Aberrant DNA methylation and expression of *EYA4* in gastric cardia intestinal metaplasia

Chenxi Li^{1,#}, Zhaohui Liu^{3,#}, Guohua Xu^{4,#}, Shibin Wu^{4,#}, Yunhui Peng⁴, Ruinuan Wu⁵, Shukun Zhao¹, Xiaoqi Liao^{1*}, Runhua Lin^{1,2*}

¹Department of Pathology, Shantou University Medical College, Shantou, ²Guangdong Provincial Key Laboratory of Infectious Diseases and Molecular Immunopathology, Shantou, ³Department of Gastroenterology, Shenzhen Second People's Hospital/The First Affiliated Hospital of Shenzhen University Health Science Center, Shenzhen, ⁴Department of Gastroenterology, Huiyang Sanhe Hospital, Huizhou, ⁵Department of Pathology, Shenzhen Second People's Hospital/The First Affiliated Hospital of Shenzhen University Health Science Center, Shenzhen, P.R. China "These authors contributed equally to this work.

*These authors are co-corresponding authors

Abstract Background: Intestinal metaplasia (IM) of the gastric cardia is an important premalignant lesion. However, there is limited information concerning its epidemiological and molecular features. Herein, we aimed to provide an overview of the epidemiological data for gastric cardiac IM and evaluate the role of EYA transcriptional coactivator and phosphatase 4 (*EYA4*) as an epigenetic biomarker for gastric cardiac IM.

Methods: The study was conducted in the context of the gastric cardiac precancerous lesion program in southern China, which included 718 non-cancer participants, who undertook endoscopic biopsy and pathological examination in three endoscopy centers, between November 2018 and November 2021. Pyrosequencing and immunohistochemistry were performed to examine the DNA methylation status and protein expression level of *EYA4*.

Results: Gastric cardiac IM presented in 14.1% (101/718) of participants and was more common among older (>50 years; 22.0% [95% CI: 17.8–26.8]) than younger participants (\leq 50 years; 6.7% [95% CI: 4.5–9.9]; *P* < 0.001). IM was more common in male participants (16.9% [95% CI: 13.2–21.3] vs. 11.3% [95% CI: 8.3–15.1]; *P* = 0.04). Pyrosequencing revealed that IM tissues exhibited significantly higher DNA methylation levels in *EYA4* gene than normal tissues (*P* = 0.016). Further, the protein expression level of *EYA4* was reduced in IM and absent in intraepithelial neoplasia tissues compared to normal tissues (*P* < 0.001).

Conclusions: Detection rates of gastric cardiac IM increase with age and are higher in men. Our findings highlight the important role of promoter hypermethylation and downregulation of *EYA4* in gastric cardiac IM development.

Keywords: DNA methylation, EYA4, gastric cardia, intestinal metaplasia

Address for correspondence: Dr. Runhua Lin, Department of Pathology, Shantou University Medical College, No. 22, Xinling Road, Shantou 515041, P.R. China. E-mail: rhlin2010@163.com ORCID: 0000-0001-7014-9829 Submitted: 28-May-2022 Revised: 29-Aug-2022 Accepted: 05-Sep-2022 Published: 04-Nov-2022

Access this article online					
Quick Response Code:	Wabsita:				
	www.saudijgastro.com				
	DOI: 10.4103/sjg.sjg_228_22				

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Li C, Liu Z, Xu G, Wu S, Peng Y, Wu R, *et al.* Aberrant DNA methylation and expression of *EYA4* in gastric cardia intestinal metaplasia. Saudi J Gastroenterol 2022;28:456-65.

INTRODUCTION

Intestinal metaplasia (IM) of the stomach is characterized by the replacement of normal gastric epithelium and gastric glands by intestinal epithelium and intestinal glands. Histologically confirmed gastric IM often confers an increased risk of progression towards gastric cancer.^[1-3] Thus, gastric IMs are generally considered to be precursor lesions for the development of dysplasia, and ultimately gastric carcinoma.^[4] However, little attention has been given to this premalignant lesion in the gastric cardia. Limited data are available on the prevalence of IM in gastric cardia, especially in cancer-free subjects. Yet, these data may be informative for further molecular investigations. Given the preneoplastic nature of the lesion, it is crucial to improve our understanding of the regulation of genes contributing to the intestinal metaplastic phenotype. Hence, identifying informative biomarkers for gastric cardiac IM could be useful to guide further research regarding gastric cardiac carcinogenesis, that may have preventive implications.

DNA methylation is a key regulator of gene expression that does not alter the DNA sequence. Although it is well known that aberrant DNA methylation is associated with multiple cancer types,^[5-7] there is less clarity regarding its role in early neoplastic progression. Indeed, these changes to DNA methylation are critical to understanding whether altered DNA methylation may contribute to premalignant lesion formation. Recent epigenetic studies have shown that altered DNA methylation occurs even in precancerous tissues, and these changes, therefore, may serve as promising early indicators of existing disease, and of risk prediction.^[1] More recently, our methylation array-based (Illumina EPIC/850K array) study showed that gastric cardiac IM exhibits distinct DNA methylation profiling and identified some candidate genes that were significantly hypermethylated in their promoter regions in IM tissues. In this work, using a stringent filtering strategy, we found that the EYA4 promoter region exhibited the largest number of differentially methylated probes (DMPs) in gastric cardiac IM tissue. Thus, we hypothesized that gastric cardia IM presents as a common event in cancer-free subjects, and that promoter hypermethylation of EYA4 may reduce its expression level in gastric cardiac IM tissues.

For the present study, we aimed to: 1) investigate the detection rate of gastric cardiac IM in cancer-free individuals, 2) verify the DNA methylation level of candidate DMPs in the *EYA4* promoter region, and 3) evaluate the protein expression level of EYA4 in gastric cardiac IM and normal gastric cardiac mucosa.

PATIENTS AND METHODS

Biopsy specimens

Gastric cardiac biopsies were obtained from 718 participants undergoing upper endoscopy for gastrointestinal symptoms, between November 2018 and November 2021, in three centers (Shantou, Huizhou, and Shenzhen) in Guangdong Province, China. All the subjects included in this study were cancer-free at the time of endoscopic examination. Informed written consent was obtained from the participants according to the protocol approved by the Institutional Review Board of Shantou University Medical College (Approval Number: SUMC-2020-23); (Approval date: April 3, 2020). Upper endoscopic examinations were conducted by four experienced endoscopists (attending physician or above, having experiences of gastrointestinal endoscopy for at least 5 years). Biopsies of the endoscopically normal gastric cardiac mucosae were included in this study. Following histopathological assessment, we divided these biopsy samples into four histological categories, including normal gastric cardiac tissue, chronic carditis, chronic atrophic carditis, and IM tissue [Figure 1a and b]. For bisulfite pyrosequencing, the following subjects were included: n = 10 (normal tissue, n = 5; IM tissue, n = 5). For the immunostaining of EYA4, the following samples were included: n = 88 (normal tissue, n = 38; IM tissue, n = 47; and IM with intraepithelial neoplasia [IEN], n = 3). A total of four IEN samples were used for histopathological analyses in our study, but finally only three IEN samples were available for immunohistochemistry (IHC).

Histopathological evaluation

Briefly, the endoscopic specimens were fixed with 10% neutral formalin for 24 h, and then specimens were embedded in paraffin. Paraffin-embedded tissues were cut into 4- μ m thick sections and stained with hematoxylin and eosin (HE). The pathological diagnosis was independently determined by two pathologists. For the specimens showing IM lesions with routine HE staining on initial examination, two additional tissue sections were stained with Alcian blue/periodic acid-Schiff (AB-PAS; Cat# BA-4121, Lot: C210401, BaSO Biotechnologies, China) staining, and immunohistochemical staining of mucin 2 (MUC2; goblet cell marker) for further validation [Figure 1c].

In this study, we classified all the endoscopic specimens into four categories (normal gastric cardiac tissue, chronic carditis, atrophic carditis, and IM tissue) based on the following histological criteria.^[8] Briefly, (a) normal gastric cardiac tissues, represented by normal morphology of the mucosa, namely, foveola, glands, gland necks, and



Figure 1: Overview of gastric cardiac biopsy specimens and detection rate of gastric cardiac IM. (a) schematic diagram showing the collection of gastric cardiac tissues from cancer-free individuals. A total of 718 participants underwent upper endoscopy were recruited from three endoscopy centers (Shantou, Huizhou, and Shenzhen) in Guangdong Province (b) hematoxylin and eosin staining of representative gastric cardiac tissues from participants with different pathological changes. Scale bars, 50 µm (c) representative images of Alcian blue-periodic acid-Schiff (AB-PAS) staining and immunostaining of mucin-2 (MUC2) in gastric cardiac IM tissues. The AB staining method stains goblet cell mucus as blue color (as indicated by a closed red arrowhead show AB-positive cells); the open red arrowhead denotes epithelial cells expressing MUC2. Scale bars, 50 um (d) pie chart depicting the detection rates of 718 individuals in our cohort depending on their pathological changes. Left panel: Histologically, gastric cardiac IM was found in 14.1% (101/718) cancer-free individuals. Right panel: A total of four subjects show low-grade intraepithelial neoplasia of the gastric cardiac mucosa in the context of IM. There is mild irregularity of the glands with crowding of epithelial cells. Most of the glandular cells show mucus depletion, the nuclei are elongated, hyperchromatic, and irregular in size. Scale bar, 50 µm (e) boxplot showing the median age in different histological categories. Statistical significance was analyzed by the Kruskal-Wallis test, followed by Dunnett's post hoc test for multiple comparisons between groups. The black horizontal dashed line indicates the median age of all the study participants. The upper and lower limits of the box represent 25th and 75th quartile age distribution with whiskers extending to 1.5 times the range from top/bottom of the box, the center line within the box corresponds to the median age in each group, outliers are not shown (f) line graph showing detection rates of gastric cardiac IM measured in different age and sex groups. Indicated P values were calculated by Pearson's chi-square test. Error bar represents the 95% confidence interval around the detection rate. The overall detection rate of gastric cardiac IM (14.1%) in study participants is depicted by the horizontal dashed black line. Source data (d and f) are provided in Supplementary Table S2

stroma are well-preserved and keep their position and proportions intact. Inflammatory infiltration is minimal or absent. (b) Chronic carditis, characterized by the infiltration of the lamina propria by plasma cells and lymphocytes (with occasional formation of follicles) without glandular atrophy. (c) Chronic atrophic carditis, defined as the reduction or disappearance of native gastric cardiac glands: a reduction in the number of layers of subepithelial glands, often with decrease in the size and number of the glands within the lamina propria. There is also a chronic inflammation of the lamina propria with abundant lymphocytes, macrophages, and plasma cells. This evaluation is possible in samples that include the muscularis mucosae, being completely represented in the full thickness of the mucosae. (d) IM tissues, recognized as the presence of goblet cells (special staining [AB-PAS] and immunostaining of MUC2 reveal that they secrete specific mucus), columnar absorptive cells with well-defined brush borders, or Paneth cells. Additionally, IEN refers to morphologically dysplastic epithelium (tortuous glandular structures are lined by mucus-depleted epithelial cells with irregular, elongated, and hyperchromatic nuclei) without breaching the basement membrane.^[9]

DNA isolation and bisulfite pyrosequencing

Genomic DNA was isolated from fresh-frozen tissues using the AllPrep DNA/RNA Mini Kit (Cat# 80824, Lot: 166018916, QIAGEN, Germany), according to the manufacturer's protocols. The concentration and purity of the extracted genomic DNA were measured with an ND-2000 spectrophotometer (Thermo Fisher Scientific, USA). Extracted DNA was stored at -80°C until further analysis.

To quantify the methylation levels of candidate cytosine-phosphate-guanosine (CpG) sites in the promoter of EYA4, quantitative bisulfite pyrosequencing was performed. Briefly, 500 ng of total genomic DNA from each of the gastric cardiac tissues was used for bisulfite conversion, using the EZ DNA Methylation Gold Kit (Zymo Research, USA). The primers used were designed using PyroMark Assay Design Software 2.0.2 (QIAGEN) [Supplementary Table S1]. One of the PCR primers was biotin labeled. The sequence for analysis is localized to the promoter region of the EYA4 gene. The PCR product was checked by 1% agarose gel electrophoresis (showing one clear band). According to the manufacturer's instructions, the biotinylated PCR product was purified as single-stranded DNA to be used as the template in a pyrosequencing reaction, using the Vacuum Prep Workstation (QIAGEN), and pyrosequencing was then performed using PyroMark Gold Q96 reagent (QIAGEN). The methylation percentage for each CpG site was generated automatically using PyroMark Q96 (QIAGEN), and the results were displayed as a pyrogram with the methylation percentage.

IHC

Immunohistochemical staining was conducted in 38 normal gastric cardiac mucosae, 47 IM tissues, and three IEN tissues. Briefly, the paraffin-embedded tissues were sectioned into $4-\mu m$ thickness and placed on adhesion

glass slides, followed by baking at 65°C for 1 h. Slides were then deparaffinized in xylene (10 min \times 3) and rehydrated with descending concentrations of ethanol (100%, 5 min; 95%, 5 min; 85%, 5 min; and 75%, 5 min) and rinsed with phosphate-buffered saline (PBS). Then, antigen retrieval was performed by heating the slides (immersed in 10 mM sodium citrate buffer, pH = 6.0 [for *EYA4*] or EDTA, pH = 9.0 [for MUC2]) for 3 min in a pressure cooker. Slides were washed with PBS (3 min \times 3), and the endogenous peroxidase activity was quenched with 3% hydrogen peroxide for 10 min at room temperature, followed by three PBS washes (3 min each). Slides were incubated with EYA4 primary antibody (Cat# ab251675, rabbit polyclonal, Lot: GR3304276-1, Abcam, UK; 1:100 dilution) or MUC2 primary antibody (Cat# ZM-0392, Lot: 21082308, clone Ccp58, ZSGB-Bio, China; ready-to-use) overnight at 4°C. Then, the slides were thoroughly washed in PBS $(3 \min \times 3)$ and incubated with horseradish peroxidase labeled goat anti-mouse/rabbit secondary antibody (Cat# KIT-5020, Lot: 210224S407c, Maixin Biotechnologies, China) for 30 min at 37°C, followed by an additional three washes in PBS (3 min each). Staining was visualized using the 3,3'-diaminobenzidine (Cat# DAB-0031, Maixin Biotechnologies, China) and counterstained with hematoxylin for 5 min. Finally, slides were dehydrated with series ethanol (75%, 5 min; 85%, 5 min; 95%, 5 min; and 100%, 5 min \times 2) and cleared in xyline (5 min \times 3). Images were captured on the OLYMPUS B ×53 microscope using Olympus cellSens imaging software (version 1.14).

The protein expression level of EYA4 was estimated semi-quantitatively, based on both staining intensity and proportion of stained cells, according to the following criteria. The intensity of immunostaining was graded from 0 to 3 (0: negative; 1: slightly brown staining; 2: moderately brown staining; and 3: darkly brown staining). The percentage of positively stained cells was graded on a scale of 0-4 (0: negative; 1: 1-10%; 2: 11-50%; 3: 51-80%; and 4: >80%). A final immunostaining score (ranging from 0 to 12) was calculated by multiplying the scores of nuclear staining intensity and percentage of positively stained cells.

