A Genetic Screen for Genes That Impact Peroxisomes in Drosophila Identifies Candidate Genes for Human Disease

Hillary K. Graves,* Sharayu Jangam,* Kai Li Tan,* Antonella Pignata,* Elaine S. Seto,* Shinya Yamamoto,*†,‡,§,1 and Michael F. Wangler*†,‡,§,1

*Department of Molecular and Human Genetics, †Department of Neuroscience, ‡Program in Developmental Biology, Baylor College of Medicine, Houston, TX 77030, and §Jan and Dan Duncan Neurological Research Institute, Texas Children Hospital, Houston, TX 77030

ORCID IDs: 0000-0001-7389-0890 (S.J.); 0000-0002-1450-7916 (K.L.T.); 0000-0003-2172-8036 (S.Y.)

ABSTRACT Peroxisomes are subcellular organelles that are essential for proper function of eukaryotic cells. In addition to being the sites of a variety of oxidative reactions, they are crucial regulators of lipid metabolism. Peroxosome loss or dysfunction leads to multi-system diseases in humans that strongly affect the nervous system. In order to identify previously unidentified genes and mechanisms that impact peroxisomes, we conducted a genetic screen on a collection of lethal mutations on the X chromosome in Drosophila. Using the number, size and morphology of GFP tagged peroxisomes as a readout, we screened for mutations that altered peroxisomes based on clonal analysis and confocal microscopy. From this screen, we identified eighteen genes that cause increases in peroxisome number or altered morphology when mutated. We examined the human homologs of these genes and found that they are involved in a diverse array of cellular processes. Interestingly, the human homologs from the X-chromosome collection are under selective constraint in human populations and are good candidate genes particularly for dominant genetic disease. This in vivo screening approach for peroxisome defects allows identification of novel genes that impact peroxisomes in vivo in a multicellular organism and is a valuable platform to discover genes potentially involved in dominant disease that could affect peroxisomes.

KEYWORDS Drosophila peroxisomes BRD4 fs(1)h

Peroxisomes are subcellular organelles that mediate crucial biological processes in eukaryotic cells, including oxidative reactions, catabolism of very-long-chain fatty acids, catabolism of branched chain fatty acids, synthesis of bile acids, and biosynthesis of plasmalogen lipids (Fujiki et al. 2014; Wanders 2014). Human diseases caused by lack of peroxisomes are devastating multisystem diseases that result in severe brain, liver, bone and kidney disease (Wanders and Waterham 2005). These conditions, called peroxisome biogenesis disorders Zellweger-spectrum disorders (PBD-ZSD), are a group of multi-system autosomal recessive disorders with severe central nervous system (CNS) manifestations and as yet no effective treatments exist for the hearing, visual and CNS phenotypes (Klouwer et al. 2015; Braverman et al. 2016).

Historically, genetic screens for biochemical phenotypes have identified genes implicated in peroxisome biogenesis, pexophagy, and peroxisomal biochemistry (Subramani 1998; Kao et al. 2018). More recently, microscopy-based screens have uncovered genes implicated in peroxisome morphology (Baron et al. 2016; Yofe et al. 2017). These studies have shown that the pathways that regulate peroxisome dynamics (i.e., peroxisome size and number) remain incompletely understood (Mast et al. 2015). This is especially true with regards to the information gained from multicellular organisms as opposed to yeast and cell models because most essential genes are not amenable to peroxisomal studies due to early lethality in development. Although null alleles in some of the Pex genes in the fruit fly Drosophila melanogaster produce viable
**Methods & Materials**

