considered the potential of this remarkable insect for exploring issues of therapeutics and of personalized medicine. Most fly biologists are trained as developmental biologists and are not familiar with the different ways the clinical researchers approach problems. In turn, clinical researchers are often not familiar with the power of *Drosophila* genetics, often emphasizing their differences.

To be sure, *Drosophila* physiology is considerably different from human physiology. While they have most of the organs we do, the details of how these organs are constructed and the manner in which they function have changed over the past 300 million years. But these issues hold equal weight for development. Yet *Drosophila* research has perhaps been the single most successful tool for exploring development-related aspects of genetics, morphology and signal transduction. While the details of development differ between flies and mammals, the former was strongly successful in laying out the basic questions, pathways and mechanisms. A remarkable amount of what was learned from flies turned out to be correct for mammals as well, more than was initially anticipated. The 'master regulators' of the heart, eye, kidney and so on, proved remarkably well conserved. Even

those aspects that were different benefited from asking the right questions derived from fly research.

Future perspective

Our knowledge of complex diseases, such as cancer and diabetes, is at a fairly early stage, mirroring the state of developmental biology two decades ago. We predict that the success of model systems in developmental studies including *Drosophila* will be repeated for personalized medicine: many assumed differences will disappear as we move from fly knowledge to mammalian exploration. We anticipate that the rapid synergy that emerged in developmental studies will be repeated for disease, and that the fly community further embraces its potential for initiating knowledge in personalized medicine. This will occur because disease studies need to embrace complexity, a strength of *Drosophila*, and because the science community is increasingly expected to account for issues of disease. This shift in priority is already being felt in the *Drosophila* community, and we anticipate that multiple disease fields will significantly benefit as a result.

Executive summary

Challenge of current genomic studies

- The identification of genetic variants associated with human disease process is only the first step. We need to determine the impact of the genetic variation and the biological function of the gene, as well as identify therapeutics that treat the aberrations, all in the context of the whole body.

***Drosophila* is a biological system well suited for studies in genomics & personalized medicine**

- *Drosophila* is inexpensive to maintain in large numbers.

- Its development has been well characterized and most mechanisms and pathways involved have been conserved throughout the animal kingdom.

There are many powerful molecular tools available to manipulate genes in *Drosophila* that include the ability to generate transgenic lines, to alter the levels & to direct site-specific changes in gene expression

- These tools include the following:

  - Genetic modifier screen;

  - Targeted expression, knockdown;

  - RNAi, both *in vivo* and *in vitro*.

Many human disease states can be modeled in *Drosophila* thus allowing mechanistic studies to be performed in the whole animal

- The *Drosophila* disease models covered are the following:

  - Cancer – multiple endocrine neoplasia type 2 and multigenic cancers including glioma;

  - Heart disease – impact by the insulin signaling pathway and long QT syndrome;

  - Diabetes – Type 1 and 2.

Functional genomics performed in *Drosophila* will lend biological context to the myriad of 'hits' coming out of the current wave of genomic studies

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- ▪ These studies include the characterization of susceptibility loci and genome-wide array studies.

Drosophila holds great promise for drug screening by combining sophisticated multigenic models with the ability to be used in a moderately high-throughput drug screen

- ▪ In preclinical studies, the ZD6474/vandetanib tyrosine kinase inhibitor was shown to be efficacious when fed to a _Drosophila_ transgenic model of MEN2 cancer.

- ▪ Several compounds were identified in a small screen when the compounds rescued a _Drosophila_ transgenic model of Fragile X syndrome.

- ▪ Another screen identified a factor that had a positive impact on a Parkinson's _Drosophila_ model.

There are still challenges to utilizing _Drosophila_ in a manner that has a greater impact on the field of personalized medicine

- ▪ _Drosophila_ community and clinical researchers need to better understand the different ways they approach their respective fields.

- ▪ Although the physiology of _Drosophila_ and humans are quite different, the molecular details are very well conserved.

