Characterization of prickle isoform-specific $pk^{pk1}$ and $pk^{sple1}$ mutations in Drosophila melanogaster

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Abstract

We used paired-end next generation sequencing (NGS) to characterize the classic isoform-specific $pk^{pk1}$ and $pk^{sple1}$ mutations of the prickle gene in Drosophila melanogaster. Here we provide evidence that these previously reported null mutations are caused by either a tirant transposon insertion into the 5' UTR of $pk^{pk1}$ or a premature stop codon in the second exon of $pk^{sple1}$. Additional likely benign missense mutations were identified in both mutant isoforms.

Figure 1. Mapping of exonic alterations in $pk^{pk1}$ and $pk^{sple1}$ Drosophila mutants.

(A) Table describing all missense mutations identified in exons of pk transcript variant A ($pk^{pk1}$) for the $pk^{pk1}$ mutant. Mutation locations are indicated by number in relation to the reported mRNA sequence. Both exons indicated (2 and 6) are shared between isoforms. (B) Table describing all mutations in the exons of pk transcript variant C ($pk^{sple1}$) for the $pk^{sple1}$ mutant.
Mutation locations are indicated by number in relation to the reported mRNA sequence. Exons include the pk\textsuperscript{sple} isoform-specific exons (1 and 2) as well as the shared exon 7. (C) Graphical representation showing alterations in pk transcript variant A for the pk\textsuperscript{pk1} mutant. The red box in the 5’ UTR region depicts the tirant insertion, while the yellow lines and arrowheads indicate the missense mutation sites described in panel A in relation to the PET (pink) and LIM (green) domains. (D) Graphical representation showing alterations in pk transcript variant C for the pk\textsuperscript{sple1} mutant. The red and yellow lines and arrowheads indicate nonsense and missense mutations, respectively (exon 2). (E) Magnified view of the tirant insertion site in pk transcript variant A for pk\textsuperscript{pk1}. The boxes below the tirant schematic show an enlarged view of the PCR-confirmed DNA sequences adjacent to and within the transposon insertion with yellow boxes highlighting the first translation start codon along with in-frame stop codon. A duplication of the CGCG region normally found at the insertion position is indicated in the 5’ UTR flanking the tirant sequences. (F – H) Comparison of amino acid sequence among several Drosophilidae family members in addition to mosquito (Aedes aegypti) in region of the missense and nonsense mutations of pk\textsuperscript{pk1} and pk\textsuperscript{sple1}.

Description

The prickle gene in Drosophila melanogaster has been studied for decades for its involvement in planar cell polarity (PCP), and more recently, epilepsy (Tao et al. 2011, Matis and Axelrod 2013, Ehaideb et al. 2014, Ehaideb et al. 2016). prickle expresses three isoforms, prickle-prickle (pk\textsuperscript{pk}; pk-RA, FBr0089042), prickle-M (pk\textsuperscript{M}; pk-RB, FBr0089043), and prickle-spiney-legs (pk\textsuperscript{sple}; pk-RC, FBr0089044). While pk\textsuperscript{M} expression is initiated during embryogenesis, expression ceases prior to the end of pupation (Gubb et al. 1999). On the other hand, both pk\textsuperscript{pk} and pk\textsuperscript{sple} are only expressed post-embryonically (Gubb et al. 1999). All isoforms arise from alternative start sites and differ based on 5’ coding or non-coding exons (Gubb et al. 1999). Mutations that affect pk\textsuperscript{pk} or pk\textsuperscript{sple} isoforms have been shown to alter development of the fruit fly body plan including the orientation of eye ommatidia as well as positioning of epidermal hairs and bristles of the wing and body (Gubb 1998, Gubb et al. 1999, Green et al. 2000, Strutt and Strutt 2007, Lin and Gubb 2009, Jenny 2010, Matis and Axelrod 2013, Carvajal-Gonzales and Mlodzik 2014). In addition, pk isoforms are required in the nervous system to ensure proper neuronal wiring and function (Mrksusich et al. 2011, Ng 2012, Ehaideb et al. 2014), while mutations specifically affecting pk\textsuperscript{M} have not been identified (Gubb et al. 1999). Although some mutations affecting Drosophila prickle isoforms have been approximately defined (pk\textsuperscript{pk}\textsuperscript{-sple13}; Gubb et al. 1999) or mapped (pk\textsuperscript{pk30}; Green et al. 2000), the original pk\textsuperscript{sple1} and pk\textsuperscript{pk1} mutations (Gubb and García-Bellido 1982) have heretofore remained uncharacterized.

