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ORIGINAL ARTICLE

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Enhancing the diagnostic accuracy of the IgE crosslinking-induced luciferase expression (EXiLE) method for walnut allergy

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Abstract
Background: Walnut (*Juglans regia*) frequently triggers nut allergies in the United Kingdom and in the United States, with increasing cases in Japan. While oral food challenges (OFCs) are the definitive method for diagnosing these allergies, they pose the risk of symptom provocation, necessitating safer alternative tests. Our aim here was to evaluate the diagnostic utility of IgE (immunoglobulin E) crosslinking-induced luciferase expression (EXiLE) for walnut allergy compared with the walnut-specific IgE (sIgE) test, Jug r 1-sIgE test, and skin prick test (SPT).
Methods: This retrospective study analyzed 55 patients tested for walnut allergy (WA) at Fujita Health University Bantane Hospital from January 2021 to December 2023. Among them, 38 had allergic reactions to walnuts based on history or OFCs and 17 did not. We evaluated the sensitivity, specificity, positive predictive value, negative predictive value, and the area under the curve (AUC) of the receiver operating characteristic curve.
Results: The EXiLE method (AUC = 0.938) exhibited superior diagnostic accuracy compared to the walnut-sIgE and comparable performance to Jug r 1-sIgE and SPT. The optimal cutoff value of 1.26-fold change demonstrated high sensitivity (0.92), specificity (0.88), positive predictive value (0.92), and negative predictive value (0.82). The EXiLE method yielded positive results in all three cases with negative Jug r 1-sIgE (< 0.35 U_A/mL).
Conclusion: The EXiLE method showed high sensitivity and specificity for diagnosing WA, indicating its potential clinical utility. Furthermore, the combination of Jug r 1-sIgE and EXiLE may enhance diagnostic accuracy. Future large-scale studies are warranted to confirm these findings and establish comprehensive diagnostic protocols.

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Introduction

Walnut (*Juglans regia*) is the common cause of nut allergies in the United Kingdom and the United States.[1] In Japan, there has been an increase in the number of patients with walnut allergy. Tree nuts are the third most common cause of food allergies, with walnuts being the most common culprit.[2] Moreover, walnuts are frequently associated with severe allergic reactions.[2] Accordingly, early diagnosis is crucial to avoid symptoms triggered by accidental ingestion.

Oral food challenge (OFC) is an essential diagnostic test for food allergies; however, it involves a risk of provoking symptoms, highlighting the need for alternative tests. In routine clinical practice, sensitization to walnuts is confirmed by measuring walnut-specific immunoglobulin E (sIgE) using ImmunoCAP and performing skin prick tests (SPTs). However, the diagnostic accuracy for symptomatic allergy remains limited.[3-6] Furthermore, SPT is an in vivo test and carries the risk of inducing allergic symptoms due to allergen exposure, albeit to a limited extent.[7]

The basophil activation test (BAT) and component-resolved diagnosis (CRD) have demonstrated utility with respect to enhanced in vitro diagnostic accuracy.[3,4,6] However, BAT requires fresh patient blood, which makes it difficult to routinely perform the test; further, there remains limited evidence regarding BAT for walnut allergy.[6] The clinical importance of CRD using Jug r 1-sIgE measured by the ImmunoCAP method has been demonstrated.[3,8] Nonetheless, even patients with Jug r 1-sIgE levels below 0.35 U_A/mL may exhibit symptoms,[9] indicating the need for further investigation.

IgE crosslinking-induced luciferase expression (EXiLE), which uses rat basophil leukemia cells (RS-ATL8 mast cell line), has demonstrated diagnostic utility for egg and shrimp allergies.[10,11] The EXiLE method can confirm cross-linking reactions to multivalent antigens and requires only a small amount of patient serum for testing.

The aim of this study was to investigate the diagnostic utility of the EXiLE method for walnut allergy.

Methods

Study design

This retrospective study evaluated the accuracy of the EXiLE method in diagnosing walnut allergy in comparison with existing methods, including the SPT, walnut-sIgE test, and Jug r 1-sIgE test. Patient data and test results were obtained by reviewing medical records.

Walnut allergy

The diagnosis of walnut allergy was established on the basis of walnut-sIgE level of 0.35 U_A/mL or higher, in conjunction with either a positive OFC or a documented history of positive symptoms, defined as allergic reactions of grade 2 or higher, or persistent grade 1. Symptoms were graded according to the method reported by Yanagida et al.[12]

Walnut tolerance

Walnut tolerance was defined as the consumption of more than 10 g of walnuts, either during the OFC in a clinical setting or through regular daily intake at home, with no symptoms.

