Fruit flies as a powerful model to drive or validate pain genomics efforts

Chronic pain is a disabling condition that persists even after normal healing processes are complete and presents considerable physical, psychological and financial burdens for patients globally. However, current analgesic treatments do not meet clinical needs. Here, we review genomic and pharmacogenomic studies of pain in humans and nociception in the fruit fly *Drosophila melanogaster*, and provide evidence supporting the use of fly genetics to compliment genome-wide and pharmacogenomic studies of human conditions, such as pain. Combining genomic and pharmacogenomic techniques to study chronic pain in humans with functional genomic assessment in model organisms may provide molecular rationale for developing more personalized or improving generalized chronic pain therapies.

KEYWORDS: analgesics ■ chronic pain ■ conserved genetics ■ *Drosophila* ■ functional genomics ■ neuropathic pain ■ nociception ■ pain ■ personalized medicine

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Nociception is a conserved biological process that is responsible for the detection and transmission of noxious input [1]. In humans, noxious stimuli can produce pain, defined as 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage', usually protecting an organism from possible harm [2]. However, maladaptive changes to this nociceptive system can lead to chronic pain, which lingers even after the healing process is complete. There are many forms of chronic pain, including back, cancer and amputation pain, and it is estimated that 5–10% of the global human population currently suffer from variations of this disorder [3]. Instead of playing a protective role, chronic pain becomes a disease in its own right [4], promoting patient morbidity without conferring a protective advantage.

Persistent or chronic pain disorders are clinically characterized by hyperalgesia (increased sensitivity to noxious stimuli) and allodynia (sensing innocuous stimuli as noxious) [4]. These conditions can arise from structural or functional alterations at multiple points of the nociceptive sensory pathway [5]. Importantly, population-based studies have established that an individual's likelihood of developing chronic pain after injury is significantly influenced by their underlying genetics [6].

While major progress has been made in defining the molecular machinery required for developing or maintaining chronic pain [5], it has nevertheless been difficult to develop new blockbuster analgesics for treating chronic pain. As Woolf describes, pain disorders are multifactorial diseases and current 'one-size-fits-all' treatments may not always be realistic [7]. While opioids and NSAIDs are potent for treating acute and inflammatory pain, they are ineffective in treating long-term neuropathic pain [8]. The first-line medications for pain relief in neuropathic pain disorders are repurposed antiepileptic (gabapentin and pregabalin) or antidepressant (tricyclic antidepressants [TCAs] and serotonin-noradrenaline reuptake inhibitors [SNRIs]) drugs, but these treatments are limited by side effects [9,10]. In addition, individual patient responses to these treatments are highly variable and likely influenced by genetics [11]. The successful design of next-generation 'precision' analgesic medicine will therefore require identification and functional validation of major patient-specific genomic idiosyncrasies (identified from human genetic and genomic studies) in animal models. Since fruit fly models have been useful in pharmacological discovery for other human diseases (see below) it is tempting to speculate that they will have utility for the development and pharmacogenomic assessment of analgesic drugs with a conserved mode of action.

Human pain disorders

Before considering the application of fruit flies in analgesic drug discovery, it is useful to consider what is currently known about the genetics of pain in humans. Many rare monogenic pain disorders are associated with coding mutations that result in channelopathies, that is, the dysfunction of ion channels (reviewed in [12]).

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For example, loss-of-function mutations of the *SCN9A* gene ($Na_v1.7$ voltage-gated sodium channel) have been reported in individuals with the disorder known as congenital indifference to pain [2,13]. Conversely, gain-of-function mutations of *SCN9A* result in hyperexcitability of the $Na_v1.7$ channel, and this is associated with the development of two distinct pain disorders: primary erythromelalgia [14] and paroxysmal extreme pain disorder [15]. Linkage analysis has also shown that a gain-of-function mutation in *TRPA1*, a nonselective cation channel, causes familial episodic pain syndrome [16]. Similarly, susceptibility to familial hemiplegic migraine subtypes 1, 2 and 3 is associated with mutations in *CACNA1A* (α_1-subunit of the $Ca_v2.1$ [P/Q-type] voltage-gated calcium channel) [17], *ATP1A2* (α_2 subunit of the Na^+/K^+-ATPase) [18] and *SCN1A* ($Na_v1.1$ voltage-gated sodium channel) [19], respectively.

Complex pain genetics

More common genetic determinants affecting pain sensitivity involve subtle genetic variants that may contribute to complex pain phenotypes. While environmental contexts clearly play a role in chronic pain, animal [20,21], population [22,23] and especially twin studies [24] on the heritability of pain place the genetic contribution between approximately 20 and 70%, depending on the study and type of pain assessed. Targeted assessment of pain genetics has helped to unravel the complex heritability of various pain diseases in the general population (reviewed in [25]). Common pain-relevant variants that have been identified by a targeted approach include *COMT* (reviewed in [26]), a catechol-*O*-methyltransferase [21]; *GCH1* (reviewed in [27]), an upstream enzyme in the dopamine and serotonin synthesis pathway [28]; the opioid receptor *OPRM1* [29]; *P2X7R*, an ionotropic ATP-gated receptor [30]; *SLC6A4*, a serotonin transporter [31]; and multiple loci of *HLA* [32].

