ORIGINAL RESEARCH

Human epididymis protein 4 (HE4) is a novel biomarker for fibrosis in IgG4-related disease and can predict poor prognosis

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ABSTRACT

Objectives IgG4-related disease (IgG4-RD) is an immune-mediated fibroinflammatory disorder with heterogeneous manifestations. This study aimed to investigate the utility of human epididymis protein 4 (HE4) as a potential clinical biomarker of fibrosis in IgG4-RD.

Methods Plasma HE4 levels were estimated in 136 patients with IgG4-RD and 73 healthy individuals (controls) by electrochemical luminescence. HE4 expression levels and the degree of fibrosis in pancreatic tissues from 15 patients with IgG4-RD and 10 controls were compared using immunohistochemistry and Masson trichrome staining. Correlation between HE4 levels and laboratory parameters was determined, and the efficacy of HE4 as a biomarker of fibrosis and prognosis in IgG4-RD was also evaluated.

Results Plasma HE4 levels were significantly higher in patients with IgG4-RD compared with controls. Optimal HE4 cut-off value for identifying patients with IgG4-RD was determined to be 50.8 pmol/L with an AUC (area under curve) of 0.791. HE4 levels were positively correlated with diverse laboratory parameters, and indicators of organ function impairment. Additionally, HE4 was highly expressed in the affected organs in patients with IgG4-RD and its plasma levels were closely correlated with degree of fibrosis, indicating the utility of HE4 in assessing internal organ damage and fibrosis. Further analysis showed that patients in the HE4 high expression group had poor prognosis.

Conclusions Our results demonstrate that HE4 can be used as a biomarker for IgG4-RD as it is correlated with diverse baseline clinical features, internal organ damage and degree of fibrosis in affected organs, and can predict poor prognosis.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The precise roles of some of IgG4-related disease (IgG4-RD) biomarkers remain controversial, and biomarkers that are directly related to organ fibrosis or damage are currently lacking.

WHAT THIS STUDY ADDS

⇒ This is the first study to demonstrate that human epididymis protein 4 (HE4) levels can function as a useful biomarker and predict prognosis in IgG4-RD.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ HE4 can be used as a biomarker for IgG4-RD as it is correlated with diverse baseline clinical features, internal organ damage and degree of fibrosis in affected organs, and can predict poor prognosis

Of interest, the hallmarks of IgG4-RD are an irregular, whorled pattern of fibrosis, called storiform-type fibrosis, and abundant infiltration of IgG4 positive plasma cells in the affected organs.[3] However, the mechanisms that promote this characteristic damage are currently unclear. Traditional biomarkers of IgG4-RD, like serum IgG4[4] and IgE,[5] as well as the newly recognised immune cell indicators, including plasmablasts[6] and follicular helper T cells,[7] have been reported to be closely correlated with disease activity and treatment response. However, the precise roles of some of these biomarkers remain controversial, and biomarkers that are directly related to organ fibrosis or damage are currently lacking.

Human epididymis protein 4 (HE4) is a secreted member of the whey acidic protein domain-putative extracellular protease inhibitor protein family[8] and has been widely used as a biomarker for evaluating malignancy risk in multiple neoplastic diseases, especially ovarian cancer.[9] Given the increasing

INTRODUCTION

IgG4-related disease (IgG4-RD) is recognised as an immune-mediated fibroinflammatory disorder that can affect almost any organ. It is characterised by the formation of tumour-like masses,[1 2] and patients often manifest with swelling and fibrosis of affected organs, accompanied by elevated serum IgG4 levels.

risk of cancer in patients with autoimmune diseases,[10] neoplastic biomarkers, including HE4, are now being used to estimate cancer risk. Congruently, HE4 was found to be elevated in some autoimmune diseases, including systemic lupus erythematosus (SLE), primary Sjögren's syndrome (pSS) and rheumatoid arthritis (RA), and notably, this increase was greater in patients with SLE with lupus nephritis[11] patients with pSS and patients with RA with interstitial lung disease.[12 13] HE4 has also been recently recognised as a biomarker for fibrotic disease and is reportedly involved in the aetiopathology of fibrotic diseases because fibroblast-derived HE4 has protease inhibitor activity that can promote the aggregation of collagen and cause fibrosis.[14 15] Based on these observations, and the fact that IgG4-RD is a recognised immune-mediated fibroinflammatory disease, we hypothesised that HE4 may play a role in IgG4-RD fibrosis and that it may be a potential biomarker for this disease. Therefore, this study evaluated the utility of HE4 as a biomarker for IgG4-RD, and assessed the correlation between HE4, clinical features and prognosis in IgG4-RD.

METHODS
Participant enrolment
This study was based on a prospective cohort (*registered on ClinicalTrials.gov, NCT01670695; study start date: June 2012*) that was recruited at the Peking Union Medical College Hospital (PUMCH). Treatment-naïve patients with IgG4-RD who met the 2019 American College of Rheumatology/European League Against Rheumatism classification criteria,[16] as well as age/gender-matched healthy controls (HCs) were enrolled in this study. Patients with other autoimmune diseases or malignancies were excluded.

Laboratory tests
Laboratory tests, including complete blood cell count, liver and renal function, erythrocyte sedimentation rate (ESR), hypersensitive C-reactive protein, serum IgG, IgM, IgA, IgE, IgG subclass and complement, were performed at recruitment.