Statistical analysis and data visualization

All statistical analyses and data visualization were performed in RStudio (version 2021.09.1.372) (http:// www.rstudio.com/) within the R statistical environment (version 4.1.2) (https://www.R-project.org) using the packages dplyr (version 1.0.7), ggplot2 (version 3.3.5), ggrepel (version 0.9.1), factoextra (version 1.0.7), reshape2 (version 1.4.4), pheatmap (version 1.0.12), Gviz (version 1.38.0), GenomicFeatures (version 1.46.1), BSgenome (version 1.62.0), TxDb.Hsapiens.UCSC.hg19. knownGene (version 3.2.2), and stats (version 4.1.2). All continuous variables with non-normal distribution were presented as median with interquartile range (IQR) for the indicated number of biological replicates, and categorical variables were presented as frequency with proportion. Continuous data were assessed for normality using Shapiro-Walk test (shapiro.test R function) before statistical tests of significance were run. Differences in median age and immunostaining score of EYA4 among multiple groups (did not pass normality test; Figures 1e and 3b) were assessed using the Kruskal-Wallis test, followed by Dunnett's post hoc test for multiple comparisons between groups (R package FSA, version 0.9.1). We used Pearson's Chi-square test (chisq.test R function) to compare the detection rates of gastric cardiac IM in each group (stratified by median age [≤ 50 vs. >50 years] and sex [female vs. male]. For the methylation rate of candidate CpG sites in normal and IM tissues, a Mann-Whitney U non-parametric test (wilcox.test R function) was used [Figure 2b]. For hierarchical cluster analysis of DNA methylation, 7-scaled values were used to calculate Euclidean distance that was applied for clustering using Ward's method [Supplementary Figure S1b]. The statistical tests used in each experiment are described in their respective figure legends. The exact sample size ("n") in each group, where applicable, was provided in the figures or figure legends. Two-tailed P values < 0.05 were considered statistically significant. Adobe Illustrator CC2015 (Adobe Systems, San Jose, CA) is used to organize figures. The micrographs (such as those in Figure 3a) are a magnification of a representative area shown adjacent to them.

RESULTS

Higher detection rate of gastric cardiac IM in elder and male participants

The median age of the study participants was 50 (IQR: 42–57) years, there were slightly more female than male participants (50.6% vs. 49.4%). Baseline characteristics of participants are presented in Table 1. The most common pathological findings were chronic carditis (494 participants [68.8%; 95% CI: 65.2–72.2]), followed by IM (101 participants [14.1%; 95% CI: 11.7–16.9]) and

Table 1: Basic characteristics of the participants enrolled ingastric cardiac biopsy cohort

Characteristics	Study cohort (n=718)
Age at diagnosis (years), Median (IQR)	50 (42-57)
Gender, <i>n</i> (%)	
Male	355 (49.4)
Female	363 (50.6)

IQR=interquartile range

chronic atrophic carditis (85 participants [11.8%; 95% CI: 9.6–14.5]; Figure 1d). Of note, foci of IEN occurred in the context of IM in four (4.0%; 95% CI: 1.3–10.4) of the 101 participants with IM. As shown in Figure 1d, the intestinal metaplastic glands are closely packed and lined by irregular cells with elongated and hyperchromatic nuclei.

Among these four histological categories, participants with IM were older than those in other categories (P < 0.001; Figure 1e). We then categorized the participants into two age groups using the median age (50 years) of the overall participants in this study as a cutoff. As expected, the detection rates of IM in participants ≤ 50 years increased from 6.7% (95% CI: 4.5–9.9) to 22.0% (95% CI: 17.8–26.8) in those > 50 years (P < 0.001). Subgroup analysis by sex showed a significantly higher detection rate of gastric cardiac IM in male participants compared to that of female participants (16.9% [95% CI: 13.2–21.3] vs. 11.3% [95% CI: 8.3–15.1], P = 0.04; Figure 1f). Individual participant characteristics are detailed in Supplementary Table S2.

Hypermethylation of EYA4 promoter region in gastric cardiac IM tissues

To identify differentially methylated genes in gastric cardiac IM compared with normal gastric cardiac tissue, we reanalyzed a dataset from our group that had originally been generated to assess methylation alterations in gastric cardiac IM.^[10] We assigned differentially methylated CpG sites found between normal and IM tissues to candidate genes, which were identified based on a false discovery rate <0.01, the difference in methylation (β value difference) $\geq 20\%$, and 19 or more differentially CpG sites located in promoter regions per gene. Through this analysis, we were able to identify five candidate genes showing significant hypermethylation in their promoter regions [Supplementary Figure S1a]. Unsupervised clustering analysis using all CpG sites in the candidate genes confirmed an explicit segregation and a clear epigenetic difference between normal and IM tissues [Supplementary Figure S1b]. Among these candidate genes, EYA4 has been reported to be hypermethylated in Barrett's esophagus,^[11,12] we thus, selected EYA4 as a promising biomarker for downstream analysis.

Next, we used a quantitative pyrosequencing assay to assess the methylation status of the five candidate CpG sites for the EY.44 promoter region [Figure 2a, Table 2] on an independent set of gastric cardiac samples with sufficient DNA. As shown in Figure 2b [Supplementary Table S3], IM tissues showed a significant higher DNA methylation level (mean methylation level of five CpG sites) than normal gastric



Figure 2: Quantitative pyrosequencing analysis reveals DNA methylation of candidate CpG sites in the *EYA4* promoter region. (a) schematic diagram showing the genomic location of a 34-bp region (Chr: 133562244-133562277) within *EYA4* promoter (indicated by a red vertical bar) and a close-up view of the examined CpG sites (red dashed rectangle) (b) a total of 10 fresh-frozen gastric cardiac tissues (*n* = 5 for normal and IM tissues, respectively) were used for quantification of DNA methylation levels of five candidate CpG sites (cg11518846, cg20980055, cg20286200, cg05062333, and cg01162672) by pyrosequencing. Box plot showing the median methylation level in normal and IM tissues. Each data dot denotes the average methylation of five candidate CpG sites mentioned above. Indicated *P* value was calculated by the Mann–Whitney *U* non-parametric test (c and d) representative pyrograms showing the methylation level of cg01162672, cg05062333, cg20286200, cg20980055, and cg11518846 in normal (c) and IM tissues (d). The percentage of DNA methylation at each CpG site is indicated by shaded areas, and the quality of the result is shown in blue (good quality) or yellow (pass quality). On the *x-axis*, the nucleotides injected by the pyrosequencer are shown, "E" represents the moment when the enzyme was added to the reaction followed by the substrate "S" that is documented by a small spike. The pyrosequencer added a "T" that is not present in the sequence, thus, no spike was observed. The actual sequence to analyze starts with a "C," followed by the methylated cytosine here marked with "R." On the *y-axis*, luminescence detected by the LCD camera is shown. Source data (b) are provided in Supplementary Table S3



Figure 3: EYA4 protein expression in normal and IM tissues. (a) representative images of immunohistochemical staining for EYA4 in normal gastric cardiac tissues and IM tissues. As shown in the upper panel, normal gastric cardiac epithelial cells demonstrate strong nuclear immunostaining for EYA4. The middle panel of immunostaining section of IM tissue highlighting areas of intestinal metaplastic glands (outlined by red dashed line) and normal glands (outlined by blue dashed line). The intestinal metaplastic cells show weak to absent immunostaining compared with normal glandular cells. Prominent loss of EYA4 expression in low-grade intraepithelial neoplasia (IEN) tissue in the context of IM is shown in the lower panel. Scale bars, 50 µm (b) quantitative comparison of immunohistochemical staining for EYA4 in normal (n = 38), IM (n = 47), and IM with IEN (n = 3) tissues. Gray dots represent the immunostaining score for EYA4 in each sample. Statistical significance was analyzed by the Kruskal-Wallis test, followed by Dunnett's post hoc test for multiple comparisons between groups. The upper and lower limits of the box represent 25th and 75th quartile immunostaining score distribution with whiskers extending to 1.5 times the range from top/bottom of the box, the center line within the box corresponds to the median value of the immunostaining score in each group, outliers are not shown. Source data (b) are provided in Supplementary Table S4

cardiac tissues (P = 0.016), demonstrating the association between gastric cardiac IM and hypermethylation in the

 Table 2: Candidate hypermethylated CpG sites in the promoter of EYA4

•		
Illumina Id†	Delta beta (IM vs. Normal tissues)	Chromosome location (GRCh37/hg19)
cg11518846	0.31	Chr6: 133562246
cg20980055	0.26	Chr6: 133562258
cg20286200	0.36	Chr6: 133562267
cg05062333	0.31	Chr6: 133562269
cg01162672	0.32	Chr6: 133562275

 $IM\!=\!intestinal$ metaplasia. $^{\dagger}IIlumina$ ID is the unique identification number in the HumanEPICBeadChip

EYA4 promoter region. Representative pyrograms for normal and IM tissues are depicted in Figure 2c and d.

Decreased EYA4 protein expression in gastric cardiac IM tissues

The intriguing observation of significant hypermethylation of the EYA4 promoter region in gastric cardiac IM tissues led us to hypothesize that these metaplastic lesions may have lower levels of EYA4 protein compared to the normal gastric cardiac tissues. Formalin-fixed paraffin-embedded biopsy samples were then subjected to immunostaining for EYA4. As shown in Figure 3a, EYA4 was uniformly expressed in the nuclei of normal gastric cardiac epithelial cells (upper panel) and its expression was significantly reduced in IM lesions (middle panel). As expected, loss expression of EYA4 was noted in all the three IEN samples included in this study (lower panel). Following image quantification, the immunostaining score of EYA4 was significantly decreased in IM and IEN tissues compared with that in normal gastric cardiac tissues (P < 0.001; Figure 3b); [Supplementary Table S4]. These results demonstrated that the downregulated expression of EYA4 is a promising biomarker for gastric cardiac IM as well as early gastric cardiac carcinogenesis.

DISCUSSION

It is becoming clear that alterations in DNA methylation are associated with precancerous lesions.^[13-16] However, the role of DNA methylation in gastric cardiac IM has received relatively less attention. Previously, we reported that DNA methylation profiles of gastric cardiac IMs significantly differed from those of normal gastric cardiac mucosa. In this study, we researched the methylation status of the *EYA4* promoter region and gained insights on epigenetic markers in gastric cardiac IM. In our earlier study, obvious hypermethylation of gene promoter regions was shown in gastric cardiac IM samples. By conducting pyrosequencing in the candidate CpG sites of *EYA4* and IHC analysis of *EYA4* protein, our present study revealed that gastric cardiac IM lesions exhibited hypermethylation in the promoter of *EYA4* and showed a reduced protein expression. This work provides evidence for promoter hypermethylation of *EYA4* as a promising epigenetic biomarker in gastric cardiac IM.

To date, the data regarding the detection rate of gastric cardiac IM in cancer-free individuals are limited. In this study, we assessed the pathology of the gastric cardiac mucosae covering more than 700 cancer-free individuals in Guangdong Province, China. We specifically focused on the detection rate of gastric cardiac IM to provide baseline data for further investigation of this premalignant lesion. Based on pathological assessment, the overall gastric cardiac IM detection rate in our study population was 14.1% (95% CI: 11.7-16.9). However, there are wide variations in the detection rates of gastric IMs among different studies,^[17,18] partly because of the unavoidable sampling bias, because most IMs showed only focal involvement. It deserves to be noted that in our study, the detection rates for gastric cardiac IM increased with age and were higher for men than for women, as shown in Figure 1f. These findings were consistent with that of previous studies on gastric IMs.^[19,20] Most likely, this age-dependent rise reflects the natural history of gastric cardiac IM and can be explained by the long duration of exposure to environmental risk factors. Therefore, it is necessary to consider the age and sex for future screening and surveillance for premalignant lesions of gastric cardia. Indeed, not all patients with gastric cardiac IMs will progress to cancer ultimately. Of note, we found IEN in the context of IM in four subjects (4/101; 4.0%) of the study participants. Thus, further work should be done to identify a subset of IM subjects at high risk of neoplastic progression.

DNA methylation changes have been studied in multiple cancer types.^[21-24] Furthermore, increasing evidence shows that aberrant DNA methylation events occur even in samples of precancerous lesions.^[25] Thus, such epigenetic events are poised to become ideal biomarkers for early stage cancer.^[26] However, only a few studies have focused on DNA methylation biomarkers in gastric non-cardiac IMs,^[15,27,28] and even less on IMs arising from gastric cardia. We recently conducted array-based genome-wide DNA methylation analysis in gastric cardiac IMs compared with normal gastric cardiac mucosae^[10] and found that EYA4 gene was significantly hypermethylated in gastric cardiac IMs. In this study, we further confirmed that hypermethylation of the EYA4 gene promoter was evident in gastric cardiac IMs [Figure 2]. This aligns with a previous report highlighting a link between EYA4 promoter hypermethylation and Barrett's esophagus, a condition of IM in the distal esophagus.^[12] Furthermore, several studies reported the hypermethylation of EYA4 in different cancer types.^[29-31] These findings support the claim that promoter DNA methylation of EYA4 is closely associated with IM development and is a very early event during multistage gastric cardiac carcinogenesis. Therefore, it will be worth investigating how well an EYA4 methylation test can detect dysplastic and cancerous lesions in future studies.

DNA hypermethylation plays an important role in carcinogenesis because it could cause the silencing of some pivotal genes, especially tumor suppressor genes.^[32,33] In this study, we observed that EYA4 protein was significantly reduced or not present in gastric cardiac IMs and dysplastic lesions, compared with normal tissues [Figure 3a], supporting our hypothesis that EYA4 expression reduction might be, at least partly, because of promoter DNA hypermethylation. These data indicate that EYA4 is a promising biomarker for gastric cardiac IMs. EYA4 is one of the four members of EYA gene family that was initially identified in Drosophila.^[34] Of note, interesting clues about the role of EYA4 protein in tumorigenesis are emerging rapidly, namely, its tumor suppressor role in esophageal squamous cell carcinoma,^[31] colorectal carcinoma,^[35] pancreatic adenocarcinoma,^[36] hepatocellular carcinoma,^[37] and bladder cancer.^[38] Thus, it is possible that reduced expression of EYA4 protein contributes to IM development and is an early molecular event during gastric cardiac carcinogenesis. Future investigations should aim to elucidate the functional role and the exact mechanism of EYA4 in IM development.

The major limitation of this study lies in the small sample size for pyrosequencing validation, for the methylation status of *EYA4* promoter. Therefore, continued efforts to investigate the frequency of *EYA4* methylation and its functional relevance, will improve our understanding of the role of *EYA4* in gastric cardiac IM. Another limitation of the study is the small sample size of IEN (n = 3) used for IHC.

In summary, the promoter hypermethylation of *EYA4* may contribute to the downregulation of protein expression in gastric cardiac IM, a precursor lesion of gastric cardiac cancer. This finding highlights the important role of aberrant DNA methylation in the *EYA4* promoter region in the pathogenesis of gastric cardiac IM [Figure 4].

Financial support and sponsorship

This study was supported by Project of Educational Commission of Guangdong Province of China (Grant No. 2017KQNCX068), the research grants from the Shantou Science and Technology Bureau (Grant No. 210712186880511; Grant No. 220507236491772),



Figure 4: Schematic model depicting the role of hypermethylation in the *EYA4* promoter region in gastric cardiac IM development. Hypermethylation-related reduced expression of the EYA4 protein may contribute to the development of gastric cardiac IM and appears to be a promising biomarker for this premalignant lesion

the Open Fund of Guangdong Provincial Key Laboratory of Infectious Diseases and Molecular Immunopathology (Grant No. GDKL202209), Medical Scientific Research Foundation of Guangdong Province of China (Grant No. A2022329), and the Huizhou Science and Technology Bureau (Grant No. 2021WC0106069).

Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Fan J, Li J, Guo S, Tao C, Zhang H, Wang W, *et al.* Genome-wide DNA methylation profiles of low- and high-grade adenoma reveals potential biomarkers for early detection of colorectal carcinoma. Clin Epigenetics 2020;12:56.
- den Hoed CM, Holster IL, Capelle LG, de Vries AC, den Hartog B, Ter Borg F, *et al.* Follow-up of premalignant lesions in patients at risk for progression to gastric cancer. Endoscopy 2013;45:249-56.
- Kim N, Park RY, Cho SI, Lim SH, Lee KH, Lee W, et al. Helicobacter pylori infection and development of gastric cancer in Korea: Long-term follow-up. J Clin Gastroenterol 2008;42:448-54.
- Whiting JL, Sigurdsson A, Rowlands DC, Hallissey MT, Fielding JW. The long term results of endoscopic surveillance of premalignant gastric lesions. Gut 2002;50:378-81.
- Morris MR, Latif F. The epigenetic landscape of renal cancer. Nat Rev Nephrol 2017;13:47-60.
- Saghafinia S, Mina M, Riggi N, Hanahan D, Ciriello G. Pan-cancer landscape of aberrant DNA methylation across human tumors. Cell Rep 2018;25:1066-80.e8.
- Natale F, Vivo M, Falco G, Angrisano T. Deciphering DNA methylation signatures of pancreatic cancer and pancreatitis. Clin Epigenetics 2019;11:132.

- 8. Rosai J.Rosai and Ackerman's Surgical Pathology e-book. Elsevier Health Sciences; 2011.
- Rugge M, Correa P, Dixon MF, Hattori T, Leandro G, Lewin K, *et al.* Gastric dysplasia: The Padova international classification. Am J Surg Pathol 2000;24:167-76.
- Lin R, Li C, Liu Z, Wu R, Lu J. Genome-wide DNA methylation profiling identifies epigenetic signatures of gastric cardiac intestinal metaplasia. J Transl Med 2020;18:292.
- Kaz AM, Wong CJ, Luo Y, Virgin JB, Washington MK, Willis JE, *et al.* DNA methylation profiling in Barrett's esophagus and esophageal adenocarcinoma reveals unique methylation signatures and molecular subclasses. Epigenetics 2011;6:1403-12.
- Zou H, Osborn NK, Harrington JJ, Klatt KK, Molina JR, Burgart LJ, et al. Frequent methylation of eyes absent 4 gene in Barrett's esophagus and esophageal adenocarcinoma. Cancer Epidemiol Biomarkers Prev 2005;14:830-4.
- Schmitz M, Eichelkraut K, Schmidt D, Zeiser I, Hilal Z, Tettenborn Z, et al. Performance of a DNA methylation marker panel using liquid-based cervical scrapes to detect cervical cancer and its precancerous stages. BMC Cancer 2018;18:1197.
- Moinova HR, LaFramboise T, Lutterbaugh JD, Chandar AK, Dumot J, Faulx A, *et al.* Identifying DNA methylation biomarkers for non-endoscopic detection of Barrett's esophagus. Sci Transl Med 2018;10:eaao5848. doi: 10.1126/scitranslmed.aao5848.
- Huang KK, Ramnarayanan K, Zhu F, Srivastava S, Xu C, Tan ALK, *et al.* Genomic and epigenomic profiling of high-risk intestinal metaplasia reveals molecular determinants of progression to gastric cancer. Cancer Cell 2018;33:137-50.e5.
- Oh TJ, Oh HI, Seo YY, Jeong D, Kim C, Kang HW, et al. Feasibility of quantifying SDC2 methylation in stool DNA for early detection of colorectal cancer. Clin Epigenetics 2017;9:126.
- Da B, Jani N, Gupta N, Jayaram P, Kankotia R, Yao Yu C, *et al.* High-risk symptoms do not predict gastric cancer precursors. Helicobacter 2019;24:e12548.
- Kang KP, Lee HS, Kim N, Kang HM, Park YS, Lee DH, *et al.* Role of intestinal metaplasia subtyping in the risk of gastric cancer in Korea. J Gastroenterol Hepatol 2009;24:140-8.
- McNamara D, Buckley M, Crotty P, Hall W, O'Sullivan M, O'Morain C. Carditis: All Helicobacter pylori or is there a role for gastro-oesophageal reflux?Scand J Gastroenterol 2002;37:772-7.
- Felley C, Bouzourene H, VanMelle MB, Hadengue A, Michetti P, Dorta G, *et al.* Age, smoking and overweight contribute to the development of intestinal metaplasia of the cardia. World J Gastroenterol 2012;18:2076-83.
- Baylin SB, Jones PA.A decade of exploring the cancer epigenome-biological and translational implications. Nat Rev Cancer 2011;11:726-34.
- Zhao SG, Chen WS, Li H, Foye A, Zhang M, Sjöström M, et al. The DNA methylation landscape of advanced prostate cancer. Nat Genet 2020;52:778-89.
- Klughammer J, Kiesel B, Roetzer T, Fortelny N, Nemc A, Nenning KH, et al. The DNA methylation landscape of glioblastoma disease progression shows extensive heterogeneity in time and space. Nat Med 2018;24:1611-24.
- Hovestadt V, Jones DT, Picelli S, Wang W, Kool M, Northcott PA, *et al.* Decoding the regulatory landscape of medulloblastoma using DNA methylation sequencing. Nature 2014;510:537-41.
- Peng DF, Kanai Y, Sawada M, Ushijima S, Hiraoka N, Kitazawa S, et al. DNA methylation of multiple tumor-related genes in association with overexpression of DNA methyltransferase 1 (DNMT1) during multistage carcinogenesis of the pancreas. Carcinogenesis 2006;27:1160-8.
- Dor Y, Cedar H. Principles of DNA methylation and their implications for biology and medicine. Lancet 2018;392:777-86.
- 27. Shin CM, Kim N, Lee HS, Park JH, Ahn S, Kang GH, *et al.* Changes in aberrant DNA methylation after Helicobacter pylori eradication:

A long-term follow-up study. Int J Cancer 2013;133:2034-42.

- Sugimoto R, Habano W, Yanagawa N, Akasaka R, Toya Y, Sasaki A, *et al.* Molecular alterations in gastric cancer and the surrounding intestinal metaplastic mucosa: An analysis of isolated glands. Gastric Cancer 2021;24:382-91.
- Emmett RA, Davidson KL, Gould NJ, Arasaradnam RP.DNA methylation patterns in ulcerative colitis-associated cancer: A systematic review. Epigenomics 2017;9:1029-42.
- Hou X, Peng JX, Hao XY, Cai JP, Liang LJ, Zhai JM, et al. DNA methylation profiling identifies EYA4 gene as a prognostic molecular marker in hepatocellular carcinoma. Ann Surg Oncol 2014;21:3891-9.
- Luo M, Li Y, Shi X, Yang W, Zhou F, Sun N, *et al.* Aberrant methylation of EYA4 promotes epithelial-mesenchymal transition in esophageal squamous cell carcinoma. Cancer Sci 2018;109:1811-24.
- 32. Sharma G, Mirza S, Parshad R, Srivastava A, Gupta SD, Pandya P, et al. Clinical significance of promoter hypermethylation of DNA repair genes in tumor and serum DNA in invasive ductal breast carcinoma patients. Life Sci 2010;87:83-91.

- Jones PA. Functions of DNA methylation: Islands, start sites, gene bodies and beyond. Nat Rev Genet 2012;13:484-92.
- Borsani G, DeGrandi A, Ballabio A, Bulfone A, Bernard L, Banfi S, et al. EYA4, a novel vertebrate gene related to Drosophila eyes absent. Hum Mol Genet 1999;8:11-23.
- Kim SJ, Tae CH, Hong SN, Min BH, Chang DK, Rhee PL, et al. EYA4 acts as a new tumor suppressor gene in colorectal cancer. Mol Carcinog 2015;54:1748-57.
- Mo SJ, Liu X, Hao XY, Chen W, Zhang KS, Cai JP, *et al*. EYA4 functions as tumor suppressor gene and prognostic marker in pancreatic ductal adenocarcinoma through β-catenin/ID2 pathway. Cancer Lett 2016;380:403-12.
- Zhu XX, Li JH, Cai JP, Hou X, Huang CS, Huang XT, *et al.* EYA4 inhibits hepatocellular carcinoma by repressing MYCBP by dephosphorylating β-catenin at Ser552. Cancer Sci 2019;110:3110-21.
- Dong W, Bi J, Liu H, Yan D, He Q, Zhou Q, et al. Circular RNA ACVR2A suppresses bladder cancer cells proliferation and metastasis through miR-626/EYA4 axis. Mol Cancer 2019;18:95.



Supplementary Figure S1: Candidate hypermethylated genes in gastric cardiac IM. These data were from Lin *et al.* 2020 (a) Bar plots displaying the DNA methylation levels of the hypermethylated genes in gastric cardiac IM compared with normal gastric cardiac tissue (b) Heatmap demonstrating unsupervised hierarchical clustering of individuals based on the methylation levels of the candidate hypermethylated genes mentioned in (a)

Supplementary Table S1: List of PCR primers,

pyrosequencing primers, and sequence for analysis

Gene	Primer description	Primer sequence	Sequence to analyze
EYA4	PCR_Forward	5'-biotin-TTTTTTTTAGTTTG GAGGTTGTAGT-3'	CRATCTCRCRTAAA AACCRCCC
	PCR_Reverse	5'-TTTATTTAACCTCRAATA ACTACCTATTATC-3'	AAAACCTCR
	Pyrosequencing_ Reverse	5'-AATAACTACCTATTATC ATAATTTA-3'	

Supplementa	upplementary Table S2: Participant characteristic			Supplementary Table S2: Contd			
Subject ID	Sex	Age	Pathological diagnosis	Subject ID	Sex	Age	Pathological diagnosis
HY-1	Male	55	Chronic carditis	HY-151	Male	55	Chronic atrophic carditis
Y-3	Female	43	Chronic carditis	HY-153	Female	45	Chronic atrophic carditis
IY-5	Male	50	Chronic carditis	HY-155	Female	33	IM
łY-7	Male	37	Chronic carditis	HY-157	Male	49	Chronic atrophic carditis
-1Y-9	Male	35	Chronic carditis	HY-159	Male	42	Chronic atrophic carditis
HY-11	Female	47	Chronic atrophic carditis	HY-161	Female	62	IM
HY-13	Female	71	Chronic carditis	HY-163	Male	58	Chronic carditis
HY-15	Female	71	Chronic carditis	HY-165	Male	29	Chronic carditis
HY-17	Female	51	Chronic carditis	HY-167	Male	34	Chronic carditis
HY-19	Female	32	Chronic carditis	HY-169	Female	48	IM
HY-21	Female	63	Chronic carditis	HY-171	Male	53	Chronic carditis
HY-23	Male	42	Chronic carditis	HY-173	Female	41	Chronic carditis
HY-25	Male	30	Chronic carditis	HY-175	Male	32	Chronic carditis
HY-29	Female	34	Chronic carditis	HY-177	Female	46	Chronic carditis
HY-31	Female	39	Chronic carditis	HY-181	Male	47	Chronic carditis
HY-33	Male	57	Chronic carditis	HY-183	Female	46	Chronic carditis
HY-35	Male	33	Chronic carditis	HY-187	Male	40	Chronic carditis
HY-37	Male	57	Chronic carditis	HY-189	Female	68	Normal tissue
HY-39	Male	53	Chronic carditis	HY-193	Female	47	Chronic carditis
HY-41	Male	35	Chronic carditis	HY-195	Male	38	Chronic carditis
HY-43	Male	53	Chronic carditis	HY-199	Male	52	IM
HY-45	Male	48	Chronic atrophic carditis	HY-201	Female	45	Chronic carditis
HY-47	Male	54	Chronic carditis	HY-203	Male	49	Chronic carditis
HY-49	Female	48	Chronic carditis	HY-205	Female	52	IM
HY-51	Male	56	IM	HY-207	Male	45	Chronic carditis
HY-53	Male	47	Chronic carditis	HY-209	Female	54	Chronic carditis
HY-55	Female	64	IM	HY-211	Female	45	Chronic carditis
HY-57	Male	29	Chronic atrophic carditis	HY-213	Female	55	Chronic carditis
HY-61	Male	31	Chronic carditis	HY-215	Female	59	Chronic carditis
HY-63	Female	69	Chronic carditis	HY-217	Male	60	Chronic carditis
HY-65	Male	26	Chronic carditis	HY-219	Female	24	Chronic atrophic carditis
HY-67	Female	45	Chronic carditis	HY-221	Male	60	Chronic atrophic carditis
HY-69	Female	25	Chronic carditis	HY-223	Female	48	Chronic carditis
HY_71	Male	47	Normal tissue	HY-225	Male	48	Chronic carditis
HY-73	Male	28	Chronic carditis	HY-227	Female	63	Chronic carditis
HY-75	Male	61	Chronic carditis	HY-229	Female	59	Chronic carditis
HV_77	Male	58	IM	HY-231	Female	50	Chronic carditis
HV_70	Male	10	Chronic carditis	HY-233	Male	37	Chronic carditis
HY-83	Male	58	IM	HY-237	Male	35	Chronic carditis
HY-85	Male	53	Chronic carditis	HY-239	Male	37	Chronic carditis
HV_87	Female	18	Chronic carditis	HY-241	Male	49	Chronic atrophic carditis
HV_80	Female	36	Chronic carditis	HY-243	Male	64	Normal tissue
HV_01	Male	46	Chronic carditis	HY-245	Male	28	Chronic carditis
-1V-03	Male	40 52	Chronic carditis	HV_247	Male	50	Chronic carditis
11-95 JV 05	Fomalo	56	Chronic carditis	HV-253	Male	52	IM
UV 07	Malo	15	Chronic carditis	HV_255	Female	11	Chronic carditis
	Malo	40	Chronic carditis	HV_257	Malo	47	Chronic carditis
UV 102	Male	50 62	Chronic carditis	LV 250	Fomalo	47	Chronic carditis
	Male	02	Chronic carditis	LV 262	Malo	43	Chronic carditis
	Male	38 55		LV 265	Malo	24	Chronic acrophic carditis
	Male	22	IIVI Chronic conditio		Fomolo	24	Chronic carditis
H Y- 109	Iviale	30	Chronic carditis		Female	24	Chronic carditis
HY-113	Female	20	Chronic carditis		Female	55	Chronic carditis
	Male	41	Chronic atrophic carditis		Female	57	Chronic carditis
HY-11/	Male	41	Chronic carditis	HI-2/5	Male	42	Chronic carditis
HY-IZI	Female	43	Chronic carditis	HI-2//	Iviale Familia	49	Chronic atrophic carditis
HY-123	Female	38	Chronic carditis		Female	50	
HT-12/	iviale	4/	Chronic atrophic carditis		Iviale	00	livi Obropis sauditi
HY-129	Male	49	Chronic carditis	HY-283	Female	46	Unronic carditis
HY-131	Male	62	Chronic atrophic carditis	HY-285	remale	44	
HY-133	Male	57	Chronic carditis	HY-287	Female	46	Chronic atrophic carditis
HY-137	Male	25	Chronic atrophic carditis	HY-289	Male	47	IM
-1Y-141	Male	51	Chronic carditis	HY-293	Male	58	IM
-143	Male	39	IM	HY-295	Male	55	Normal tissue
HY-145	Male	49	Chronic carditis	HY-297	Female	34	Chronic carditis
HY-147	Male	43	Chronic carditis	HY-299	Female	36	IM
HY-149	Female	61	Chronic carditis	HY-301	Male	52	IM