Inclusion and exclusion criteria for participants

At the Department of Pediatrics, Bantane Hospital, Fujita Health University, a study was conducted involving 112 patients with a history of walnut ingestion who underwent walnut-sIgE blood tests between January 2021 and December 2023. Cases were excluded if stored serum was unavailable (n = 30), if there was no documented history of an allergic reaction (exceeding grade 2 or persistent grade 1 symptoms) due to walnut ingestion within the past two years (n = 16), or if the presence or absence of symptoms after walnut ingestion was indeterminate, specifically in instances where subjects had consumed less than 10 g of walnuts and experienced no symptoms (n = 11).[12] After a thorough examination of medical records and diagnostic findings, the study incorporated a total of 55 participants (Figure 1).

Study flowchart: We analyzed the data of 55 individuals after applying selection and exclusion criteria. Based on the presence or absence of symptoms induced by walnut consumption, participants were divided into two groups—the walnut allergy group and the walnut tolerant group.

Evaluation of patient characteristics

The attending physician was responsible for diagnoses of food allergy, atopic dermatitis, bronchial asthma, and allergic rhinitis.

Ethical considerations

In accordance with the principles of the Helsinki Declaration, the study design and potential risks of symptom induction were thoroughly explained to the patients and their guardians, both orally and in writing. Written informed consent was obtained from all participants for both the allergy tests and data publication. The study design was approved by the Institutional Review Board of Fujita Health University Hospital (Approval No. HM23-458).

OFC

The OFC was conducted using an open challenge method. Roasted walnuts were used to facilitate consumption. The attending physician determined the starting dose by considering the patient's clinical history and laboratory findings, in accordance with the Japanese Food Allergy Guidelines 2020, to maintain patient safety.[13] The total challenge dose was set between 0.1 g and 10 g and was administered in 1–3 divided doses. In cases of multiple administrations, the doses were given at 30-minute intervals. The total

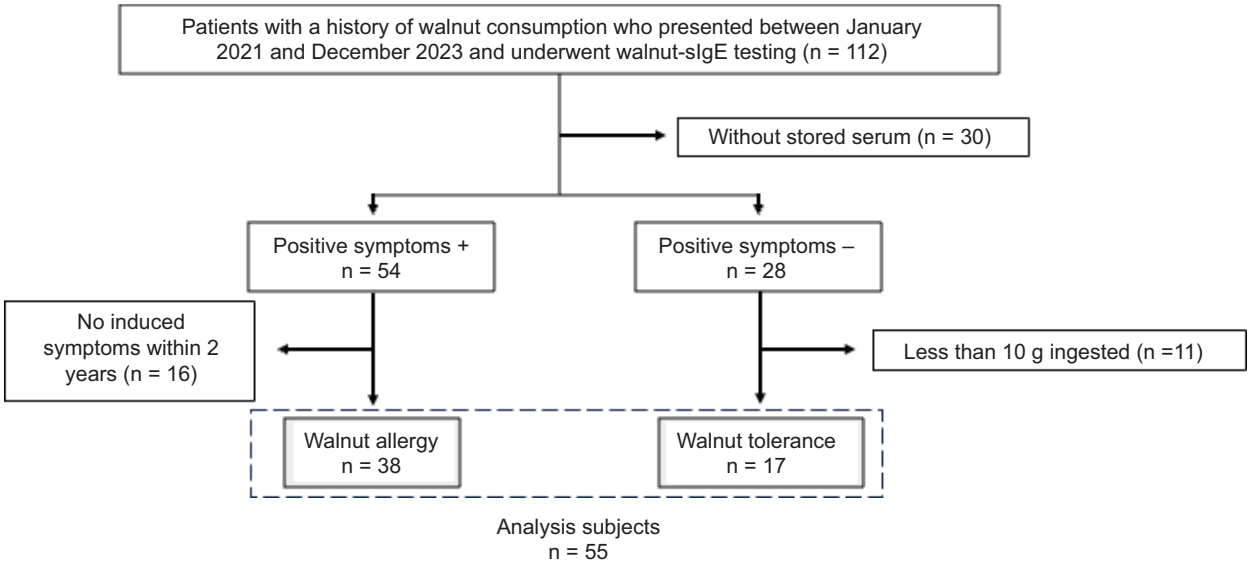

Figure 1 Study flowchart.

challenge dose was incrementally increased, with the goal of consuming 10 g. The challenge was terminated if positive symptoms occurred, the total challenge dose was consumed, or the subject was unable to continue consumption. A positive result was defined as grade 2 or higher, or persistent grade 1 symptoms occurring within 2 hours of consumption. A negative result was defined as the absence of any positive symptoms for ≥ 2 hours following the final dose.

Definition of anaphylaxis

The definition of anaphylaxis as established by the 2020 Anaphylaxis Guidance from the World Allergy Organization was used for the purposes of this study.[14]

Measurement of serum-specific IgE

Blood samples were collected during patient visits and stored at −30°C in a laboratory freezer. Specific IgE levels for walnut and Jug r 1 were measured using the ImmunoCAP assay system (Thermo Fisher Scientific, Uppsala, Sweden).