Drosophila X-Chromosome peroxisome (X-Pex) screen

All X-linked recessive lethal mutant alleles utilized in this paper listed in Table 1 and Supplemental Material, Table 1 were generated on an isogenized y¹ w¹ FRT19A chromosome using ethyl methanesulfonate (EMS) mutagenesis as described (Haeltzman et al. 2014; Yamamoto et al. 2014; Deal and Yamamoto 2019). These fly strains are publicly available from the Kyoto Stock Center (https://kyotofly.kit.jp/stocks/documents/EMS_X_lethals.html) or the Bloomington Drosophila Stock Center (https://bdsc.indiana.edu/stocks/chemically_induced_mutations/xlethals.html). Heterozygous females (y¹ w¹ mut¹ FRT19A/FM7c Kr-GALA, UAS-GFP, mut¹ indicates the mutation of interest) from these lethal lines were crossed to males of the genotype hsFLP, Ubi-RFP FRT19A; Actin-GALA, UAS-GFP-SKL/CyO, and their embryonic progeny were heat shocked at 0-4 hr after egg laying at 37°C for 1 hr. Third larval instar wandering larvae were dissected in PBS and fixed in 4% paraformaldehyde for 20-30 min. Fat bodies were mounted in DAPI and screened for peroxisomal phenotypes using GFP tagged peroxisomes (Chao et al. 2016; Yamagata et al. 2017a) in conjunction with clonal analysis, allowing generation of homozygous mutant cells within Drosophila larval fat body in an otherwise heterozygous animal to bypass early lethality. Our screen identified a number of genes not previously implicated in peroxisome dynamics or regulation. In previous studies, we’ve shown that the genes from this collection are enriched for human disease genes (Yamamoto et al. 2014). Based on this we propose our screen results as identifying candidate human disease genes particularly for dominant disease that may impact peroxisomes.

**Table 1** Hits from the Peroxisome X-pex screen

| Fly Gene | Allele(s) | Peroxisomal Phenotype | Human Gene(s) | Biological Function in Fly (UniProt) |
|----------|-----------|-----------------------|---------------|-------------------------------------|
| fs(1)h (FBgn0004656) | fs(1)[hA], [B], [C] | Category A & B | BRD2, BRD2, BRD3, BRD4 | Transcriptional regulation |
| Rbcn-3B (FBgn0023510) | Rbcn-3B[A], [B] | Category A | WDR7 | Vacuolar acidification, Notch signaling |
| Coq8 (FBgn0052649) | Coq8[A],[B] | Category A | COQ8B | Protein kinase, electron transport |
| Usp16-45 (FBgn0029763) | Usp16-45[A],[B], [C] | Category A | USP45 | Protein deubiquitination |
| mxc (FBgn0260789) | mxc[A],[B],[C],[D],[E] | Category A | No Human Ortholog | Transcriptional, hemocyte differentiation and proliferation |
| Cp7Fb (FBgn0014465) | Cp7Fb[A] | Category A | No Human Ortholog | Chorion |
| Upf1 (FBgn0033524) | Upf1[A],[B] | Category A | UPF1 | Nononsense mediated decay |
| Upf2 (FBgn0029992) | Upf2[A],[B] | Category A | UPF2 | Nononsense mediated decay |
| Nrg (FBgn0264975) | Nrg[XB] | Category A | NRCAM, NFASC, L1CAM, CHL1 | Cell adhesion |
| Fum1 (FBgn0286222) | Fum1[A] | Category A | FH | TCA cycle enzyme (mitochondria) |
| Coq7 (FBgn0029502) | Coq7[B] | Category A | COQ7 | Ubiquinone biosynthesis (mitochondria) |
| sgg (FBgn0003371) | sgg[A],[B], [E] | Category A | GSK3B, GSK3A | Protein kinase |
| CG17829 (FBgn0025635) | CG17829[A],[B] | Category A | HIF1 | Transcriptional regulation |
| CG3149 (FBgn0027564) | CG3149[B] | Category A | RFT1 | Glycolipid translocation |
| Smox (FBgn0025800) | Smox[B] | Category A | SMAD3 | Transcriptional regulation |
| Pl4Kika (FBgn0267350) | Pl4Kika [E], [W] | Category A | Pl4KA | Synaptic growth, cell polarity, membrane organization |
| MTPAP (FBgn0024360) | MTPAP[A],[B] | Category A | MTPAP | Mitochondrial transcription |
| temp (FBgn0027296) | temp [A], [B] & [D] | Category A | P7AR1 | Rab geranylgeranyltransferase activity |
| **TOTAL** | 37 hits | 18 fly genes | 23 human genes | |

