

## Original Article

# P1-hepatocyte nuclear factor 4 $\alpha$ expression significantly correlates with the severity of chronic gastritis

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Received March 6, 2017; Accepted May 3, 2017; Epub June 1, 2017; Published June 15, 2017

**Abstract:** To evaluate the expression pattern of hepatocyte nuclear factor 4 $\alpha$  (HNF4 $\alpha$ ) in chronic gastritis and reveal the relationship between HNF4 $\alpha$  expression and severity of chronic gastritis. Biopsy samples from the gastric mucosa of 225 patients and 5 control subjects were collected. The expressions of P1 promoter-driven HNF4 $\alpha$  (P1-HNF4 $\alpha$ ) and P2 promoter-driven HNF4 $\alpha$  (P2-HNF4 $\alpha$ ) in chronic gastritis were analyzed by immunohistochemical staining. Significant differences in P1-HNF4 $\alpha$  expression levels were observed between the different severity of chronic gastritis groups without intestinal metaplasia (IM) ( $P < 0.05$ ), while there was no difference in IM area for the P1-HNF4 $\alpha$  expression, no matter how far the severity of chronic inflammation. But another HNF4 $\alpha$  isoform P2-HNF4 $\alpha$  expression was widespread. The P2-HNF4 $\alpha$  strongly expressed in any gastric tissues, including normal epithelia, gastritis with various degrees of severity, and gastric IM. High level expression of P1-HNF4 $\alpha$  was associated with the severity of chronic gastritis. The P1-HNF4 $\alpha$  could be potentially useful as a diagnostic marker for chronic gastritis.

**Keywords:** HNF4 $\alpha$ , chronic gastritis, intestinal metaplasia

## Introduction

Chronic gastritis, a common disease in human beings, is considered to be a multistep, progressive and lifelong inflammation [1]. Relevant factors of chronic gastritis were identified, including chemicals, immunologic, genetic activities and pathogenic bacteria [2]. *Helicobacter pylori* (*H. pylori*), which usually colonizes near the pyloric antrum, has been implicated as a principal cause of chronic gastritis [1, 3]. As a result of long-lasting reaction directed against *H. pylori*, gene expression would be shifts in the human gastric antrum, and a cascade of intestinal metaplasia (IM) events could occur [4].

Hepatocyte nuclear factor 4 $\alpha$  (HNF4 $\alpha$ ), which is one of the key regulators of hepatocyte differentiation in mammals, is implicated in the differentiation of the gastrointestinal tract during embryogenesis [5]. Several isoforms of HNF4 $\alpha$  have been cloned and characterized which result from alternative promoter (P1 and P2) usage and splicing [6, 7]. The distribution pat-

tern of P1 and P2 promoter-driven HNF4 $\alpha$  differs among digestive tract organs: P1-HNF4 $\alpha$  is expressed in intestinal tissue and gastric intestinal metaplasia, while P2-HNF4 $\alpha$  is expressed in gastrointestinal tract [6-9].

Although numerous reports have shown that the expression patterns of isoforms P1 and P2-HNF4 $\alpha$  in gastrointestinal tract and pathologically changed tissues are different [6-10], the exact correlations between the expression patterns of various HNF4 $\alpha$  isoforms and the severity of inflammation in chronic gastritis have yet to be well defined. The aim of this study was to investigate the associations between the expression of P1-HNF4 $\alpha$  or P2-HNF4 $\alpha$  and the degree of inflammation in the pyloric mucosa.

## Materials and methods

### Tissue samples

225 patients (age 55.7 $\pm$ 12.4 years) with functional dyspepsia admitted to endoscopy at

## HNF4α expression correlates with chronic gastritis

**Table 1.** Correlation between activity and chronic inflammation in gastritis samples

Chronic inflammation	Case	Activity				P-value
		0+	1+	2+	3+	
Mild	65	38	21	6	0	P<0.05
Moderate	101	30	39	21	11	
Severe	59	0	10	26	23	

**Table 2.** Correlation between activity and intestinal metaplasia in gastric mucosa samples

Chronic inflammation	Case	IM*				P-value
		0+	1+	2+	3+	
Mild	65	41	17	7	0	P<0.05
Moderate	101	53	23	19	6	
Severe	59	28	7	10	14	

\*IM: Intestinal metaplasia.