SPT

Commercially available raw walnuts (*Juglans regia*) were ground into a homogeneous paste, and the prick-by-prick test was performed using SmartPractice® prick lancets (SmartPractice, AZ, USA).[15] Saline solution was used as a negative control, with histamine solution (10 mg/mL; Torii Pharmaceutical, Tokyo, Japan) being used as a positive control. The size of the wheal was measured 15 minutes after the test began. If the shape of the wheal was irregular, the average of the longest diameter and the diameter perpendicular to its midpoint was taken as the measurement.[16]

Walnut protein extract

Commercial raw walnuts were ground into a paste and ≈50 mg was placed in a test tube. Subsequently, 1 mL of the Mammalian Cell Lysis Kit (Sigma-Aldrich, St. Louis, MO, USA) was added and mixed for 15 minutes while cooling. Next, the mixture was centrifuged at 12,000 × g for 10 minutes at 4°C, and the supernatant was filtered through a Millex-HP 0.45 μm filter (Merck Millipore Ltd., Burlington, MA, USA) and stored at −30°C. Protein concentration was measured using the Pierce BCA protein assay (Thermo Scientific, Uppsala, Sweden).

EXiLE method

The EXiLE method was performed following the protocol reported by Nakamura et al.[17] Specifically, RS-ATL8 cells, a rat mast cell line, were sensitized with serum diluted at 1:100 at a concentration of 5 × 10^4 cells/50 μL/well, followed by stimulation with walnut extract antigen diluted in a culture medium to a final concentration of 10 ng/mL. The optimal stimulation concentration was determined using pooled serum from 24 patients with walnut allergy (Figure S1). Luciferase luminescence was measured using a Nivo S Multimode Plate Reader (PerkinElmer Inc., Waltham, MA, USA). In cases where measurements were taken under identical conditions in multiple wells, the median value was used for the analysis. The same experiments were repeated on different days under similar conditions. The samples used were the same sera tested for walnut and Jug r 1-sIgE levels.

Statistical analysis

Patient characteristics and immunological measurements were evaluated using Fisher's exact test for categorical

variables and Mann–Whitney U test for continuous variables. The Kruskal–Wallis test was used to compare the severity between groups. To compare the diagnostic accuracy of each test, receiver operating characteristic (ROC) curves were generated. Subsequently, the area under the curve (AUC), sensitivity, and specificity at each cutoff value were calculated. Statistical significance was defined as a two-sided P-value < 0.05. Jug r 1-sIgE values, with a measurement range of 0.1–100 U_A/mL, were statistically processed as 0.05 U_A/mL if below 0.1 U_A/mL and as 101 U_A/mL if above 100 U_A/mL. Walnut-sIgE values were similarly processed for statistical analysis. The comparison of ROC AUCs was performed using EZR, a graphical user interface for R (version 4.2.3; R Foundation for Statistical Computing, https://www.R-project.org/), utilizing the method described by DeLong et al.[18] All other statistical analyses were performed using GraphPad Prism 10 (GraphPad Software Inc., CA, USA).

Results

Patient characteristics

Table 1 presents the characteristics and immunological parameters of the included participants. The median age at the time of blood sampling in the walnut tolerance group was significantly higher at 9 years compared to 4.5 years in the walnut allergy group (p = 0.0063). Furthermore, the walnut tolerance group demonstrated a significantly elevated median total IgE level, with values of 1673.9 kU/L compared to 505 kU/L in the walnut allergy group (p = 0.0003).

No significant between-group differences were observed in the rates of past immediate-type reactions to foods other than walnuts, atopic dermatitis, bronchial asthma, or allergic rhinitis.

SPT data were missing for 14 patients (6 in the walnut tolerance group and 8 in the walnut allergy group). Further clinical information regarding age, sex, non-walnut food allergies, and other allergic conditions is presented in Table S1.

Between-group comparison of test values

Walnut-sIgE, Jug r 1-sIgE, SPT, and EXiLE test values were compared between the walnut allergy and walnut tolerance groups (Figure 2). Compared with the walnut tolerance group, the walnut allergy group showed significantly higher values for all tests: walnut-sIgE (14.35 vs. 1.42 U_A/L, P < 0.0001), Jug r 1-sIgE (8.85 vs. 0.1 U_A/L, P < 0.0001), SPT (7.5 vs. 4.0 mm, P < 0.0001), and EXiLE (2.17- vs. 1.15-fold change, P < 0.0001).

Responses of walnut-sIgE (A), Jug r 1-sIgE (B), SPT (C), and EXiLE (D) in walnut-allergic and walnut-tolerant participants. The median response for each test is indicated by a line. Between-group differences in responses were assessed using the Mann–Whitney U test. There were significant between-group differences in all test values.

sIgE: specific IgE; SPT: skin prick test; EXiLE: IgE cross-linking-induced luciferase expression.

Induced symptoms and severity

Among the 38 individuals in the walnut allergy group, 12 had positive OFC results and 26 had a documented history of allergic reactions. Table S2 summarizes the symptoms and severity of reactions in the walnut allergy group. The most common symptoms were skin-related (76%), followed

Table 1 Patient characteristics.