We used paired-end Illumina next generation sequencing to characterize and map pk\textsuperscript{pk1} and pk\textsuperscript{sple1} mutations. On average, for the pk\textsuperscript{pk1} (and pk\textsuperscript{sple1}) mutants, 96.5% (and 96.8%, respectively) of the genome had at least 1X read coverage, 92.3% (and 93%, respectively) had at least 10X read coverage, and 84.3% (and 85.8%, respectively) had at least 30X read coverage. For the genomic region encompassing the prickle locus, both mutants had at least 30X read coverage. No mutations affecting predicted splice-sites were found in either mutant. Two nonsynonymous mutations, C902A and T2690C, were identified in pk\textsuperscript{pk1} (Figure 1A), neither of which fall within the evolutionarily conserved protein-protein-interaction PET and LIM domains (Figure 1C; Gubb et al. 1999, Sweede et al. 2008). Additionally, alignment of paired-end reads to the dm6 D. melanogaster genome revealed a region 13 bases downstream of the transcription start site of prickle transcript variant A that only showed alignment of one out of the two reads of numerous paired-end sequences. Analysis of the unmapped read of these pairs identified sequences from a tirant transposon (Figure 1C; Cañizares et al. 2000) which was confirmed with PCR followed by Sanger sequencing (partial sequence for the tirant insertion is shown in Figure 1E). This transposon insertion is thus likely responsible for the loss-of-function phenotype in the pk\textsuperscript{pk1} mutant, particularly given the numerous start and stop codons at the 5’ end of the tirant sequence which would be predicted to be utilized in favor of the correct distal downstream pk\textsuperscript{pk} start codon (see Figure 1E). In pk\textsuperscript{sple1} homozygous mutants, we identified nonsense (C1593T) and missense (A1601C) mutations in the coding sequence, both of which were located at the 3’ end of the pk\textsuperscript{sple}-specific second exon of prickle transcript variant C and upstream of the PET and LIM domain exons (Figures 1B, 1D). These data suggest that the premature stop codon is likely responsible for loss-of-function of pk\textsuperscript{sple} in the pk\textsuperscript{sple1} mutant.

In order to assess whether the nonsynonymous mutations found in the pk\textsuperscript{pk1} or pk\textsuperscript{sple1} sequences could significantly affect prickle gene function, we examined the conservation of amino acids in these regions. In pk\textsuperscript{pk1}, while both mutations fall in conserved regions (as predicted by PhyloP in the UCSC Genome Browser; Figures 1F and 1G), they are likely tolerated and non-deleterious. C902A of pk\textsuperscript{pk1} (Figure 1F) replaces proline with threonine, an amino acid that is similar in structure to the serine found at that position in a close outgroup to the Drosophilidae family (Aedes aegypti, the mosquito), although this region of the protein shows relatively poor conservation between D. melanogaster and A. aegypti. T2690C also is likely non-
deleterious, as *Drosophila melanogaster* is the only member of family *Drosophilidae* shown to contain a serine at this location while all others carry the amino acid that is inserted due to the missense mutation (proline; Figure 1G). Further evidence is found in *D. melanogaster* sequences submitted to GenBank (www.ncbi.nlm.nih.gov/genbank/) that indicate a proline at this position (GenBank identifiers AJ243708.3, BT126167.1). In *pk^{sple1}*, the nonsynonymous mutation occurs immediately downstream from the premature stop site and replaces a glutamine with proline, the amino acid found at the analogous position in another member of the *Drosophilidae*, *D. ananassae* (Figure 1H). Additionally, this region of *pk^{sple}* is poorly conserved. Collectively, these results argue that the missense mutations found in *pk^{pk1}* and *pk^{sple1}* are less likely to be deleterious.

**Methods**

**Outcrossing of pk Lines**

Both *pk^{pk1}* and *pk^{sple1}* were outcrossed for 10 generations into a *w^{1118}* line obtained from Dr. Andy Frank (University of Iowa) prior to whole genome sequencing. This line was chosen because it has robust neurotransmission and growth properties at the neuromuscular junction (Yeates et al. 2017). *prickle* mutant bristle phenotypes were confirmed at each relevant step.

**Whole Genome Sequencing:**

Genomic DNA (from 5 whole male and 5 whole female flies) was extracted with the DNeasy Blood & Tissue Kit (Qiagen). Library preparations and whole Genome Sequencing (paired-end 2 x 150 bp reads, Illumina NovaSeq 6000) was performed by the Iowa Institute of Human Genetics (IIHG) Genomics Division at the University of Iowa (https://medicine.uiowa.edu/humangenetics/genomics-division).