The Fly gene and specific allele are listed along with the phenotype observed in the screen (Category A, B and C). The human homologs of each gene were identified using DIOPT or HCOP (Hu et al. 2011; Braschi et al. 2019). Known biological function of the fly protein is listed according to the annotation in UniProt (Uniprot Consortium 2019).
Similar secondary screens were performed on the same X-lethal collection to identify regulators of other biological processes such as ring canal formation and somatic stem cell maintenance during oogenesis (Yamamoto et al. 2013; Cook et al. 2017) demonstrating the value of this collection in screening for genes involved in diverse cellular processes. We named our screen the “X-Pex” putting the fly X-chromosome together with an abbreviation of peroxisome. We will refer to the gene set and the screen as “X-Pex” throughout the manuscript.

ImageJ Protocol for peroxisomal area counting
Different scans of the original Z-stack images were used to calculate the area of the peroxisomes. The individual scan is divided in GFP-SKL(green layer) and RFP(red layer), converted to black and white and then by finding the edges the area of each peroxisome is calculated with freehand selection tool or wand tool. The detailed protocol is presented in the supplementary file.

Human gene candidate analysis
Human homologs from the fly genes were determined using the Human Gene Nomenclature Orthology Prediction (HCOP, https://www.genenames.org/tools/hcop/) and the Drosophila RNAi Screening center Integrative Ortholog Prediction Tool (DIOPT, https://www.flyrai.org/cgi-bin/DRSC_orthologs.pl) tools (Hu et al. 2011; Gray et al. 2015). These genes were further examined in a series of public human and model organism databases using the MARRVEL tool (http://marrvel.org/) to gather information about the homologous proteins in human and other model organisms (Wang et al. 2017). Human gene nomenclature was confirmed using the HGNC (HUGO Gene Nomenclature Committee, https://www.genenames.org) database (Braschi et al. 2019). Mendelian disease links were explored in the OMIM (https://www.omim.org/) database (Amberger et al. 2015), and each gene was
examined using the gnomAD (https://gnomad.broadinstitute.org/) browser (Lek et al. 2016). Each gene was also examined using the DOMINO tool for predicted likelihood of a gene having dominant impact on disease (Quinodoz et al. 2017). In addition, de novo events were examined in denovo-db (http://denovo-db.gs.washington.edu/denovo-db/) website (Turner et al. 2017).

**Data availability**

All the supplemental data files are available on the GSA figshare portal including the Supplemental Tables listed in the manuscript. Supplemental Table I lists all the Drosophila reagents that are available from the X-screen through public stock centers including the Bloomington stock center and Kyoto Stock center. Supplemental material available at figshare: https://doi.org/10.25387/g3.8872547.

---

**RESULTS AND DISCUSSION**

**Identification of genes involved in peroxisomal dynamics**

To identify new regulators of peroxisomal morphology and dynamics, we performed a screen with the collection of recessive lethal X-chromosome mutants that have been extensively studied by our group (Yamamoto et al. 2014). We had previously generated a large collection of recessive lethal mutant lines on an isogenized chromosome and these lines have been extensively screened for developmental and neurological phenotypes (Yamamoto et al. 2014; Deal and Yamamoto 2019). Moreover, we previously utilized this collection of mutations to uncover new human disease genes and showed that human orthologs of these fly essential genes are enriched for...
genes listed in the Online Mendelian Inheritance in Man (OMIM) disease database (Yamamoto et al. 2014; Yoon et al. 2017; Tan et al. 2018). We took 215 lines from this collection that correspond to 100 genes (98 mapped genes and 2 unmapped complementation group) and screened for peroxisomal phenotypes using GFP tagged peroxisomes (Chao et al. 2016; Wangler et al. 2017a) in conjunction with clonal analysis, allowing generation of homozygous mutant cells within Drosophila larval fat body in an otherwise heterozygous animal to bypass early lethality.