Description	Tolerant	Allergic	P value
Number of subjects	17	38	
Male sex	13 (24%)	23 (42%)	0.3602
Age (y), median (min, max)			
At the time of blood collection	9 (4, 14)	4.5 (1, 13)	**0.0063**
Total IgE (kU/L), median (min, max)	1673.9 (294.2, 6682)	505 (10.5, 19,813)	**0.0003**
In the past, immediate reactions to other foods	14 (82%)	21 (55%)	0.0719
History of allergic disease			
Atopic dermatitis	11 (65%)	33 (86%)	0.0758
Bronchial asthma	6 (35%)	8 (21%)	0.3219
Allergic rhinitis	14 (82%)	16 (42%)	>0.9999
sIgE to walnut			
Level (kU_A/L), median (min, max)	1.42 (0.23, 19.8)	14.35 (0.9, >100)	**<0.0001**
sIgE to Jug r 1			
Level (kU_A/L), median (min, max)	0.1 (< 0.1, 9.82)	8.85 (< 0.1, >100)	**<0.0001**
SPT			
Wheal size (mm), median (min, max)	4 (0, 6)[a]	7.5 (3, 20)[b]	**<0.0001**

Data are expressed as n (%), unless otherwise noted.
To compare continuous and categorical data between groups, researchers employed the Mann–Whitney U test and Fisher's exact test, respectively. Both tests were conducted using a two-tailed approach.
[a]Missing values: 6.
[b]Missing values: 8.
sIgE: specific IgE; SPT: skin prick test.

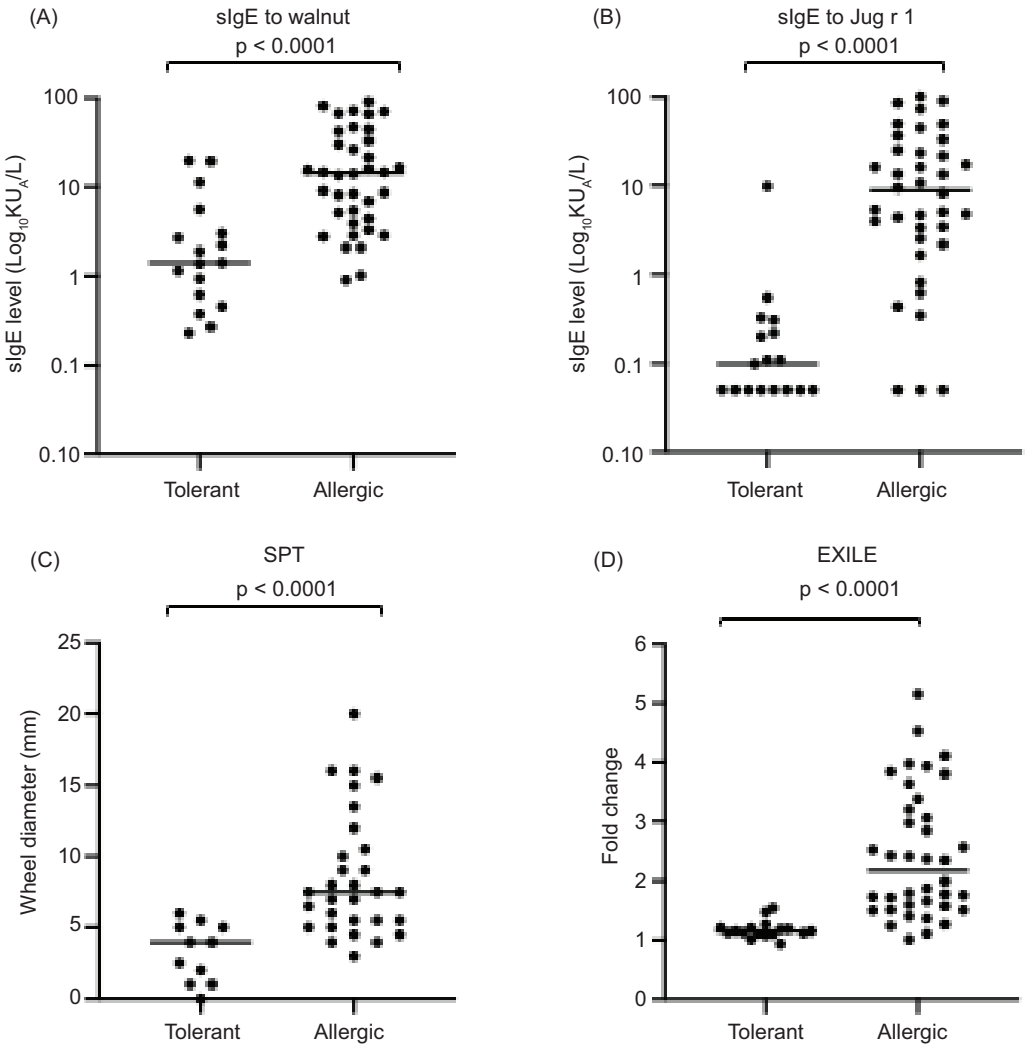

Figure 2 Between-group comparison of test values.

by respiratory (61%), gastrointestinal (39%), and neurological symptoms (13%). None of the patients presented cardiovascular symptoms. Of the patients, eight (21%) met the criteria for anaphylaxis. Regarding the severity of the reactions, 2 individuals were classified as having mild symptoms (5%), while 30 (79%) and 6 (16%) were classified as having moderate and severe reactions, respectively. There were no significant differences in the test results among the three groups (Figure S2).

