

Letters

CLINICAL EFFICACY OF ASIAN LADYBUG WHOLE-BODY EXTRACT SKIN TESTING

Nakazawa et al¹ isolated 2 major allergens of the ladybug *Harmonia axyridis* and identified a 5% to 10% cross-reactivity of *H axyridis* and cockroach extracts by inhibition assay. This limited ladybug-cockroach cross-reactivity was consistent with a subsequent report of skin test concordance between ladybug and cockroach.² More recently, Clark et al³ examined the in vitro cross-reactivity of ladybug and cockroach using a whole-body *H axyridis* extract. Their inhibition studies demonstrated maximal 75% in vitro inhibition of cockroach by ladybug and 95% inhibition of ladybug by cockroach. They concluded that when testing for ladybug allergy, "a positive result may or may not indicate that exposure to actual ladybug allergens is causing sensitization"^{3(p432)} because in vitro assays may poorly differentiate between ladybug and cockroach sensitization. In contrast, our experience with routine in vivo whole-body ladybug skin testing for more than 10 years suggests that skin tests efficiently identify true ladybug hypersensitivity.

This West Virginia clinical skin test experience used a locally prepared 1:20 wt/vol glycerinated whole-body *H axyridis* extract prepared as described by Goetz.² In that report, aeroallergen skin test results were recorded as positive or negative. Original skin prick tests had been read 15 minutes after placement and were recorded as 0 (no wheal or erythema), 1+ (erythema <20 mm), 2+ (wheal <3 mm in diameter with surrounding erythema), 3+ (wheal ≥3 mm in diameter with surrounding erythema), or 4+ (wheal ≥3 mm in diameter with pseudopods and surrounding erythema). A positive aeroallergen skin test result was recorded if the result was either (1) 3+ or 4+ and the histamine control was 4+ or (2) 2+, 3+, or 4+ and the histamine control was 2+ or 3+. The 1,400 routine clinical skin tests (58 aeroallergens in the adult panel) performed between 2001 through 2004 differentiated ladybug- and cockroach-positive skin test results (1:20 wt/vol glycerinated skin tests) (ALK-Abello, Round Rock, Texas): 185 tests were positive for both ladybug and cockroach, 227 were cockroach positive and ladybug negative, and 114 were ladybug positive and cockroach negative.

The patient pairs of skin tests were compared using the κ statistic ($\kappa = 1$ if skin test results are identical [ie, concordant] for the 2 antigens in every patient and $\kappa = 0$ if skin test results differ [ie, are discordant] for the 2 antigens in every patient).⁴ Ladybug-cockroach skin tests were the most concordant of all arthropod pairs ($\kappa = 0.36$), with dust mite-cockroach ($\kappa = 0.29$) and dust mite-ladybug ($\kappa = 0.20$) concordance somewhat smaller. Although cross-reactivity may account for a modest portion of the concordance between ladybug and cockroach skin tests, the overall concordance was far smaller than that of a strongly cross-reactive pair of allergens, such as short and tall ragweed ($\kappa = 0.67$), rye and timothy grass ($\kappa = 0.83$), or the 2 dust mite species ($\kappa = 0.83$). The independence of the ladybug and cockroach skin test responses was also illustrated by the large number of single-positive skin tests (ie, only 1 positive skin test result among all 58 allergens in the standard panel) seen for 6%

of ladybug- and 6% of cockroach-positive skin test results. Only dust mites had a higher single-positive skin test frequency (10%).