Treatment strategies
The treatment strategies of the enrolled patients were categorised into one of the following four types: glucocorticoid (GC) monotherapy, GC combined with immunosuppressants (IMs, GC+IMs), sparce GC use (treated only with IMs) and watchful waiting. The IMs prescribed included cyclophosphamide, mycophenolate mofetil, azathioprine, methotrexate, leflunomide and iguratimod.

Assessment of disease activity and prognostic outcome definitions
Disease activity was assessed using IgG4-RD Responder Index (RI) scores[17] at every visit during the follow-up period. Patients with IgG4-RD were divided into two groups based on prognostic outcomes, namely, remission

and deterioration (poor prognosis). Poor prognosis was defined as the disease relapse or the need to switch to a stronger treatment due to disease fluctuation. Disease relapse was defined as the recurrence or worsening of clinical symptoms or imaging findings[18]; however, a mere elevation in serum IgG4 level was not considered as disease relapse. Remission was defined as the improvement of affected organs and symptoms and the decrease of IgG4-RD RI score with the successful reduction of treatment dose to maintenance dose.

HE4 measurements
Blood samples from treatment-naïve patients with IgG4-RD, patients with IgG4-RD after treatment and matched HCs were collected in 5 mL vacutainer tubes containing the chelating agent EDTA on an early morning fast. Each participant was required to fast for at least 8 hours prior to sample collection. Plasma was separated from 5 mL of peripheral blood by centrifugation at room temperature within 30 min of collection and was stored at −80°C until analysis. Plasma HE4 levels were measured on a Roche Cobas electrochemical luminescence analyser (Hoffmann-La Roche, Basel, Switzerland), at the Department of Clinical Laboratory, PUMCH, according to manufacturer's instructions.

Immunohistochemistry
Tissue samples from patients with IgG4-RD were obtained on biopsy or surgery at PUMCH and were histopathologically confirmed. These tissues were retrospectively identified, retrieved and analysed separately from this observational study. Control tissues used for comparison included the healthy parts of the pancreas (solid pseudopapillary neoplasm, serous cystic tumour or mucinous cystic neoplasm), which were also obtained by biopsy or surgery.

Tissue array slides were deparaffinised with two changes of xylene, each for 10 min, and the slides were rehydrated in graded concentrations of ethanol (100%, 95%, 85%, 75%) for 5 min each. Next, they were subjected to heat-mediated antigen retrieval in Tris/EDTA buffer (pH 9.0) in a pressure cooker. Endogenous peroxidase activity was quenched using 3% H_2O_2 at room temperature for 10 min. After rinsing thrice with distilled water, each time for 3 min, slides were blocked in 5% goat serum for 30 min at room temperature and then incubated overnight with 1:1000 diluted recombinant anti-HE4 monoclonal antibody (Abcam, ab200828) at 4°C. Following extensive washing with phosphate buffered saline buffer, slides were incubated for 1 hour with a biotinylated secondary antibody from a test kit (PV-9001, ZSGB-BIO, Beijing, China), the colour developed with a DAB kit (ZLI-9017, ZSGB-BIO, Beijing, China), the reaction stopped using distilled water and the slides counterstained with haematoxylin. Slides were dehydrated in graded concentrations of ethanol, cleared with two changes of xylene, each time for 10 min and then dried and mounted using Permount Mounting Medium.

HE4 expression measurement

HE4 expression in each slide was scored based on staining intensity as 0—negative, 1—weak, 2—moderate and 3—strong, and multiplied by the percentage of positive cells, which was scored as 0, 1 (1%–24%), 2 (25%–49%), 3 (50%–74%) or 4 (75%–100%). Tissue samples were categorised as negative (score ≤4) or positive (score ≥5) based on the final staining score.

Masson trichrome stain

Tissue array slides were deparaffinised and rehydrated as described above, and all slides with IgG4-RD were stained with Masson trichrome. Collagen area and total tissue area were measured using ImageJ (V.1.53c), and collagen volume fraction (CVF) was expressed as percentage of total area.

Analysis of HE4 expression in the Human Protein Atlas

We used the Human Protein Atlas (https://www.protein-atlas.org) website to obtain data on the expression levels of HE4 in different organs. Data on protein expression was available for 44 normal human tissue types based on antibody-based protein profiling using immunohistochemistry and messenger RNA expression data based on deep sequencing included information on 256 different normal tissue types.[19]

Statistical analyses

Patient clinical data are described based on distribution patterns, that is, normally distributed variables are shown as mean±SD, while non-normally distributed data are displayed as median with IQR. SPSS, V.25.0 for Windows (SPSS, Armonk, USA) was used for all statistical analyses. The Mann-Whitney test was used for comparison between two groups. The receiver operator characteristic (ROC) curve was used to determine the cut-off value of HE4 that can distinguish between patients with IgG4-RD and HCs. Spearman's correlation was used to determine the relationship between HE4 and other variables. Kaplan-Meier curves and the log-rank test were used to compare relapse rate and prognosis between patients with HE4-positive (HE4+) and HE4-negative (HE4−) IgG4-RD. A p value of <0.05 was considered to indicate statistical significance.