ROC curves

Figure 3 shows the ROC curves for each test. The AUC values were highest for EXiLE (0.938; 95% CI, 0.873–1.000), followed by Jug r 1-sIgE (0.910; 95% CI, 0.825–0.996), SPT (0.894; 95% CI, 0.796–0.992), and walnut-sIgE (0.844; 95% CI, 0.728–0.959). When AUCs of the ROC curves for each test were compared, a significant difference was observed between EXiLE and walnut-sIgE levels, while no significant differences were found among the other tests. Table 2 presents the cutoff values, sensitivities, and specificities of each test. For EXiLE, a cutoff of 1.26-fold change resulted in a sensitivity of 0.92, specificity of 0.88, PPV of 0.92, and NPV of 0.82.

ROC curves illustrate the proportions of walnut allergic and walnut tolerant participants. Each type of allergy test (walnut-sIgE, Jug r 1-sIgE, SPT, and EXiLE using walnut extract) is represented by a different line pattern. AUC values are indicated.

sIgE: specific IgE; SPT: skin prick test; EXiLE: IgE crosslinking-induced luciferase expression; ROC: receiver operating characteristic; AUC: area under the curve.

Discussion

In this study, we evaluated the utility of the EXiLE method for diagnosing walnut allergy, comparing it with standardized diagnostic tests such as walnut-sIgE, Jug r 1-sIgE, and SPT. The results demonstrated that the EXiLE method showed higher diagnostic accuracy than walnut-sIgE and comparable accuracy to Jug r 1-sIgE and SPT.

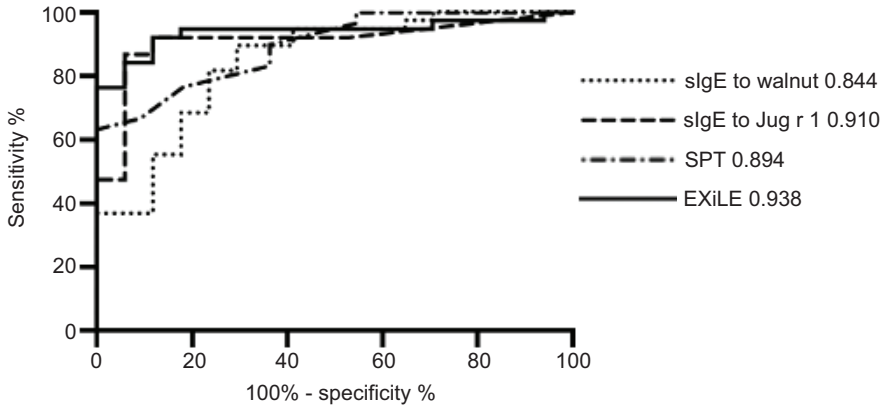

Figure 3 ROC curves.

Table 2 Diagnostic accuracy of walnut and Jug r 1 ImmunoCAP, SPT, and EXiLE method at individual cutoff values.

Allergy test	AUC	Cutoff	Sensitivity	Specificity	PPV	NPV	Positive likelihood ratio	P value by Fisher's exact test
sIgE to walnut	0.844 (0.728–0.959)	0.35 U_A/mL	1	0.12	0.69	1	1.13	0.0916
		3.30 U^A/mL	0.82	0.76	0.89	0.65	3.47	<0.0001
sIgE to Jug r 1	0.910 (0.825–0.996)	**0.35 U^A/mL**	0.92	0.88	0.92	0.82	7.83	<0.0001
SPT	0.894 (0.796–0.992)	3.0 mm	1	0.45	0.83	1	1.83	0.0006
		5.5 mm	0.77	0.82	0.83	0.83	4.22	0.0012
EXiLE	0.938 (0.873–1.000)	**1.26-fold change**	0.92	0.88	0.92	0.82	7.83	<0.0001

The optimal cutoff values estimated from ROC are highlighted in bold, with 95% confidence intervals within parentheses. ROC: receiver operating characteristic; AUC: area under the curve; PPV: positive predictive value; NPV: negative predictive value; sIgE: specific IgE; EXiLE: IgE crosslinking-induced luciferase expression.

