Hybrid kappa\lambda antibody is a new serological marker to diagnose autoimmune pancreatitis and differentiate it from pancreatic cancer

Mingju Hao¹,²,³, Wenli Li⁴, Lang Yi¹,², Songlin Yu⁵, Gaowei Fan¹,², Tian Lu¹,², Xin Yang¹,², Guojing Wang¹,², Dong Zhang¹,², Jiansheng Ding¹,², Kuo Zhang¹, Rui Zhang¹, Guigao Lin¹, Yanxi Han¹, Lunan Wang¹ & Jinming Li¹

The only generally accepted serological marker currently used for the diagnosis of autoimmune pancreatitis (AIP) is IgG4. Our aim was mainly to determine whether hybrid κ\lambda antibody can help to diagnose AIP and to differentiate it from pancreatic cancer. We established an enzyme-linked immunosorbent assay (ELISA) system to measure the levels of hybrid κ\lambda antibodies in human sera. Sera were obtained from 338 patients, including 61 with AIP, 74 with pancreatic cancer, 50 with acute pancreatitis, 40 with ordinary chronic pancreatitis, 15 with miscellaneous pancreatic diseases, and 98 with normal pancreas. Our study showed levels of hybrid κ\lambda antibodies in the AIP group were significantly higher than in the non-AIP group (P < 0.001). The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the diagnosis of AIP were 80.3%, 91%, 66.2% and 95.5% respectively. Furthermore, the combined measurement of serum hybrid κ\lambda antibody and IgG4 tended to increase the sensitivity although the difference was not statistically significant (90.2% vs. 78.7%, P = 0.08), compared to measurement of IgG4 alone. Our findings suggest that hybrid κ\lambda antibody could be a new serological marker to diagnose AIP and differentiate it from pancreatic cancer.

Autoimmune pancreatitis (AIP) is a form of chronic pancreatitis characterized by an autoimmune inflammatory process in which prominent lymphoplasmacytic infiltrates with associated fibrosis of the pancreas cause organ dysfunction. Historically, AIP was used to describe the clinical profiles associated with lymphoplasmacytic sclerosing pancreatitis (LPSP), which is now called type 1 AIP. Accordingly, type 2 AIP is used to describe idiopathic duct-centric chronic pancreatitis (IDCP). Considering the well-known and long-standing association with serum IgG4 elevation, some experts suggested using “AIP” solely for type 1 AIP and referring to type 2 AIP as IDCP. Steroid therapy often leads to the rapid and sustained resolution of pancreatic mass lesions, pancreatic-duct strictures, and biliary obstruction, and therefore the diagnosis of AIP has a significant impact on the prognosis and treatment of the patient. The characteristic features of dense lymphoplasmacytic infiltration and fibrosis in the pancreas are the gold standard for the diagnosis of AIP. However, it is usually difficult to obtain specimens from the pancreas for histological confirmation.

In 2001, Hamano et al. first reported that serum IgG4 concentrations were highly sensitive and highly specific for AIP. In 2006, IgG4 was first added to the serological criteria for the diagnosis of AIP by the Japan Pancreas Society. The HISORt criteria and the International Consensus Diagnostic Criteria (ICDC) allow the use of serum IgG4 as a serological criterion. However, although serum IgG4 is useful for screening, it is not reliable as

¹National Center for Clinical Laboratories, Beijing Hospital, Beijing, People’s Republic of China. ²Graduate School, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, People’s Republic of China. ³Department of Clinical Laboratory, Qianfo Mountain Hospital of Shandong University, Jinan, People’s Republic of China. ⁴China-Japan Friendship Hospital, Beijing, People’s Republic of China. ⁵Department of Clinical Laboratory, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, People’s Republic of China. Correspondence and requests for materials should be addressed to J.L. (email: jmli@nccl.org.cn)