It is important to note that X-chromosome contains ~15% of protein coding genes in Drosophila, and there is no correlation between X-linked genes in flies and humans. In this screen, we created homozygous mutant clones in the fat body of developing Drosophila larvae in an otherwise heterozygous animal and assayed for changes in the distribution pattern of a peroxisomal reporter, GFP-SKL (Figure 1A). GFP-SKL is a GFP with a C-terminal peroxisomal localization signal, which we have previously shown to be an accurate marker for peroxisomal dynamics (Chao et al. 2016; Wangler et al. 2017a). We have previously observed examples of Category B in mutants with peroxisomal fission defects (Chao et al. 2016) and Category C in biogenesis defects (Wangler et al. 2017a) in Drosophila. We screened 215 lethal mutant lines from the already available collection that was mapped to a complementation group or to a gene (Figure 1D, Supplemental Table 1). We considered a hit to be positive when multiple independent reporters could differentiate a clear difference in the mutant clone compared to the surrounding tissue (heterozygous or homozygous wild-type) cells (Supplementary Figure 1). In total we identified 37 alleles corresponding to 18 genes (Table 1). For these hits, when possible we assayed two or more alleles per gene to confirm a change in GFP-SKL if possible. Some of these mutations led to an inconsistent increase of the peroxisome population, suggesting that perdurance of the wild type protein or other unmeasured factors may mask a change in peroxisomal dynamics in some clones. Interestingly, an overwhelming majority of the lines fell into Category A (Table 1). Although there are a few known peroxisomal related genes that are located on the Drosophila X-chromosome including Pex5 (homolog of human PEX5) and Mfe2 (homolog of human HSD17B4) (Faust et al. 2012), these were not part of the collection we screened here (Yamamoto et al. 2014). We

---

**Figure 3** Peroxisomal morphological quantification. A. Higher magnification images of fat bodies of Usp16-45 Fig. 3A-A” were taken on Zeiss710-LSM microscope with 63X objective of NA 1.4 with 16 bit depth and 512x512 resolution. The scale bars noted in the image are 10µm. Note A is the image shown in Figure 1C to illustrate the Category A phenotype, and A” is the GFP channel while A’” is the GFP channel. B. Higher magnification images of fat bodies of fs(h)1 Fig 3B-B” were taken on Zeiss710-LSM microscope with 63X objective of NA 1.4 with 16 bit depth and 512x512 resolution. The scale bars noted in the image are 10µm. B is the image shown in Figure 1C to illustrate the Category B phenotype, and B” is the GFP channel. C. Quantification of number of peroxisomes present per µm² of Clonal and non-clonal area. This data represents the number of peroxisomes present per µm² of clonal/non-clonal area. Peroxisomes from five different stacks were counted with ImageJ and then divided with the total clonal/non-clonal area in that individual stack to get these data points. D. Quantification of percent area of total peroxisomes. The areas covered by peroxisomes in clonal as well as non-clonal regions from five different stacks were counted with ImageJ. The total of those peroxisomal areas in the same stack is divided by clonal/non-clonal area as shown in A’ and B’ of the same stack. The percent value of this is counted as one data point.
Table 2 Human Gene Candidate Analysis