RESULTS

Plasma HE4 levels are significantly higher in IgG4-RD

Detailed clinical characteristics of the 136 treatment-naïve patients with IgG4-RD and 73 age-matched and gender-matched HCs enrolled in this study are listed in online supplemental table S1, and the study flow chart is shown in figure 1. Plasma HE4 levels were significantly elevated in patients with IgG4-RD (58.8 pmol/L;
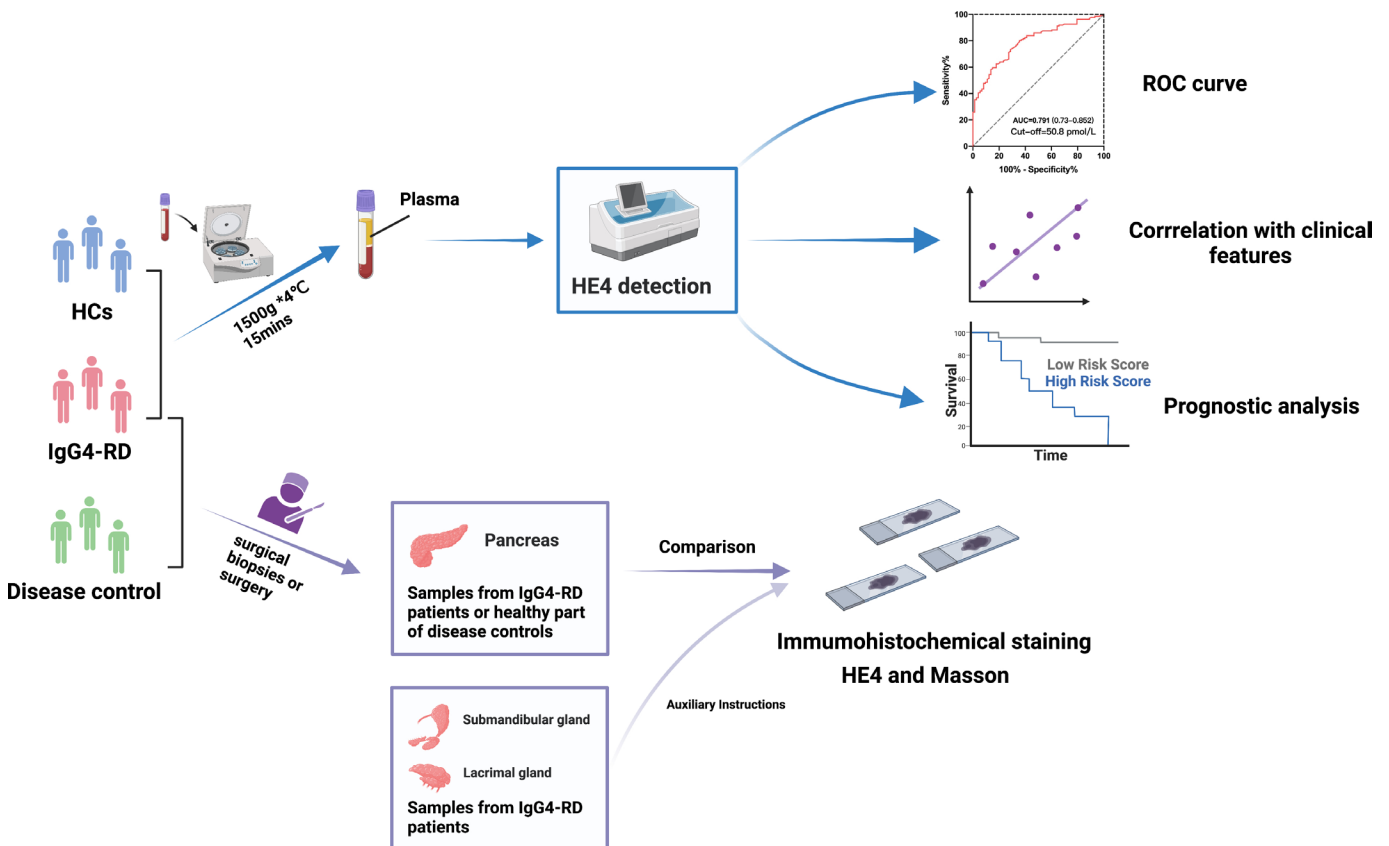
Figure 1 The workflow diagram of this study (created with BioRender.com). Disease control, control tissues from the healthy parts of the pancreas (solid pseudopapillary neoplasm, serous cystic tumour or mucinous cystic neoplasm). HCs, healthy controls; HE4, human epidermis protein 4; IgG4-RD, IgG4-related disease; ROC, receiver operator characteristic; AUC: area under curve.
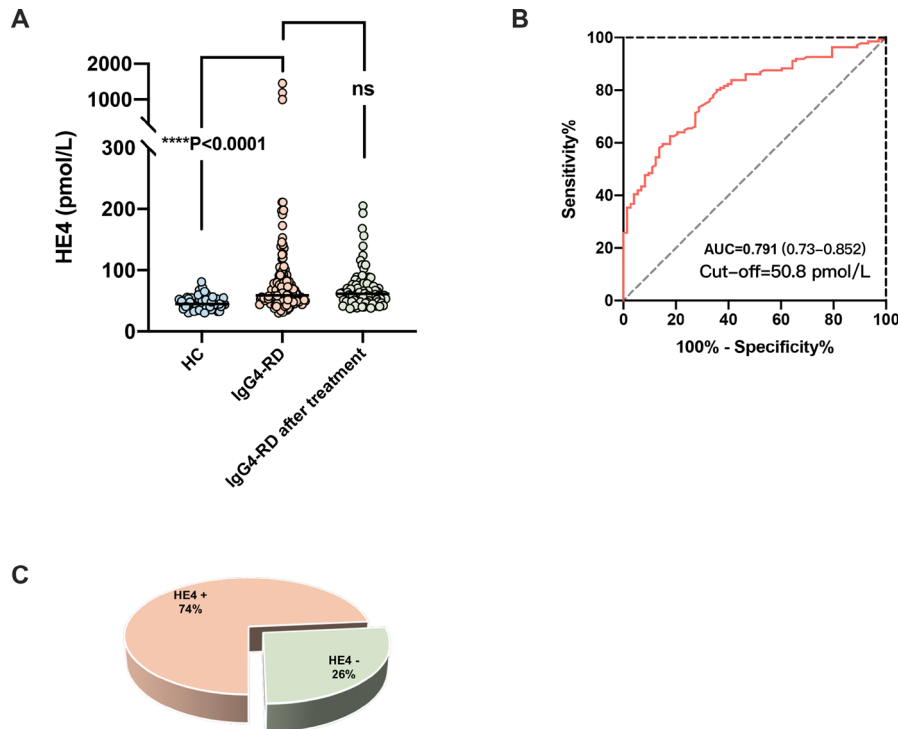
Figure 2 The plasma HE4 levels in patients with IgG4-RD. Comparison of the plasma HE4 levels in patients with IgG4-RD and HCs. ROC curve for the HE4 cut-off value between patients with IgG4-RD and HCs. The distribution of patients with HE4-positive (HE4+) and HE4-negative (HE4−) IgG4-RD according to the cut-off value (*p<0.05, **p<0.01, ***p<0.001). HCs, healthy controls; HE4, human epididymis protein 4; IgG4-RD, IgG4-related disease; ROC, receiver operator characteristic; AUC: area under curve.