**Genome Assembly**

FastQC program v0.10.0 (Wingett and Andrews 2018) was used to perform quality control analysis of the paired end sequencing data for the *pk^{pk1}* and *pk^{sple1}* samples. Illumina adapter contaminants were removed from the sequencing data using the Trimmomatic v0.32 (Bolger et al. 2014) with the following settings ILLUMINACLIP: <file of ADAPTERS.txt>:4:40:12 HADCROP:15 SLIDINGWINDOW:5:30 AVGQUAL:30 CROP:130 MINLEN:36. Both single and paired end reads retained after adapter trimming were mapped to the dm6 genome using bwa v0.7.5 (Li and Durbin 2009) and samtools v0.1.18 (Li et al. 2009). Reads with one pair mapping to a given genomic interval in chr2R of the dm6 genome and their associated unmapped pair were identified using a bedtools v2.26 (Quinlan et al. 2010), samtools v1.31 (Li et al. 2009), awk programming language, and bash scripting. This data was used to identify the insertion of a transposon sequence in the *pk^{pk1}* mutants in the neighboring region of these mapped single end reads.

Total reads were counted from the fastq.gz files of every sample using Linux shell commands. Trimmed read and mapped read counts were generated from .bam alignment files of every sample using samtools flagstat command. Total bases of the dm6 genome were computed from the chromosome sizes file that was generated from the FASTA index file of the dm6 genome using samtools v0.1.18 (Li et al. 2009) and Linux shell commands. The chrUn and chromosomes with ‘random’ in their sequence identifiers were filtered out. Coverage for every sample was generated by counting the number of bases in the dm6 genome covered by at least 1, 10, or 30 mapped reads independently using bedtools v2.26 (Quinlan et al. 2010) genomcov program and shell scripting.

Sanger Sequencing using an ABI3500 Sanger Sequencer was performed on PCR-amplified fragments to confirm both the insertion of the *tirant* transposon of *pk^{pk1}* and the premature stop codon of *pk^{sple1}* mutants as well as all other missense mutations. The Multiz Alignment tool (Blanchette et al. 2004) in the USCS Genome Browser (Kent et al. 2002, Karolchik et al. 2004) was used to compare amino acid sequences.

**Reagents**

| *Drosophila Melanogaster* | 
|--------------------------|
| **Stock Number, Bloomington** | **Genes Affected** | **Genotype** |
| *Drosophila* Stock Center | *w, pk, cn* | *w^{1118};pk[1] cn[1]* |
| BDSC 367, FBst0000367 | | |
| BDSC 422, FBst0000422 | w, pk | w[1118];pk[sple-1] |
|-----------------------|--------|-------------------|
| n/a; line obtained from Dr. Andy Frank | w | w[1118] |

**Acknowledgements:** We thank David Gubb for his insightful information on prickle alleles.

**References**

Blanchette M, Kent WJ, Riemer C, Elnitski L, Smit AF, Roskin KM, et al., Miller W. 2004. Aligning multiple genomic sequences with the threaded blockset aligner. Genome Res 14: 708-15. PubMed ID: [15060014](https://pubmed.ncbi.nlm.nih.gov/15060014/)

Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30: 2114-20. PubMed ID: [24695404](https://pubmed.ncbi.nlm.nih.gov/24695404/)

Cañizares J, Grau M, Paricio N, Moltó MD. 2000. Tirant is a new member of the gypsy family of retrotransposons in Drosophila melanogaster. Genome 43: 9-14. PubMed ID: [10701107](https://pubmed.ncbi.nlm.nih.gov/10701107/)

Carvajal-Gonzalez JM, Mlodzik M. 2014. Mechanisms of planar cell polarity establishment in Drosophila. F1000Prime Rep 6: 98. PubMed ID: [25580252](https://pubmed.ncbi.nlm.nih.gov/25580252/)

Ehaideb SN, Iyengar A, Ueda A, Iacobucci GJ, Cranston C, Bassuk AG, et al., Manak JR. 2014. prickle modulates microtubule polarity and axonal transport to ameliorate seizures in flies. Proc Natl Acad Sci U S A 111: 11187-92. PubMed ID: [25024231](https://pubmed.ncbi.nlm.nih.gov/25024231/)

Ehaideb SN, Wignall EA, Kasuya J, Evans WH, Iyengar A, Koerselman HL, et al., Manak JR. 2016. Mutation of orthologous prickle genes causes a similar epilepsy syndrome in flies and humans. Ann Clin Transl Neurol 3: 695-707. PubMed ID: [27648459](https://pubmed.ncbi.nlm.nih.gov/27648459/)

Green C, Levashina E, McKimmie C, Dafforn T, Reichhart JM, Gubb D. 2000. The necrotic gene in Drosophila corresponds to one of a cluster of three serpin transcripts mapping at 43A1.2. Genetics 156: 1117-27. PubMed ID: [11063688](https://pubmed.ncbi.nlm.nih.gov/11063688/)

Gubb D. 1998. Cellular polarity, mitotic synchrony and axes of symmetry during growth. Where does the information come from? Int J Dev Biol 42: 369-77. PubMed ID: [9654021](https://pubmed.ncbi.nlm.nih.gov/9654021/)