| Human Gene | Known Disease in OMIM | pLI | pLI o/e | Missense Z score | Missense Z score o/e | Domino |
|------------|-----------------------|-----|---------|------------------|----------------------|--------|
| GSK3A      | None                  | 0   | 1       | 3.2              | 0.43                 | 1A     |
| BRD4       | None                  | 1   | 0       | 3.74             | 0.63                 | 0.997A |
| URF1       | None                  | 1   | 0.07    | 5.7              | 0.41                 | 0.996A |
| L1CAM      | # 304100, # 303350, # 307000 | 1   | 0.04    | 2.84             | 0.66                 | n/a    |
| NFASC      | # 618356              | 1   | 0.12    | 2.59             | 0.74                 | 0.871A |
| BRD3       | None                  | 0.98| 0.14    | 3.76             | 0.64                 | 0.893A |
| GSK3B      | None                  | 0.96| 0.14    | 2.91             | 0.48                 | 0.999A |
| SMAD3      | # 613795              | 0.84| 0.17    | 3.47             | 0.39                 | 0.723B |
| URF2       | None                  | 1   | 0.03    | 3.3               | 0.65                 | 0.636B |
| WDR7       | None                  | 1   | 0.10    | 2.63             | 0.75                 | 0.636B |
| BRD2       | None                  | 1   | 0.08    | 0.5              | 0.93                 | 0.474C |
| MTPAP       | # 613672              | 1   | 0.04    | 1.05             | 0.84                 | 0.242D |
| PTAR1      | None                  | 0.85| 0.15    | 1.51             | 0.71                 | 0.275D |
| NRCAM       | None                  | 0.18| 0.24    | 2.05             | 0.79                 | 0.191E |
| FH         | # 606812 - # 150800   | 0.09| 0.28    | 1.39             | 0.77                 | 0.371D |
| HIFP       | None                  | 0.03| 0.29    | 1.73             | 0.73                 | 0.719B |
| PI4KA      | # 616531              | 0   | 0.36    | 3.53             | 0.72                 | 0.589C |
| BRDT       | # 617644              | 0   | 0.46    | 0.47             | 0.94                 | 0.209D |
| CHL1       | None                  | 0   | 0.47    | −1.92            | 1.21                 | 0.152E |
| USP45      | None                  | 0   | 0.74    | 0.75             | 0.90                 | 0.168E |
| COQ7       | # 616733              | 0   | 0.88    | −0.42            | 1.10                 | 0.091E |
| RFT1       | # 612015              | 0   | 0.77    | 0.97             | 0.84                 | 0.063E |
| COQ8B (a.k.a. ADCK4) | # 615573 | 0   | 0.78    | 0.89             | 0.87                 | 0.056E |

The human homologs of the X-Pex genes were examined for known Mendelian disease association (OMIM # entries) with genes that are not known to cause disease shown in red (Amberger et al. 2015). These are further sorted using data from the public human database gnomAD and the DOMINO scoring system for dominant disease. “pLI” score shows the probability (from 0-1) of a gene having intolerance to loss-of-function variation in the population of individuals represented in gnomAD data. “Missense z-score” show a z-score value for rates of missense variation in a gene. “pLI-o/e” is the observed / expected for loss-of-function variants in a gene, while “Missense o/e” is a similar ratio for missense variants. For DOMINO scores the code shows A = “Very likely dominant (0.8-1)”, B = “Likely dominant (0.6-0.7)”, C = “Either dominant or recessive (0.4-0.5)”, D = “Likely recessive (0.2-0.3). E = “Very Likely recessive (0-0.1)”

In our previous studies from the X-screen collection we showed that the process of identification and screening for lethal mutations in Drosophila enriches for human Mendelian disease genes (Yamamoto et al. 2014). Indeed, our subsequent studies on this collection continued to yield novel human disease genes (Deal and Yamamoto 2019). We therefore wanted to assess this potential for genes from our peroxisome screen within public human databases.

First, we determined the human homolog of each fly gene with the highest predicted score using the Drosophila RNAi Screening center Integrative Ortholog Prediction Tool (DIOPT) tool (Hu et al. 2011; Gray et al. 2015) (Supplemental Table 2). We also utilized the MARRVEL tool, MARRVEL allows for simultaneous display of public human genomic data and model organism phenotypes and conservation (Wang et al. 2017). For the eighteen fly genes that we considered hits, we found that sixteen of the eighteen had human homologs (88.9%). Thirteen out of sixteen fly genes had a single human homolog while remaining three of the genes had multiple human homologs (Table 1). We also wanted to know if any of the genes were already linked to a human single gene disorder. We examined the twenty human homologs from the X-Pex screen in a Mendelian disease using the Online Mendelian Inheritance in Man (OMIM) database (Amberger et al. 2015). Of the twenty-three human genes, nine were listed in relation to at least one Mendelian phenotype where the gene is causative for a
DOMINO score of 0.53 ± 0.08, n = 20, while the known peroxisomal disease genes had a mean DOMINO score of 0.17 ± 0.04, n = 24, and the difference was statistically significant (P < 0.0001)**. This was also compared to all the homologs of the X screen genes.