Supplementa	ry Table S2: C	ontd		Supplementa	ary Table S2:
Subject ID	Sex	Age	Pathological diagnosis	Subject ID	Sex
HY-303	Male	43	Chronic carditis	HY-469	Male
HY-305	Male	34	Chronic carditis	HY-473	Female
HY-307	Female	55	Normal tissue	HY-475	Male
HY-309	Female	48	IM	HY-477	Male
HY-313	Female	53	Chronic atrophic carditis	HY-481	Female
HY-315	Male	49	Chronic carditis	HY-483	Male
HY-319	Male	48	Chronic carditis	HY-485	Female
HY-321	Male	48	Chronic carditis	HY-489	Male
HY-323	Male	50	Chronic carditis	HY-491	Male
HY-325	Female	63		HY-493	Iviale
HY-327	Female	42	Normal tissue	HY-497	Female
HI-329	Male	41	Chronic carditis	H1-499	Fomolo
HV-333	Male	33 //1	Chronic carditis	HV-503	Male
HV_335	Male	22	Chronic carditis	HV-505	Female
HV_337	Female	66	Chronic carditis	HY-507	Male
HY-339	Female	41	IM	HY-509	Male
HY-341	Female	46	Chronic carditis	HY-511	Male
HY-343	Female	40	Chronic carditis	HY-513	Male
HY-345	Male	72	Chronic carditis	HY-517	Female
HY-347	Male	49	Chronic carditis	HY-519	Female
HY-349	Female	60	IM	HY-521	Female
HY-351	Male	54	Chronic carditis	HY-523	Male
HY-353	Male	49	Normal tissue	HY-525	Female
HY-355	Female	52	Chronic carditis	HY-527	Male
HY-361	Male	45	Chronic carditis	HY-529	Female
HY-365	Female	54	Chronic carditis	HY-531	Male
HY-367	Male	32	Chronic carditis	HY-537	Male
HY-369	Female	54	Chronic carditis	HY-539	Male
HY-371	Male	33	Chronic atrophic carditis	HY-543	Male
HY-373	Female	68	Normal tissue	HY-545	Male
HY-375	Male	50	Normal tissue	HY-547	Male
HY-377	Male	53	Chronic carditis	HY-549	Male
HY-3/9	Male	39	Chronic carditis	HY-551	Male
HY-3/9	Female	55	Chronic carditis	HY-553	Iviale
H I-383	Female	58	Chronic carditis	HY-55/	Female
H1-383	Female	7 I 5 6	Chronic atrophic carditis	HT-339	Female
HV_380	Malo	51	Chronic carditis	HV-563	Female
HV_301	Male	44	Chronic atrophic carditis	HV-565	Female
HY-303	Male	66	Normal tissue	HY-567	Male
HY-395	Male	56	Chronic atrophic carditis	HY-569	Female
HY-397	Male	58	Chronic carditis	HY-571	Male
HY-399	Male	47	Chronic atrophic carditis	HY-573	Male
HY-403	Female	49	Chronic carditis	HY-575	Male
HY-405	Female	54	Chronic atrophic carditis	HY-577	Female
HY-407	Female	52	Chronic atrophic carditis	HY-579	Female
HY-411	Male	52	Chronic carditis	HY-581	Female
HY-413	Female	41	Chronic carditis	HY-583	Male
HY-415	Male	40	Chronic carditis	HY-585	Male
HY-417	Male	49	Chronic atrophic carditis	HY-587	Female
HY-421	Female	42	Chronic carditis	HY-601	Female
HY-423	Male	29	Chronic carditis	HY-607	Male
HY-425	Male	51	Chronic atrophic carditis	HY-609	Female
HY-427	Male	51	IM	HY-611	Male
HY-429	Male	49	Chronic carditis	HY-613	Male
HY-431	Male	37	Chronic carditis	HY-615	Female
HY-433	Male	52	Chronic carditis	HY-617	Female
HY-437	Male	51	IM	HY-619	Male
HY-439	Male	51	Chronic carditis	HY-621	Male
HY-443	Female	42	Chronic carditis	HY-623	Male
HY-445	Male	49	Chronic carditis	HY-625	Female
HY-455	Male	40	Chronic carditis	HY-627	Female
HY-459	Female	49	Normal tissue	HY-629	Female
HY-463	Male	4/	Chronic carditis	HY-631	Male
H1-40/	Female	51	Unronic carditis	HY-033	iviale

-	-		-	-
C		Table	C 2.	Contd
SUDD	ешентат	/ lable	OZ.	Conta

Subject ID	Sex	Age	Pathological diagnosis
HY-469	Male	48	Chronic carditis
HY-473	Female	71	Chronic carditis
HY-475	Male	46	Chronic carditis
HY-477	Male	39	IM
HY-481	Female	43	Chronic carditis
HY-483	Male	33	Normal tissue
HY-485	Female	47	Chronic carditis
HT-489	Male	48 52	Chronic carditis
HY-493	Male	58	Chronic carditis
HY-497	Female	53	Chronic carditis
HY-499	Male	64	Chronic atrophic carditis
HY-501	Female	55	Chronic carditis
HY-503	Male	54	Chronic atrophic carditis
HY-505	Female	50	Chronic carditis
HY-507	Male	52	Chronic carditis
HY-509	Male	53	Chronic carditis
	Malo	51	Chronic caronics
HY-517	Female	62	Chronic carditis
HY-519	Female	59	Chronic carditis
HY-521	Female	47	Chronic carditis
HY-523	Male	38	Chronic carditis
HY-525	Female	57	Chronic atrophic carditis
HY-527	Male	46	Chronic carditis
HY-529	Female	51	Chronic atrophic carditis
HY-531	Male	54	Chronic carditis
HY-53/	Male	64 52	
HV-543	Male	52 50	IIVI Chronic carditis
HY-545	Male	59	Chronic carditis
HY-547	Male	51	Normal tissue
HY-549	Male	55	IM
HY-551	Male	38	Chronic carditis
HY-553	Male	38	Chronic atrophic carditis
HY-557	Female	64	Chronic carditis
HY-559	Female	61	Chronic carditis
HY-561	Female	52	Chronic atrophic carditis
HY-503	Female	30 54	Chronic carditis
HY-567	Male	46	Chronic carditis
HY-569	Female	47	Normal tissue
HY-571	Male	41	IM
HY-573	Male	40	Normal tissue
HY-575	Male	54	IM
HY-577	Female	58	Chronic carditis
HY-579	Female	45	Chronic carditis
HY-581	Female	59	Chronic carditis
HV-585	Male	36 36	Chronic carditis
HY-587	Female	37	Chronic carditis
HY-601	Female	49	Normal tissue
HY-607	Male	46	Chronic carditis
HY-609	Female	38	Chronic atrophic carditis
HY-611	Male	45	Chronic carditis
HY-613	Male	51	Chronic carditis
HY-615	Female	51	Chronic carditis
HY-61/	Female	40	Chronic carditis
HY-019	Male	58	IM Chronic corditio
HY-623	Male	48	Chronic atrophic carditie
HY-625	Female	62	Chronic carditis
HY-627	Female	48	Chronic carditis
HY-629	Female	44	Chronic atrophic carditis
HY-631	Male	76	Chronic carditis
HY-633	Male	49	Chronic atrophic carditis
			C 1

Supplementa	ry Table S2: C	Contd		Supplementa	ry lable S2: 0	Cont
Subject ID	Sex	Age	Pathological diagnosis	Subject ID	Sex	
HY-635	Female	61	Chronic atrophic carditis	HY-809	Male	
HY-637	Male	59	Chronic carditis	HY-811	Female	
HY-639	Male	46	Chronic carditis	HY-813	Female	
HY-641	Male	46	Chronic atrophic carditis	HY-815	Male	
HY-643	Female	46	Chronic carditis	HY-817	Female	
HY-645	Female	50	Chronic carditis	HY-819	Male	
HY-647	Female	27	Chronic carditis	HY-821	Male	
HY-649	Female	42	Chronic atrophic carditis	HY-823	Female	
HY-651	Female	60	Chronic atrophic carditis	HY-825	Male	
HY-653	Male	61	IM	HY-827	Male	
HY-655	Male	50	Chronic carditis	HY-829	Male	
HY-657	Female	45	Chronic carditis	HY-831	Female	
HY-659	Male	42	Chronic atrophic carditis	HY-833	Male	
HY-661	Female	66	Chronic carditis	HY-835	Male	
HY-663	Male	60	Chronic carditis	HY-837	Female	
HY-665	Female	72	Chronic carditis	HY-839	Female	
HY-667	Male	60	Chronic carditis	HY-841	Male	
HY-669	Female	52	Chronic carditis	HY-843	Male	
HY-671	Female	57	Chronic carditis	HY-845	Female	
HY-673	Female	49	Chronic carditis	HY-847	Male	
HY-675	Male	37	Chronic carditis	HY-849	Female	
HY-677	Male	35	Chronic carditis	HY-851	Male	
HY-679	Female	52	Chronic carditis	HY-853	Female	
HY-681	Male	48	Chronic carditis	HY-857	Female	
HY-683	Female	58	Chronic carditis	HY-859	Male	
HY-685	Female	55	Chronic carditis	HY-861	Male	
HY-687	Male	52	IM	HY-863	Male	
HY-691	Male	61	Chronic carditis	HY-865	Female	
HY-693	Female	50	Chronic carditis	HY-867	Female	
HY-695	Female	71	IM	HY-871	Male	
HY-697	Female	35	Chronic atrophic carditis	HY-873	Male	
HY-699	Male	47	Chronic atrophic carditis	HY-875	Female	
HY-734	Male	64	IM	HY-877	Male	
HY-736	Female	38	Chronic carditis	HY-879	Male	
HY-738	Male	58	Chronic carditis	HY-883	Male	
HY-740	Female	57	Chronic carditis	HY-885	Male	
HY-742	Female	55	Chronic carditis	HY-887	Female	
HY-744	Female	42	Chronic atrophic carditis	HY-889	Female	
HY-746	Female	41	Chronic carditis	HY-891	Female	
HY-749	Male	57	Chronic carditis	HY-893	Male	
HY-751	Female	47	Chronic carditis	HY-895	Male	
HY-753	Female	39	Normal tissue	HY-897	Female	
HY-755	Male	43	Chronic carditis	HY-901	Male	
HY-757	Female	51	Normal tissue	HY-903	Male	
HY-759	Female	48	Normal tissue	HY-905	Female	
HY-761	Female	53	Chronic carditis	HY-907	Female	
HY-765	Female	51	Chronic carditis	HY-909	Female	
HY-767	Female	56	Chronic carditis	HY-911	Female	
HY-769	Female	58	IM	HY-915	Female	
HY-771	Female	40	Chronic carditis	HY-917	Female	
HY-773	Female	57	IM	HY-919	Male	
HY-775	Male	58	Normal tissue	HY-921	Female	
HY-777	Male	65	IM	HY-923	Male	
HY-779	Female	45	Chronic carditis	HY-925	Female	
HY-781	Female	44	IM	HY-929	Male	
HY-783	Female	44	Chronic carditis	HY-933	Male	
HY-785	Female	40	Chronic carditis	HY-935	Male	
HY-787	Male	60	IM	HY-937	Female	
HY-791	Male	23	Chronic carditis	HY-939	Female	
HY-795	Male	57	Chronic carditis	HY-941	Male	
HY-797	Female	55	Chronic carditis	HY-943	Female	
HY-799	Female	49	Chronic carditis	HY-945	Male	
HY-801	Male	62	Chronic carditis	HY-947	Male	
HY-803	Female	49	Chronic carditis	HY-949	Female	
111/005	Famala	15	Normal tioqua		Mala	
HY-805	Female	40	Normal ussue	111-9.51	IVIAIE	

