Occupational Rhinoconjunctivitis Induced by Flaxseed: Identification of Novel Allergenic Proteins From Cupin, Chaperonin, and Enzyme Families

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Flaxseed (*Linum usitatissimum*) is widely incorporated in food and pharmaceutical products by virtue of its content in protein, dietary fiber, polyunsaturated fatty acids, and other compounds that seem to have anti-inflammatory, antioxidant, and cardioprotective properties. It is also consumed because of its laxative effects, although severe allergic reactions have been reported after ingestion [1,2]. Hypersensitivity to flaxseed was first described in 1930, and, since then, clinical cases have mainly been related to ingestion of seeds and oil rather than inhalation of powder. Flaxseed contains many potential allergens. In 2002, Leon et al [3] reported the case of a patient sensitized to a 56-kDa dimeric protein from flaxseed, possibly malate dehydrogenase, and proposed it as a major allergen. A 53-kDa protein was subsequently identified as a major flaxseed allergen [4], similar to the protein described by Leon et al. Other authors have suggested that protein bands of around 22 and 20 kDa [4] or lipid transfer proteins (LTPs) [5] were involved in flaxseed hypersensitivity. Previous studies [1,6] identified conlinin, 2S storage protein, and 11S globulin [7] as flaxseed allergens. Conlinin was included as allergen Lin u1.01 in the official allergen database maintained by the World Health Organization and the International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee [8].

Sensitization to flaxseed proteins related to occupational exposure was first described in the early 1960s in seamen, dockers, and oil processing plant workers in Marseilles and documented by skin prick test, and in 2008, Vandenplas et al [9] reported a case of an oleochemical plant worker who developed occupational asthma and rhinitis after inhaling flaxseed oilcake dust. Occupational asthma and rhinitis were diagnosed by specific inhalation challenge and skin prick test performed with a flaxseed extract prepared from linseed oilcake, and by measuring serum IgE level against linseed.

We report the case of 30-year-old man who was referred to our Occupational Medicine Unit because of work-related rhinoconjunctivitis symptoms, namely, nasal obstruction, rhinorrhea, sneezing, itching, and burning eye (his symptoms improved or disappeared outside work). The patient was a nonsmoker who worked for a company that produced pharmaceutical and herbal products. The symptoms started 6 months after he took charge of phytomedication production and was exposed to *L usitatissimum* powder. The results of skin prick tests with common aeroallergens were negative. Prick-by-prick tests were carried out using some of the components of the tablets made by the company the patient worked for; the test with linseed was highly positive (20-mm wheal lasting about 2 hours, as well as a transient exacerbation of his ocular and rhinitis symptoms [histamine, 7 mm]).

The control prick-by-prick tests carried out in 3 controls were negative. Nasal provocation testing performed as indicated by Cho et al [10] with flaxseed powder was highly positive, and it was impossible to measure resistance after the provocation test owing to evident clinical rhinoconjunctivitis symptoms triggered by exposure to flaxseed dust. The basophil activation test performed with a flaxseed extract at a dilution of 1:1000 yielded a positive result, with 26% of basophils expressing CD63 (Figure 1S, supplemental). As reported in the literature, flaxseed contains many potential allergens (eg, storage proteins) [1] (Table 1S).

The 2-D colloidal Coomassie stained gel analysis of the tablets highlighted a mean (SD) total of 203 (15) valid protein spots (Figure, A), although only 11 of them immunoreacted with the patient's serum (Figure, B). These spots were excised and underwent protein identification with mass spectrometry. As the *L usitatissimum* protein sequence database used is highly redundant, data were analyzed with Scaffold using the "protein cluster analysis" option. The liquid chromatography tandem mass spectrometry analysis (Supplemental materials and methods) enabled the identification of several proteins per sample. Thus, we focused on the major proteins, which were identified based on the number of total spectrum counts. A cluster of cupins (legumin, 11S globulin seed storage protein, 48-kDa glycoprotein precursor) were detected as the major proteins in spots 1-6, as was a second cluster (alanine-glyoxylate aminotransferase). In spots 7 and 8, a cluster of ATP-dependent CLP protease proteins was detected as the major protein in both samples. Moreover, a further 2 protein clusters corresponding to aconitate hydratase and oxoglutarate dehydrogenase were also robustly identified in these samples, as was a fourth protein cluster, chaperone protein CLPB3. As for spots 9-11, a cluster of ß glucosidase proteins was detected as the major allergen. A further 3 clusters from the chaperonin protein family were also robustly detected in the 3 ranges of spots. The patient gave his consent for his medical data to be published.