IQR, 50.3–82.2) compared with HCs (45.0 pmol/L; IQR, 39.8–52.6; p<0.0001), as shown in figure 2A. Next, ROC analysis to determine the cut-off level of HE4 for identifying patients with IgG4-RD, based on the Youden index, yielded a value of 50.8 pmol/L with an AUC (area under curve) of 0.791 (95% CI 0.730 to 0.852, p<0.0001, figure 2B). However, plasma HE4 levels (n=94) remained stable after treatment during the follow-up period (61.5 pmol/L; IQR, 50.4–76.7, figure 2A).

Next, patients with IgG4-RD were categorised as either HE4+ or HE4−, based on this cut-off value of 50.8 pmol/L (figure 2C), and the results of a comparison of the clinical features between these two groups are displayed in online supplemental table S2 and figure 3. We show that, compared with HE4− patients, HE4+ patients were older at disease onset (p<0.001, online supplemental table S2) and that this group contained more men than women (p=0.004, online supplemental table S2). Meanwhile, the elevated HE4 levels in male subjects were only seen in patients with IgG4-RD, but not in age-matched and sex-matched HCs (online supplemental figure S1). Multiple serum immunoglobulin levels (figure 3A) and other laboratory test results (figure 3B) were elevated in HE4+ patients compared with HE4− patients, and these included IgG4, IgA, IgM, ESR, C-reactive protein (CRP), eosinophils and creatinine (Cr). C3 was significantly decreased in HE4+ patients. Next, lacrimal gland involvement was lower in HE4+ patients (38% vs 61%, p=0.017, figure 3C), but interestingly, they displayed significantly

more internal organ involvement, including in the pancreas, bile duct, lung, retroperitoneum and kidney. In contrast, HE4− patients had prominent superficial organ involvement (including lacrimal gland, submandibular gland, parotid gland and paranasal sinus) and the proportion of internal organ involvement in HE4− and HE4+ patients was 63% and 36.11% (p=0.005), respectively figure 3D. Patients were further divided into different subgroups according to the presence or absence of affected organs to compare plasma HE4 levels and we found that patients with IgG4-RD with pancreas, bile duct and retroperitoneum involvements had higher plasma HE4 levels than patients without corresponding tissue involvement (online supplemental table S3). Thus, elevated HE4 levels can predict potential damage to internal organs in patients with IgG4-RD.

Plasma HE4 levels are closely correlated to baseline clinical features and organ damage