ject IDSexAgePathological diagnosis309Male28Chronic carditis311Female38Chronic carditis313Female38Chronic carditis314Male58IM317Female38Chronic carditis319Male46Chronic carditis321Male46Chronic carditis323Female35Chronic carditis324Male41Normal tissue325Male41Normal tissue326Male30Normal tissue327Male30Normal tissue333Male50Chronic carditis334Female30Normal tissue335Male52Normal tissue341Male52Normal tissue343Male42Chronic carditis344Female70Normal tissue351Male70Normal tissue353Female72IM364Female72IM374Female72IM385Female72IM386Female72IM387Female72IM388Female72IM389Female73Chronic carditis380Female74Chronic carditis381Male40Chronic carditis385Female38	plementary	Table S2: Con	td	
809Male28Chronic carditis811Female53Chronic carditis813Female53Chronic carditis814Male58IM815Male46Chronic carditis817Female35Chronic carditis818Male46Chronic carditis823Female35Chronic carditis824Male76Normal tissue825Male76Normal tissue829Male30Normal tissue833Male50Chronic carditis834Female30Normal tissue835Male42Chronic carditis836Female41Normal tissue837Female41Normal tissue838Female67Chronic carditis844Male52Normal tissue845Female43Chronic carditis846Female72IM857Female44Chronic carditis858Male34Chronic carditis859Male34Chronic carditis865Female38Chronic carditis877Female49Chronic carditis866Female38Chronic carditis873Male40Chronic carditis874Male53Chronic carditis875Female49Chronic carditis876Female<	ject ID	Sex	Age	Pathological diagnosis
811Female53Chronic carditis813Female38Chronic carditis815Male58IM817Female53Chronic carditis821Male46Chronic carditis822Male41Normal tissue823Female35Chronic carditis824Male76Normal tissue825Male39Normal tissue826Male39Normal tissue833Female30Normal tissue833Male50Chronic carditis833Male50Chronic carditis834Male52Normal tissue835Female41Normal tissue836Female67Chronic carditis837Female67Chronic carditis843Male58Chronic carditis844Male58Chronic carditis855Male34Chronic carditis856Female42Chronic carditis857Female44Chronic carditis865Female49Chronic carditis877Male40Chronic carditis863Male41Chronic carditis877Male52Chronic carditis878Male40Chronic carditis877Male52Chronic carditis878Male53Chronic carditis879M	309	Male	28	Chronic carditis
313Female38Chronic Carditis315Male53Chronic carditis319Male38IM321Male46Chronic carditis322Male41Normal tissue323Female35Chronic carditis324Male41Normal tissue327Male39Normal tissue328Male30Normal tissue329Male30Normal tissue331Female30Chronic carditis333Male42Chronic carditis334Female50Chronic carditis335Male42Chronic carditis344Male42Chronic carditis345Female67Chronic carditis346Female70Normal tissue353Female72IM354Female70Normal tissue355Male34Chronic carditis366Female44Chronic carditis375Female44Chronic carditis366Female49Chronic carditis371Male25Chronic carditis373Male40Chronic carditis374Male53Chronic carditis375Female53IM376Female54IM377Male38Chronic carditis378Male53Chronic carditi	311	Female	53	Chronic carditis
Ansatz Ansatz<	313	Female	38	Chronic carditis
AndChronic carditis319Male36IM321Male35Chronic carditis323Female35Chronic carditis324Male76Normal tissue325Male39Normal tissue326Male39Normal tissue327Male39Normal tissue333Male50Chronic carditis334Female30Normal tissue335Male38Chronic carditis336Female41Normal tissue337Female67Chronic carditis341Male52Normal tissue343Male42Chronic carditis344Male58Chronic carditis345Female67Chronic carditis346Female70Normal tissue357Female44Chronic carditis358Female44Chronic carditis359Male34Chronic carditis361Male40Chronic carditis363Female38Chronic carditis364Alae40Chronic carditis365Female38Chronic carditis371Male53Chronic carditis373Male43Chronic carditis374Male53Chronic carditis375Female53IM386Male53Chronic carditis </td <td>5 ID R 17</td> <td>Female</td> <td>53 53</td> <td>Chronic carditis</td>	5 ID R 17	Female	53 53	Chronic carditis
AndAndAnd221Male41Normal tissue2325Male41Normal tissue2327Male39Normal tissue239Male39Normal tissue231Female30Normal tissue333Male50Chronic carditis334Female41Normal tissue335Male52Normal tissue336Female41Normal tissue337Female41Normal tissue341Male52Normal tissue343Male42Chronic carditis344Male58Chronic carditis345Female67Chronic carditis346Female72IM357Female44Chronic carditis353Female72IM354Female43Chronic carditis355Female44Chronic carditis366Female49Chronic carditis367Female49Chronic carditis363Male41Chronic carditis371Male25Chronic carditis373Male40Chronic carditis374Female52Infonic carditis375Female39IM376Female53IM377Male52Chronic carditis378Male53Chronic carditis379<	R 19	Male	38	IM
323Female35Chronic carditis325Male41Normal tissue327Male39Normal tissue329Male39Normal tissue331Female30Normal tissue333Male50Chronic carditis334Male50Chronic carditis335Male41Normal tissue336Male42Chronic carditis337Female41Normal tissue343Male42Chronic carditis344Male58Chronic carditis345Female67Chronic carditis346Female72IM347Male58Chronic carditis348Male70Normal tissue353Female72IM354Female72IM355Female44Chronic carditis361Male41Chronic carditis363Male41Chronic carditis364Male40Chronic carditis365Female38Chronic carditis375Female39IM386Male53Chronic carditis377Male53IM383Male53Chronic carditis384Male53Chronic carditis385Male53IM386Male53IM387Female59	321	Male	46	Chronic carditis
325Male41Normal tissue327Male76Normal tissue329Male39Normal tissue331Female30Normal tissue333Male50Chronic carditis334Male38Chronic carditis335Male38Chronic carditis336Male42Chronic carditis337Female67Chronic carditis341Male52Normal tissue343Male42Chronic carditis344Male58Chronic carditis345Female43Chronic carditis346Female70Normal tissue353Female72IM354Female44Chronic carditis355Male34Chronic carditis366Female49Chronic carditis371Male40Chronic carditis365Female49Chronic carditis373Male40Chronic carditis374Male38Chronic carditis375Female39IM377Male38Chronic carditis388Male53Chronic carditis374Male52Normal tissue375Female52Chronic carditis387Female52Chronic carditis388Male53Chronic carditis397Female	323	Female	35	Chronic carditis
327Male76Normal tissue329Male39Normal tissue331Female30Normal tissue333Male50Chronic carditis334Male38Chronic carditis337Female41Normal tissue339Female30Chronic carditis341Male52Normal tissue343Male42Chronic carditis344Male58Chronic carditis345Female67Chronic carditis346Female72IM357Female44Chronic carditis358Female72IM357Female44Chronic carditis363Male41Chronic carditis364Male40Chronic carditis365Female49Chronic carditis366Female49Chronic carditis371Male25Chronic carditis373Male33Chronic carditis374Male33Chronic carditis375Female39IM383Male53Chronic carditis374Male52Normal tissue375Female53Chronic carditis386Male53Chronic carditis387Male52Normal tissue388Male53Chronic carditis389Female54C	325	Male	41	Normal tissue
229Male39Normal tissue331Female30Normal tissue333Male50Chronic carditis334Male38Chronic carditis337Female41Normal tissue339Female30Chronic carditis341Male52Normal tissue343Male42Chronic carditis344Male58Chronic carditis345Female67Chronic carditis346Female43Chronic carditis351Male70Normal tissue353Female72IM354Female72IM355Female44Chronic carditis366Female38Chronic carditis367Female49Chronic carditis368Chronic carditis36366Female3837Male4037Male2537Male4037Male37Male37Male37Male37Male37Male38Chronic carditis37Male38Chronic carditis37Male38Ghronic carditis37Male38Chronic carditis39Male39IM393Male394Female395Male	327	Male	76	Normal tissue
331Female30Normal tissue333Male50Chronic carditis334Male38Chronic carditis335Male31Normal tissue336Female41Normal tissue337Female67Chronic carditis341Male52Normal tissue343Male42Chronic carditis344Male58Chronic carditis345Female43Chronic carditis351Male70Normal tissue353Female72IM354Female44Chronic carditis355Female34Chronic carditis361Male40Chronic carditis363Male44Chronic carditis364Male49Chronic carditis365Female38Chronic carditis373Male49Chronic carditis374Male25Chronic carditis375Female39IM376Female39IM387Female53IM388Male53Chronic carditis387Female53IM388Male53Chronic carditis387Female52Normal tissue388Male53Chronic carditis389Female52Normal tissue380Male73Chronic carditis<	329	Male	39	Normal tissue
335Male30Chronic carditis335Male38Chronic carditis337Female30Chronic carditis339Female30Chronic carditis341Male52Normal tissue343Male42Chronic carditis344Male52Normal tissue345Female43Chronic carditis347Male70Normal tissue348Female72IM357Female72IM358Female72IM359Male34Chronic carditis361Male40Chronic carditis363Female49Chronic carditis364Female49Chronic carditis365Female49Chronic carditis366Female49Chronic carditis371Male25Chronic carditis373Male53Chronic carditis374Male38Chronic carditis375Female39IM386Male53Chronic carditis387Female53IM388Male53Chronic carditis389Female52Normal tissue391Female52Normal tissue392Male53Chronic carditis393Male73Chronic carditis394Female53Chronic carditis <td>331</td> <td>Female</td> <td>30 50</td> <td>Normal tissue</td>	331	Female	30 50	Normal tissue
ActionActionAction337Female41Normal tissue339Female30Chronic carditis341Male52Normal tissue343Male42Chronic carditis344Female67Chronic carditis347Male58Chronic carditis349Female43Chronic carditis351Male70Normal tissue353Female72IM354Female44Chronic carditis355Female44Chronic carditis361Male40Chronic carditis363Male41Chronic carditis364Male40Chronic carditis365Female38Chronic carditis366Female49Chronic carditis371Male25Chronic carditis373Male40Chronic carditis374Male57IM385Male53Chronic carditis377Male57IM388Female52Chronic carditis389Female52Chronic carditis389Female52Chronic carditis391Female54IM383Male73Chronic carditis3845Male53Chronic carditis385Male53Chronic carditis386Female59Chronic carditis	335	Male	38	Chronic carditis
339Female30Chronic carditis341Male52Normal tissue343Male52Normic carditis344Female67Chronic carditis345Female43Chronic carditis346Female43Chronic carditis351Male72IM355Female72IM356Female72IM357Female44Chronic carditis363Male41Chronic carditis364Male41Chronic carditis365Female38Chronic carditis366Female49Chronic carditis371Male40Chronic carditis373Male40Chronic carditis374Male38Chronic carditis375Female39IM377Male38Chronic carditis385Male53Chronic carditis386Male53Chronic carditis387Female52Chronic carditis388Female52Chronic carditis397Female59Chronic carditis397Female59Chronic carditis398Female59Chronic carditis397Female59Chronic carditis397Female50Chronic carditis397Female50Chronic carditis398Female<	337	Female	41	Normal tissue
841Male52Normal tissue843Male42Chronic carditis844Female67Chronic carditis847Male58Chronic carditis849Female43Chronic carditis851Male70Normal tissue853Female72IM856Female72IM857Female44Chronic carditis863Male34Chronic carditis863Male40Chronic carditis863Male49Chronic carditis864Female38Chronic carditis873Male25Chronic carditis874Male25Chronic carditis875Female39IM877Male38Chronic carditis879Male53Chronic carditis885Male53Chronic carditis886Female52Chronic carditis887Female53IM888Female52Normal tissue897Female54IM893Male73Chronic carditis894Female55Normal tissue897Female67Chronic carditis893Male52Normal tissue894Female55Normal tissue897Female67Chronic carditis898Female52Normal tissue </td <td>339</td> <td>Female</td> <td>30</td> <td>Chronic carditis</td>	339	Female	30	Chronic carditis
343Male42Chronic carditis345Female67Chronic carditis347Male58Chronic carditis349Female43Chronic carditis351Male70Normal tissue353Female72IM357Female44Chronic carditis359Male34Chronic carditis361Male40Chronic carditis363Male41Chronic carditis364Female49Chronic carditis365Female49Chronic carditis367Female49Chronic carditis373Male25Chronic carditis374Male38Chronic carditis375Female39IM377Male38Chronic carditis387Male53Chronic carditis388Male53Chronic carditis389Female52Chronic carditis381Male53Chronic carditis382Male53Chronic carditis383Male53Chronic carditis384Female54IM385Male52Normal tissue386Female59Chronic carditis387Female50Chronic carditis388Female53Chronic carditis391Female54Chronic carditis392Female </td <td>341</td> <td>Male</td> <td>52</td> <td>Normal tissue</td>	341	Male	52	Normal tissue
845Female67Chronic carditis847Male58Chronic carditis849Female43Chronic carditis851Male70Normal tissue853Female72IM854Chronic carditis859855Male34Chronic carditis856Male40Chronic carditis863Male41Chronic carditis864Male40Chronic carditis865Female49Chronic carditis866Female49Chronic carditis877Male25Chronic carditis873Male40Chronic carditis874Female39IM875Female39IM877Male38Chronic carditis879Male53Chronic carditis885Male53Chronic carditis886Male53Chronic carditis887Female52Chronic carditis889Female52Normal tissue897Female59Chronic carditis893Male73Chronic carditis894Male73Chronic carditis895Male52Normal tissue896Female54Chronic carditis897Female55Normal tissue996Female67Chronic carditis997Female56IM	343	Male	42	Chronic carditis
344Male58Chronic carditis3849Female43Chronic carditis3851Male70Normal tissue3853Female72IM3857Female44Chronic carditis3861Male34Chronic carditis3861Male41Chronic carditis3863Male41Chronic carditis3864Female49Chronic carditis3865Female49Chronic carditis3873Male40Chronic carditis3873Male40Chronic carditis3874Female39IM3875Female39IM3876Female39IM3883Male53Chronic carditis387Female53IM3885Male53Chronic carditis387Female54IM3889Female52Normal tissue3971Female56IM3885Male52Normal tissue3973Male52Normal tissue3974Female52Normal tissue3975Female59Chronic carditis396Male35Chronic carditis397Female59Chronic carditis396Male33Chronic carditis397Female57Normal tissue397Female53Chronic carditis <td>345</td> <td>Female</td> <td>67</td> <td>Chronic carditis</td>	345	Female	67	Chronic carditis
449Female4.3Chronic carditis351Male70Normal tissue353Female72IM354Male34Chronic carditis355Female34Chronic carditis361Male41Chronic carditis363Male41Chronic carditis364Female38Chronic carditis365Female49Chronic carditis366Female49Chronic carditis371Male25Chronic carditis373Male40Chronic carditis374Male38Chronic carditis375Female39IM376Female39IM377Male38Chronic carditis378Male53Chronic carditis379Male53Chronic carditis386Male53Chronic carditis387Female54IM388Male53Chronic carditis393Male52Normal tissue394Female59Chronic carditis395Male52Normal tissue396Female46Chronic carditis397Female54Chronic carditis398Male53Chronic carditis397Female55Normal tissue398Male53Chronic carditis397Female55Nor	347	Male	58	Chronic carditis
353Female70Normal tissue353Female72IM357Female44Chronic carditis359Male34Chronic carditis361Male41Chronic carditis363Male41Chronic carditis3645Female38Chronic carditis365Female49Chronic carditis367Female49Chronic carditis373Male40Chronic carditis374Male25Chronic carditis375Female39IM387Male57IM388Male53Chronic carditis379Male53Chronic carditis385Male53Chronic carditis386Female52Chronic carditis387Female52Normal tissue388Female52Normal tissue391Female56IM393Male73Chronic carditis394Female52Normal tissue395Male35Chronic carditis396Female67Chronic carditis397Female67Chronic carditis396Female67Chronic carditis397Female53Chronic carditis396Female67Chronic carditis397Female53Chronic carditis398Female69 </td <td>349</td> <td>Female</td> <td>43 70</td> <td>Unronic carditis</td>	349	Female	43 70	Unronic carditis
ActionFemale72Int357Female44Chronic carditis359Male34Chronic carditis361Male40Chronic carditis363Male41Chronic carditis364Female49Chronic carditis365Female49Chronic carditis367Female49Chronic carditis371Male25Chronic carditis373Male40Chronic carditis374Male38Chronic carditis375Female39IM387Male33Chronic carditis388Male53Chronic carditis389Female53IM389Female54IM389Female56IM389Female52Chronic carditis391Female56IM393Male73Chronic carditis394Aale35Chronic carditis395Male49Chronic carditis396Female67Chronic carditis397Female64Chronic carditis398Female53Chronic carditis399Female53Chronic carditis390Female67Chronic carditis391Female53Chronic carditis392Female55Normal tissue393Male33Normal tiss	353	Female	70	IM
359Male34Chronic carditis361Male40Chronic carditis363Male41Chronic carditis365Female38Chronic carditis367Female49Chronic carditis371Male25Chronic carditis373Male40Chronic carditis374Male38Chronic carditis375Female39IM377Male38Chronic carditis383Male53Chronic carditis3845Male53Chronic carditis385Male53IM386Female52Chronic carditis387Female53IM388Male73Chronic carditis397Female52Normal tissue397Female59Chronic carditis393Male35Chronic carditis394Male35Chronic carditis395Male35Chronic carditis396Female67Chronic carditis397Female69Chronic carditis396Female53Chronic carditis397Female55Normal tissue397Female55Normal tissue397Female55Normal tissue397Female56IM398Male45Chronic carditis399Female51N	357	Female	44	Chronic carditis
361Male40Chronic carditis363Male41Chronic carditis364Female38Chronic carditis367Female49Chronic carditis371Male25Chronic carditis373Male40Chronic carditis374Male39IM375Female39IM377Male38Chronic carditis383Male33Chronic carditis384Male53Chronic carditis385Male53Chronic carditis386Female53IM387Female52Chronic carditis388Female52Normal tissue397Female52Normal tissue393Male73Chronic carditis394Female52Normal tissue395Male35Chronic carditis396Female67Chronic carditis397Female67Chronic carditis396Female53Chronic carditis397Female67Chronic carditis396Female53Chronic carditis397Female54Chronic carditis396Female55Normal tissue397Female67Chronic carditis396Female55Normal tissue397Female56IM398Female56<	359	Male	34	Chronic carditis
363Male41Chronic carditis365Female38Chronic carditis367Female49Chronic carditis371Male25Chronic carditis373Male40Chronic carditis374Male39IM375Female39IM377Male38Chronic carditis379Male57IM383Male53Chronic carditis384Female53IM385Male53Chronic carditis386Female52Chronic carditis387Female56IM389Female52Normal tissue391Female56IM393Male73Chronic carditis395Male52Normal tissue396Male35Chronic carditis397Female67Chronic carditis390Male35Chronic carditis391Female67Chronic carditis392Male33Normal tissue393Male33Normal tissue394Female55Normal tissue395Female67Chronic carditis396Female55Normal tissue397Female56IM398Semale55Normal tissue399Male36Normal tissue391Female <td>361</td> <td>Male</td> <td>40</td> <td>Chronic carditis</td>	361	Male	40	Chronic carditis
365Female38Chronic carditis367Female49Chronic carditis371Male25Chronic carditis373Male40Chronic carditis374Male39IM375Female39IM376Male38Chronic carditis377Male38Chronic carditis379Male57IM383Male53Chronic carditis385Male53Chronic carditis386Female52Chronic carditis387Female56IM389Female52Normal tissue391Female59Chronic carditis395Male73Chronic carditis396Male35Chronic carditis397Female59Chronic carditis396Male35Chronic carditis397Female67Chronic carditis396Male35Chronic carditis397Female67Chronic carditis390Female67Chronic carditis391Female53Chronic carditis392Male35Chronic carditis393Male46Chronic carditis394Female53Chronic carditis395Male33Normal tissue396Female55Normal tissue397Female56 <td< td=""><td>363</td><td>Male</td><td>41</td><td>Chronic carditis</td></td<>	363	Male	41	Chronic carditis
367Female49Chronic carditis371Male25Chronic carditis373Male40Chronic carditis375Female39IM377Male38Chronic carditis379Male57IM383Male33Chronic carditis385Male53Chronic carditis386Male53Chronic carditis387Female52Chronic carditis389Female52Chronic carditis391Female56IM393Male73Chronic carditis395Male52Normal tissue397Female59Chronic carditis393Male35Chronic carditis394Pemale49Chronic carditis395Male35Chronic carditis396Female67Chronic carditis397Female67Chronic carditis396Female67Chronic carditis397Female67Chronic carditis390Female41Chronic carditis391Female53Chronic carditis392Female55Normal tissue393Male33Normal tissue394Female31Chronic carditis395Male56IM396Female51Chronic carditis397Female51 <td>365</td> <td>Female</td> <td>38</td> <td>Chronic carditis</td>	365	Female	38	Chronic carditis
371Male25Chronic carditis373Male40Chronic carditis375Female39IM377Male38Chronic carditis379Male57IM383Male53Chronic carditis385Male53Chronic carditis387Female53IM389Female52Chronic carditis391Female56IM393Male73Chronic carditis394Female59Chronic carditis395Male52Normal tissue396Female59Chronic carditis397Female59Chronic carditis396Male35Chronic carditis397Female67Chronic carditis396Female67Chronic carditis397Female67Chronic carditis390Male35Chronic carditis391Female67Chronic carditis392Female67Chronic carditis393Male55Normal tissue394Female51Chronic carditis395Female89Chronic carditis396Female55Normal tissue397Female55Normal tissue398Male56IM399Female51Chronic carditis391Female51Chronic ca	367	Female	49	Chronic carditis
And the second	3/1	Male	25	Chronic carditis
AllAllAllAll377Male38Chronic carditis379Male57IM383Male33Chronic carditis385Male53Chronic carditis387Female53IM389Female52Chronic carditis391Female56IM393Male73Chronic carditis394Male52Normal tissue395Male52Normal tissue396Female59Chronic carditis397Female59Chronic carditis393Male35Chronic carditis394Male49Chronic carditis395Male35Chronic carditis396Female67Chronic carditis397Female67Chronic carditis393Male35Chronic carditis394Female53Chronic carditis395Female67Chronic carditis396Female53Chronic carditis397Female53Chronic carditis398Female55Normal tissue399Female56IM391Female56IM392Male56IM393Male56IM394Semale50Chronic carditis395Male50Chronic carditis396Female </td <td>875</td> <td>Female</td> <td>30</td> <td>IM</td>	875	Female	30	IM
379Male57IM383Male33Chronic carditis385Male53Chronic carditis387Female53IM389Female52Chronic carditis391Female56IM393Male73Chronic carditis395Male52Normal tissue397Female59Chronic carditis396Male49Chronic carditis397Female59Chronic carditis393Male35Chronic carditis394Male35Chronic carditis395Male35Chronic carditis396Male35Chronic carditis397Female67Chronic carditis203Male35Chronic carditis204Female67Chronic carditis205Female69Chronic carditis206Female55Normal tissue207Female55Normal tissue208Chronic carditis209209Male36Normal tissue211Female51Chronic carditis222Female59Chronic carditis223Male56IM233Male59Chronic carditis241Male50Chronic carditis255Male50Chronic carditis243Female51Normal tissue	377	Male	38	Chronic carditis
383Male33Chronic carditis385Male53Chronic carditis387Female53IM389Female52Chronic carditis391Female56IM393Male73Chronic carditis395Male52Normal tissue397Female59Chronic carditis393Male35Chronic carditis394Male35Chronic carditis395Male35Chronic carditis396Male35Chronic carditis397Female67Chronic carditis393Male35Chronic carditis394Female67Chronic carditis395Female67Chronic carditis396Female46Chronic carditis397Female53Chronic carditis398Female53Chronic carditis399Female55Normal tissue391Female31Chronic carditis392Male36Normal tissue393Male56IM394Female51Chronic carditis395Male59Chronic carditis396Female51Normal tissue397Female51Normal tissue398Male50Chronic carditis399Female51Normal tissue393Male50<	379	Male	57	IM
385Male53Chronic carditis387Female53IM389Female52Chronic carditis391Female56IM393Male73Chronic carditis395Male52Normal tissue397Female59Chronic carditis393Male49Chronic carditis394Male49Chronic carditis395Male35Chronic carditis396Female67Chronic carditis397Female67Chronic carditis393Male35Chronic carditis394Female67Chronic carditis395Female67Chronic carditis396Female46Chronic carditis397Female53Chronic carditis390Female53Chronic carditis391Female53Chronic carditis392Pemale55Normal tissue393Male33Normal tissue394Male36Normal tissue395Male59Chronic carditis396Female51Chronic carditis397Female51Normal tissue398Male50Chronic carditis399Female51Normal tissue393Male50Chronic carditis394Female51Normal tissue395Mal	383	Male	33	Chronic carditis
387Female53IM389Female52Chronic carditis391Female56IM393Male73Chronic carditis395Male52Normal tissue397Female59Chronic carditis393Male35Chronic carditis394Male35Chronic carditis395Male35Chronic carditis396Female67Chronic carditis397Female67Chronic carditis393Male35Chronic carditis394Semale41Chronic carditis395Female46Chronic carditis396Female53Chronic carditis397Female53Chronic carditis399Female53Chronic carditis391Female53Chronic carditis392Pemale55Normal tissue393Male33Normal tissue394Male36Normal tissue395Male56IM393Male56IM394Female51Chronic carditis395Male50Chronic carditis396Female51Normal tissue397Female51Normal tissue398Female50Chronic carditis399Female51Normal tissue393Male50Chron	385	Male	53	Chronic carditis
389Female52Chronic carditis391Female56IM393Male73Chronic carditis395Male52Normal tissue397Female59Chronic carditis390Male49Chronic carditis391Male35Chronic carditis392Male35Chronic carditis393Male35Chronic carditis394Semale67Chronic carditis395Female46Chronic carditis396Female41Chronic carditis397Female53Chronic carditis397Female53Chronic carditis399Female53Chronic carditis391Female55Normal tissue391Female31Chronic carditis391Female31Chronic carditis392Male36Normal tissue393Male56IM393Male56IM393Male59Chronic carditis393Male50Chronic carditis393Male50Chronic carditis393Male50Chronic carditis393Male50Chronic carditis394Female51Normal tissue395Male50Chronic carditis394Female50Chronic carditis395Male5	387	Female	53	IM
And BaysMale50Inv393Male73Chronic carditis395Male52Normal tissue397Female59Chronic carditis3901Male49Chronic carditis203Male35Chronic carditis203Male35Chronic carditis204Semale67Chronic carditis205Female67Chronic carditis206Female46Chronic carditis207Female46Chronic carditis209Female41Chronic carditis201Female53Chronic carditis201Female55Normal tissue201Female31Chronic carditis202Male36Normal tissue203Male45Chronic carditis223Male36Normal tissue224Female51Chronic carditis233Male56IM243Female51Chronic carditis241Male50Chronic carditis243Female40Normal tissue244Male50Chronic carditis245Male50Chronic carditis246Male50Chronic carditis247Male43Chronic carditis249Female35Chronic carditis244Female35Chronic carditis245<	389	Female	52	Chronic carditis
AllFormat issue395Male52Normal tissue397Female59Chronic carditis397Female59Chronic carditis203Male35Chronic carditis203Male35Chronic carditis204Female67Chronic carditis205Female46Chronic carditis206Female41Chronic carditis207Female46Chronic carditis209Female41Chronic carditis201Female53Chronic carditis201Female55Normal tissue201Female31Chronic carditis202Male36Normal tissue203Male45Chronic carditis203Male56IM203Male56IM203Male56IM203Male50Chronic carditis204Male50Chronic carditis205Female51Normal tissue204Male50Chronic carditis205Male50Chronic carditis206Male50Chronic carditis207Female51Normal tissue208Female50Chronic carditis209Female50Chronic carditis209Female50Chronic carditis209Female50Chronic carditis<	303	Male	73	Chronic carditis
397Female59Chronic carditis201Male49Chronic carditis203Male35Chronic carditis205Female67Chronic carditis207Female46Chronic carditis209Female41Chronic carditis201Female53Chronic carditis205Female69Chronic carditis206Female53Chronic carditis207Female53Chronic carditis209Female53Chronic carditis201Female55Normal tissue201Female31Chronic carditis202Female28Chronic carditis203Male45Chronic carditis204Male36Normal tissue205Female51Chronic carditis205Male56IM205Male59Chronic carditis203Male56IM203Male50Chronic carditis204Male50Chronic carditis205Male50Chronic carditis204Male50Chronic carditis205Male50Chronic carditis206Male50Chronic carditis207Female35Chronic carditis208Female40Normal tissue209Female50Chronic carditis209 </td <td>395</td> <td>Male</td> <td>52</td> <td>Normal tissue</td>	395	Male	52	Normal tissue
201Male49Chronic carditis203Male35Chronic carditis205Female67Chronic carditis207Female46Chronic atrophic carditis209Female41Chronic carditis209Female41Chronic carditis201Female53Chronic carditis2015Female69Chronic carditis2017Female55Normal tissue2019Male33Normal tissue2021Female31Chronic carditis203Male45Chronic carditis203Male36Normal tissue203Male56IM203Male59Chronic carditis203Male50Chronic carditis203Male50Chronic carditis203Male50Chronic carditis2041Male50Chronic carditis2055Male50Chronic carditis2041Male50Chronic carditis2043Female35Chronic carditis2044Male50Chronic carditis2045Male50Chronic carditis2047Male43Chronic carditis2049Female35Chronic carditis2041Male62Chronic carditis2042Female35Chronic carditis2043Female43Chronic c	397	Female	59	Chronic carditis
203Male35Chronic carditis205Female67Chronic carditis207Female46Chronic atrophic carditis209Female41Chronic atrophic carditis209Female41Chronic atrophic carditis201Female53Chronic atrophic carditis205Female69Chronic carditis207Female55Normal tissue207Female31Chronic carditis207Female31Chronic carditis208Male45Chronic carditis209Male36Normal tissue203Male56IM203Male56IM203Male50Chronic carditis203Male50Chronic carditis203Male50Chronic carditis203Male50Chronic carditis203Male50Chronic carditis203Female51Normal tissue2041Male50Chronic carditis203Female40Normal tissue2041Male50Chronic carditis2045Male50Chronic carditis2047Male43Chronic carditis2049Female35Chronic carditis2041Male62Chronic carditis2042Female35Chronic carditis2043Female43Ch	901	Male	49	Chronic carditis
205Female67Chronic carditis207Female46Chronic atrophic carditis209Female41Chronic atrophic carditis201Female53Chronic atrophic carditis205Female69Chronic carditis207Female55Normal tissue207Female33Normal tissue208Male33Normal tissue209Male36Chronic carditis203Male45Chronic carditis203Male36Normal tissue203Male56IM203Male59Chronic carditis203Male56IM203Male50Chronic carditis203Male50Chronic carditis2041Male50Chronic carditis203Female51Normal tissue2041Male50Chronic carditis2043Female40Normal tissue2044Male50Chronic carditis2045Male50Chronic carditis2047Male43Chronic carditis2049Female35Chronic carditis2041Male62Chronic carditis2042Female35Chronic carditis2043Female43Chronic carditis2044SSChronic carditis2045Male43Chronic carditis	903	Male	35	Chronic carditis
207Female46Chronic atrophic carditis209Female41Chronic carditis209Female53Chronic atrophic carditis201Female53Chronic carditis2017Female55Normal tissue2019Male33Normal tissue2021Female31Chronic carditis2023Male45Chronic carditis2023Male45Chronic carditis203Male36Normal tissue203Male56IM203Male56IM203Male50Chronic carditis203Female51Chronic carditis203Female51Chronic carditis203Female50Chronic carditis2041Male50Chronic carditis2043Female40Normal tissue2044Male50Chronic carditis2045Male50Chronic carditis2047Male43Chronic carditis2049Female35Chronic carditis2041Male62Chronic carditis2042Female35Chronic carditis2043Female43Chronic carditis2044Female43Chronic carditis2045Male42Chronic carditis	905	Female	67	Chronic carditis
2009Female41Chronic carditis2011Female53Chronic atrophic carditis2015Female69Chronic carditis2017Female55Normal tissue2019Male33Normal tissue2021Female31Chronic carditis2023Male45Chronic carditis2023Male28Chronic carditis2029Male36Normal tissue2033Male56IM2045Male59Chronic carditis2037Female51Chronic carditis2039Female51Normal tissue2041Male50Chronic carditis2043Female51Normal tissue2044Male50Chronic carditis2045Male50Chronic carditis2047Male43Chronic carditis2049Female35Chronic carditis2049Female35Chronic carditis2049Female42Chronic carditis	<i>407</i>	Female	40	Chronic atrophic carditis
P15Female60Chronic carditisP15Female69Chronic carditisP17Female55Normal tissueP19Male33Normal tissueP21Female31Chronic carditisP23Male45Chronic carditisP24Female28Chronic carditisP25Female28Chronic carditisP29Male36Normal tissueP33Male56IMP35Male59Chronic carditisP37Female51Chronic carditisP39Female51Normal tissueP41Male50Chronic carditisP43Female40Normal tissueP45Male50Chronic carditisP47Male43Chronic carditisP49Female35Chronic carditisP49Female35Chronic carditisP49Female42Chronic carditis	909 911	Female	41 53	Chronic atrophic carditis
P17Female55Normal tissueP19Male33Normal tissueP21Female31Chronic carditisP23Male45Chronic carditisP25Female28Chronic carditisP29Male36Normal tissueP33Male56IMP35Male59Chronic carditisP37Female51Chronic carditisP39Female51Normal tissueP41Male50Chronic carditisP43Female40Normal tissueP45Male50Chronic carditisP47Male50Chronic carditisP49Female35Chronic carditisP49Female35Chronic carditisP49Female42Chronic carditis	915	Female	69	Chronic carditis
P19Male33Normal tissueP21Female31Chronic carditisP23Male45Chronic carditisP25Female28Chronic carditisP29Male36Normal tissueP33Male56IMP35Male59Chronic carditisP37Female51Chronic carditisP39Female51Normal tissueP41Male50Chronic carditisP43Female40Normal tissueP45Male50Chronic carditisP47Male43Chronic carditisP49Female35Chronic carditisP49Female42Chronic carditis	917	Female	55	Normal tissue
P21Female31Chronic carditisP23Male45Chronic carditisP25Female28Chronic carditisP29Male36Normal tissueP33Male56IMP35Male59Chronic carditisP37Female51Chronic carditisP39Female51Normal tissueP41Male50Chronic carditisP43Female40Normal tissueP44Male50Chronic carditisP45Male50Chronic carditisP47Male43Chronic carditisP49Female35Chronic carditisP49Female42Chronic carditis	919	Male	33	Normal tissue
923Male45Chronic carditis925Female28Chronic carditis929Male36Normal tissue933Male56IM935Male59Chronic carditis937Female51Chronic carditis939Female51Normal tissue941Male50Chronic carditis943Female40Normal tissue944Male50Chronic carditis945Male50Chronic carditis947Male50Chronic carditis947Male50Chronic carditis949Female35Chronic carditis949Female42Chronic carditis	921	Female	31	Chronic carditis
925Female28Chronic carditis929Male36Normal tissue933Male56IM935Male59Chronic carditis937Female51Chronic carditis939Female51Normal tissue941Male50Chronic carditis943Female40Normal tissue945Male50Chronic carditis945Male50Chronic carditis947Male50Chronic carditis949Female35Chronic carditis949Female35Chronic carditis951Male42Chronic carditis	923	Male	45	Chronic carditis
229Male36Normal tissue233Male56IM235Male59Chronic carditis237Female51Chronic carditis239Female51Normal tissue241Male50Chronic carditis243Female40Normal tissue245Male50Chronic carditis247Male50Chronic carditis249Female35Chronic carditis247Male43Chronic carditis249Female35Chronic carditis255Male42Chronic carditis	925	Female	28	Chronic carditis
AllSoInf935Male59Chronic carditis937Female51Chronic carditis939Female51Normal tissue941Male50Chronic carditis943Female40Normal tissue945Male50Chronic carditis947Male43Chronic carditis949Female35Chronic carditis949Female35Chronic carditis951Male62Chronic carditis955Male42Chronic carditis	729 D33	Male	30 56	IM
237Female51Chronic carditis239Female51Normal tissue241Male50Chronic carditis243Female40Normal tissue245Male50Chronic carditis247Male43Chronic carditis249Female35Chronic carditis251Male62Chronic carditis255Male42Chronic carditis	935	Male	59	Chronic carditis
P39Female51Normal tissueP41Male50Chronic carditisP43Female40Normal tissueP45Male50Chronic carditisP47Male43Chronic carditisP49Female35Chronic carditisP41Male62Chronic carditisP43Chronic carditisChronic carditisP44Male42Chronic carditis	937	Female	51	Chronic carditis
941Male50Chronic carditis943Female40Normal tissue945Male50Chronic carditis947Male43Chronic carditis949Female35Chronic carditis951Male62Chronic carditis955Male42Chronic carditis	939	Female	51	Normal tissue
P43Female40Normal tissueP45Male50Chronic carditisP47Male43Chronic carditisP49Female35Chronic carditisP51Male62Chronic carditisP55Male42Chronic carditis	941	Male	50	Chronic carditis
Wale50Chronic carditisWale43Chronic carditisWale43Chronic carditisWale35Chronic carditisWale62Chronic carditisWale42Chronic carditis	943	Female	40	Normal tissue
var male 4.3 Chronic carolitis 949 Female 35 Chronic carditis 951 Male 62 Chronic carditis 955 Male 42 Chronic carditis	945	Male	50	Chronic carditis
251 Male 62 Chronic carditis 255 Male 42 Chronic carditis	747 D/0	Female	40 35	Chronic carditis
955 Male 42 Chronic carditis	951	Male	62	Chronic carditis
	955	Male	42	Chronic carditis

