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Sarcoplasmic calcium-binding protein and alcohol dehydrogenase, new occupational allergens in the fruit fly *Drosophila melanogaster*.

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Contribution statement:

Betancor D and Fernández-Nieto M attended the patient and did the occupational study. López-Matas MA, González-Ruiz A, Martín-López L and Carnés J. designed and performed the experiments. All authors discussed the results and contributed to the final manuscript.

Key messages:

-We reported a case of occupational allergy to *Drosophila melanogaster* mediated by four allergens.

- Two of them, sarcoplasmic calcium-binding protein and alcohol dehydrogenase have been identified for the first time as *Drosophila* occupational allergens.

- Alcohol dehydrogenase has been identified as a new insect allergen.

To the Editor,

An urban lifestyle has led to changes in the way we interact with insects. They have been taken from their natural habitat and used in research, to feed pets or as a new food source. Insect allergy is increasing globally and affects 1% to 7% of the global population involving food, respiratory and sting allergy¹.

Drosophila melanogaster, also known as common fruit fly or vinegar fly, is an insect with a short life and well-defined biology. It has been widely used in biological and genetic research as a model organism. It has also become an important allergen source in laboratory environments with sensitization rates between 6% to 31% in laboratory workers^{2,3}. Different proteins have been identified as presumable *D. melanogaster* allergens^{4,5}, three are included in Allergome database (www.allergome.org) tropomyosin, arginine kinase and Mn superoxide dismutase. All of them were identified by indirect methods, not in patients sensitized to *D. melanogaster*. The only *D. melanogaster* allergen confirmed until now is LSP-2 (hexamerin)⁵, however the identification of this allergen was only shown by comparing the IgE immunoblot band pattern with the binding profile of an anti-LSP-2 antibody.

We report a case of occupational rhinoconjunctivitis to *D. melanogaster* fly in which alcohol dehydrogenase (ADH) and sarcoplasmic calcium-binding protein 1 (SCP), have been identified as new allergens in this insect. The patient was a 50-year-old woman without previous

atopic history who has worked in a research laboratory for six years manipulating and analysing the *D. melanogaster* fly's wings with no nasal protection. For two to three years she has presented rhinoconjunctivitis symptoms (nasal congestion, rhinorrhoea, sneezing and nasal and ocular stinging) intermittently at work with worsening during the spring months. Symptoms usually began after working one hour and improved with oral antihistamine. No asthma symptoms were associated.

Skin prick test to a battery of common aeroallergens (ALK-Abelló, Madrid, Spain) was positive to grass (6x5 mm) and *Platanus* pollen (5x4 mm) and negative to other pollens, dust mites (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*) and animal epithelium (cat, dog and horse epithelium). Prick-by-prick with different *D. melanogaster* parts was positive to body (7x5 mm) and wings (6x5 mm). Prick-by-prick with *D. melanogaster* was also performed on three non-atopic patients (controls) with a negative result. During time off work spirometry test was performed with a normal result: forced vital capacity (FVC) 4.26 (122%), forced expiratory volume in 1 second (FEV1) 3.29 (110%), FEV1/FVC 76.97%, mid expiratory flow (MEF50) 6.09 (102%). Exhaled nitric oxide (FeNO) was normal at 21 ppb. FeNO was measured using the online method (NiOX; AerocrineAB; Solna, Sweden).

Nasal allergen challenge (NAC) was performed as previously reported⁶. Nasal symptom scores and objective nasal patency challenge were performed before and after NAC as recommended⁶. Serial spirometry and FeNO were measured during and after NAC to rule out the asthmatic component. After obtaining written informed consent, the patient was challenged in a 7 mm³ dynamic exposure chamber washing down a container of 100 µg of *D. melanogaster* flies with an accumulated time of 20 minutes. After four minutes exposure, the patient presented rhinorrhoea and sneezing and after 10 minutes, she showed erythema and ocular stinging. FeNO increased 45% from the basal value. There was an increase on the visual analogue scale (VAS) of 65 mm (from 20 mm to 85 mm) and total nasal symptom score (TNSS) after NAC was seven points (from one to eight points). This marked rise in nasal symptom scores indicates a positive NAC and a link between exposure to *D. melanogaster* and the allergic response. Serial rhinomanometry measures did not reveal differences in transverse area. FEV1 values were monitored with a computerized asthma monitor (AMOS, Jaeger) every hour with no asthmatic response.

Fruit flies collected from the patient's workplace were used to prepare a *D. melanogaster* extract by homogenization in PBS (phosphate buffered saline) 0.01 M, pH 7.4 overnight at 4°C. The sample was centrifuged and the supernatant collected, dialyzed, filtered, frozen and freeze-dried. Protein concentration in the extract was measured using the Bradford technique (Thermo Scientific, Waltham, MA, USA) with a result of 45.7 µg of protein/mg of lyophilized extract.