Figure 4 Comparison of known human peroxisomal disease genes to the new X-Pex candidates. A. The Probability of Loss of Function intolerance score (pLi) calculated from human data from the gnomAD database (Lek et al. 2016). The X-Pex genes displayed a mean pLi score of 0.55 ± 0.11, n = 20, while the known peroxisomal disease genes had a mean pLi of 0.14 ± 0.06, n = 25, which was statistically significant (P = 0.0016)**. This was also compared to all the homologs of the X screen genes. B. The observed over expected (o/e) loss of function scores calculated from public human data from the gnomAD database. The X-Pex genes had a mean o/e score of 0.29 ± 0.06, n = 20, while the known peroxisomal disease genes had an o/e score of 0.50 ± 0.06, n = 25, which was statistically significant (P = 0.0218)*. This was also compared to all the homologs of the X screen genes. C. The missense constrain z-score calculated from public human data from the gnomAD database. The X-Pex genes had mean missense constrain z-scores of 2.16 ± 0.34, n = 20, while the known peroxisomal genes had z-scores of 0.67 ± 0.23, n = 25, which was statistically significant (P = 0.0005)**. This was also compared to all the homologs of the X screen genes. D. DOMINO scores calculated from public human data from the gnomAD database. The X-Pex genes had a mean o/e score of 0.73 ± 0.04, n = 20, compared to the known peroxisomal disease genes o/e score of 0.90 ± 0.03, n = 25, also statistically significant (P = 0.0025)**. This was also compared to all the homologs of the X screen genes. E. DOMINO scores calculated for the gene sets. The X-Pex gene set had a mean DOMINO score of 0.50 ± 0.08, n = 20, while the known peroxisomal disease genes had an o/e score of 0.29 ± 0.06, n = 20, while the known peroxisomal disease genes had an o/e score of 0.50 ± 0.06, n = 25, which was statistically significant (P = 0.0016)**. This was also compared to all the homologs of the X screen genes.

DOMINO score of 0.53 ± 0.08, n = 20, while the known peroxisomal disease genes had a mean DOMINO score of 0.17 ± 0.04, n = 24, and the difference was statistically significant (P < 0.0001)**. This was also compared to all the homologs of the X screen genes.

described disease (Supplemental Table 3). Fourteen human genes have no known single gene disorder (Table 2 and Supplementary Table 3). In our previous work in this collection, we observed that by screening for lethality, we enrich for essential genes in flies that are homologs of disease genes in humans (Wangler et al. 2015). We therefore hypothesized that these fourteen human genes from our screen, not currently associated with human disease could be considered good candidates for undiagnosed cases.

One way to assess whether these genes could be good candidates for undiagnosed disease is to examine whether damaging or deleterious variants in the genes occur in the population at large. If variants in these fourteen human genes from our screen, not currently associated with human disease could be considered good candidates for undiagnosed cases.

One way to assess whether these genes could be good candidates for undiagnosed disease is to examine whether damaging or deleterious variants in the genes occur in the population at large. If variants in these four genes from our screen, not currently associated with human disease could be considered good candidates for undiagnosed cases.

In order to explore evidence for this we examined public human genomic databases, we were looking for evidence of selective constraint, or lack of damaging variation indicating that gene is under selection in humans, in all twenty-three human genes identified by our screen. To do this we examined the gnomAD database which is a large genome and exome aggregation largely selected for healthy or adult-onset disease cases (Lek et al. 2016). For each gene we examined the constraint metrics or evidence that damaging variants in the gene are absent from these “control” individuals (Table 2, Supplemental Table 3). This type of information would point to a gene being under strong selection because it is much easier to observe a single damaging allele than homozygous or compound heterozygous. Therefore we wondered if the X-Pex gene set could be considered good candidates for dominant disease and we examined this through two strategies: 1) comparing the gene metrics to known recessive peroxisomal genes and 2) use of the DOMINO tool for predicting dominant disorders.
In the first approach we compared these same characteristics of these genes to two other sets of genes, first we compared the X-Pex gene set to all the other homologs of the genes that we screened. We also compared to a group of twenty-five well known human peroxisomal disease genes that encode proteins in the peroxisome biogenesis machinery and enzymes involved in very-long-chain fatty acid oxidation, plasmalogen synthesis and reactive oxygen species (Supplemental Table 4). Comparing these three gene sets we found that the human homologs of the essential fly genes had significantly higher probability of loss of function intolerance (Figure 4A–B), and higher missense constraint (Figure 4C–D) in the human databases compared to the known peroxisomal disease genes. This was consistent across both genes that were positive in the peroxisome secondary screen as well as negative. These intolerance scores apply more to dominant disorders than autosomal recessive. Consistent with that, all known PEX gene-related Peroxisome biogenesis disorders are autosomal recessive (Braverman et al. 2016). (Supplemental Table 4). We therefore hypothesized that the selection of lethals in our original fly screen pointed us to a set of human genes that are more likely to underlie dominant disease.