Genome-wide association studies (GWAS) have revolutionized the study of human diseases, including pain or pain-related diseases (reviewed in [6]). For example, significant progress has been made by multiple recent studies to identify genetic determinants associated with migraine, a chronic neurological disorder that can involve pain as well as nonpainful visual 'aura'. These studies found a strong association at chromosome 8q22 [33], in a SNP located between the genes *MTDH* (a predicted transmembrane adhesion protein with no reported knockout [KO] mice currently, but targeted embryonic stem [ES] cells are available; see [34]) and *PGCP* (a glutamate carboxypeptidase). A similar study reported variations near *TRPM8* (sensory ion channel involved in cold perception [35]), *LRP1* (low-density lipoprotein receptor-related protein gene also associated with Alzheimer's disease [36]), and *PRDM16* (a transcriptional cofactor that regulates TGF-β signaling [37]), all of which showed significant association with migraine [38]. Another GWAS for migraine [39] found an additional association with the TGF-β receptor (*TGFBR2*), sequences near the muscle transcription factor *MEF2D* [40], as well as the relatively uncharacterized genes *PHACTR1* and *ASTN2* (no reported KO mice for either; targeted ES cells available for *PHACTR1* but not *ASTN2*; see [34]). Finally, a very recent meta-analysis of 29 migraine GWAS identified an additional five candidate migraine susceptibility loci [41], four of which have KO mice or ES cells available. Beyond migraine, a recent meta-analysis for chronic widespread pain found some association at chromosome 5p15.2, a locus upstream of *CCT5* and downstream of *FAM173B* [42]. Neither gene appears to be particularly 'druggable', and no KO mouse has been published for either candidate, although targeted ES cells are available for both genes (again see [34]). Interestingly, *CCT5* interacts with PP4c, a phosphatase involved in central sensitization of pain signaling [43]. While the pain field anxiously awaits the publication of multiple ongoing nociception/pain GWAS studies, these reports on migraine genetics are encouraging. The majority of genes implicated in these studies are likely to be druggable, and further basic and translational investigation may lead to novel therapeutic targets for migraine.

Pain exome sequencing

As technologies have continued to evolve, the focus has also shifted from population GWAS aimed at identifying common genetic variation to exome sequencing targeted at identifying rare gene variants of high effect. The first of these exome-sequencing studies has now been published for subjects that exhibit extreme pain sensitivity or insensitivity [44]. Although no *bona fide* 'significant' rare variants were identified, the data analysis pipelines for these types of studies are still in their infancy. This study or similar studies may represent a rich source of information if combined with meta-analysis protocols that incorporate independent *a priori* knowledge. For example, one could target collection

or analyses of sequencing data to focus on conserved functional pain genes that are also identified in the fly, or pain GWAS loci as these are reported. Nevertheless, this study did highlight granzyme M, an immune protease involved in immunity against the herpes family cytomegalovirus [45]. While the mechanism, or even functional relevance, of this observation has not yet been confirmed, herpes virus infections are well known to alter pain perception in some patients (for example, see [46]).

Pain pharmacogenetics

On the other hand, pharmacogenetic approaches have helped to identify multiple coding variants that exert a strong effect on drug efficacy in humans [47]. One of the best-characterized analgesic pharmacogenetic genes is *CYP2D6*. *CYP2D6* is a member of the CYP450 family of mixed function oxidases. This liver enzyme acts on multiple drugs and, among other actions, converts codeine to bioactive morphine. There are over 80 allelic variants of *CYP2D6* [48], and this polymorphic locus explains a large portion of individual drug response variance within the general population. Other genes implicated in the pharmacogenetics of opioids include the morphine receptor *OPRM1* [49,50]; *COMT*, which controls the breakdown of epinephrine and dopamine [51]; the multidrug resistance transporter *ABCB1* [50]; and the melanocortin-1 receptor *MC1R* [52]. While major progress has been made towards identifying candidate pharmacogenetic 'diagnostic' tools to guide patient treatment, in which we now have over 2000 disease-linked variants [53], these techniques have not translated to improvements in the clinic. For example, in a study commissioned by the CDC examining the pharmacogenetic 'gold standard' CYP450 superfamily, it was reported that CYP450 genotyping did not improve patient outcome [54], and the use of testing for this variant was discouraged until further clinical trials were completed [55]. Given that individual patients will have hundreds of thousands of variations, strategies must be developed to prioritize and validate the key variations, if possible.

Interestingly, the first pain-related GWAS published was a pharmacogenomic study [56]. This study addressed the genomics of analgesic dosing and effect (lidocaine with epinephrine) following oral surgery in 60 women and 52 men of European descent. Despite being relatively small for a GWAS, a significant signal was found near an uncharacterized zinc-finger protein (predicted transcriptional regulator) on chromosome 19 (*ZNF429*). While *zfp160,* a mouse ortholog of this gene, is currently uncharacterized, targeted ES cell lines are available (see [34]). More recently, a GWAS for opioid sensitivity was performed on 355 people following cosmetic jaw surgery, and this effort highlighted a strong association at chromosome 2 ~q33.3, involving SNPs between *CREB1* and *METTL21A* [57]. *METTL21A* is uncharacterized and while no KO mice have been reported, ES cells are available (see [34]). *CREB1* is a component of the cAMP signaling pathway and has been long associated with opioid response [58]. These data help support the concept of pharmacogenomics as applied to analgesics. The clinical utility of these efforts, however, are not yet apparent, and in most cases genotyping provides no major advantages [58]; therefore, personalized opioid responses are currently titrated empirically based on the patients' needs [59]. While progress in identifying the genetics of pain disease and analgesic action have been promising, these results are just at the 'tip of the iceberg', and continued and substantial government investment are required to map the genetics of this complex process.

Functional validation of genomic & pharmacogenomic approaches

Despite the explosion of excitement for genomic mapping of complex traits, including pharmacogenomic profiles, these data on pain in humans will require functional validation before they can be applied to drug development or emerging efforts to initiate precision medicine strategies. Depending on the situation, validation of these data may be achieved through *in vitro* biochemical techniques, cell culture experiments, or in some cases using *ex vivo* or *in vivo* animal models. Given that pain or nociception is a whole-animal process, meaningful validation of large-scale GWAS and sequencing data may require *in vivo* models. Mice are powerful tools for pain research; however, large-scale systematic use is limited by ethical, time and cost issues. The use of the fruit fly to evaluate functionality in pain genomics and pharmacogenomics is an alternate option. Approximately 60% of human disease genes have orthologs in the fly [60,61], and flies are routinely used to model human diseases ranging from metabolic disorders, such as diabetes [62] or galactosemia [63], to neurological disorders such as Parkinson's disease (PD) [64], amyotrophic lateral sclerosis [65], Alzheimer's disease [66], Huntington's disease (HD) [67], fragile

X syndrome [68], seizure disorders [69], sleep disorders [70], cognitive dysfunctions such as learning and memory [71] or aggression [72], and pain [73–81]. Importantly, human genes and mutation variants are often functional in the fruit fly [82,83], highlighting the exquisite conservation of core physiological and pathophysiological elements.