Acknowledgments

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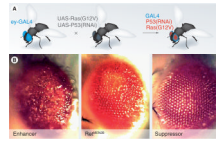
Figure 1. Tools used to generate disease models
(A) Crossing a fly line containing eye-targeted GAL4 to a fly line with one or more transgenes results in progeny that express the transgenes in the tissue of interest (e.g., the eye). **(B)** Targeting expression of the oncogenic isoform Ret^{MEN2B} to the eye results in a 'rough eye' phenotype, best observed with misplaced outer lenses. Genetic enhancers (left panel) lead to a more extreme phenotype including outgrowths, while genetic suppressors (right panel) bring the eye closer to its normally smooth lens array.
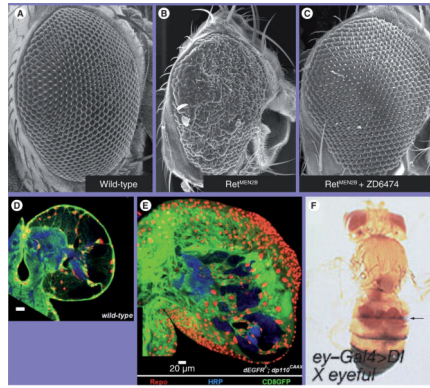Data taken from [Read R, Cagan R, Unpublished Data].

Figure 2. *Drosophila* **cancer models**
Compared to wild-type adult eyes **(A)**, expressing oncogenic *Drosophila* Ret led to a small 'rough eye' phenotype **(B)**. Feeding the compound ZD6474/vandetanib rescued the effects of oncogenic Ret [85] **(C). (D–E)** *Drosophila* glioma model based on activated EGF receptor/P110 isoforms targeted to glia. Glial nuclei are labeled with Repo (red), cell bodies with CD8GFP (green); neurons are labeled with HRP (blue) driven by repo-Gal4. These 'repo>dEGFR$^\lambda$;dp110CAAX' brains **(E)** showed a dramatic increase in glial number and an enlarged brain relative to wild-type **(D)** [47]. **(F)** Expressing δ within the developing fly eye led to both enlarged eyes and eye tissue at distant sites (arrow).
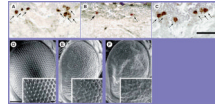HRP: Horseradish peroxidase.
Reproduced with permission from [34].

Figure 3. *Drosophila* **neurodegenerative models**
(A–C) *Drosophila* Parkinson's model protected by Hsp70 expression. **(A)** Dopaminergic neurons in dorsomedial neuronal clusters were visible with an antibody to tyrosine hydroxylase (arrows). **(B)** Overexpressing α-synuclein – linked to human Parkinson's – led to fewer dopaminergic neurons. **(C)** A screen [84] identified Hsp70 as protective of dopaminergic neurons when coexpressed with α-synuclein [73]. **(C–E)** The triplet-repeat disease spinocerebellar ataxia type 1 was modeled in flies. This disease is linked to an expanding glutamine tract within the ataxin-1 protein. **(D)** Scanning electron micrograph of a control eye. **(E)** Targeting expression of an ataxin-1 protein containing 30 consecutive glutamine repeats to the developing eye led to a 'rough eye' phenotype. **(F)** Increasing the glutamine repeats to 82 led to a still more severe rough eye phenotype. Reproduced with permission from [73].

Table 1

Comparing commonly used models.

	Cell culture	*Caenorhabditis elegans* (worm)	*Drosophila* (fly)	*Danio rerio* (zebrafish)	*Musca* (mouse)
Lifecycle (days)	~1	3	9	>90	>50
Epithelia?	No	±	Yes	Yes	Yes
Multigenic (>3) cancer models?	Yes	No	Yes	No	No
Large-scale genetic screening?	Yes	Yes	Yes	No	No
Whole-animal drug screening?	No	Yes	Yes	Limited	No
Approximate cost/month/100 animals	Low	12¢	20¢	US$180	US$2100

Drosophila sits in the 'sweet spot' of linking genes to therapeutics. It is rapid and inexpensive to work with, but retains important aspects of whole-animal complexity.