The AUC of the EXiLE method was 0.938 (95% CI, 0.873–1.000), which was the highest among the methods tested. The sensitivity, specificity, PPV, and NPV were 0.92, 0.88, 0.92, and 0.82, respectively, confirming the excellent performance of the EXiLE method for diagnosing walnut allergy. The AUC for Jug r 1-sIgE was 0.910, showing similarly high diagnostic accuracy, which is consistent with previous studies reporting its effectiveness in CRD of walnut allergy.[3]

SPT and BAT have been reported to be useful tools for detecting IgE crosslinking reactions to multivalent antigens.[19] While SPT is widely used in clinical practice due to its simplicity, it carries the risk of inducing allergic reactions, with systemic reactions occurring in 15–23 cases per 100,000 tests and an anaphylaxis incidence rate of approximately 0.02%.[7] BAT, however, requires fresh blood samples and the use of flow cytometry to measure cell surface markers, which limits its clinical application.[19,20] In comparison, the EXiLE method shares a similar diagnostic principle with BAT, as both are in vitro tests designed to measure IgE crosslinking reactions. However, the EXiLE method offers several practical advantages compared to BAT. Unlike BAT, the EXiLE method utilizes a small amount of stored serum, providing greater flexibility in sample handling and storage,[10] which makes it suitable for multisample studies and delayed testing. Although the EXiLE method requires the cultivation of RS-ATL8 cells and the measurement of luciferase luminescence, it can process multiple samples simultaneously in a two-day workflow, enabling efficient high-throughput testing. This feature may make the EXiLE method more cost-effective in high-volume settings, despite requiring specialized equipment for cell culture and luminescence measurement. These attributes, including its reliance on stored serum, capacity for high-throughput testing, and cost-effectiveness in large-scale testing, may minimize patient risk, enhance diagnostic accuracy, and make the EXiLE method particularly advantageous for both research and clinical environments where safety, scalability, and efficiency are essential.

Jug r 1, which is a 2S albumin, is one of the major allergens in walnuts; however, other allergens such as lipid transfer proteins, 11S globulin, and vicilin fractions have also been reported to be involved in systemic reactions.[9,21] In this study, three cases in the walnut allergy group were negative for Jug r 1-sIgE (< 0.35 U_A/mL), whereas the EXiLE method yielded positive results in all three cases. Conversely, among the three cases that tested negative with the EXiLE method, all three had positive results for

Jug r 1-sIgE. This suggests that the EXiLE method is useful in diagnosing walnut allergy even when Jug r 1-sIgE is negative. These findings are summarized in Table S3, which provides detailed information on the diagnostic test results for these patients. The discrepancies between EXiLE-positive and Jug r 1-sIgE-negative results suggest the involvement of additional allergenic components beyond Jug r 1. For instance, LTPs or other cross-reactive markers may contribute to these cases. Molecular testing, such as CRD, plays a critical role in diagnosing complex allergies by identifying specific allergenic components and enabling a more precise understanding of cross-reactivity patterns. Incorporating CRD alongside the EXiLE method could enhance diagnostic accuracy, particularly in cases where traditional diagnostic tools may be insufficient. By combining the high sensitivity of EXiLE with the precision of CRD, future diagnostic protocols may offer a more comprehensive assessment of walnut allergy, potentially improving patient management and treatment strategies.

In this study, significant differences were observed in age at blood sampling and total IgE levels between the walnut allergy and walnut tolerance groups. Although the total IgE levels were consistent with a previous report by Sato et al.,[3] the retrospective nature of the study may have introduced selection bias. Notably, most of the walnut tolerance group in this study consisted of patients who were attending the hospital for other food allergies, which may have influenced the results. Further multicenter studies are necessary to validate these findings.

This study has several limitations. First, the relatively small sample size necessitates caution when generalizing the results. Additionally, the retrospective design and setting within a specialized allergy hospital may have introduced bias in data collection and interpretation. Second, instances where the total walnut consumption was below 10 g and no symptoms were observed were excluded, as this quantity may not be representative of the threshold for symptom induction. This exclusion resulted in a reduced number of cases available for analysis, which may limit the generalizability of the findings. Moreover, missing data for SPT further limited the scope of the analysis. The EXiLE method demonstrated high sensitivity and specificity in diagnosing walnut allergy. However, its clinical applicability depends on addressing certain limitations. In this study, crude walnut extracts were used as the antigen source. Given the high sensitivity of the EXiLE method, with antigen stimulation concentrations as low as ng/mL, the quality and consistency of these extracts are critical for ensuring reproducibility and diagnostic precision. To overcome this limitation, we recommend that future studies develop and use standardized extraction protocols, ensuring careful attention to avoid contamination with other proteins throughout the extraction to measurement process. These measures could reduce variability and optimize the performance of the EXiLE method, particularly in highly specific diagnostic settings. Such improvements would further enhance its reliability and utility in both research and clinical environments. In this study, walnut allergens other than Jug r 1 were not measured and therefore could not be evaluated. We recognize this limitation and propose that future research should include a broader range of allergenic components to provide a more comprehensive understanding of walnut allergy. This approach could also contribute to the elucidation of cross-reactivity mechanisms and further improve diagnostic precision.