Supplementary Table S2: Contd				Supplementa	ry Table S2: C	Contd
Subject ID	Sex	Age	Pathological diagnosis	Subject ID	Sex	Age
	Fomalo	20	Chronic carditic	C14	Mala	62
111-937	Mala	29	Chronic carditis	044	Male	62
	Iviale	80	Chronic carditis	045	Iviale	07
HY-903	Female	04	Chronic carditis	647	Male	60
HY-967	Female	31	Chronic atrophic carditis	C49	Male	54
HY-969	Female	63	Chronic carditis	C5	Male	72
HY-971	Female	52	IM	C50	Male	74
HY-973	Female	47	Chronic carditis	C6	Male	63
HY-975	Male	55	Chronic carditis	C7	Male	66
HY-977	Male	48	Chronic carditis	C8	Female	44
HY-979	Female	58	Chronic carditis	S1	Male	46
HY-981	Female	46	Chronic carditis	S 10	Male	34
HY-985	Male	36	Chronic atrophic carditis	S 100	Male	62
HY-987	Female	43	Chronic carditis	S101	Male	51
HY-989	Female	33	Chronic carditis	S102	Female	57
HY_001	Female	57	Chronic carditis	\$103	Female	54
	Fomalo	61	Chronic carditis	S 105	Malo	12
П1-993 UV 005	Female	50	Chronic carditis	3103	Iviale Family	42
	Female	50		5100	Female	55
HY-997	Female	49	Chronic carditis	S 107	Female	42
HY-999	Female	51	Normal tissue	S 108	Male	57
HY-1001	Female	20	Chronic carditis	S 109	Male	52
HY-1003	Female	48	Chronic carditis	S11	Female	55
HY-1005	Male	55	Chronic carditis	S110	Female	43
HY-1007	Female	53	Normal tissue	S111	Female	41
HY-1009	Female	64	Chronic carditis	S112	Male	54
HY-1013	Male	56	IM	S113	Female	55
HY-1015	Male	58	IM	S11/	Male	61
HV_1017	Malo	67	Chronic carditis	S 1 14 S 1 15	Male	46
	Male	60	Chronic carditis	S 1 15	Famala	40
	Famala	60	Chronic caruttis	0117	Female	57
HY-1023	Female	09	Chronic atrophic carditis	511/	Female	50
\$323	Female	53	Chronic carditis	S118	Male	51
\$324	Female	64	IM	S119	Female	50
S325	Female	51	Chronic carditis	S 12	Female	31
S326	Female	46	Chronic carditis	S120	Female	63
S327	Female	58	Chronic carditis	S121	Female	61
S328	Female	44	Chronic carditis	S122	Female	56
S329	Male	60	IM	S123	Female	50
S414	Male	34	Chronic carditis	S124	Female	47
S415	Female	53	Chronic carditis	S125	Male	56
S416	Female	20	Chronic carditis	S126	Male	54
S417	Female	60	Chronic carditis	S127	Male	40
\$418	Female	58	IM	\$128	Male	40
S/10	Male	30	IM	S 120	Male	30
\$420	Malo	46	IM	S 12 9	Fomolo	27
5420	Male	40	livi Chronic corditic	5150	Feilidie	57
5421	Male	27	Chronic carditis	0100	Iviale	53
3422	wale	32		S 13Z	Iviale	35
8423	Male	52	IM	\$133	Male	51
S424	Male	4/	IM	S 134	Male	48
C1	Female	71	IM	S 135	Female	48
C 10	Male	36	Chronic atrophic carditis	S 136	Female	55
C11	Male	54	IM	S 137	Female	51
C12	Male	46	IM	S 138	Male	53
C 13	Male	58	Chronic carditis	S 139	Male	37
C 15	Female	25	Chronic carditis	S140	Female	56
C 16	Female	70	Chronic atrophic carditis	S141	Male	38
C 17	Female	59	Chronic carditis	S143	Male	35
C3	Male	63	IM	S144	Male	55
C34	Male	66	Chronic carditis	S 1/1 5	Fomale	20
004	Malo	42	Chronic strophic carditic	S 145 S 146	Malo	Z 7 // 1
C37	Male	42	Chronic atrophic carditic	C 1/7	Formala	25
C20	Formal-	40		0 147	Female Female	30
038	remale	0/		5 148	Female	08
039	Female	00	Unronic carditis	5149	⊦emale	4/
C4	Female	68	Chronic carditis	\$150	Male	46
C40	Male	62	Chronic carditis	S151	Female	39
C41	Female	33	Chronic carditis	S152	Male	65
C42	Female	62	Chronic carditis	S153	Female	54
C43	Male	42	Chronic atrophic carditis	S 154	Female	32