We further assessed the relationship between HE4 levels and patient clinical features to determine the efficacy of HE4 as a biomarker for IgG4-RD (figure 4A). Consistent with our previous observations, HE4 levels were closely correlated with patient baseline clinical features (figure 4A–C), namely, ESR (r=0.5534, p<0.0001), CRP (r=0.3607, p<0.0001), IgG (r=0.3144, p=0.0004), IgG4 (r=0.3144, p=0.0002), IgE (r=0.2374, p=0.0097), C3 (r=−0.2441, p=0.0073) and C4 (r=−0.203, p=0.0439), and with biochemical indicators of organ impairment
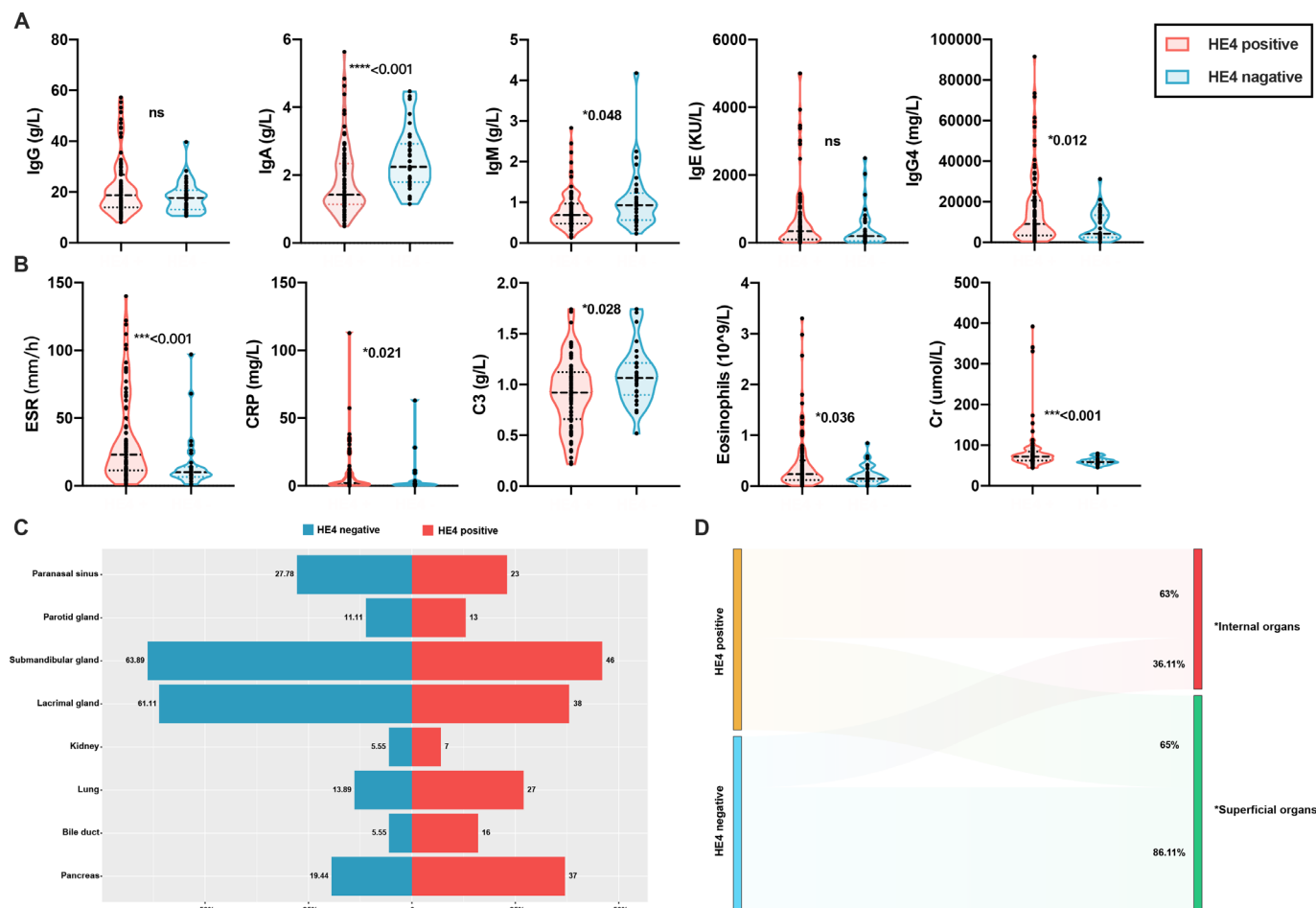
Figure 3 Comparison of the clinical features between patients with HE4-positive (HE4+) and HE4-negative (HE4–) IgG4-RD. Violin plots for the comparison of the serum immunoglobulin levels between HE4+ and HE4– patients. Violin plots for the comparison of laboratory test results (ESR, CRP, C3, C4, Cr) between HE4+ and HE4– patients. Comparison of the organ involvements between HE4+ and HE4– patients. Sanky plot that summarise and compare the internal and superficial organ involvement between HE4+ and HE4– patients (*p<0.05, **p<0.01, ***p<0.001). Cr, creatinine; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HE4, human epididymis protein 4; IgG4-RD, IgG4-related disease.

(figure 4A and D), viz., Cr (r=0.547, p<0.0001), gamma-glutamyl transpeptidase (GGT) (r=0.3021, p=0.0117), alanine aminotransferase (ALT) (r=0.2027, p=0.0291) and aspartate aminotransferase (AST) (r=0.2816, p=0.0151). However, there was not statistically correlation between HE4 levels and IgG4-RD RI scores or affected organ numbers (online supplemental figure S2). These results indicated that HE4 levels might have little relationship with disease activity but suggested the potential organ damage in patients with IgG4-RD.

Greater expression of HE4 in IgG4-RD-affected organs is closely related to fibrosis

Previous studies have confirmed higher HE4 expression in the affected tissues in various malignancies and autoimmune diseases,[20 21] and epithelial-derived or fibroblast-derived HE4 could aggravate tissue fibrosis and thereby promote tissue damage.[14 22] As HE4 levels were elevated in patients with IgG4-RD and were associated with internal organ involvement, we measured HE4 expression in affected tissue samples from patients. Results from the Human Protein Atlas (https://www.proteinatlas.org)

showed that HE4 expression was significantly different in the organs of healthy humans but that it was highly expressed in the nasopharynx, the bronchus, the colon, the epididymis and the seminal vesicles. Further, it was moderately expressed in the thyroid, salivary glands and the duodenum, among other tissues. While none of these organs, except for the salivary gland, are commonly affected in patients with IgG4-RD, HE4 protein expression was minimal in the lacrimal gland, the pancreas, the lung, the liver and the lymph nodes (figure 5A), and RNA expression patterns were similar to the protein expression profile (figure 5B).