Fruit flies for pain research

In a ground-breaking study conducted a decade ago, several fly models of acute nociception (heat and mechanical) were reported [73]. Currently, the nociceptive behaviors exhibited by *Drosophila* are used to study the genetics of mechanical [73,77,80], thermal [73,81], chemical [84] and chronic pain after UV exposure [74,78]. Although pain is an emotional sensation that is unique to humans, genes that regulate nociception in the fly have been identified, some of which are conserved through to humans. For example, *TRPA1* [77–79,81,84,85], *α2δ3* [76], *Piezo* [80,86], and homologs of TNF and TNFR74 [74], among other genes [76], contribute to abnormal nociception phenotypes in the fly when mutated, and also appear to play a role in mammalian or human pain. The completion of an *in vivo* transgenic fly RNAi library covering approximately the entire fly genome has further increased the utility of the fruit fly model for nociception research [87], and we have published a full-genome functional dissection of loss of nociception using this fly library [76]. As novel candidate pain genes or mutations are identified by conventional genetic or GWAS approaches, fly researchers can rapidly validate these genes as conserved 'pain' genes in the relevant fly nociception paradigm.

Flies for pharmacological research

Sequencing of the human and fly genome has highlighted the degree of conservation at both sequence and pathway levels [60,61]. This similarity is reflected by the fact that some bioactive compounds show activity in flies and humans. For example, *Drosophila* and humans respond similarly to wake-promoting compounds, such as modafinil [88], caffeine [89,90], crack cocaine [91] and methamphetamine [92], as well as sedatives such as antihistamines [89], antiepileptic treatments [93,94] and antipsychotic drugs [95]. Moreover, the ability to perform high-throughput screening in *Drosophila* via random mutagenesis or targeted RNAi-mediated knockdown protocols can further facilitate the identification of novel drug targets or drugs (**Figure 1**) [96]. To this end, a pilot fly screen evaluating 2000 compounds for novel antitumor drugs demonstrated that acivicin, a glutamine analogue that shows activity against human tumor cells, can also inhibit tumorigenesis in *Drosophila* [97]. Using RNAi-mediated knockdown of candidate acivicin target genes, CTP synthase was isolated as an important component of acivicin-mediated inhibition of tumor formation [97].

Several studies of PD have also exploited the utility of fruit flies for drug studies. Studies on the effects of anti-PD drugs in α-synuclein transgenic flies demonstrated that L-DOPA, pergolide, bromocriptine, 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine and atropine were effective in restoring normal locomotor function in flies suffering from PD-like disease [64]. In another study, reduced mitochondrial activity caused by the *pink1* mutation in a *Drosophila* PD model was rescued by vitamin K_2 [98]. Alternatively, screening of approximately 1000 known aminergic drugs for an ability to suppress a *Drosophila* model of PD resulted in the identification of 13 novel potential anti-PD drugs already used to treat other indications [99].

Similarly, in a fly model of fragile X syndrome (*Fmr1* mutant flies), a screen of 2000 compounds identified nine drugs that could block mutation-associated lethality [68]. Moreover, from a library of 4000 biologically active compounds, screening of a *Drosophila* model of HD identified five new drugs that block fly HD [67]. Of note, three out of these five drugs are already US FDA approved, indicating that pharmacological studies in flies may also lead to the repurposing of current available drug compounds. In addition, the *Drosophila* HD model has been used to confirm the effectiveness of C2–8, a potential HD drug, in reducing neurodegeneration *in vivo* [100]. While there is a current lack of published work on testing analgesics in flies, it is tempting to speculate that *Drosophila* will also be valuable for studying or evaluating candidate analgesics, especially with regard to mode of action, given the conserved nature of nociception across phyla.

Fruit fly GWAS

In human populations, GWAS approaches have proven useful for identifying novel candidate genetic components of disease or physiological phenotypes. Similar genotype/phenotype association studies can also be performed in flies. The *Drosophila melanogaster* Genetic Reference Panel (DGRP) is one example of an available resource to perform *Drosophila* GWAS, and consists of 192 inbred strains derived from a single natural population [101]. Since the complete sequences of each of these strains are available, phenotyping