Conclusions

Our findings suggest that the EXiLE method is beneficial for the diagnosis of walnut allergy. By incorporating the EXiLE method into routine diagnostic practice, healthcare providers may improve the accuracy of walnut allergy diagnosis, thereby enhancing patient management and care. To further enhance diagnostic accuracy, we propose expanding diagnostic protocols to incorporate molecular markers and cross-reactivity analyses in subsequent investigations. These methodologies could elucidate sensitization patterns and cross-reactivity mechanisms, particularly in patients presenting with complex walnut allergies. To address the limitations identified in this study, future large-scale multicenter prospective studies are essential to further validate these findings and establish the role of the EXiLE method in comprehensive allergy assessment.

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Disclosure

The abstract of this paper is planned to be presented at the Pediatric Academic Societies Meeting 2025.

Authors Contribution

Hikaru Sugita was responsible for planning the study, preparation of the walnut extract, data acquisition, analysis, and interpretation, and drafting the manuscript. Yuji Mori contributed to data acquisition, interpretation, and manuscript drafting. Tetsushi Yoshikawa contributed to data interpretation. Yasuto Kondo participated in the study design, data interpretation, and manuscript drafting. All authors have read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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Supplementary

Table S1 Clinical characteristics and allergy history of patients, including their age, sex, history of non-walnut food allergies, and other allergic conditions.

Patient ID	Age at the time of blood collection (y)/sex	Other food allergy history	Other allergy history
WT1	9/F	Egg, milk, kiwi, sesame, tomato	AD, BA, AR
WT2	4/M	Milk	None
WT3	11/F	Peanut, sesame, kiwi, orange, pineapple, buckwheat	AR
WT4	4/M	Egg	None
WT5	7/M	None	AR
WT6	14/M	Kiwi, peach, melon, cherry, egg	AD, AR
WT7	4/M	Milk	AD, BA
WT8	4/M	Egg	AD, BA, AR
WT9	7/M	None	AD
WT10	9/F	None	AD, AR
WT11	9/M	Egg	AD
WT12	9/M	Peanut, milk	AR
WT13	5/M	Peanut, wheat	AD, BA, AR
WT14	6/F	Peanut, milk, fish	AD, AR
WT15	11/M	Egg, milk	AD, BA, AR
WT16	10/M	Egg, milk	AD, BA, AR
WT17	10/M	Egg, milk	AR
WA1	6/M	Cashew, pistachio	AD, BA, AR
WA2	2/F	Egg, milk	AD
WA3	5/F	None	None
WA4	4/M	None	AD, AR
WA5	4/M	None	AD, AR
WA6	6/M	Peanut	AD, AR
WA7	3/F	Pecan	AD, AR
WA8	4/F	None	AD, AR
WA9	6/F	Egg	AD, BA
WA10	6/F	None	AD, AR
WA11	7/M	Wheat	AD, AR
WA12	2/F	Cashew, egg	AD
WA13	13/M	Peanut	AD, AR
WA14	6/M	Hazelnut, melon, peach, watermelon	AD, AR
WA15	4/F	None	AD, AR
WA16	2/M	None	None
WA17	3/M	None	None
WA18	11/M	Egg, milk	AD, AR
WA19	13/M	Milk, salmon roe, apple, pineapple, tomato, eggplant	AD, AR
WA20	9/M	Cashew, egg	AD, BA, AR
WA21	6/M	None	BA, AR
WA22	3/F	Egg	AD, AR
WA23	4/F	None	AD, AR
WA24	2/M	None	AD
WA25	9/M	Hazelnut, banana, lotus root, fish	AD
WA26	1/M	None	AD
WA27	2/F	Cashew	AD, AR
WA28	7/M	Cashew, pistachio	AD
WA29	9/M	None	AD, AR
WA30	7/M	None	AD, BA, AR
WA31	7/F	Milk	AD, BA, AR
WA32	3/M	None	None
WA33	9/F	Egg	AD, AR
WA34	9/M	None	AD, AR
WA35	3/M	Shrimp	AD, BA
WA36	4/F	None	AD, AR
WA37	3/F	Egg	AD, BA, AR
WA38	4/M	Cashew	AD

WA: walnut allergy; WT: walnut tolerance; AD: atopic dermatitis; BA: bronchial asthma; AR: allergic rhinitis.

Table S2 Symptoms and severity of walnut allergy group (n = 38).

Symptoms	Number of positive cases (%)
Skin	29 (76)
Respiratory	23 (61)
Gastrointestinal	15 (39)
Cardiovascular	0 (0)
Nervous	5 (13)
Anaphylaxis	8 (21)
Symptom severity	
Mild	2 (5)
Moderate	30 (79)
Severe	6 (16)

Table S3 Diagnostic test results for patients with discrepancies between EXiLE and Jug r 1-sIgE.