As HE4 is mainly secreted by activated/damaged epithelial cells or fibroblasts, and the above results showed that patients with higher HE4 levels appeared to have greater internal organ involvement, including of the pancreas, the bile duct and the lung, (figure 3D) we performed HE4 and Masson immunohistochemical staining of pancreatic, submandibular and lacrimal gland tissue from patients with IgG4-RD and controls to understand the potential correlation between HE4 expression

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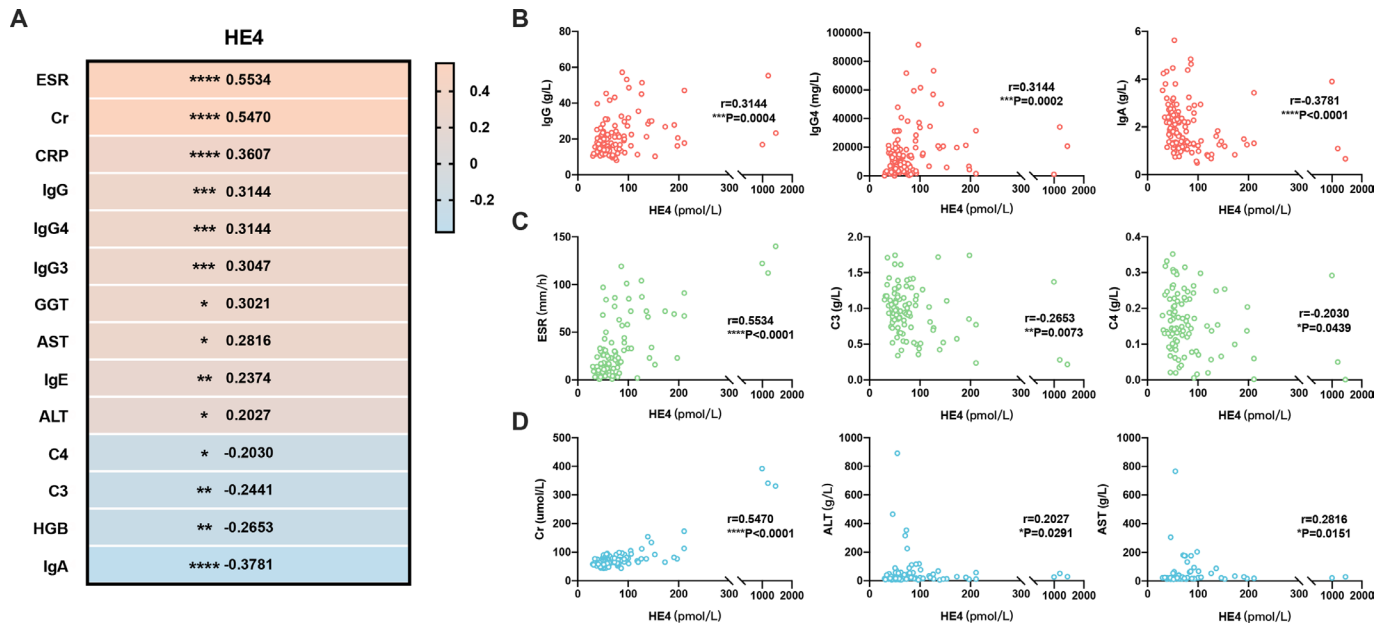
Figure 4 The correlation between HE4 levels and clinical features. Heatmap for the correlation between HE4 levels and other clinical features. Typical scatter plots display of the correlation between the serum immunoglobulin levels and HE4 levels. Typical scatter plots display of the correlation between laboratory parameters and HE4 levels. Typical scatter plots display of the correlation between the blood biochemical tests and HE4 levels (*p<0.05, **p<0.01, ***p<0.001). IgG, IgA, IgE, IgG3 and IgG4 were immunoglobulin G, A, E, G3 and G4. C3/4 represented for complement 3/4. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; Cr, creatinine; GGT, gamma-glutamyl transpeptidase; HE4, human epididymis protein 4.

and affected organ fibrosis. We found that 13 out of 15 (86.67%) IgG4-RD pancreatic samples were positive for HE4 staining, while all control samples were negative (figure 5C). Further, HE4 staining score was significantly higher in IgG4-RD pancreas than in controls (8.73±3.13 vs 1.80±1.69, p<0.0001, figure 5D) and it was accompanied by a high degree of the Masson's trichrome staining (CVF 0.78±0.17 vs 0.09±0.02, p<0.0001, figure 5D). Additionally, intense HE4 expression was also detected in the submandibular and lacrimal glands of patients with IgG4-RD (online supplemental figure S3), and a significant correlation was seen between HE4 score and CVF (r=0.7916, p<0.0001), suggesting that HE4 expression levels in tissues might be correlated with the degree of fibrosis (figure 5E).