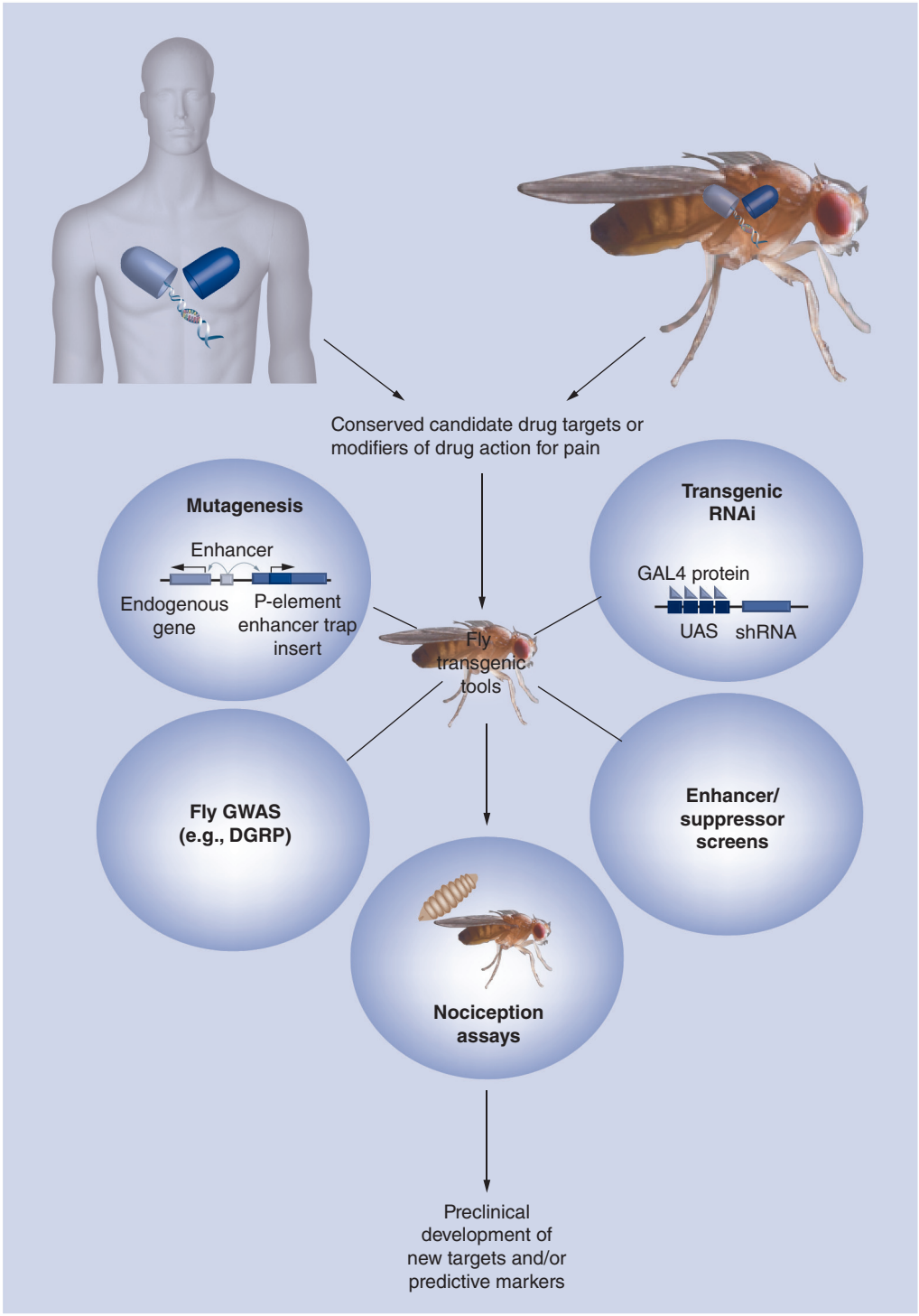

Figure 1. Human and fly pharmacogenomics can complement each other for the studying of pain and analgesia. Genomic and pharmacogenomics efforts in human and fly populations can help us to identify candidate analgesic drug targets or modifiers of analgesic drug action that are conserved across species. The functions of the identified targets can then be rapidly validated in the fly by the use of its diverse genetic tools, including examples such as P-element mutagenesis and enhancer trapping, the UAS–GAL4 method of tissue-specific RNA interference-mediated knockdown of target genes, the manipulation of available genome databases such as the DGRP [101], and enhancer/suppressor screening protocols. Nociception assays such as noxious heat avoidance in adult flies and larvae can be employed in parallel to validate the functional relevance of identified genes in nociception behaviors. This will ultimately lead to the preclinical identification and development of possible new targets or markers for pain that can be further explored in other surrogate models. DGRP: *Drosophila melanogaster* Genetic Reference Panel; GWAS: Genome-wide association study.

these populations can allow for rapid fly GWAS for inheritable traits. For example, these DGRP lines have been used to perform a fly GWAS to identify genetic variants that contribute to differences in resistance to starvation stress, life span, startle-induced locomotor response, mating speed and chill-induced coma recovery times [102].

Pharmacogenomics with fruit flies

This fly GWAS tool can also be used to perform pharmacogenomic GWAS on compounds that are bioactive in both humans and flies. An example is the investigation of genetic variants associated with oxidative stress resistance in *Drosophila* [103]. DGRP lines were used to perform a GWAS for variation associated with resistance to two oxidizing agents (paraquat and menadione sodium bisulfite). In this case, hundreds of candidate variants involved in resistance or susceptibility to these compounds were identified (some coding), and many of these genes are conserved through to humans. Another DGRP fly GWAS identified 13 genes associated with variations in sensitivity to oxidative stress induced by menadione sodium bisulfite [104]. From a pharmacogenomics perspective, it is relatively easy to screen for genetic variants that affect any compound that can exert a clear biological effect on a fruit fly using this fly GWAS tool, and thus allow rapid generation of pharmacogenomic profiles in flies. Moreover, pharmacogenomic prescreening in the fly may allow for targeted pharmacogenomics validation in human populations, and in this way avoid the stringent statistical requirements of a GWAS.

Pharmacogenomic studies in humans and genome scanning in flies have also been performed in parallel to identify genes involved in the modulation of the action of drug compounds. A recent investigation used a combined fly/human approach to address genes that may play a role in the protective role of nicotine in PD [105]. In this study, the investigators performed an underpowered GWAS for nicotine's protective role in human PD, and in concert performed a microarray for gene expression in flies treated with paraquat (a toxin used to model PD) and cotreated with varying doses of nicotine. Remarkably, both independent approaches highlighted fly and human versions of a synaptic vesicle protein, *SV2C*. Interestingly, the *SV2* family of genes has been implicated in the regulation of neurotransmitter storage and release [106], and is highly expressed in dopaminergic neurons in the substantia nigra [107]. It is currently unclear what impact *a priori* should have when analyzing GWAS results, and these pipelines are developing [108]. Had the researchers investigated fly expression data first, and then performed targeted analysis on human PD smokers versus nonsmokers, the *SV2C* variant would be considered a *bona fide* pharmacogenetic allele for the protective effects of nicotine on PD. Thus, fruit fly tools can be used to validate GWAS datasets or, alternatively, to instruct targeted assessment of cohorts to empower pharmacogenomics efforts for various human cohorts.

Conclusion & future perspective

Nociception, pain diseases and patient responses to various analgesics all have a heritable genetic component. As population genomics data flows *en masse*, strategies are required to assess factors ranging from function to interindividual variations, and, in cases such as pain, these validation strategies will likely require *in vivo* animal models. Fruit flies are a powerful tool for functional genomics research, and are well positioned to provide validation of GWAS or sequencing datasets, including pharmacogenomics efforts. This could be through target validation of new candidate human pain genes, or through small-molecule pharmacogenomics mapping using fly GWAS techniques (Figure 1). Moreover, in cases where reoccurring coding variants of known pain genes are observed, these mutant variants could be ectopically expressed in various central or peripheral nerves within the fly pain circuit, and effects on various nociceptive responses evaluated. This strategy could prove particularly effective for *in vivo* screening of small molecules that can alter function of rare mutant (or common) pain genes. Efforts to identify and validate genetic variation that affect pain thresholds or sensitivity to analgesics at the genome level can therefore help in future rational drug design and may one day instruct precision therapies.