Guizhou Provincial People's Hospital were included in the study.

All patients were positive for *H. pylori* infection based on the positive results of the histology and/or C14 urea breath test. Patients were excluded if they had any of the following: gastrointestinal hemorrhage, atrophy, malignancy, immunosuppression, severe concomitant diseases, and use of any antibiotics or proton-pump inhibitors in the 4 weeks prior to the study. Normal pylorus tissues were obtained from patients undergoing duodenal switch gastric bypass surgery. This study was approved by the ethics committee of Guizhou provincial people's hospital. Informed consent is obtained from each research subject before that subject participates in the research study.

### Immunohistochemistry

Biopsy specimens were fixed in 10% formalin, embedded in paraffin and stained with Hematoxylin-Eosin. Immunostaining was performed using the Leica Bond-Max automation system (Leica Biosystems, Bannockburn, IL). Primary antibody Muc-2 (1:200; ZSGB-BIO, Beijing, China) was used to identify the presence of IM. Positive staining was localized to cytoplasm. Primary antibodies P1-HNF4α (K9218, 1:500; R&D Systems, Minneapolis, MN) and P2-HNF4α (H6939, 1:500; R&D Systems, Minneapolis, MN) were used to inves-

tigate the expression pattern in gastric epithelial cells. Nuclear staining was considered a positive reaction. The extent of HNF4α expression in the area of gastric epithelium was graded semi-quantitatively as follows: 0+, no; 1+, extended to 1/10; 2+, from 1/10 to 5/10; 3+, more than 5/10 of the area. The assessment was made by a single senior pathologist (Z.Z).

### Statistical analysis

The chi-square test was used to verify a significant association between activity, chronic inflammation, intestinal metaplasia, and P1-HNF4α protein expression. Data were analyzed using statistical software (SPSS, version 13.0; SPSS Inc., Chicago, IL). A value of  $P<0.05$  was considered as statistically significant.