10.1038/srep27415
scientific reports | 6:27415 | DOI: 10.1038/srep27415

Figure 1. Arbitrary units (AU) of hybrid κ\(\lambda\) antibody in study groups. Arbitrary units (AU) of hybrid κ\(\lambda\) antibody in patients with autoimmune pancreatitis (AIP, n = 61), acute pancreatitis (AP, n = 50), ordinary chronic pancreatitis (CP, n = 40), pancreatic cancer (Pca, n = 74), miscellaneous pancreatic diseases (MP, n = 15) and normal pancreas (NP, n = 98). Each horizontal bar represents the median value of serum hybrid κ\(\lambda\) antibody in each study group. Significance: *P < 0.001 for AIP with respect to each non-AIP group, **P = 0.66 between groups of non-AIP.

In this study, we attempted to investigate the diagnostic utility of hybrid κ\(\lambda\) antibody in patients with AIP. Studies have shown that 4–10% of both healthy controls and controls with other diseases have high serum IgG4 concentrations\(^9,10\). In addition, about 20% of patients with AIP have normal serum IgG4 concentrations at presentation\(^11,12\). The most important disease that should be differentiated from AIP is pancreatic cancer\(^13,14\). Both diseases tend to occur in elderly men, presenting with similar clinical features, such as painless obstructive jaundice, weight loss, and recent-onset diabetes\(^15–17\). However, many studies reported that moderate elevations in serum IgG4 cannot be used alone to distinguish AIP from pancreatic cancer due to its low sensitivity and specificity\(^9,18\). CA19-9 is considered as a biomarker in pancreatic cancer, but this can also be elevated in other pancreatic diseases such as chronic pancreatitis, or in other pathological states\(^19\). So far, a simple serological test for the diagnosis and differentiation of AIP from pancreatic cancer is still lacking.

The classic antibody paradigm is that a single mature plasma cell produces symmetrical antibodies composed of one type of immunoglobulin heavy chain and one type of immunoglobulin light chain, either kappa (κ) or lambda (λ)\(^20\). Within the last 10 years, it has been shown that human IgG4 is a dynamic antibody, which is involved in a continuous process of half-molecule exchange. This process, also referred to as "Fab-arm exchange", can result in asymmetric antibodies with two different antigen-combining sites\(^21–24\). Recently, one report showed that hybrid κ\(\lambda\) antibodies compose a substantial portion of IgG4 in normal human serum\(^25\). These molecules are formed by two IgG4 heavy chains plus one κ and one λ light chain. Because AIP is characterized by an elevated serum IgG4 concentration, we attempted to investigate the diagnostic utility of hybrid κ\(\lambda\) antibody in the diagnosis of AIP and its differentiation from pancreatic cancer.

The principal aim of this study was to establish a light-chain capture sandwich ELISA system to quantify the relative hybrid κ\(\lambda\) antibody concentrations in patients with a variety of pancreatic diseases. We aimed to determine the sensitivity, specificity and predictive values of serum hybrid κ\(\lambda\) antibody for the diagnosis of AIP, and compare them with those of serum IgG4. Because the principal differential diagnosis of AIP is pancreatic cancer, we also aimed to evaluate the use of serum hybrid κ\(\lambda\) antibody and combined measurement with serum IgG4 in differentiating between the two diseases.

**Results**

High prevalence of serum hybrid κ\(\lambda\) antibodies in patients with AIP in comparison with other study groups. After the four-parameter equation of the standard serum was determined, the arbitrary units in test sera were calculated from their O.D. values. Using the double sandwich ELISA system and the standard reference curve we determined the prevalence of hybrid κ\(\lambda\) antibody in all groups (Fig. 1). The median of hybrid κ\(\lambda\) antibody in patients with AIP, acute pancreatitis, ordinary chronic pancreatitis, pancreatic cancer, miscellaneous pancreatic diseases and normal pancreas were 6.75 AU/mL (range, 1.53–55.5 AU/mL), 2.06 AU/mL (range, 0.26–4.97 AU/mL), 2.06 AU/mL (range, 0.86–4.94 AU/mL), 2.04 AU/mL (range, 0.93–6.1 AU/mL), 1.61 AU/mL (range, 0.94–4.87 AU/mL) and 2.05 AU/mL (range, 0.42–7.2 AU/mL), respectively. Levels of hybrid κ\(\lambda\) antibody in AIP group were significantly higher than in the non-AIP groups (P < 0.001). There was no significant difference between the non-AIP groups (P = 0.66).