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Executive summary

Pain genetics

- Targeted and genome-wide population studies have established multiple genes that are associated with pain or analgesic response; however, much more investment into human genome-wide association studies (GWAS) and sequencing efforts is required.
- In parallel, general research must validate the large amount of pain genomic and pharmacogenomic data in cell systems or model organisms.

Fruit flies for pain research

- Fruit fly nociception models can be used to test conserved functions of candidate pain genetics and analgesics.
- There are multiple fruit fly nociception paradigms available.

Fruit fly GWAS

- It is now possible to perform GWAS and genome-wide RNAi screening in flies.
- Targeted human SNP genotyping or sequencing in combination with fruit fly functional validation may be a powerful genomic or pharmacogenomic approach.

References

Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

1 Sherrington CS. Flexion-reflex of the limb, crossed extension-reflex, and reflex stepping and standing. *J. Physiol.* 40(1–2), 28–121 (1910).

2 Cox JJ, Reimann F, Nicholas AK *et al.* An SCN9A channelopathy causes congenital inability to experience pain. *Nature* 444(7121), 894–898 (2006).

▪▪ **Shows that chronic pain disorders can result from single gene mutations.**

3 Bouhassira D, Lanteri-Minet M, Attal N, Laurent B, Touboul C. Prevalence of chronic pain with neuropathic characteristics in the general population. *Pain* 136(3), 380–387 (2008).

4 Woolf CJ, Mannion RJ. Neuropathic pain: aetiology, symptoms, mechanisms, and management. *Lancet* 353(9168), 1959–1964 (1999).

5 Costigan M, Scholz J, Woolf CJ. Neuropathic pain: a maladaptive response of the nervous system to damage. *Annu. Rev. Neurosci.* 32, 1–32 (2009).

6 Young EE, Lariviere WR, Belfer I. Genetic basis of pain variability: recent advances. *J. Med. Genet.* 49(1), 1–9 (2012).

7 Woolf CJ. Overcoming obstacles to developing new analgesics. *Nat. Med.* 16(11), 1241–1247 (2010).

8 Ballantyne JC, LaForge KS. Opioid dependence and addiction during opioid treatment of chronic pain. *Pain* 129(3), 235–255 (2007).

9 Kingery WS. A critical review of controlled clinical trials for peripheral neuropathic pain and complex regional pain syndromes. *Pain* 73(2), 123–139 (1997).

10 Ogawa S, Satoh J, Arakawa A, Yoshiyama T, Suzuki M. Pregabalin treatment for peripheral neuropathic pain: a review of safety data from randomized controlled trials conducted in Japan and in the west. *Drug Saf.* 35(10), 793–806 (2012).

11 Dworkin RH, O'Connor AB, Backonja M *et al.* Pharmacologic management of neuropathic pain: evidence-based recommendations. *Pain* 132(3), 237–251 (2007).

12 Raouf R, Quick K, Wood JN. Pain as a channelopathy. *J. Clin. Invest.* 120(11), 3745–3752 (2010).

13 Goldberg YP, MacFarlane J, MacDonald ML *et al.* Loss-of-function mutations in the *Nav1.7* gene underlie congenital indifference to pain in multiple human populations. *Clin. Genet.* 71(4), 311–319 (2007).

14 Yang Y, Wang Y, Li S *et al.* Mutations in *SCN9A*, encoding a sodium channel alpha subunit, in patients with primary erythermalgia. *J. Med. Genet.* 41(3), 171–174 (2004).

15 Fertleman CR, Baker MD, Parker KA *et al.* *SCN9A* mutations in paroxysmal extreme pain disorder: allelic variants underlie distinct channel defects and phenotypes. *Neuron* 52(5), 767–774 (2006).

16 Kremeyer B, Lopera F, Cox JJ *et al.* A gain-of-function mutation in *TRPA1* causes familial episodic pain syndrome. *Neuron* 66(5), 671–680 (2010).

17 Ophoff RA, Terwindt GM, Vergouwe MN *et al.* Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca^{2+} channel gene CACNL1A4. *Cell* 87(3), 543–552 (1996).

18 De Fusco M, Marconi R, Silvestri L *et al.* Haploinsufficiency of ATP1A2 encoding the Na^+/K^+ pump alpha2 subunit associated with familial hemiplegic migraine type 2. *Nat. Genet.* 33(2), 192–196 (2003).

19 Dichgans M, Freilinger T, Eckstein G *et al.* Mutation in the neuronal voltage-gated sodium channel SCN1A in familial hemiplegic migraine. *Lancet* 366(9483), 371–377 (2005).

20 Mogil JS, Wilson SG, Bon K *et al.* Heritability of nociception II. 'Types' of nociception revealed by genetic correlation analysis. *Pain* 80(1–2), 83–93 (1999).

21 Mogil JS, Wilson SG, Bon K *et al.* Heritability of nociception I: responses of 11 inbred mouse strains on 12 measures of nociception. *Pain* 80(1–2), 67–82 (1999).

22 Diatchenko L, Slade GD, Nackley AG *et al.* Genetic basis for individual variations in pain perception and the development of a chronic pain condition. *Hum. Mol. Genet.* 14(1), 135–143 (2005).

23 Kato K, Sullivan PF, Evengard B, Pedersen NL. Importance of genetic influences on chronic widespread pain. *Arthritis Rheum.* 54(5), 1682–1686 (2006).

24 Norbury TA, MacGregor AJ, Urwin J, Spector TD, McMahon SB. Heritability of responses to painful stimuli in women: a classical twin study. *Brain* 130(Pt 11), 3041–3049 (2007).

25 Fernandez Robles CR, Degnan M, Candiotti KA. Pain and genetics. *Curr. Opin. Anaesthesiol.* 25(4), 444–449 (2012).

26 Belfer I, Segall S. COMT genetic variants and pain. *Drugs Today (Barc.)* 47(6), 457–467 (2011).

27 Costigan M, Latremoliere A, Woolf CJ. Analgesia by inhibiting tetrahydrobiopterin synthesis. *Curr. Opin. Pharmacol.* 12(1), 92–99 (2012).