Contd...

IM

Chronic carditis Chronic carditis

Pathological diagnosis Chronic atrophic carditis

Chronic carditis

Chronic carditis

Chronic carditis Chronic carditis

Chronic carditis Chronic carditis Chronic carditis Chronic atrophic carditis Chronic atrophic carditis Chronic atrophic carditis Chronic carditis Normal tissue Chronic carditis Chronic atrophic carditis Chronic carditis Chronic carditis Chronic carditis Chronic carditis

IM IM IM

IM

IM

IM IM IM

Supplementa	ry Table S2: C	ontd		Supplementa	ary Table S2: Con
Subject ID	Sex	Age	Pathological diagnosis	Subject ID	Sex
S 156	Female	51	Chronic atrophic carditis	S237	Female
S 157	Female	56	Chronic atrophic carditis	S238	Male
S 158	Female	53	Chronic carditis	S239	Male
S 159	Female	54	Chronic atrophic carditis	S240	Female
S 160	Female	40	Chronic carditis	S241	Female
S 16 1	Female	59	Chronic atrophic carditis	S242	Female
S 162	Female	59	Chronic atrophic carditis	S243	Female
S 163	Female	33	Chronic atrophic carditis	S244	Female
S 164	Female	40	Chronic carditis	S245	Female
S 165	Female	53	Chronic carditis	S246	Female
S 166	Female	48	Chronic carditis	S247	Female
S 167	Female	45	Chronic carditis	S249	Male
S 168	Male	66	IM	S250	Male
S 169	Male	57	Chronic carditis	S251	Male
S 170	Female	38	Chronic carditis	S252	Female
S 17 1	Female	53	Chronic atrophic carditis	S253	Male
S 172	Female	51	IM	S254	Female
S 173	Male	50	Chronic carditis	S255	Female
S 174	Female	46	Chronic atrophic carditis	S256	Female
S 175	Female	55	Chronic carditis	S257	Male
S 176	Female	51	Chronic carditis	S258	Female
S 177	Female	42	Chronic carditis	S259	Female
S 178	Male	54	Chronic atrophic carditis	S260	Female
S 179	Female	53	Chronic carditis	S261	Female
S 180	Female	33	Chronic carditis	S262	Male
S 181	Male	43	Chronic atrophic carditis	S263	Female
S 182	Female	64	Chronic atrophic carditis	S264	Male
S 183	Male	52	Chronic carditis	S265	Female
S 184	Male	62	IM	S266	Female
S 185	Male	57	IM	S267	Female
S 186	Female	40	Chronic carditis	S268	Female
S 187	Male	50	Chronic carditis	S269	Male
S 188	Female	40	Chronic atrophic carditis	S271	Female
S 189	Male	64	Chronic carditis	S272	Female
S 190	Female	36	Chronic carditis	S273	Male
S 191	Male	41	Chronic carditis	S275	Female
S 192	Male	45	Chronic carditis	S276	Female
S206	Male	51	Chronic carditis	S277	Male
S207	Female	53	Chronic carditis	S278	Female
S208	Male	49	Chronic carditis	S280	Female
S200	Female	49	Chronic carditis	S281	Female
S210	Male	54	Chronic carditis	S282	Female
S210	Female	56	Chronic carditis	\$283	Male
S211 S212	Female	57	Chronic carditis	S284	Male
SZ 12 S2 13	Female	54	Chronic carditis	S285	Male
S215 S21/	Female	18	Chronic carditis	S286	Female
S214 S215	Male	40	Chronic carditis	S287	Female
SZ 1J S2 14	Male	43	Chronic carditis	5207	Mala
SZ 10 S2 17	Fomalo	57	Chronic carditis	3200 82	Fomalo
021/ 0210	Molo	57	Chronic carditis	SS S21	Mala
5210 5210	Iviale	01	Chronic carditis	531 5215	Male
5219	Female	48	Chronic carditis	5315	Iviale
5220	Female	03		5310	Female
5221	Male	42	Chronic carditis	531/	Male
S222	Male	55	Chronic carditis	\$318	Male
S223	Male	61	IM ol i iii	S319	Female
S224	Male	53	Chronic carditis	\$32	Female
8225	Male	44	Chronic carditis	\$320	Male
S226	Male	32	Chronic carditis	S321	Male
S227	Female	64	IM	S322	Male
S229	Female	45	Chronic carditis	S33	Female
S231	Female	64	IM	S34	Male
S232	Male	72	IM	S36	Male
S233	Male	35	Chronic carditis	S37	Female
S234	Male	52	Chronic carditis	S38	Female
S235	Female	55	Chronic carditis	S39	Female
				- ·	- ·

 Supplementary Table S2: Contd...

 Subject ID
 Sex
 Age
 Pathological diagnosis

 5237
 Female
 61
 IM

 5238
 Male
 45
 Chronic carditis

S238	Male	45	Chronic carditis
S239	Male	46	Chronic carditis
S240	Female	56	Chronic carditis
S241	Female	51	Chronic carditis
S242	Female	53	Chronic carditis
S243	Female	49	Chronic carditis
S244	Female	52	IM
S245	Female	46	Chronic atrophic carditis
S246	Female	50	IM .
S247	Female	50	Chronic carditis
S249	Male	62	Chronic carditis
S250	Male	64	Chronic carditis
S251	Male	56	Chronic carditis
S252	Female	56	Chronic carditis
S253	Male	52	Chronic carditis
S254	Female	33	Chronic carditis
S255	Female	40	Chronic carditis
S256	Female	56	Chronic carditis
S257	Male	53	Chronic carditis
S258	Female	61	Chronic carditis
S259	Female	41	Chronic carditis
S260	Female	43	Chronic carditis
S261	Female	40	Chronic carditis
S262	Male	50	Chronic carditis
S263	Female	58	Chronic carditis
S264	Iviale	42	Chronic carditis
S265	Female	50	
5200	Female	04	Chronic carditis
5207	Female	4Z //1	Chronic carditis
S200	Male	41	Chronic carditis
S209 S271	Female	40 57	IM
S272	Female	48	IM
S273	Male	53	Chronic carditis
S275	Female	47	Chronic carditis
S276	Female	49	Chronic carditis
S277	Male	65	IM
S278	Female	51	Chronic carditis
S280	Female	63	Chronic carditis
S281	Female	55	Chronic carditis
S282	Female	58	Chronic carditis
S283	Male	40	Chronic carditis
S284	Male	56	Chronic carditis
S285	Male	41	Chronic carditis
S286	Female	49	Chronic carditis
S287	Female	55	Chronic carditis
S288	Male	64	Chronic carditis
S3	Female	52	Chronic carditis
S31	Male	31	Chronic atrophic carditis
S315	Male	45	Chronic carditis
S316	Female	56	Chronic carditis
\$31/	Male	44	Chronic carditis
\$318	Male	5/	Chronic carditis
5319	Female	31	Chronic carditis
33Z	Female	30 62	Chronic carditis
S320	Iviale	02	Chronic carditis
5321 5322	Male	49 71	Chronic carditis
532Z 533	Fomalo	51	Chronic carditis
S34	Malo	68	IM
S36	Malo	62	Chronic carditis
S37	Female	4.3	Chronic carditis
S38	Female	59	IM
S39	Female	60	Chronic carditis
S4	Female	28	Chronic carditis

Supplementary Table S2: Contd				
Subject ID	Sex	Age	Pathological diagnosis	
S40	Female	37	Chronic carditis	
S42	Male	38	Chronic carditis	
S43	Male	49	IM	
S44	Male	56	Chronic carditis	
S45	Female	66	IM	
S47	Female	59	Chronic carditis	
S48	Female	45	Chronic carditis	
S49	Female	63	IM	
S5	Female	29	Chronic carditis	
S50	Female	52	Chronic carditis	
S51	Male	54	Chronic atrophic carditis	
S52	Male	31	Chronic atrophic carditis	
S53	Female	62	Chronic carditis	
S55	Female	44	Chronic carditis	
S56	Male	45	Chronic carditis	
S57	Male	63	IM	
S58	Male	44	Chronic atrophic carditis	
S59	Male	71	IM	
S6	Female	22	Chronic carditis	
S60	Female	48	Chronic atrophic carditis	
S61	Female	33	IM	
S62	Male	39	Chronic carditis	
S63	Female	27	Chronic carditis	
S64	Female	35	Chronic carditis	
S65	Male	39	Chronic carditis	
S67	Female	35	Chronic carditis	
S68	Female	36	Chronic carditis	
S69	Female	59	Chronic atrophic carditis	
S7	Female	66	IM	
S70	Female	37	IM	
S71	Female	61	IM	
S72	Male	49	Chronic atrophic carditis	
S73	Female	45	Chronic atrophic carditis	
S74	Male	44	IM	
S75	Female	60	Chronic carditis	
S76	Female	59	Chronic carditis	
S77	Female	60	Chronic carditis	
S78	Female	62	Chronic carditis	
S79	Female	42	Chronic atrophic carditis	
S8	Male	53	Chronic carditis	
S80	Female	37	Chronic carditis	
S81	Male	43	Chronic carditis	
\$82	Male	5/	IM	
S83	Male	68	Chronic carditis	
S84	Female	35	Chronic carditis	
S85	Male	51	Chronic carditis	
S86	Female	27	Chronic carditis	
S8/	Female	53	Chronic atrophic carditis	
300	remale	40	Unronic carditis	
287	remaie	55		
3Y 500	iviale	20 26	Normal tissue	
37U 502	Female	50 54	Chronic carultis	
37Z	remaie	34 70		
373 805	iviale	/ ð 4 1		
070 072	remaie	01		
370 507	Ividle	4U 45		
57/ 508	Female	55	Chronic carditis	
070	i ciliaic	55		

Supplementary Table S3: DNA mehylation levels detected by pyrosequencing in different histological categories

Histological categories	DNA methylation	
Normal	1.8	
Normal	1.4	
Normal	4.8	
Normal	1.2	
Normal	2.2	
IM	43.2	
IM	6.6	
IM	21.8	
IM	16.8	
IM	2.4	

Supplementary Table S4: Immunostaining score for *EYA4* in different histological categories

Supplementary Table S4: Contd...