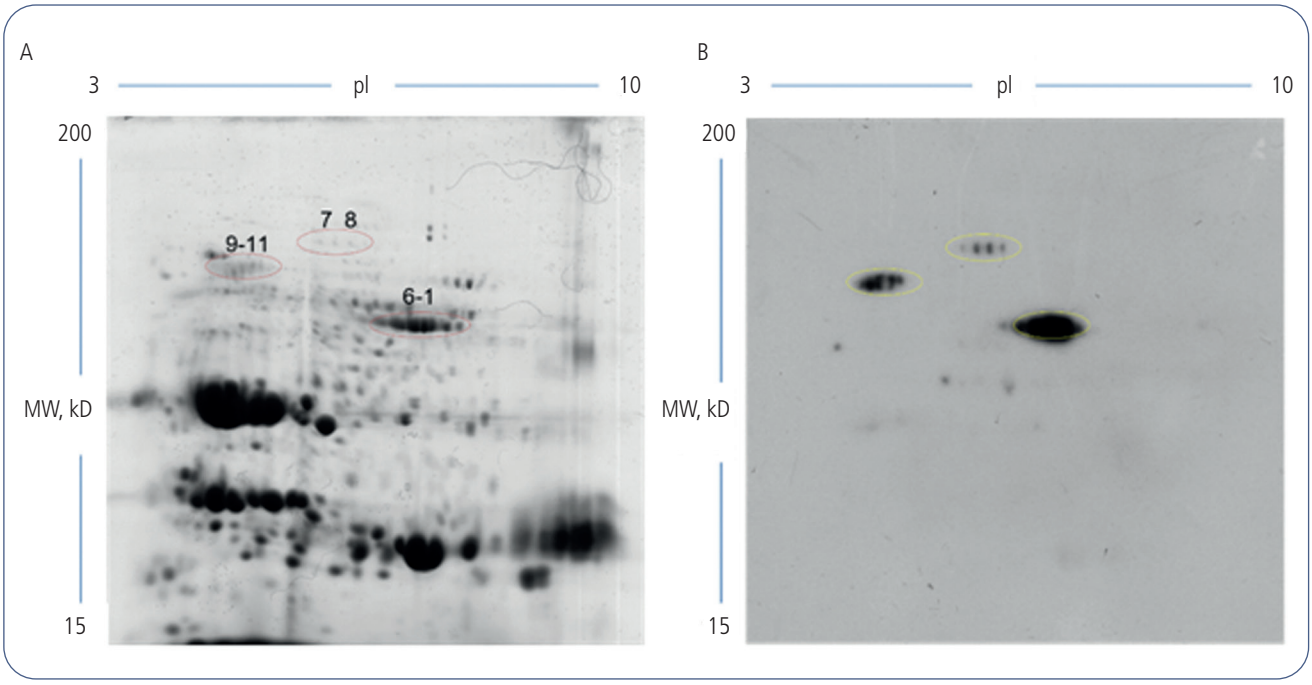

Figure. Representative images of 2D gel (A) and 2D Western blot (B) of flaxseed proteins immunodetected by the patient's serum.

To our knowledge, this is the first reported case of occupational rhinoconjunctivitis induced by flaxseed in which the allergenic proteins were identified. Only 2 previous studies have reported occupational respiratory allergy due to *L usitatissimum*. In one, the disease was confirmed by specific inhalation challenge [9]. The major flaxseed allergen included in the allergen nomenclature is Lin u 1, a 2s albumin. The 11S globulin seed storage protein and other cupins that we identified have already been considered probable allergens in flaxseed [10]. Moreover, LTP proteins have been described as possible culprit allergens in cases of anaphylaxis induced by accidental ingestion of linseed with coffee [5]. In the present case of occupational rhinoconjunctivitis, we identified a new, previously unreported airway allergen, alanine-glyoxylate aminotransferase. We also identified new flaxseed airway allergens of chaperonins and an enzyme family (ie, ATP-dependent CLP protease, aconitate hydratase, oxoglutarate dehydrogenase, ß glucosidase), which have been reported as airway allergens in other sources (Table 1S) [8], thus suggesting an allergic effect of these proteins due to inhalation, with major value in terms of possible cross-reactivity and in terms of preventing exposure at work.

The patient changed his job function to avoid exposure to flaxseed powder, and his rhinoconjunctivitis symptoms improved significantly.

In conclusion, since flaxseed is widely used in the food and pharmaceutical industry, workplace exposure to flaxseed powder must be considered a risk factor for occupational respiratory allergy. Preventive measures should be taken to avoid occupational airborne exposure to flaxseed powder.

The patient's clinical and occupational history, as well as the results of diagnostic tests and procedures, are detailed in the supplemental material.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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