28 Tegeder I, Costigan M, Griffin RS *et al.* GTP cyclohydrolase and tetrahydrobiopterin regulate pain sensitivity and persistence. *Nat. Med.* 12(11), 1269–1277 (2006).

29 Shabalina SA, Zaykin DV, Gris P *et al.* Expansion of the human mu-opioid receptor gene architecture: novel functional variants. *Hum. Mol. Genet.* 18(6), 1037–1051 (2009).

30 Sorge RE, Trang T, Dorfman R *et al.* Genetically determined P2X7 receptor pore formation regulates variability in chronic pain sensitivity. *Nat. Med.* 18(4), 595–599 (2012).

31 Herken H, Erdal E, Mutlu N *et al.* Possible association of temporomandibular joint pain and dysfunction with a polymorphism in the serotonin transporter gene. *Am. J. Orthod. Dentofacial Orthop.* 120(3), 308–313 (2001).

32 van Rooijen DE, Roelen DL, Verduijn W *et al.* Genetic HLA associations in complex regional pain syndrome with and without dystonia. *J. Pain* 13(8), 784–789 (2012).

33 Anttila V, Stefansson H, Kallela M *et al.* Genome-wide association study of migraine implicates a common susceptibility variant on 8q22.1. *Nat. Genet.* 42(10), 869–873 (2010).

34 Skarnes WC, Rosen B, West AP *et al.* A conditional knockout resource for the genome-wide study of mouse gene function. *Nature* 474(7351), 337–342 (2011).

35 Peier AM, Moqrich A, Hergarden AC *et al.* A TRP channel that senses cold stimuli and menthol. *Cell* 108(5), 705–715 (2002).

36 Liu Q, Zerbinatti CV, Zhang J *et al.* Amyloid precursor protein regulates brain apolipoprotein E and cholesterol metabolism through lipoprotein receptor LRP1. *Neuron* 56(1), 66–78 (2007).

37 Bjork BC, Turbe-Doan A, Prysak M, Herron BJ, Beier DR. Prdm16 is required for normal palatogenesis in mice. *Hum. Mol. Genet.* 19(5), 774–789 (2010).

38 Chasman DI, Schurks M, Anttila V *et al.* Genome-wide association study reveals three susceptibility loci for common migraine in the general population. *Nat. Genet.* 43(7), 695–698 (2011).

39 Freilinger T, Anttila V, de Vries B *et al.* Genome-wide association analysis identifies susceptibility loci for migraine without aura. *Nat. Genet.* 44(7), 777–782 (2012).

40 Arnold MA, Kim Y, Czubryt MP *et al.* MEF2C transcription factor controls chondrocyte hypertrophy and bone development. *Dev. Cell* 12(3), 377–389 (2007).

41 Anttila V, Winsvold BS, Gormley P *et al.* Genome-wide meta-analysis identifies new susceptibility loci for migraine. *Nat. Genet.* 45(8), 912–917 (2013).

42 Peters MJ, Broer L, Willemen HL *et al.* Genome-wide association study meta-analysis of chronic widespread pain: evidence for involvement of the 5p15.2 region. *Ann. Rheum. Dis.* 72(3), 427–436 (2013).

43 Zhang X, Ozawa Y, Lee H *et al.* Histone deacetylase 3 (HDAC3) activity is regulated by interaction with protein serine/threonine phosphatase 4. *Genes Dev.* 19(7), 827–839 (2005).

44 Williams FM, Scollen S, Cao D *et al.* Genes contributing to pain sensitivity in the normal population: an exome sequencing study. *PLoS Genet.* 8(12), e1003095 (2012).

45 Pao LI, Sumaria N, Kelly JM *et al.* Functional analysis of granzyme M and its role in immunity to infection. *J. Immunol.* 175(5), 3235–3243 (2005).

46 Kallio-Laine K, Seppanen M, Lokki ML *et al.* Widespread unilateral pain associated with herpes simplex virus infections. *J. Pain* 9(7), 658–665 (2008).

47 Vuilleumier PH, Stamer UM, Landau R. Pharmacogenomic considerations in opioid analgesia. *Pharmacogenomics Pers. Med.* 5, 73–87 (2012).

48 Wang B, Yang LP, Zhang XZ, Huang SQ, Bartlam M, Zhou SF. New insights into the structural characteristics and functional relevance of the human cytochrome P450 2D6 enzyme. *Drug Metab. Rev.* 41(4), 573–643 (2009).

49 Chou WY, Wang CH, Liu PH, Liu CC, Tseng CC, Jawan B. Human opioid receptor A118G polymorphism affects intravenous patient-controlled analgesia morphine consumption after total abdominal hysterectomy. *Anesthesiology* 105(2), 334–337 (2006).

50 Campa D, Gioia A, Tomei A, Poli P, Barale R. Association of ABCB1/MDR1 and OPRM1 gene polymorphisms with morphine pain relief. *Clin. Pharmacol. Ther.* 83(4), 559–566 (2008).

51 Reyes-Gibby CC, Shete S, Rakvag T *et al.* Exploring joint effects of genes and the clinical efficacy of morphine for cancer pain: OPRM1 and COMT gene. *Pain* 130(1–2), 25–30 (2007).

52 Mogil JS, Wilson SG, Chesler EJ *et al.* The melanocortin-1 receptor gene mediates female-specific mechanisms of analgesia in mice and humans. *Proc. Natl Acad. Sci. USA* 100(8), 4867–4872 (2003).

53 Mirnezami R, Nicholson J, Darzi A. Preparing for precision medicine. *N. Engl. J. Med.* 366(6), 489–491 (2012).

54 Katsanis SH, Javitt G, Hudson K. Public health. A case study of personalized medicine. *Science* 320(5872), 53–54 (2008).

55 Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group. Recommendations from the EGAPP Working Group: testing for cytochrome P450 polymorphisms in adults with nonpsychotic depression treated with selective serotonin reuptake inhibitors. *Genet. Med.* 9(12), 819–825 (2007).