Sensitivity, specificity, and predictive values of hybrid κ\(\lambda\) antibody in the diagnosis of AIP. In order to evaluate the utility of hybrid κ\(\lambda\) antibody in the diagnosis of AIP, cutoff values were established to determine the sensitivity and specificity. The sensitivity and specificity of hybrid κ\(\lambda\) antibody differed at various cutoff values. Using the cutoff value of 4.04 AU/mL from the ROC curve (Fig. 2), we calculated that the sensitivity and specificity were 80.3% (49/61) and 91% (252/277), respectively. The area under the ROC curve was 0.93
P < 0.001). The PPV and NPV of serum hybrid κ\λ antibody for diagnosis of AIP were 66.2% (49/74) and 95.5% (252/264) when calculated using the 61 AIP patients diagnosed during the study period (Table 1).

### Sensitivity, specificity and predictive values of serum IgG4 in the diagnosis of AIP.

The median of serum IgG4 level was 3340 mg/L (range, 164–21100 mg/L), 616 mg/L (range, 22–2594 mg/L), 653 mg/L (range, 81–1409 mg/L), 746.5 mg/L (range, 81–1452 mg/L), and 460.5 mg/L (range, 21–2930 mg/L) in patients with AIP, acute pancreatitis, ordinary chronic pancreatitis, pancreatic cancer, miscellaneous pancreatic diseases, and normal pancreas, respectively. Serum IgG4 concentration was significantly higher in patients with AIP than in the other study groups (P < 0.001 for each comparison, Fig. 3). When a serum IgG4 concentration of 1350 mg/L was used as a cut-off value, the sensitivity was calculated at 78.7% (48/61) with a specificity of 87.7% (243/277). The sensitivity, specificity, PPV and NPV of serum IgG4 were not significantly different to those of serum hybrid κ\λ antibody (78.7% vs. 80.3%, P = 0.823; 87.7% vs. 91%, P = 0.215; 58.5% vs. 66.2%, P = 0.323; 94.9% vs. 95.5%, P = 0.777, Table 2).

### The combined measurement of hybrid κ\λ antibody and IgG4 in the diagnosis of AIP.

Seropositivity was defined as the elevation of serum IgG4 or hybrid κ\λ antibody levels. For the AIP group, seven patients (7/61, 11.5%) showed elevation of serum hybrid κ\λ antibody with normal serum IgG4, whereas six patients (6/61, 9.8%) showed elevation of serum IgG4 in spite of normal hybrid κ\λ antibody levels (Table 3). As a result, the combined measurement could increase the diagnostic sensitivity from 78.7% (243/277) to 90.2% (55/61), although this difference was not statistically significant (P = 0.08). The specificity of the combined measurement was not sacrificed significantly (85.9% vs. 87.7%, P = 0.53).

### Serum hybrid κ\λ antibody and IgG4 in the differentiation of AIP from pancreatic cancer.

Among patients with pancreatic cancer, nine patients had elevated levels of hybrid κ\λ antibodies (9/74), and eight patients had elevated levels of serum IgG4 (8/74). Hybrid κ\λ antibody (≥4.04 AU/mL) showed a specificity of 87.8%. In the case of IgG4 (≥1350 mg/L), the specificity was 89.2%. There was no significant difference between the two tests (P = 0.797, Table 4).