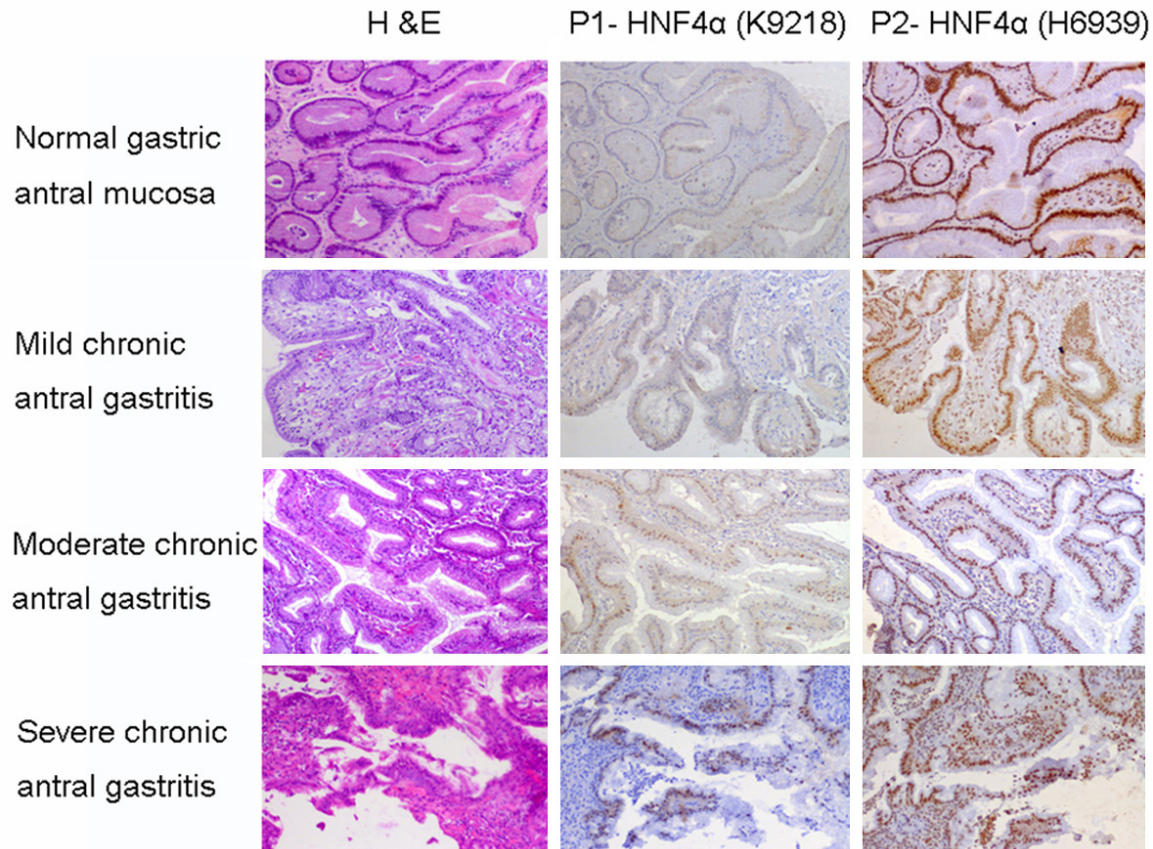
## Results

### Inflammation activity and intestinal metaplasia in chronic gastritis

According to the updated Sydney System grading [11], 65 mild chronic gastritis, 101 moderate chronic gastritis and 59 severe chronic gastritis were included in the study. Meanwhile, 5 normal pylorus tissues were used as controls. Chronic gastritis is often accompanied by the activity. The findings showed a high grade of activity in severe chronic gastritis, and it was strongly correlated to the severity of chronic gastritis (Table 1,  $P<0.05$ ). IM status was characterized by the appearance of goblet cells and expression of Muc-2. IM was not detected in any of 5 normal tissues, while IM was present in 24 mild inflammation samples (24/65, 36.9%), 48 moderate inflammation samples (48/101, 47.5%) and 31 severe inflammation samples (31/59, 52.5%). The frequency of IM was significantly associated with the severity of inflammation in chronic gastritis samples (Table 2,  $P<0.05$ ).

### HNF4α expression in chronic gastritis without intestinal metaplasia

P1-HNF4α expression was not detectable in normal pylorus epithelia, while the inflamed epithelia had various expression levels of P1-HNF4α in gastritis samples without IM (Figure 1). Among the 41 mild inflammation without IM cases, P1-HNF4α expression was detectable in 8/41 cases (19.5%) (Table 3). In



**Figure 1.** P1-HNF4α and P2-HNF4α expression in gastric antral tissues. Chronic antral gastritis was categorized into mild, moderate and severe. Gastric antral epithelial cases were stained with hematoxylin and eosin (H&E) and immunohistochemistry of P1-HNF4α and P2-HNF4α. (Magnification, 200×).

**Table 3.** Correlation between P1-HNF4α protein expression and chronic inflammation in samples without intestinal metaplasia

Chronic inflammation (without IM*)	Case	P1-HNF4α expression				P-value
		0+	1+	2+	3+	
Mild	41	33	7	1	0	P<0.05
Moderate	53	15	18	12	8	
Severe	28	1	3	9	15	

\*IM: Intestinal metaplasia.