Elevated HE4 levels predict poor prognosis in IgG4-RD
Under the premise that HE4 is closely related to affected organ damage and fibrosis, we also explored the correlation between HE4 expression levels and patient prognosis. As described above, patients with IgG4-RD were categorised as either HE4+ and HE4− based on plasma HE4 levels, and a subset of patients with a follow-up period of >12 months were included in this analysis. First, we compared treatment regimens between the two groups of patients, namely, the four types described in methods, and found that, in the presence of diverse higher baseline clinical parameters and internal organ involvement, HE4+ patients were more likely to be treated with GCs monotherapy/GCs+IMs than HE4− patients (73.58% vs 47.83%, p=0.03). In contrast, HE4− patients were

more likely to be treated with watchful waiting (33.33% vs 6.12%, p=0.028) or milder therapies (figure 5F). A comparison of prognosis between two groups indicated that, even under stronger treatment regimens, HE4+ and HE4− patients still had comparable relapse rate and that HE4+ patients were more likely to suffer poor prognosis (relapse and/or enhancement treatment) during the follow-up period (HR 2.741, 95% CI (1.250 to 4.887), p=0.0033, figure 5G). Further comparison of the poor prognosis rate under the different treatment regime also showed a higher incidence of poor prognosis in HE4+ patients under all the treatment regime, especially under GCs sparing and watchful waiting (online supplemental figure S4). As with the correlation between internal organ damage and fibrosis, higher HE4 levels predicted poorer prognosis in IgG4-RD.

DISCUSSION
HE4 is a widely used and high-value biomarker for diverse neoplastic diseases, and recent reports indicate that elevated HE4 levels occur in a variety of autoimmune diseases, including SLE,[11] RA[13] and pSS.[12] Available evidence also indicates that it is closely related to concurrent interstitial lung disease and renal involvement. As IgG4-RD is an immune-mediated fibroinflammatory disease,[21] we hypothesised that HE4 might be a potential biomarker for IgG4-RD and evaluated its utility. To the best of our knowledge, this is the first study to demonstrate that HE4 levels can function as a useful biomarker and predict prognosis in IgG4-RD.
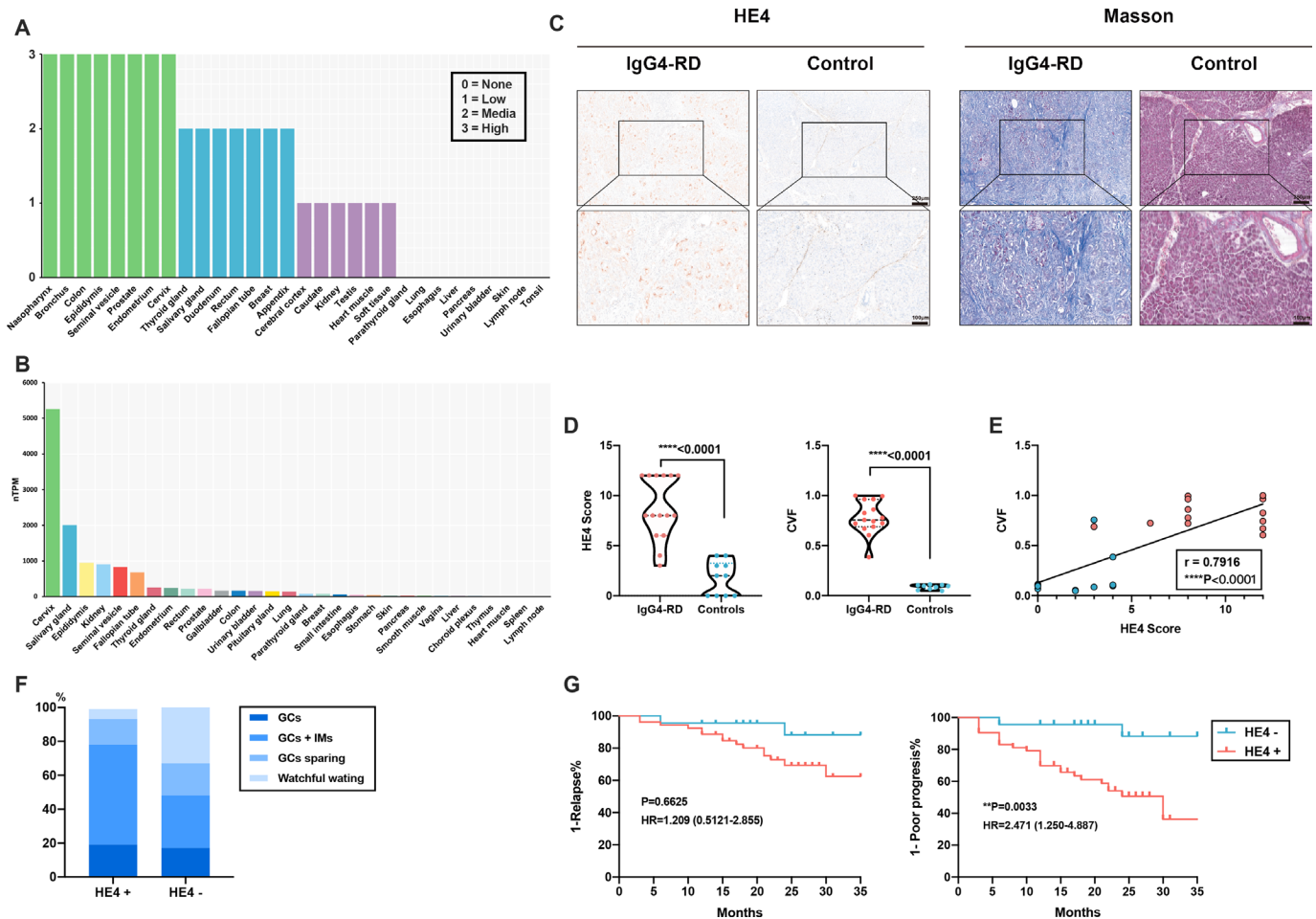
Figure 5 The relationship between patients with HE4 levels and IgG4-RD's fibrosis degree or disease prognosis. Histograms showing the immunohistochemical results of HE4 in different tissues in the Human Protein Atlas (https://www.proteinatlas.org). Histograms showing RNA sequencing results of HE4 in different tissues in the Human Protein Atlas (https://www.proteinatlas.org). Immunohistochemistry and Masson trichrome stain for HE4 expression and fibrosis degree in patients and controls. Comparison of HE score and collagen volume fraction (CVF) between patients and controls. Scatter plot showing correlation between HE score and CVF in patients and controls. Comparison of the treatment regime between HE4+ and HE4– patients. Kaplan-Meier curves for the comparative analysis of relapse and poor prognosis of HE+ group and HE– group in patients (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001). nTPM, normalized protein-coding transcripts per million; GCs, glucocorticoids; HE4, human epididymis protein 4; HE4+, HE4-positive; HE4–, HE4-negative; IgG4-RD, IgG4-related disease; IMs, immunosuppressants.