Analogous to the sensitivity in the diagnosis of AIP from all non-AIP groups, the combined measurement showed a specificity of 87.8% (65/74) in the differentiation of AIP from pancreatic cancer, which was almost the same as that (89.2%, 66/74) of IgG4 alone (P = 0.797, Table 4).
In a large cohort of patients with a wide variety of pancreatic diseases, we showed that levels of hybrid κ\λ antibodies in patients with AIP were significantly higher than in patients with non-AIP conditions. The sensitivity and specificity for hybrid κ\λ antibody in the diagnosis of AIP were 80.3% and 91% respectively. A proportion (12/61, 19.7%) of AIP patients had normal hybrid κ\λ antibody levels, and a proportion (25/277, 9%) of non-AIP patients had elevated hybrid κ\λ antibody levels. For serum IgG4 levels, the sensitivity and specificity were 78.7% and 87.7%, respectively, which are not as high as the initial study by Hamano et al.\textsuperscript{26}, but are close to the reports by Ghazale et al.\textsuperscript{9} and other previous reports\textsuperscript{10–12}. The differences in the diagnostic values of serum IgG4 for AIP

**Table 2.** Sensitivity, specificity and predictive value of IgG4 in patients with autoimmune pancreatitis (AIP) and controls (non-AIP).

| Levels of serum IgG4 | AIP (n = 61) | Non-AIP (n = 277) | Total | Predictive value (%) |
|----------------------|-------------|-------------------|-------|----------------------|
| ≥1350 mg/L           | 48          | 34                | 82    | Positive 58.5%       |
| <1350 mg/L           | 13          | 243               | 256   | Negative 94.9%       |
| Total                | 61          | 277               | 338   |                      |

Sensitivity 78.7% Specificity 87.7%

**Table 3.** The elevation of serum hybrid κ\λ antibody and IgG4 in AIP.

| Hybrid κ\λ antibody elevation (≥4.04 AU/mL) | IgG4 elevation (≥1350 mg/L) |
|--------------------------------------------|-----------------------------|
| Yes                                        | Yes                        |
| No                                         | No                          |
| 42 (68.8%)                                 | 7 (11.5%)                   |
| 6 (9.8%)                                   | 6 (9.8%)                    |

**Table 4.** Sensitivity and specificity of hybrid κ\λ antibody and IgG4 in the differentiation of AIP from pancreatic cancer.

| Hybrid κ\λ antibody elevation (≥4.04 AU/mL) | AIP (n = 61) | Pancreatic cancer (n = 74) | Sensitivity (%) | Specificity (%) |
|--------------------------------------------|-------------|-----------------------------|-----------------|-----------------|
| Yes                                        | 49          | 9                           | 80.3%           | 87.8%           |
| No                                         | 12          | 65                          |                 |                 |

| IgG4 elevation (≥1350 mg/L) | Yes | No | Sensitivity (%) | Specificity (%) |
|-----------------------------|-----|----|-----------------|-----------------|
| 48                          | 8   | 78.7% | 89.2%           |
| 13                          | 66  |       |                 |                 |

| Hybrid κ\λ antibody or IgG4 elevation | Yes | No | Sensitivity (%) | Specificity (%) |
|---------------------------------------|-----|----|-----------------|-----------------|
| 55                                    | 9   | 90.2% | 87.8%           |
| 6                                     | 65  |       |                 |                 |

**Discussion**

In a large cohort of patients with a wide variety of pancreatic diseases, we showed that levels of hybrid κ\λ antibodies in patients with AIP were significantly higher than in patients with non-AIP conditions. The sensitivity and specificity for hybrid κ\λ antibody in the diagnosis of AIP were 80.3% and 91% respectively. A proportion (12/61, 19.7%) of AIP patients had normal hybrid κ\λ antibody levels, and a proportion (25/277, 9%) of non-AIP patients had elevated hybrid κ\λ antibody levels. For serum IgG4 levels, the sensitivity and specificity were 78.7% and 87.7%, respectively, which are not as high as the initial study by Hamano et al.\textsuperscript{26}, but are close to the reports by Ghazale et al.\textsuperscript{9} and other previous reports\textsuperscript{10–12}. The differences in the diagnostic values of serum IgG4 for AIP
could be attributed to differences in the patients enrolled in the studies, or differences in the disease activity of the cases selected \(^{27-28}\).