Gubb D, García-Bellido A. 1982. A genetic analysis of the determination of cuticular polarity during development in Drosophila melanogaster. J Embryol Exp Morphol 68: 37-57. PubMed ID: [6809878](https://pubmed.ncbi.nlm.nih.gov/6809878/)

Gubb D, Green C, Huen D, Coulson D, Johnson G, Tree D, Collier S, Roote J. 1999. The balance between isoforms of the prickle LIM domain protein is critical for planar polarity in Drosophila imaginal discs. Genes Dev 13: 2315-27. PubMed ID: [10485852](https://pubmed.ncbi.nlm.nih.gov/10485852/)

Jenny A. 2010. Planar cell polarity signaling in the Drosophila eye. Curr Top Dev Biol 93: 189-227. PubMed ID: [20959167](https://pubmed.ncbi.nlm.nih.gov/20959167/)

Karolchik D, Hinrichs AS, Furey TS, Roskin KM, Sugnet CW, Haussler D, Kent WJ. 2004. The UCSC Table Browser data retrieval tool. Nucleic Acids Res 32: D493-6. PubMed ID: [14681465](https://pubmed.ncbi.nlm.nih.gov/14681465/)

Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, Haussler D. 2002. The human genome browser at UCSC. Genome Res 12: 996-1006. PubMed ID: [12045153](https://pubmed.ncbi.nlm.nih.gov/12045153/)

Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25: 1754-60. PubMed ID: [19451168](https://pubmed.ncbi.nlm.nih.gov/19451168/)

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al., 1000 Genome Project Data Processing Subgroup,. 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25: 2078-9. PubMed ID: [19505943](https://pubmed.ncbi.nlm.nih.gov/19505943/)

Lin YY, Gubb D. 2009. Molecular dissection of Drosophila Prickle isoforms distinguishes their essential and overlapping roles in planar cell polarity. Dev Biol 325: 386-99. PubMed ID: [19028485](https://pubmed.ncbi.nlm.nih.gov/19028485/)

Matis M, Axelrod JD. 2013. Regulation of PCP by the Fat signaling pathway. Genes Dev 27: 2207-20. PubMed ID: [24142873](https://pubmed.ncbi.nlm.nih.gov/24142873/)

Mrkusich EM, Flanagan DJ, Whittington PM. 2011. The core planar cell polarity gene prickle interacts with flamingo to promote sensory axon advance in the Drosophila embryo. Dev Biol 358: 224-30. PubMed ID: [21827745](https://pubmed.ncbi.nlm.nih.gov/21827745/)

Ng J. 2012. Wnt/PCP proteins regulate stereotyped axon branch extension in Drosophila. Development 139: 165-77. PubMed ID: [22147954](https://pubmed.ncbi.nlm.nih.gov/22147954/)
Quinlan AR, Hall IM. 2010. BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics 26: 841-2. PubMed ID: 20110278

Strutt D, Strutt H. 2007. Differential activities of the core planar polarity proteins during Drosophila wing patterning. Dev Biol 302: 181-94. PubMed ID: 17045581

Sweede M, Ankem G, Chutvirasakul B, Azurmendi HF, Chbeir S, Watkins J, et al., Capelluto DG. 2008. Structural and membrane binding properties of the prickle PET domain. Biochemistry 47: 13524-36. PubMed ID: 19053268

Tao H, Manak JR, Sowers L, Mei X, Kiyonari H, Abe T, et al., Bassuk AG. 2011. Mutations in prickle orthologs cause seizures in flies, mice, and humans. Am J Hum Genet 88: 138-49. PubMed ID: 21276947

Wingett SW, Andrews S. 2018. FastQ Screen: A tool for multi-genome mapping and quality control. F1000Res 7: 1338. PubMed ID: 30254741

Yeates CJ, Zwiefelhofer DJ, Frank CA. 2017. The maintenance of synaptic homeostasis at the Drosophila neuromuscular junction is reversible and sensitive to high temperature. eNeuro 4: e0220-17.2017. PubMed ID: 29255795

Funding: Research reported in this study was supported by the National Institutes of Health to J. Robert Manak and Alexander G Bassuk (Award Number R01NS098590)

Author Contributions: Anthony J Lilienthal: data curation, writing - original draft, writing - review editing, formal analysis, methodology. Mrutyunjaya Parida: data curation, formal analysis, methodology, software. J Robert Manak: writing - original draft, writing - review editing, conceptualization, project, supervision.

Reviewed By: Anonymous

History: Received September 15, 2022 Revision Received October 18, 2022 Accepted October 19, 2022 Published Online October 20, 2022 Indexed November 3, 2022

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Citation: Lilienthal, AJ; Parida, M; Manak, JR (2022). Characterization of prickle isoform-specific pkpk1 and pkple1 mutations in Drosophila melanogaster. microPublication Biology. 10.17912/micropub.biology.000656