56 Kim H, Ramsay E, Lee H, Wahl S, Dionne RA. Genome-wide association study of acute post-surgical pain in humans. *Pharmacogenomics* 10(2), 171–179 (2009).

57 Nishizawa D, Fukuda K, Kasai S *et al.* Genome-wide association study identifies a potent locus associated with human opioid sensitivity. *Mol. Psychiatry* 9, 34 (2012).

58 Lane-Ladd SB, Pineda J, Boundy VA *et al.* CREB (cAMP response element-binding protein) in the locus coeruleus: biochemical, physiological, and behavioral evidence for a role in opiate dependence. *J. Neurosci.* 17(20), 7890–7901 (1997).

59 Hajj A, Khabbaz L, Laplanche JL, Peoc'h K. Pharmacogenetics of opiates in clinical practice: the visible tip of the iceberg. *Pharmacogenomics* 14(5), 575–585 (2013).

60 Bier E. *Drosophila*, the golden bug, emerges as a tool for human genetics. *Nat. Rev. Genet.* 6(1), 9–23 (2005).

61 Rubin GM, Yandell MD, Wortman JR *et al.* Comparative genomics of the eukaryotes. *Science* 287(5461), 2204–2215 (2000).

62 Rulifson EJ, Kim SK, Nusse R. Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. *Science* 296(5570), 1118–1120 (2002).

63 Kushner RF, Ryan EL, Sefton JM *et al.* A *Drosophila* melanogaster model of classic galactosemia. *Dis. Model. Mech.* 3(9–10), 618–627 (2010).

64 Pendleton RG, Parvez F, Sayed M, Hillman R. Effects of pharmacological agents upon a transgenic model of Parkinson's disease in *Drosophila* melanogaster. *J. Pharmacol. Exp. Ther.* 300(1), 91–96 (2002).

▪▪ **Evidence of similar pharmacodynamic properties of drugs used for human diseases when administered to flies.**

65 Forrest S, Chai A, Sanhueza M *et al.* Increased levels of phosphoinositides cause neurodegeneration in a *Drosophila* model of amyotrophic lateral sclerosis. *Hum. Mol. Genet.* 22(13), 2689–2704 (2013).

66 Lang M, Wang L, Fan Q *et al.* Genetic inhibition of solute-linked carrier 39 family transporter 1 ameliorates a beta pathology in a *Drosophila* model of Alzheimer's disease. *PLoS Genet.* 8(4), e1002683 (2012).

67 Desai UA, Pallos J, Ma AA *et al.* Biologically active molecules that reduce polyglutamine aggregation and toxicity. *Hum. Mol. Genet.* 15(13), 2114–2124 (2006).

68 Chang S, Bray SM, Li Z *et al.* Identification of small molecules rescuing fragile X syndrome phenotypes in *Drosophila*. *Nat. Chem. Biol.* 2008; 4(4), 256–263 (2008).

69 Sun L, Gilligan J, Staber C *et al.* A knock-in model of human epilepsy in *Drosophila* reveals a novel cellular mechanism associated with heat-induced seizure. *J. Neurosci.* 32(41), 14145–14155 (2012).

70 Freeman A, Pranski E, Miller RD *et al.* Sleep fragmentation and motor restlessness in a *Drosophila* model of restless legs syndrome. *Curr. Biol.* 22(12), 1142–1148 (2012).

71 Levin LR, Han PL, Hwang PM, Feinstein PG, Davis RL, Reed RR. The *Drosophila* learning and memory gene rutabaga encodes a Ca^{2+}/calmodulin-responsive adenylyl cyclase. *Cell* 68(3), 479–489 (1992).

72 Chen S, Lee AY, Bowens NM, Huber R, Kravitz EA. Fighting fruit flies: a model system for the study of aggression. *Proc. Natl Acad. Sci. USA* 99(8), 5664–5668 (2002).

73 Tracey WD, Wilson RI, Laurent G, Benzer S. Painless, a *Drosophila* gene essential for nociception. *Cell* 113(2), 261–273 (2003).

▪▪ **First landmark gene study of pain in *Drosophila melanogaster*.**

74 Babcock DT, Landry C, Galko MJ. Cytokine signaling mediates UV-induced nociceptive sensitization in *Drosophila* larvae. *Curr. Biol.* 19(10), 799–806 (2009).

75 Kim SH, Lee Y, Akitake B, Woodward OM, Guggino WB, Montell C. *Drosophila* TRPA1 channel mediates chemical avoidance in gustatory receptor neurons. *Proc. Natl Acad. Sci. USA* 107(18), 8440–8445 (2010).

▪ **Conserved pain genetics in flies and humans.**

76 Neely GG, Hess A, Costigan M *et al.* A genome-wide *Drosophila* screen for heat nociception identifies alpha2delta3 as an evolutionarily conserved pain gene. *Cell* 143(4), 628–638 (2010).

▪ **Conserved pain genetics in flies and humans.**

77 Zhong L, Hwang RY, Tracey WD. Pickpocket is a DEG/ENaC protein required for mechanical nociception in *Drosophila* larvae. *Curr. Biol.* 20(5), 429–434 (2010).

78 Babcock DT, Shi S, Jo J, Shaw M, Gutstein HB, Galko MJ. Hedgehog signaling regulates nociceptive sensitization. *Curr. Biol.* 21(18), 1525–1533 (2011).

79 Neely GG, Keene AC, Duchek P *et al.* TrpA1 regulates thermal nociception in *Drosophila*. *PLoS ONE* 6(8), e24343 (2011).

80 Kim SE, Coste B, Chadha A, Cook B, Patapoutian A. The role of *Drosophila* Piezo in mechanical nociception. *Nature* 483(7388), 209–212 (2012).

▪ **Conserved pain genetics in flies and humans.**

81 Zhong L, Bellemer A, Yan H *et al.* Thermosensory and nonthermosensory isoforms of *Drosophila* melanogaster TRPA1 reveal heat-sensor domains of a thermoTRP channel. *Cell Rep.* 1(1), 43–55 (2012).