Our study showed that the sensitivity and specificity of serum IgG4 was not significantly different from serum hybrid κ/λ antibody in the diagnosis of AIP. In view of this, we wondered whether the combined measurement could increase the diagnostic performance for AIP. Seropositivity was defined as the elevation of serum IgG4 or of hybrid κ/λ antibody. Interestingly, seven AIP patients (7/61, 11.5%) showed elevated levels of serum hybrid κ/λ antibodies despite normal serum IgG4, and six AIP patients (6/61, 9.8%) showed elevation of serum IgG4 with normal levels of hybrid κ/λ antibodies. As a result, the combined measurement of serum IgG4 and hybrid κ/λ antibody could strongly increase the diagnostic sensitivity to 90.2% in comparison with using serum IgG4 alone. More importantly, with the combined measurement the specificity was not significantly sacrificed. This finding is similar to that of a previous report by Song T \textit{et al.} \(^{29}\). They showed that the combined measurement of total serum IgG and IgG4 might increase diagnostic sensitivity without compromising the specificity. However, the sensitivity of the combined measurement in their study was only 68.3% (56/82), which was significantly lower than our result (56/82 vs. 55/61, \(P = 0.002\)).

In our study of 61 patients with AIP, only 12 patients (19.7%) had hybrid κ/λ antibody levels below the cut-off value of 4.04 AU/mL. Therefore, the negative predictive value of hybrid κ/λ antibody levels for AIP was very high (95.5%) and the likelihood of AIP in a patient without hybrid κ/λ antibody elevation is very low, which could help rule out AIP from non-AIP diseases. The positive predictive value of a test is closely related the prevalence of the disease of interest in the studied population. AIP is uncommon with an estimated prevalence of <1/100,000 in the general population \(^{20}\). Despite the high specificity of serum hybrid κ/λ antibody for AIP, 91% in our study, it had a low PPV (66.2%). Even so, if more patients with nonspecific abdominal pain were included in our research, the proportion of AIP would be lower and so would the PPV.

When a cutoff value of 4.04 AU/mL of serum hybrid κ/λ antibody was used to differentiate AIP from pancreatic cancer, in contrast to the high prevalence of hybrid κ/λ antibody in patients with AIP only nine (9/74, 12.1%) patients with pancreatic cancer had elevated levels of hybrid κ/λ antibodies. The specificity was 87.8%, which was almost the same as that for serum IgG4 (89.2%). In keeping with the result for the diagnosis of AIP from all non-AIP groups, the specificity of the combined measurement of serum IgG4 and hybrid κ/λ antibody in the differentiation of AIP from pancreatic cancer was not significantly sacrificed, when compared with that of serum IgG4 alone.

More recently, we reported hybrid κ/λ antibody was a novel biomarker related to disease activity and inflammation in rheumatoid arthritis (RA). Levels of serum hybrid κ/λ antibody were markedly elevated in the patients with RA compared with osteoarthritis (OA) and healthy controls \(^{31}\). Nevertheless, the levels of hybrid κ/λ antibody in patients with AIP were significantly higher than those with RA (data not shown). In the present study, all the patients enrolled were suspected to have pancreatic diseases, with obstructive jaundice and abdominal pain, or a pancreatic mass. Hybrid κ/λ antibody is valuable in diagnosing or differentiating AIP from pancreatic cancer based on the clinical findings of suspected pancreatic disorders. Considering the elevation of hybrid κ/λ antibody in RA, or other possible autoimmune diseases, elevated hybrid κ/λ antibody concentrations should be interpreted in conjunction with a thorough evaluation of the clinical, radiological and histological findings for AIP diagnosis, especially for patients who have autoimmune diseases.