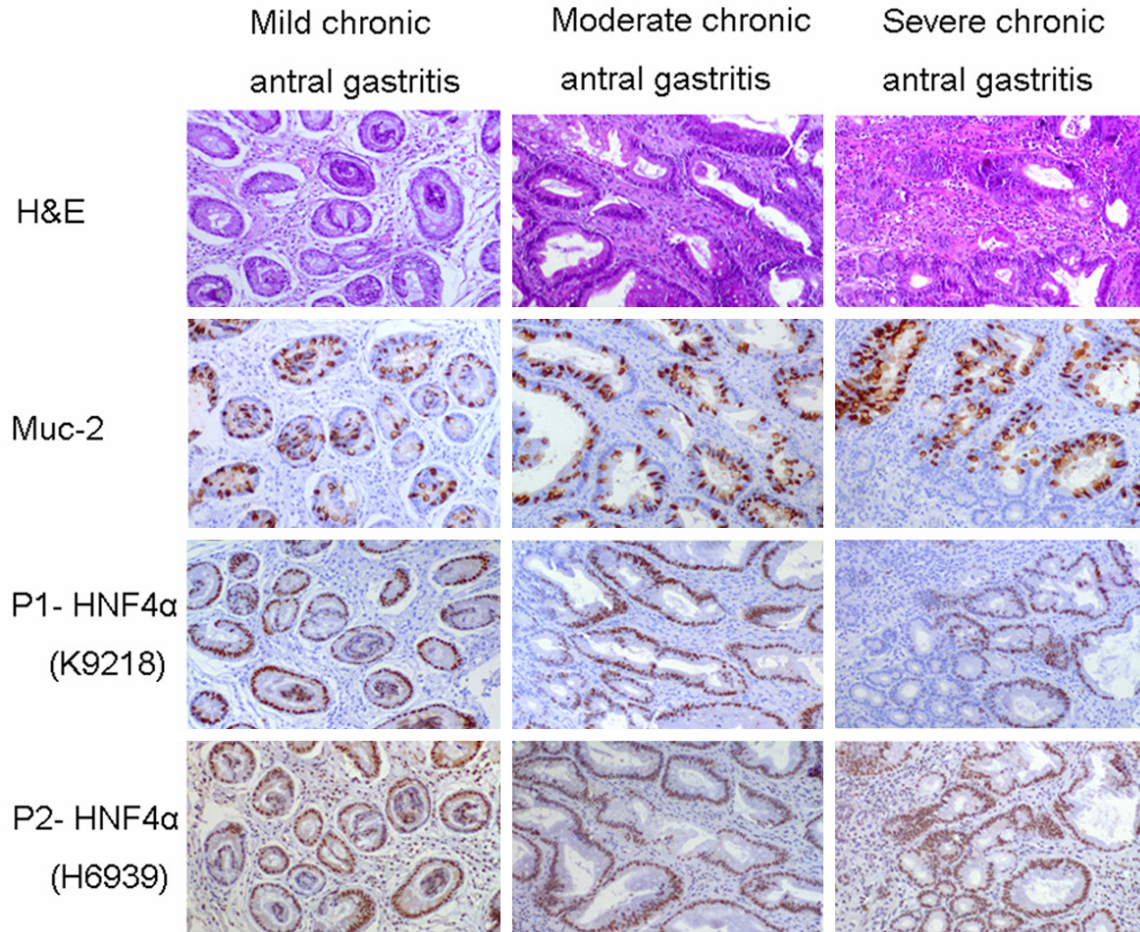
53 moderate inflammation without IM cases, the expression of P1-HNF4α was 38/53 cases (71.7%) (Table 3). Other 28 severe inflammation without IM cases, P1-HNF4α expression was found in nearly all the cases (27/28, 96.4%) (Table 3). The expression degrees of P1-HNF4α was significantly associated with the severity of inflammation in chronic gastritis samples ( $P<0.05$ ). However, there was no significant dif-

ference of P1-HNF4α expression between active or inactive in mild, moderate or severe chronic gastritis (data not shown). Unsurprisingly, the expression degrees of P2-HNF4α had no difference from normal to severe inflammation samples, which were almost strongly staining positive (Figure 1).