82 Wolf MJ, Amrein H, Izatt JA, Choma MA, Reedy MC, Rockman HA. *Drosophila* as a model for the identification of genes causing adult human heart disease. *Proc. Natl Acad. Sci. USA* 103(5), 1394–1399 (2006).

83 Yamaguchi M, Hirose F, Inoue YH *et al.* Ectopic expression of human p53 inhibits entry into S phase and induces apoptosis in the *Drosophila* eye imaginal disc. *Oncogene* 18(48), 6767–6775 (1999).

84 Kang K, Pulver SR, Panzano VC *et al.* Analysis of *Drosophila* TRPA1 reveals an ancient origin for human chemical nociception. *Nature* 464(7288), 597–600 (2010).

85 del Camino D, Murphy S, Heiry M *et al.* TRPA1 contributes to cold hypersensitivity. *J. Neurosci.* 30(45), 15165–15174 (2010).

86 Coste B, Xiao B, Santos JS *et al.* Piezo proteins are pore-forming subunits of mechanically activated channels. *Nature* 483(7388), 176–181 (2012).

87 Dietzl G, Chen D, Schnorrer F *et al.* A genome-wide transgenic RNAi library for conditional gene inactivation in *Drosophila*. *Nature* 448(7150), 151–156 (2007).

88 Hendricks JC, Kirk D, Panckeri K, Miller MS, Pack AI. Modafinil maintains waking in the fruit fly *Drosophila melanogaster*. *Sleep* 26(2), 139–146 (2003).

89 Shaw PJ, Cirelli C, Greenspan RJ, Tononi G. Correlates of sleep and waking in *Drosophila melanogaster*. *Science* 287(5459), 1834–1837 (2000).

90 Andretic R, Kim YC, Jones FS, Han KA, Greenspan RJ. *Drosophila* D1 dopamine receptor mediates caffeine-induced arousal. *Proc. Natl Acad. Sci. USA* 105(51), 20392–20397 (2008).

91 McClung C, Hirsh J. Stereotypic behavioral responses to free-base cocaine and the development of behavioral sensitization in *Drosophila*. *Curr. Biol.* 8(2), 109–112 (1998).

92 Andretic R, van Swinderen B, Greenspan RJ. Dopaminergic modulation of arousal in *Drosophila*. *Curr. Biol.* 15(13), 1165–1175 (2005).

93 Tan JS, Lin F, Tanouye MA. Potassium bromide, an anticonvulsant, is effective at alleviating seizures in the *Drosophila* bang-sensitive mutant bang senseless. *Brain Res.* 1020(1–2), 45–52 (2004).

94 Reynolds ER, Stauffer EA, Feeney L, Rojahn E, Jacobs B, McKeever C. Treatment with the antiepileptic drugs phenytoin and gabapentin ameliorates seizure and paralysis of *Drosophila* bang-sensitive mutants. *J. Neurobiol.* 58(4), 503–513 (2004).

▪▪ **Evidence of similar pharmacodynamic properties of drugs used for human diseases when administered to flies.**

95 Dokucu ME, Yu L, Taghert PH. Lithium- and valproate-induced alterations in circadian locomotor behavior in *Drosophila*. *Neuropsychopharmacology* 30(12), 2216–2224 (2005).

96 Bell AJ, McBride SM, Dockendorff TC. Flies as the ointment: *Drosophila* modeling to enhance drug discovery. *Fly* 3(1), 39–49 (2009).

97 Willoughby LF, Schlosser T, Manning SA *et al.* An *in vivo* large-scale chemical screening platform using *Drosophila* for anti-cancer drug discovery. *Dis. Model. Mech.* 6(2), 521–529 (2013).

98 Vos M, Esposito G, Edirisinghe JN *et al.* Vitamin K2 is a mitochondrial electron carrier that rescues pink1 deficiency. *Science* 336(6086), 1306–1310 (2012).

99 Lawal HO, Terrell A, Lam HA *et al. Drosophila* modifier screens to identify novel neuropsychiatric drugs including aminergic agents for the possible treatment of Parkinson's disease and depression. *Mol. Psychiatry* doi:10.1038/mp.2012.170 (2012) (Epub ahead of print).

100 Zhang X, Smith DL, Meriin AB *et al.* A potent small molecule inhibits polyglutamine aggregation in Huntington's disease neurons and suppresses neurodegeneration *in vivo*. *Proc. Natl Acad. Sci. USA* 102(3), 892–897 (2005).

101 Mackay TF, Richards S, Stone EA *et al.* The *Drosophila* melanogaster genetic reference panel. *Nature* 482(7384), 173–178 (2012).

▪▪ **A landmark study that constructed a library of fly strains that allows for the investigation of genotype/phenotype associations of complex traits in flies.**

102 Ayroles JF, Carbone MA, Stone EA *et al.* Systems genetics of complex traits in *Drosophila* melanogaster. *Nat. Genet.* 41(3), 299–307 (2009).

103 Weber AL, Khan GF, Magwire MM, Tabor CL, Mackay TF, Anholt RR. Genome-wide association analysis of oxidative stress resistance in *Drosophila* melanogaster. *PLoS ONE* 7(4), e34745 (2012).

104 Jordan KW, Craver KL, Magwire MM, Cubilla CE, Mackay TF, Anholt RR. Genome-wide association for sensitivity to chronic oxidative stress in *Drosophila* melanogaster. *PLoS ONE* 7(6), e38722 (2012).

105 Hill-Burns EM, Singh N, Ganguly P *et al.* A genetic basis for the variable effect of smoking/nicotine on Parkinson's disease. *Pharmacogenomics J.* (2012).

106 Nowack A, Yao J, Custer KL, Bajjalieh SM. SV2 regulates neurotransmitter release via multiple mechanisms. *Am. J. Physiol. Cell Physiol.* 299(5), C960–C967 (2010).

107 Janz R, Sudhof TC. SV2C is a synaptic vesicle protein with an unusually restricted localization: anatomy of a synaptic vesicle protein family. *Neuroscience* 94(4), 1279–1290 (1999).

108 Minelli C, De Grandi A, Weichenberger CX *et al.* Importance of different types of prior knowledge in selecting genome-wide findings for follow-up. *Genet. Epidemiol.* 37(2), 205–213 (2013).