Our study has two main limitations. First, the results are limited by a shortage of type 2 AIP patients. Because type 2 AIP is not part of the spectrum of IgG4-related diseases, and does not have definitive serological autoimmune markers, we can speculate that hybrid κ/λ antibody concentrations may be normal in these patients. Nevertheless, this does not change its significance in clinical practice because most cases of AIP in Asia fit the profile of type 1 AIP \(^{32,33}\) and patients with type 2 AIP differ significantly in their demography, other organ involvement, and disease relapse \(^{3}\). Second, the relatively small number of AIP patients are included that may not be sufficient to allow for a decisive conclusion regarding our results because AIP is an uncommon pancreatic disease. To strengthen the research and pave the way for clinical use of the new biomarker, a prospective study in a different and large patient population is needed in the future.

In summary, this is the first report showing that the level of hybrid κ/λ antibody is markedly elevated in AIP. The high sensitivity and specificity indicated that it could be a new serological marker to diagnose AIP, and to differentiate it from pancreatic cancer. The combined measurement of serum hybrid κ/λ antibody and IgG4 tended to have a higher sensitivity without sacrificing specificity, compared with serum IgG4 alone. Further studies are necessary to clarify its characteristics, and to evaluate the immunoregulatory role in disease activity.

**Methods**

**Samples and materials.** All 338 patients studied were referred to Peking Union Medical College Hospital for suspected pancreatic disease, with obstructive jaundice and abdominal pain, or a pancreatic mass. Among them, 61 patients (51 men and 10 women) were diagnosed with type 1 AIP and the remaining patients were classified into the five diagnostic groups summarized in Table 5: acute pancreatitis, ordinary chronic pancreatitis, pancreatic cancer, miscellaneous pancreatic diseases, and normal pancreas. Miscellaneous conditions included other pancreatic abnormalities such as pancreatic cyst, pancreatic neuroendocrine tumors, and insulinoma. The diagnosis of AIP was based on the International Consensus Diagnostic Criteria \(^{2}\), on the basis of clinical data, imaging, and histopathological findings. All diagnostic cases are definitive cases. Diagnosis of pancreatic cancer was confirmed by pancreatic histology and/or cytology. Patients who had no identifiable pancreatic abnormality were classified as "normal pancreas". All patients were enrolled randomly between May 2014 and January 2016. Serum samples were stored at \(-80\) C until performing the assay. In addition, a pooled serum sample from 20 healthy subjects (10 men and 10 women) with normal findings on abdominal ultrasonography and without autoimmunity was used as a standard reference serum to determine the standard curve, arbitrarily assigned to contain two units of hybrid κ/λ antibodies per milliliter (AU/mL). The standard reference serum was aliquoted and stored...
Table 5. Demographic characteristics of study groups. *Miscellaneous pancreatic diseases included 4 patients with pancreatic cysts, 7 patients with pancreatic neuroendocrine tumors, and 4 patients with insulinoma.

| Study group                        | n  | Age (years) | Sex (male/female) |
|------------------------------------|----|-------------|-------------------|
| AIP                                | 61 | 57 (27–83)  | 51/10             |
| Pancreatic cancer                  | 74 | 58 (29–79)  | 58/16             |
| Acute pancreatitis                 | 50 | 42 (14–78)  | 22/28             |
| Ordinary chronic pancreatitis      | 40 | 52 (8–78)   | 28/12             |
| Miscellaneous pancreatic diseases* | 15 | 46 (26–77)  | 6/9               |
| Normal pancreas                    | 98 | 46 (33–56)  | 57/41             |

Statistical analysis. ELISA results were analyzed by a four-parameter logistic-log curve fitting program (ELISA v. 2.15; Centers for Disease Control and Prevention, USA) and expressed in arbitrary units (units per milliliter). Sensitivity, specificity, and predictive values were calculated using conventional formulae, and were compared using the χ² test. According to data distribution, values were represented as median and range. Comparisons were performed using the Mann-Whitney U test between AIP and non-AIP group. The Kruskal-Wallis nonparametric analysis is used to compare differences between non-AIP groups. Receiver operator characteristic (ROC) curves were used to judge the diagnostic utility. Statistical analyses were performed with SPSS 19.0 (SPSS Inc., USA) and GraphPad Prism version 6.0 (GraphPad, USA) as appropriate. Two-sided p-values of less than 0.05 were regarded as statistically significant.