The reliability of a whole-body *H axyridis* extract was further examined in the allergy population with the strongest history of clinical *H axyridis* allergy. Identification of true ladybug sensitivity is most critical for individuals who are tested and subsequently treated with ladybug allergen vaccine therapy. Since 1998, patients in my practice have been classified as ladybug allergic if they had a personal history of ladybug asthma, rhinoconjunctivitis, or urticaria and a positive ladybug skin test result. Ladybug-allergic individuals with significant symptoms have been offered ladybug allergen vaccine therapy.² To further investigate ladybug skin test validity, results for 89 individuals receiving ladybug allergen vaccine therapy during 2004 through 2007 were examined. As previously described, skin test reactions were graded using an integer scale from 0 to 4.² Cockroach and ladybug skin tests were examined for relative differences in size in this ladybug-allergic cohort (Fig 1). If cockroach-ladybug cross-reactivity caused significant misclassification of individuals as ladybug allergic when they were primarily cockroach sensitive, then cockroach skin test reactions in these individuals should be equal to, and more often larger than, ladybug skin test reactions. In fact, cockroach skin test reactions were larger than ladybug reactions in only 17% of patients, whereas ladybug skin test reactions were larger and by a much greater extent in 57% of patients. Skin test reactions were similar in size for 26%. In this highly *H axyridis*-allergic population, cockroach skin test reactions were undetectable in 35% of patients. The highly skewed larger ladybug skin test reaction sizes provide additional evidence that it is unlikely that ladybug skin test-positive patients were false-positives due to cross-reactivity.

When used for skin testing, whole-body *H axyridis* extract performs well in identification of individuals hypersensitive to this ladybug. Cross-reactivity between cockroach and ladybug is

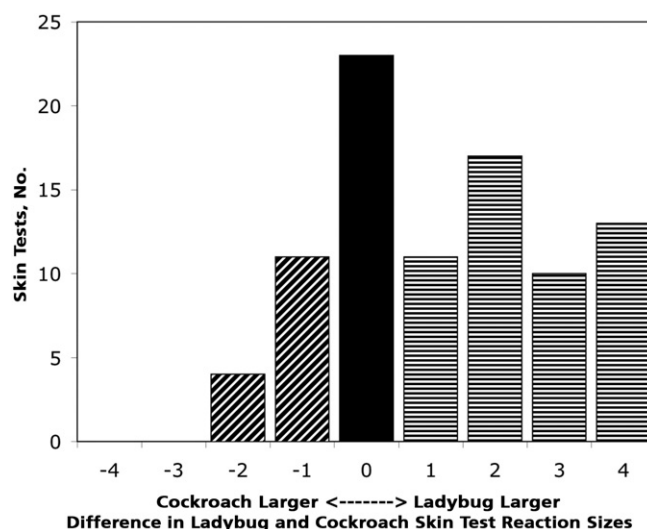


Figure 1. Relative size of ladybug and cockroach skin test reactions in 89 ladybug-allergic individuals treated with ladybug allergen vaccine therapy. Ladybug skin test minus cockroach skin test (size scale: 0–4).

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small and should rarely cause misidentification of allergy by species during skin testing.

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A NEW AUTOSOMAL RECESSIVE, HETEROZYGOUS PAIR OF MUTATIONS OF *CYBA* IN A PATIENT WITH CHRONIC GRANULOMATOUS DISEASE

CGD is an immunodeficiency disorder resulting when phagocytes cannot kill the microbes they have ingested, caused by a defect in the hydrogenated NADPH oxidase enzyme complex resulting in decreased production of reactive oxygen species.^{1,2} The NADPH oxidase comprises the membrane-bound flavocytochrome b558, a heterodimer of gp91phox and p22phox, and 4 cytosolic regulatory components, including p47phox, p67phox, p40phox, and Rac (Figure 1).³ About 2/3 of CGD cases are from mutations in the *CYBB* encoding gp91phox on chromosome Xp21.1, thus forming the X-linked (XL) recessive form of CGD. Most patients are male. Mutations in the genes encoding p47phox, p67phox, and p22phox cause the autosomal recessive (AR) forms of CGD, where males and females are equally affected, and collectively account for about 1/3 of CGD cases worldwide. A22 CGD, which indicates CGD due to a defect in p22phox, is rare and accounts for only about 6% of cases. The protein p22phox is encoded by the *CYBA* gene located on chromosome 16q24.⁴ Diagnosis of CGD is made by showing absent or sharply reduced oxidase activity in stimulated neutrophils. CGD has been screened using nitroblue tetrazolium. This test is subjective and relatively nonquantitative, being based on visual inspection of a limited number of cells, and could miss the diagnosis. Nevertheless, the dihydrorhodamine-1,2,3 (DHR) test is an easy, reliable, quick, and highly sensitive method to diagnose CGD.⁵ Wu et al⁶ also showed that the DHR test is more sensitive than the chemiluminescent test.