The significantly elevated plasma HE4 levels in patients with IgG4-RD were closely correlated with diverse baseline clinical features, indicating the close correlation between HE4 and the pathogenesis of IgG4-RD. Thus, we used ROC curve analysis to determine the cut-off value for distinguishing between patients with IgG4-RD and healthy individuals, and importantly, the suitability of this cut-off value (50.8 pmol/L) is established by the fact that, when patients with IgG4-RD were categorised as HE4+ or HE4– based on this cut-off, HE4+ patients displayed higher baseline clinical parameters (ESR, CRP and immunoglobulins) and organ function indicators (Cr, ALT, AST and GGT), indicating that the plasma HE4 levels be used to predict potential organ damage in IgG4-RD. HE4 levels remained stable after treatment during the follow-up period, like some fibrotic biomarkers,[23] they did not parallel with patients' clinical response.

Fibrosis of the affected organs is one of the main pathological features of IgG4-RD,[1 24] and the underlying mechanisms are currently unclear, potential indicators of fibrosis or organ damage in IgG4-RD are still lacking. Based on the results of a comparison between HE4– and HE4+ patients, we posit that HE4 would be a valuable biomarker for IgG4-RD because (1) higher proportion of internal organ involvement was seen in HE4+ patients, (2) immunochemistry revealed that HE4 is highly expressed in the IgG4-RD pancreas, and salivary and lacrimal glands and, (3) HE4 levels are closely related to the degree of fibrosis. HE4 is mainly secreted by activated or damaged epithelial cells and fibroblasts,[14 22] and according to the Human Protein Atlas, commonly affected organs (pancreas, lacrimal gland, salivary gland, bile duct, retroperitoneum tissue, etc) in IgG4-RD[25–27] either do not express HE4 or have low levels

under normal conditions. Although reproductive organs including cervix and epididymis could also be affected in patients with IgG4-RD, the incidence was extremely low, with only a few case reports.[28 29] Thus, the disease environment in IgG4-RD might lead to the activation and upregulation of HE4 in the epithelium or fibroblasts of the affected organs, which would lead to its secretion and accumulation in the tissues, or its release into peripheral circulation. Taken together, these observations suggest that HE4 might be a biomarker that can indicate both internal organ involvement and degree of fibrosis in IgG4-RD.

Elevated HE4 levels are currently widely used as a biomarker of poor prognosis in neoplastic diseases.[30 31] Hence, we explored the correlation between HE4 and prognosis in IgG4-RD in a subset of HE4+ and HE4– patients, that is, those with a follow-up period more than 1 year. First we found that HE4+ patients were more likely to use GC monotherapy or GCs+IMs, while HE4– patients preferred either watchful waiting or sparse GC use. Next, HE4+ patients had comparable relapse rates with poorer prognosis despite stronger treatment regimens and higher incidence of poor prognosis under each treatment regime, attesting to the potential value of elevated HE4 levels in predicting prognosis in IgG4-RD. Recently, HE4 has been reported to be involved in aetiopathology of fibrosis, with myofibroblast-derived HE4 capable of inhibiting type I collagen degradation induced by MMP2 or MMP9,[14] and tubular epithelial cell-derived HE4 promoting fibrosis in chronic kidney disease through the HIF-1α/HE4/NF-κB signalling pathway.[22] Therefore, subsequent accumulation of HE4 in the affected organs might be mechanistically involved in fibrosis during IgG4-RD, but further experimental validation is needed to verify this hypothesis.

In summary, we show, potentially for the first time that, HE4 levels are elevated in patients with IgG4-RD, and that higher HE4 levels are closely related to diverse clinical parameters and organ function indicators. Greater HE4 expression in the affected organs in IgG4-RD and its close correlation with degree of fibrosis suggest that HE4 can be used as a marker for not only internal organ damage and fibrosis but also for predicting prognosis.

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