References
1. Finkelberg, D. L., Sahani, D., Deshpande, V. & Brugge, W. R. Autoimmune pancreatitis. *N Engl J Med* 355, 2670–2676 (2006).
2. Shimosegawa, T. et al. International consensus diagnostic criteria for autoimmune pancreatitis: guidelines of the International Association of Pancreatology. *Pancreas* 40, 352–358 (2011).
3. Chari, S. T. et al. Histopathologic and clinical subtypes of autoimmune pancreatitis: the Honolulu consensus document. *Pancreas* 39, 549–554 (2010).
4. Hart, P. A., Zen, Y. & Chari, S. T. Recent Advances in Autoimmune Pancreatitis. *Gastroenterology* 149, 39–51 (2015).
5. Kamisawa, T. & Okamoto, A. Autoimmune pancreatitis: proposal of IgG4-related sclerosing disease. *J Gastroenterol Hepatol* 41, 613–625 (2006).
6. Athal, G. P., Breslin, N. P. & Gunstomb, B. High serum IgG4 concentrations in patients with sclerosing pancreatitis. *N Engl J Med* 345, 147–148 (2001).
7. Okazaki, K. et al. Clinical diagnostic criteria of autoimmune pancreatitis: revised proposal. *J Gastroenterol Hepatol* 41, 626–631 (2006).
8. Chari, S. T. et al. Diagnosis of autoimmune pancreatitis: the Mayo Clinic experience. *Clin Gastroenterol Hepatol* 4, 1010–1016; quiz 934 (2006).
9. Ghazale, A. et al. Value of serum IgG4 in the diagnosis of autoimmune pancreatitis and in distinguishing it from pancreatic cancer. *Am J Gastroenterol* 102, 1646–1653 (2007).
10. Sadler, R. et al. The diagnostic significance of serum IgG4 levels in patients with autoimmune pancreatitis: a UK study. *Eur J Gastroenterol Hepatol* **23**, 139–145 (2011).
11. Sah, R. P. & Chari, S. T. Serologic issues in IgG4-related systemic disease and autoimmune pancreatitis. *Curr Opin Rheumatol* **23**, 108–113 (2011).
12. Tabata, T. et al. Serum IgG4 concentrations and IgG4-related sclerosing disease. *Clin Chim Acta* **408**, 25–28 (2009).
13. Morselli-Labate, A. M. & Peszilli, R. Usefulness of serum IgG4 in the diagnosis and follow up of autoimmune pancreatitis: A systematic literature review and meta-analysis. *J Gastroenterol Hepatol* **24**, 15–36 (2009).
14. Kamisawa, T. et al. Strategy for differentiating autoimmune pancreatitis from pancreatic cancer. *Pancreas* **37**, e62–e67 (2008).
15. Yoshida, K. et al. Chronic pancreatitis caused by an autoimmune abnormality: Proposal of the concept of autoimmune pancreatitis. *Dig Dis Sci* **40**, 1561–1568 (1995).
16. Okazaki, K. & Chiba, T. Autoimmune related pancreatitis. *Gut* **51**, 1–4 (2002).
17. Lara, L. P. & Chari, S. T. Autoimmune pancreatitis. *Curr Gastroenterol Rep* **7**, 101–106 (2005).
18. Ngwa, T., Law, R., Hart, P., Smyrk, T. C. & Chari, S. T. Serum IgG4 elevation in pancreatic cancer: diagnostic and prognostic significance and association with autoimmune pancreatitis. *Pancreas* **44**, 557–560 (2015).
19. Chang, M. C. et al. Adiponectin as a potential differential marker to distinguish pancreatic cancer and chronic pancreatitis. *Pancreas* **35**, 16–21 (2007).
20. Bernier, G. M. & Cebra, J. J. Polypeptide Chains of Human Gamma-Globulin: Cellular Localization by Fluorescent Antibody. *Science* **144**, 1590–1591 (1964).