Patient ID	Walnut-sIgE level (kU_A/L)	Jug r 1-sIgE level (kU_A/L)	EXiLE (fold change)	SPT wheal diameter (mm)
WA6	1.02	**0.62**	1.014	**16**
WA31	0.9	**0.82**	1.110	ND
WA32	2.09	**2.55**	1.237	4.5
WA8	**3.9**	< 0.1	**1.503**	**5.5**
WA15	2.1	< 0.1	**1.509**	**6.5**
WA33	**15.6**	< 0.1	**1.718**	ND

The values that correspond to positive results for each diagnostic test, based on the optimal cutoff values determined in this study, are shown in bold. Specifically, the cutoff values for each test are as follows: walnut-sIgE (3.30 kU_A/L), Jug r 1-sIgE (0.35 kU_A/L), SPT (5.5 mm), and EXiLE (1.26-fold change).
WA: walnut allergy; sIgE: specific IgE; EXiLE: IgE crosslinking-induced luciferase expression; SPT: skin prick test; ND: not determined.

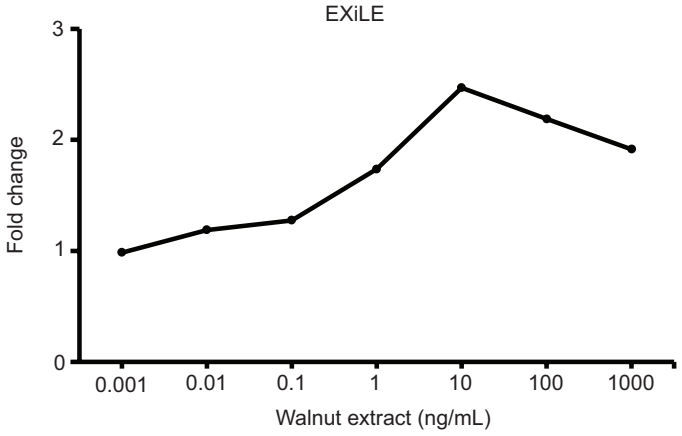

Figure S1 Dose-response curve of luciferase expression in the EXiLE test using walnut extract. Serum pools diluted 1:100 (from 24 walnut-allergic subjects) were stimulated with walnut extract at concentrations ranging from 0.001 to 1000 ng/mL. EXiLE, IgE crosslinking-induced luciferase expression.

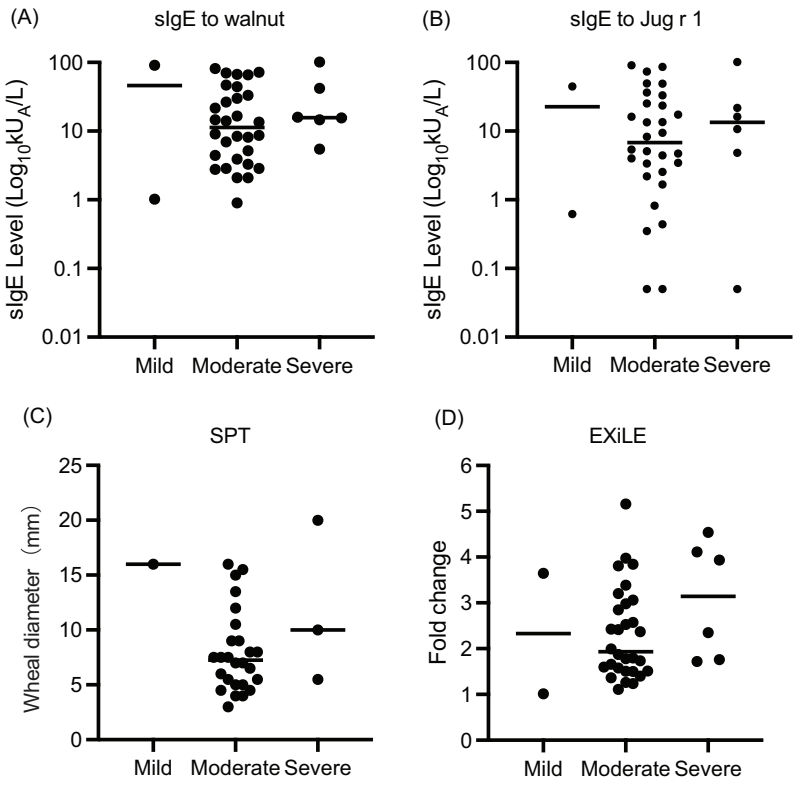

Figure S2 Responses of walnut-sIgE (A), Jug r 1-sIgE (B), SPT (C), and EXiLE (D) across the severity groups. The median response for each test is indicated by a line. Between-group differences in responses were assessed using the Kruskal–Wallis test. No significant between-group differences were observed in any of the tests. sIgE, specific IgE; SPT, skin prick test; EXiLE, IgE crosslinking-induced luciferase expression.