The protein profile was investigated by SDS-PAGE under reducing conditions. The molecular distribution of the bands ranged from 10 to 100 kDa (Figure 1, SDS-PAGE). ELISA assay was performed with *D. melanogaster* and cockroach (*Blatella germanica*) extract to study sensitization to other insects. At the same dilution of the patient serum, IgE recognized *Drosophila* extract (Optical Density 0.35) but not cockroach extract. The allergenic profile was investigated by immunoblotting with the patient serum. Twenty µg of *D. melanogaster* protein were run in SDS-PAGE, transferred to a Trans-Blot® Turbo™ Transfer Pack (Bio-Rad, Hercules, CA, USA), and incubated overnight with the patient serum diluted 1:4. After incubation with anti-IgE-HRP (Southern Biotech, Birmingham, USA) the reaction was developed with Clarity Western ECL (BioRad) and visualized by chemiluminescence.

The patient serum recognized four bands at approximately 27 kDa, 29.5 kDa, 33.5 kDa and 83.5 kDa (Figure 1, Immunoblot). The four positive bands identified by immunoblot were sequenced by LC-MS/MS (Liquid Chromatography Tandem Mass Spectrometry) for identification. Analysis revealed that 27 kDa protein corresponded to sarcoplasmic calcium-binding protein 1 (SCP), 29.5 kDa to alcohol dehydrogenase (ADH), 33.5 kDa to tropomyosin and 83.5 kDa to hexamerin LSP-2 (Table 1). Tropomyosin and hexamerin LSP-2 corresponded to allergens previously described in *D. melanogaster*⁵.

SCP corresponded to an EF-hand type Ca²⁺ binding protein. Thirty-eight peptides were identified with a sequence coverage of 77% (Table 1). This protein is a relevant allergen described in Crustacea⁷. Although patient did not refer allergic symptoms after eating shellfish, sensitization was not determined because she refused a prick-by-prick test with shrimp. SCP has also been identified in yellow fever mosquito (*Aedes* 5) in patients with respiratory allergy⁸. This is the first time that SCP is identified as allergen in other insects, and reported as a *D. melanogaster* allergen.

Regarding ADH twelve peptides were identified with a sequence coverage of 70% (Table 1). ADH has been never reported before as an allergen in insects. Only two ADHs have been described as allergens to date, both from fungi; Cand a 1, in *Candida albicans*⁹ and Cur I ADH in *Curvularia lunata*¹⁰. ADHs are glycolytic enzymes involved in NAD⁺ regeneration and in the *D. melanogaster* fruit fly the enzyme's catalytic activity contributes to adaptation of the species to its ethanol-rich ecological niche.

In summary, we reported a case of occupational allergy to *Drosophila melanogaster* mediated by four allergens. Two of them, SCP and ADH have been identified for the first time as *Drosophila* occupational allergens. Moreover, ADH has been identified as a new insect allergen. Further studies are needed to fully characterize the allergy to this insect so frequently used in laboratories.

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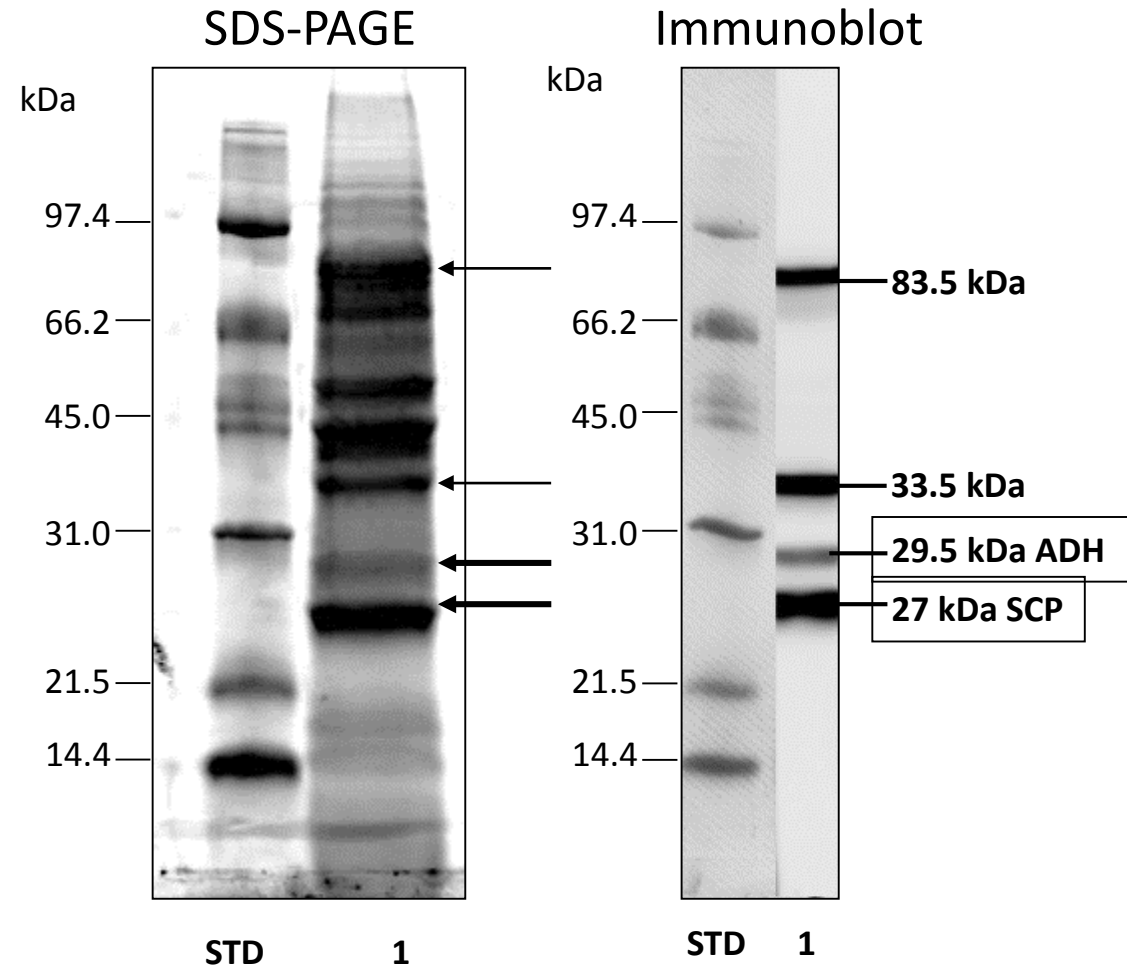
Figure Legend:

Figure 1: Protein profile by SDS-PAGE (left panel), the four bands sequenced by LC-MS/MS are marked with arrows, the new allergens SCP and ADH are marked with thicker arrows. Allergenic profile by immunoblotting (right panel), the four bands detected are marked and the positions of SCP and ADH are included in rectangles. STD: Molecular weight

Table 1: *Drosophila melanogaster* allergens identified by LC-MS/MS. Protein band corresponds to the position observed in the SDS-PAGE and immunoblot (Figure 1). Peptides identified are marked in grey.

Protein band	Accession number	Name	Sequence coverage (%)	Peptides	Aminoacid sequence and identified peptides
27 kDa	Q8MSI2	Sarcoplasmic calcium-binding protein 1	77	38	MAYSWDNRVDFVVRMYDIDNNGFLDQNDFLCMAVRACVVEGKGCSTAR LDDYKMLMKNLWDEISAIADDDKDGKISNQEFKDAVKKTCVGKKYEEFPQ AMRAFIESNFKLLDIDSDGIVGVKEYRYNCITRVAIDITPIDKAFETLL NDEDRKRGGSLDRYKELYGQFLGNTADNHPAVNLFGPL
29.5 kDa	A0A077HCQ5	Alcohol dehydrogenase	70	12	MSFTLTNKNVIFVAGLGGIGLDTSKELLKRDNLVILDRIENPAAIAEL KAINPKVTVTFFPYDVTVPPIAETTKLLKTIFAQLKTVDVVLINGAGILDDH QIERTIAVNYTGLVNTTTAILDFWDKRGKGGPGGICNIGSVTGFNAIYQV PVYSGTKAAVVNFTSSLAKLAPITGVTAYTVNPGITRRTLVTHTFNSWLDV EPQVAEKLLAHPTQPSLACAENFVKAIELNQNGAIWKL
33.5 kDa	P09491.1	Tropomyosin-2	53	25	MDAIKKKMQAMKLEKDNAIDKADTCENQAKDANSRADKLNEEVRDLEKKF VQVEIDLVTAKEQLEKANTELEEKEKLLTATESEVATQNRKVQQIEEDLE KSEERSTTAQQKLEATQSADENNRMCVKLENRSQQDEERMDQLTNQLKE ARMLAEDADTKSDEVSRKLAFVEDELEVAEDRVRSGESKIMELEELKVV GNSLKSLEVSEEKANQRVEEFKREMKTLISIKLKEAEQRAEHAEKQVKRLQ KEVDRLEDELGINKDRYKSLADEMDSTFAELAGY
83.5 kDa	Q24388	Larval serum protein 2	72	73	MKSFTVIALAAVALLATLGQAKHLDSKVADKDFLMKQKFMYQILQHIYQD DVFTTPFGGSYVEYKPWEHVADYVHPEMLEHFFELWQHQPFTDDMVWSVM YDKHEEYVVGVLVRLFYFAKNWETFQHVYWARQHVNKQLFVYAVTIASLF RDDMQGVVLPAYEIHWPWSYFDSQALEWAEHYKMHGFHHVKQMDNIYNV IRTNYSNVHGSNLNYDHDLAYYLEDVGFNAFYFFNLDYPFWTKGGEHVL NKDRRGELYLYVHWQLLARWYLERLSHDLGEVPAFNMYVPTESGYASNLR TYYGVPQWHRENHHSFYHEHNYEHIEHVEMYTQRVMDWIHKNKFDVETI NVLGNI IQGNADSVDKKFYGS�DKLYRFIVNEGHYHGHDSEFPGLFMHY

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**Figure 1**