21. van der Neut Kolfschoten, M. et al. Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange. *Science* **317**, 1554–1557 (2007).
22. Aalberse, R. C., Stapel, S. O., Schuurman, J. & Rispens, T. Immunoglobulin G4: an odd antibody. *Clin Exp Allergy* **39**, 469–477 (2009).
23. Rispens, T., Ooijevaar-de Heer, P., Bende, O. & Aalberse, R. C. Mechanism of Immunoglobulin G4 Fab-arm Exchange. *Journal of the American Chemical Society* **133**, 10302–10311 (2011).
24. Labrijn, A. E. et al. Therapeutic IgG4 antibodies engage in Fab-arm exchange with endogenous human IgG4 in vivo. *Nat Biotechnol* **27**, 767–771 (2009).
25. Young, E. et al. Estimation of polyclonal IgG4 hybrids in normal human serum. *Immunology* **142**, 406–413 (2014).
26. Hamano, H. et al. High serum IgG4 concentrations in patients with sclerosing pancreatitis. *N Engl J Med* **344**, 732–738 (2001).
27. Ohara, H., Nakazawa, T., Ando, T. & Joch, T. Systemic extrapancreatic lesions associated with autoimmune pancreatitis. *J Gastroenterol Hepatol* **22** Suppl 18, 15–21 (2007).
28. Kaji, R. et al. Serum immunoglobulin G4 associated with number and distribution of extrapancreatic lesions in type 1 autoimmune pancreatitis patients. *J Gastroenterol Hepatol* **27**, 268–272 (2012).
29. Song, T. J. et al. The combined measurement of total serum IgG and IgG4 may increase diagnostic sensitivity for autoimmune pancreatitis without sacrificing specificity, compared with IgG4 alone. *Am J Gastroenterol* **105**, 1655–1660 (2010).
30. Uchida, K., Masumune, A., Shimosegawa, T. & Okazaki, K. Prevalence of IgG4-Related Disease in Japan Based on Nationwide Survey in 2009. *Int J Rheumatol* **2012**, 358371 (2012).
31. Yi, L. et al. Increased Kappa/Lambda Hybrid Antibody in Serum Is a Novel Biomarker Related to Disease Activity and Inflammation in Rheumatoid Arthritis. *Mediators of Inflammation* **2016**, 2953072 (2016).
32. Wu, G. et al. Review of 43 patients with autoimmune pancreatitis based on the international consensus diagnostic criteria in China. *Pancreas* **43**, 810–811 (2014).
33. Meng, Q. et al. Diagnosis and Treatment of Autoimmune Pancreatitis in China: A Systematic Review. *PLos One* **10**, e0130466 (2015).

**Acknowledgements**

This work was supported by National Natural Science Foundation of China Grant 81271915 and National High Technology Research and Development Program of China Grant 2011AA02A116.

**Author Contributions**

M.H., W.L. and L.Y. designed of the experiments and wrote the main manuscript text. They contributed equally to this work; S.Y., G.F., T.L. and X.Y. collected the specimen and analyzed the data; G.W., D.Z. and J.D. prepared all critical revision of the manuscript. All authors reviewed the manuscript.

**Additional Information**

**Competing financial interests**: The authors declare no competing financial interests.

**How to cite this article**: Hao, M. et al. Hybrid kappa/lambda antibody is a new serological marker to diagnose autoimmune pancreatitis and differentiate it from pancreatic cancer. *Sci. Rep.* **6**, 27415; doi: 10.1038/srep27415 (2016).

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/