In order to test this, we used the DOMINO tool to assess the probability of dominant disease for each gene (Quinodex et al. 2017). The DOMINO score, indicating the likelihood of dominant disorders also differed between the X-Pex gene set and the known peroxisomal disease gene set (Figure 4E). The X-Pex gene set had a higher DOMINO score, while the known peroxisomal disease genes had lower DOMINO scores, thus more likelihood of relating to recessive disease and the difference was statistically significant ($P < 0.0001$). As noted, for the known peroxisomal genes this is indeed the case, as twenty-two of the twenty-five genes are disease genes for autosomal recessive disorders (Supplemental Table 4).

With this data we predict that some X-Pex genes could underlie dominant phenotypes, we sought evidence for dominant phenotypes related to alleles in the set of X-Pex genes using public databases of \textit{de novo} events from individuals with disease and controls (Turner et al. 2017). This dataset primarily focuses on neurodevelopmental phenotypes and the \textit{de novo} events from diverse cohorts. Strikingly, we observed suggestive results for six genes from the X-Pex gene set with high DOMINO scores (GSK3A, BRD4, UPF1, BRD3, GSK3B and SMAD3) and at least one individual with developmental delay, Autism, or congenital heart disease. These cases are not definitively linked to these loci and are noted to have a missense \textit{de novo} event. Interestingly no missense \textit{de novo} events are observed in control individuals for these genes (Supplemental Table 5).

Whether these genes are ultimately good candidates remains to be explored in undiagnosed cases as these database searches do not definitely link the specific gene with disease. It is also not known whether the peroxisomal phenotype that was observed in our screen would be conserved in humans. Peroxisomes are not routinely examined in clinical samples, so this data provides a key starting point for these genes of interest. Even a secondary impact on peroxisomes without direct interaction could aid in exploring these candidate genes further.

Taken together we propose the X-Pex provides a good list of candidate genes, in particular, \textit{de novo} events in these genes from patients with neurodevelopmental phenotypes should be explored. Considering that peroxisomal disease classically relates to autosomal recessive conditions, the X-Pex gene list may provide an entry point to study the role of \textit{de novo} events in genes that impact peroxisomes that have been missed in previous screens. This study therefore provides additional support for the use of forward genetic screens in model organisms in the study and identification of human disease genes (Yamamoto et al. 2014; Wangler et al. 2015).

**ACKNOWLEDGMENTS**

The authors thank the Kyoto and Bloomington \textit{Drosophila} Stock Centers for maintaining and publically distributing the \textit{X}-chromosome recessive lethal stocks used in this study. S.Y. and M.F.W thank the Junior Faculty Seed Funds from the Department of Molecular and Human Genetics at Baylor College of Medicine and Global Foundation for Peroxisomal Disorders, RhizoKids and Wynne Mattey Research Foundation to support this research.