#### *HNF4α expression in chronic gastritis with intestinal metaplasia*

The proportion of IM was different in various level of chronic samples, but the expression of P1-HNF4α was almost the same in IM area, no matter how far the severity of chronic inflammation. Almost all cells in IM region were strongly stained for P1-HNF4α (Figure 2). As for P2-HNF4α, the immunoreactivity was seen in nearly all gastric cells and IM cells. In addition, there was little expression difference between any inflammation samples and normal tissues for P2-HNF4α (Figure 2).





**Figure 2.** Muc-2, P1-HNF4 $\alpha$  and P2-HNF4 $\alpha$  expression in chronic antral gastritis with intestinal metaplasia. Patient samples were categorized into mild, moderate and severe. Gastric antral epithelial cases were stained with hematoxylin and eosin (H&E) and immunohistochemistry of Muc-2, P1-HNF4 $\alpha$  and P2-HNF4 $\alpha$ . (Magnification, 200 $\times$ ).

## Discussion

*H. pylori* infection is well known to be associated with chronic gastritis, intestinal metaplasia and carcinoma [12, 13]. After a long-lasting infection, a number of pathological changes were occurred from normal pathway of gastric differentiation to chronic inflammation, IM and carcinoma [12, 13]. During this process, there were vast changes in gene expression profiles [6, 7, 9, 14-19]. Our results demonstrated that P1-HNF4 $\alpha$  was not expressed on normal gastric mucosa, but it was associated with the severity of inflammation in chronic gastritis samples (**Figure 1**; **Table 3**). Especially, all of the IM area were strongly stained with P1-HNF4 $\alpha$ , no matter how far the severity of inflammation (**Figure 2**). Previous work has shown that IM may develop from gastric epithelium that has undergone chronic inflammation,

and our study also showed that IM is more likely to occur in severe inflammation samples (**Table 2**). All in all, our findings indicated that the expression patterns of P1-HNF4 $\alpha$  were in line with the pathological process from gastric mucosa to IM.

Infection with *H. pylori* is the most common cause of chronic active gastritis [20]. Our results showed most of the severe chronic gastritis were active, but most of the mild inflammation gastric biopsies were inactive. The activity was strongly associated with the severity of inflammation (**Table 1**). However, there is no significant difference between the activity and inactivity in the expression of P1-HNF4 $\alpha$  in the same grade chronic gastritis (data not shown). We deduced that the progressive shift of P1-HNF4 $\alpha$  expression might need a prolonged inflammation, and chronic was more

representative of long-term reaction than activity. Further studies are needed to clarify this point.

Interestingly, P2-HNF4 $\alpha$  was strongly expressed in varied gastric tissues, including normal gastric epithelia, gastritis with various degrees of severity, and gastric IM (**Figures 1, 2**). It has been reported that P2-HNF4 $\alpha$  is widely expressed in gastrointestinal tract [6-10]. Our results were in line with the previous reports and showed the expression status of P2-HNF4 $\alpha$  in chronic gastritis.

In conclusion, this study showed a different expression patterns of P1-HNF4 $\alpha$  and P2-HNF4 $\alpha$  in chronic gastritis. An increased expression of P1-HNF4 $\alpha$  was associated with the severity of inflammation in chronic gastritis, and the expression come to a head in gastric IM, while P2-HNF4 $\alpha$  expression was widespread and there was no difference in all chronic gastritis. These results suggest that the expression of P1-HNF4 $\alpha$  but not P2-HNF4 $\alpha$  may be a useful diagnostic marker for chronic gastritis.

## Acknowledgements

This work was supported by the National Natural Science Foundation of China, under Grant 81560088; Science and Technology Foundation of Guizhou Province, under Grant J[2015]2086; and Guizhou Province Science and Technology Agency-Guizhou Provincial People's Hospital Joint Fund, under Grant LH[2015]7108.

## Disclosure of conflict of interest

None.

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