REPORT OF A CASE

The patient was a 12-year-old girl. At 5 years she had a fever with diarrhea and abdominal pain. The enterocolitis resolved on ceftriaxone treatment, but 1 week after remission she relapsed. Search for an immunodeficiency began. Results of the nitroblue tetrazolium test of neutrophils with phorbol-myristate acetate (PMA) were abnormal, and the chemiluminescent test also showed decreased activity, both suggesting CGD. The DHR test with PMA stimulation on flow cytometry confirmed the diagnosis (Figure

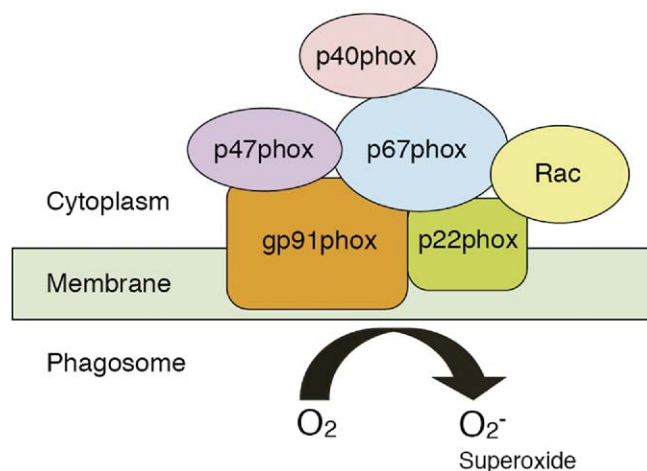


Figure 1. Diagram of the structure of the hydrogenated NADPH oxidase. Classic NADPH oxidase comprises a membrane-bound heterodimer of gp91phox and p22phox and 4 other subunits. In neutrophils, the oxidase is located on the phagosome membranes, and superoxide (O_2^-) is generated inside the phagosome. Mutations in the genes encoding these subunits of NADPH oxidase cause chronic granulomatous disease.

2A). While the patient received prophylaxis, she was repeatedly hospitalized with pulmonary TB, salmonellosis, cervical lymphadenitis, cellulitis, and osteomyelitis, complicated with septic shock once.

The DHR test of her parents showed positive respiratory bursts with intermediate peaks, indicating the heterozygous manner of her parents (Figure 2B and C). The patient and her family were tested for mutations in *CYBA* by PCR. The patient showed compound heterozygous mutations, which are substitution mutations in exon 2 and intron 3 in different alleles. The genomic DNA analysis and RT-PCR analysis of the patient showed a missense mutation in exon 2 (70G>A), resulting in a gly24-to-arg (G24R) substitution (Figure 3A), and an A>G substitution at position –2 of intron 3 (IVS3-2A>G) (Figure 3B). The mutation in intron 3 causes an mRNA splicing defect and results in skipping of exon 4 (Figure 4A and B). Her parents are carriers of 70G>A and IVS3-2A>G, respectively.

DISCUSSION

We describe a new AR, compound heterozygous mutation in the *CYBA* gene, from the parents of the CGD patient. Most previous *CYBA* mutations were homozygous. In most of these patients, the parents are heterozygous for the mutations, indicating consanguinity in the family. Our patient had a missense mutation and a splice site mutation. The missense mutation in exon 2 (G24R) was considered a mutation.⁷ Yamada et al⁷ reported the homozygous missense mutation in exon 2 (G24R) in 2 females who had a mild phenotype, impaired respiratory burst activities, and a reduced but detectable expression of p22phox protein (A22+). However, heterozygous mutations (combination of G24R mutation and splice, missense, or nonsense mutation) in other studies had an absence of p22phox protein expression (A220).^{8,9} The splice site mutation in intron 3, which causes skipping of exon 4, is a novel mutation. In one study,¹⁰ a homozygous G-to-A transition at position 1 of intron 4 (donor splice site) led to skipping

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