**LITERATURE CITED**

Amberger, J. S., C. A. Bocchini, F. Schiettecatte, A. F. Scott, and A. Hamosh, 2015 OMIM.org: Online Mendelian Inheritance in Man (OMIM®), an online catalog of human genes and genetic disorders. Nucleic Acids Res. 43: D789–D798. https://doi.org/10.1093/nar/gku1205

Baron, M. N., C. M. Klinger, R. A. Rachubinski, and A. J. Simmonds, 2016 A Systematic Cell-Based Analysis of Localization of Predicted Drosophila Peroxisomal Proteins. Traffic 17: 536–553. https://doi.org/10.1111/tra.12384

Braschi, B., P. Denny, K. Gray, T. Jones, R. Seal et al., 2019 Genenames.org: the HGNC and VGNC resources in 2019. Nucleic Acids Res. 47: D786–D792. https://doi.org/10.1093/nar/gky930

Braverman, N. E., G. V. Raymond, W. B. Rizzo, A. B. Moser, M. E. Wilkinson et al., 2016 Peroxisome biogenesis disorders in the Zellweger spectrum: An overview of current diagnosis, clinical manifestations, and treatment guidelines. Mol. Genet. Metab. 117: 313–321. https://doi.org/10.1016/j.ymgme.2015.12.009

Chao, Y.-H., L. A. Robak, F. Xia, M. K. Koenig, A. Adesina et al., 2016 Missense variants in the middle domain of DNM1L in cases of infantile encephalopathy alter peroxisomes and mitochondria when assayed in Drosophila. Hum. Mol. Genet. 25: 1846–1856. https://doi.org/10.1093/hmg/ddw059

Cook, M. S., C. Cazin, M. Amoyel, S. Yamamoto, E. Bach et al., 2017 Neutral Competition for Drosophila Follicle and Cyst Stem Cell Niches Requires Vesicle Trafficking Genes. Genetcs 206: 1417–1428. https://doi.org/10.1534/genetics.117.21202

Deal, S. L., and S. Yamamoto, 2019 Unraveling Novel Mechanisms of Neurodegeneration Through a Large-Scale Forward Genetic Screen in Drosophila. Front. Genet. 9: 700. https://doi.org/10.3389/fgene.2018.00700

Fang, X., J. Zhou, W. Liu, X. Duan, U. Gala et al., 2016 Dynamin Regulates Autophagy by Modulating Lysosomal Function. J. Genet. Genomics 43: 77–86. https://doi.org/10.1016/j.jgg.2015.10.005

Faust, J. E., A. Manisundaram, P. T. Ivanova, S. B. Milne, J. B. Summerville et al., 2014 Peroxisomes are required for lipid metabolism and muscle function in Drosophila melanogaster. PLoS One 9: e100213. https://doi.org/10.1371/journal.pone.0100213

Faust, J. E., A. Verma, C. Peng, and J. A. McNew, 2012 An inventory of peroxisomal proteins and pathways in Drosophila melanogaster. Traffic 13: 1377–1392. https://doi.org/10.1111/j.1600-0854.2012.01393.x

Florence, B. L., and D. V. Faller, 2008 Drosophila female sterile (1) homeotic is a multifunctional transcriptional regulator that is modulated by Ras signaling. Dev. Dyn. 237: 554–564. https://doi.org/10.1002/dvdy.21432

Fujiki, Y., K. Okumoto, M. Mukai, M. Honsho, and S. Tamura, 2014 Peroxisome biogenesis in mammalian cells. Front. Physiol. 5: 307. https://doi.org/10.3389/fphys.2014.00307

Gray, K. A., B. Yates, R. L. Seal, M. W. Wright, and E. A. Bruford, 2015 Genenames.org: the HGNC resources in 2015. Nucleic Acids Res. 43: D1079–D1085. https://doi.org/10.1093/nar/gku1071

Haeltzerman, N. A., L. Jiang, Y. Li, V. Bayat, H. Sandoval et al., 2014 Large-scale identification of chemically induced mutations in Drosophila melanogaster. Genome Res. 24: 1707–1718. https://doi.org/10.1101/gr.174615.114

Hu, Y., I. Flockhart, A. Vinayagam, C. Bergwitz, B. Berger et al., 2011 An integrative approach to ortholog prediction for disease-focused and other functional studies. BMC Bioinformatics 12: 357. https://doi.org/10.1186/1471-2105-12-357