Normal 8 Normal 7.2 Normal 7.2 Normal 12 Normal 11.2 Normal 11.2 Normal 11.2 Normal 11.2 Normal 11.2 Normal 12 Normal 12 Normal 8 Normal 6.8 Normal 5.6 Normal 7 Normal 12 Normal 6 Normal 5.6 Normal 5.6	Histological categories	Immunostaining score
Normal 8 Normal 8 Normal 12 Normal 11.2 Normal 11.2 Normal 11.2 Normal 11.2 Normal 12 Normal 12 Normal 8 Normal 6.2 Normal 5.6 Normal 5.6 Normal 5.6 Normal 3 Normal 5.6 Normal 5.6 Normal 5.2 Normal 5.2 Normal </td <td>Normal</td> <td>8</td>	Normal	8
Normal 7.2 Normal 12 Normal 11.2 Normal 11.2 Normal 11.2 Normal 12 Normal 12 Normal 8 Normal 4.6 Normal 7 Normal 7 Normal 7 Normal 12 Normal 12 Normal 14.6 Normal 12 Normal 14 Normal <t< td=""><td>Normal</td><td>8</td></t<>	Normal	8
Normal 7.2 Normal 12 Normal 11.2 Normal 11.2 Normal 11.2 Normal 11.2 Normal 11.2 Normal 12 Normal 8 Normal 4.6 Normal 7 Normal 5.6 Normal 7.2 Normal 7.2 Normal 12 Normal 12 Normal 6 Normal 10.4 Normal 5.6 IM <td>Normal</td> <td>8</td>	Normal	8
Normal 8 Normal 11.2 Normal 11.2 Normal 11.2 Normal 12 Normal 12 Normal 8.8 Normal 8 Normal 4.6 Normal 5.6 Normal 7 Normal 3 Normal 12 Normal 12 Normal 14 Normal 6.8 Normal 12 Normal 5.6 Normal	Normal	7.2
Normal 12 Normal 11.2 Normal 11.2 Normal 12 Normal 8 Normal 6.2 Normal 7 Normal 6.2 Normal 7.2 Normal 7.2 Normal 7.2 Normal 12 Normal 10.4 Normal 10.4 Normal 6 Normal 5.6 IM <t< td=""><td>Normal</td><td>8</td></t<>	Normal	8
Normal 11.2 Normal 11.2 Normal 11.2 Normal 8.8 Normal 8 Normal 8 Normal 9.6 Normal 8 Normal 8 Normal 8 Normal 12 Normal 8 Normal 6.2 Normal 6.2 Normal 7 Normal 6.2 Normal 12 Normal 12 Normal 12 Normal 12 Normal 12 Normal 10.4 Normal 5.6 Normal 10.4 Normal 6 Normal <td>Normal</td> <td>12</td>	Normal	12
Normal 11.2 Normal 12 Normal 8.8 Normal 8 Normal 4.6 Normal 4.8 Normal 5.6 Normal 7 Normal 3.6 Normal 7.2 Normal 12 Normal 12 Normal 12 Normal 3.6 Normal 12 Normal 12 Normal 12 Normal 5.6 Normal 6 Normal 5.2 IM <	Normal	11.2
Normal 11.2 Normal 12 Normal 8 Normal 8 Normal 9,6 Normal 8 Normal 12 Normal 8 Normal 12 Normal 8 Normal 12 Normal 8 Normal 8 Normal 9,6 Normal 8 Normal 8 Normal 8 Normal 6,2 Normal 4,6 Normal 5,6 Normal 7 Normal 3 Normal 3 Normal 3 Normal 12 Normal 12 Normal 10,4 Normal 12 Normal 6 Normal 10,4 Normal 5,6 Normal 5,6 Normal 5,6 Normal 5,6 Normal <td>Normal</td> <td>11.2</td>	Normal	11.2
Normal 12 Normal 8 Normal 8 Normal 9,6 Normal 12 Normal 8 Normal 12 Normal 6,8 Normal 10,4 Normal 8 Normal 9,6 Normal 8 Normal 8 Normal 8 Normal 8 Normal 6,2 Normal 4,6 Normal 5,6 Normal 7 Normal 3,6 Normal 3,6 Normal 12 Normal 12 Normal 12 Normal 12 Normal 12 Normal 10,4 Normal 10,4 Normal 6 Normal 5,6 Normal 10,4 Normal 10,4 Normal 5,6 Normal 10,4 <	Normal	11.2
Normal 8.8 Normal 8 Normal 9.6 Normal 8 Normal 12 Normal 6.8 Normal 8 Normal 8 Normal 8 Normal 8 Normal 9.6 Normal 8 Normal 9.6 Normal 9.6 Normal 4.6 Normal 6.2 Normal 5.6 Normal 5.6 Normal 3.6 Normal 3.6 Normal 12 Normal 3.6 Normal 12 Normal 5.6 IM 2 IM 5.2 IM 5.2	Normal	12
Normal 8 Normal 9,6 Normal 8 Normal 12 Normal 6.8 Normal 10.4 Normal 8 Normal 10.4 Normal 8 Normal 9,6 Normal 8 Normal 6.2 Normal 4.6 Normal 5.6 Normal 5.6 Normal 3.6 Normal 3.6 Normal 12 Normal 10.4 Normal 12 Normal 5.6 IM 2 IM 4 IM 5.2 IM	Normal	8.8
Normal 8 Normal 8 Normal 12 Normal 6.8 Normal 0.4 Normal 8 Normal 0.4 Normal 8 Normal 8 Normal 8 Normal 4.6 Normal 6.2 Normal 6.2 Normal 5.6 Normal 5.6 Normal 5.6 Normal 3.6 Normal 3.6 Normal 12 Normal 12 Normal 12 Normal 12 Normal 12 Normal 10.4 Normal 10.4 Normal 10.4 Normal 5.6 Normal 10.4 Normal 6 Normal 10.4 Normal 5.2 IM 5.2	Normal	8
Normal 9,6 Normal 12 Normal 6.8 Normal 8 Normal 10.4 Normal 8 Normal 9,6 Normal 8 Normal 4.6 Normal 6.2 Normal 7 Normal 5.6 Normal 5.6 Normal 3.6 Normal 3.6 Normal 3.6 Normal 12 Normal 3.6 Normal 3.6 Normal 12 Normal 12 Normal 12 Normal 12 Normal 12 Normal 10.4 Normal 10.4 Normal 10.4 Normal 5.6 IM 2 Normal 5.6 IM 2.2 IM 5.2 IM 5.2 IM 5.2 IM	Normal	8
Normal 12 Normal 6.8 Normal 10.4 Normal 9.6 Normal 9.6 Normal 9.6 Normal 9.6 Normal 8 Normal 6.2 Normal 6.2 Normal 5.6 Normal 5.6 Normal 7 Normal 5.6 Normal 7.2 Normal 3.6 Normal 7.2 Normal 12 Normal 12 Normal 12 Normal 10.4 Normal 10.4 Normal 5.6 IM 2 IM 4 Normal 5.2 IM 5.2 IM 5.2 IM 5.2 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 5.2 IM 5.2 <td>Normal</td> <td>9.6</td>	Normal	9.6
Normal 12 Normal 8 Normal 10.4 Normal 8 Normal 9.6 Normal 8 Normal 4.6 Normal 4.6 Normal 7 Normal 7.2 Normal 3.6 Normal 3.6 Normal 12 Normal 12 Normal 12 Normal 12 Normal 12 Normal 12 Normal 6.8 Normal 12 Normal 10.4 Normal 5.6 IM 2 IM 4 Normal 5.6 IM 2 IM 5.2 IM 5.2 IM 5.2 IM 5.6 IM 5.6 IM 5.6 IM 5.2 IM 5.2 IM 6	Normal	8
Normal 6.8 Normal 10.4 Normal 8 Normal 9.6 Normal 8 Normal 4.6 Normal 4.6 Normal 4.6 Normal 5.6 Normal 5.6 Normal 5.6 Normal 3.6 Normal 3.8 Normal 3.8 Normal 6.8 Normal 3.6 Normal 12 Normal 12 Normal 6 Normal 10.4 Normal 6 Normal 10.4 Normal 5.6 Normal 5.6 IM 2 IM 4 IM 0.25 IM 5.2 IM 5.2 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 5.6 <td>Normal</td> <td>12</td>	Normal	12
Normal 8 Normal 8 Normal 9,6 Normal 8 Normal 6.2 Normal 6.2 Normal 6.2 Normal 7 Normal 6.2 Normal 7 Normal 5.6 Normal 3.6 Normal 7.2 Normal 3.6 Normal 12 Normal 12 Normal 12 Normal 12 Normal 6 Normal 10.4 Normal 10.4 Normal 10.4 Normal 5.6 IM 2 IM 4 IM 2 IM 5.2 IM 5.2 IM 5.2 IM 5.2 IM 5.6 IM 5.6 IM 5.2 IM 5.2 IM 6.4	Normal	6.8
Normal 10.4 Normal 8 Normal 9.6 Normal 4.6 Normal 4.6 Normal 7 Normal 5.6 Normal 5.6 Normal 5.6 Normal 3.6 Normal 3.6 Normal 12 Normal 12 Normal 12 Normal 12 Normal 10.4 Normal 6 Normal 5.6 Normal 10.4 Normal 10.4 Normal 10.4 Normal 6 Normal 10.4 Normal 5.6 IM 2 IM 4 IM 5.2 IM 5.2 IM 5.6 IM 5.6 IM 5.6 IM 6.2 IM <	Normal	8
Normal 8 Normal 8 Normal 4.6 Normal 6.2 Normal 7 Normal 5.6 Normal 5.6 Normal 3.6 Normal 3.6 Normal 3.2 Normal 12 Normal 12 Normal 12 Normal 12 Normal 6 Normal 12 Normal 10.4 Normal 5.6 Normal 10.4 Normal 10.4 Normal 5.6 IM 2 IM 4 IM 0.25 IM 5.2 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 6.2 IM 6.2 IM 6.2	Normal	10.4
Normal 9.6 Normal 4.6 Normal 6.2 Normal 7 Normal 5.6 Normal 3.6 Normal 3.6 Normal 3.6 Normal 3.6 Normal 12 Normal 12 Normal 12 Normal 6 Normal 6 Normal 6 Normal 12 Normal 12 Normal 12 Normal 6 Normal 10.4 Normal 5.6 IM 2 IM 4 Model 5.6 IM 5.2 IM 5.2 IM 5.2 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 5.2 IM 6	Normal	8
Normal 8 Normal 6.2 Normal 7 Normal 4.8 Normal 5.6 Normal 6.8 Normal 3.6 Normal 3.6 Normal 7.2 Normal 12 Normal 12 Normal 12 Normal 12 Normal 12 Normal 12 Normal 14.4 Normal 5.6 Normal 1.4 Normal 10.4 Normal 5.2 IM 4 IM 0.25 IM 1.25 IM 5.2 IM 5.2 IM 5.6 IM 5.6 IM 5.6 IM 6 IM 6.4 IM 6.2 IM 6.2 IM 6.8	Normal	9.6
Normal 4.6 Normal 7 Normal 4.8 Normal 5.6 Normal 3.6 Normal 3.6 Normal 7.2 Normal 3 Normal 7.2 Normal 12 Normal 12 Normal 12 Normal 6.8 Normal 0.4 Normal 6.8 Normal 5.6 Mormal 10.4 Normal 6.8 Normal 5.6 IM 2 IM 4 IM 0.255 IM 5.2 IM 5.2 IM 5.2 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 6.2 IM 6.2 IM 5.2 IM 6.2 <t< td=""><td>Normal</td><td>8</td></t<>	Normal	8
Normal 6.2 Normal 7 Normal 4.8 Normal 6.8 Normal 3.6 Normal 7.2 Normal 12 Normal 12 Normal 12 Normal 12 Normal 12 Normal 6 Normal 6 Normal 10.4 Normal 6.8 Normal 0.4 Normal 5.6 IM 2 IM 4 IM 0.255 IM 5.25 IM 5.2 IM 5.6 IM 5.6 IM 5.2 IM 5.6 IM 5.2 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 5.2 IM 6.2 <tr< td=""><td>Normal</td><td>4.6</td></tr<>	Normal	4.6
Normal 7 Normal 5.6 Normal 3.6 Normal 3.6 Normal 3.6 Normal 3.1 Normal 12 Normal 12 Normal 12 Normal 12 Normal 12 Normal 6 Normal 10.4 Normal 6.8 Normal 0.4 Normal 10.4 Normal 5.6 Mormal 5.6 IM 4 Mormal 5.6 IM 4 Normal 0.25 IM 4 IM 5.2 IM 5.2 IM 5.2 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 6 IM 6 IM 6 IM 5.2 IM 6.4	Normal	6.2
Normal 4.8 Normal 5.6 Normal 3.6 Normal 7.2 Normal 12 Normal 12 Normal 12 Normal 12 Normal 6 Normal 10.4 Normal 6.8 Normal 0.4 Normal 6.8 Normal 6.8 Normal 0.4 Normal 6.8 Normal 5.6 IM 2 IM 4 IM 0.25 IM 1.25 IM 5.2 IM 5.2 IM 5.2 IM 5.2 IM 5.2 IM 6 IM 5.6 IM 5.6 IM 6 IM 6 IM 6.2 IM 6.2 IM 5.2 IM 6.4	Normal	7
Normal 5.6 Normal 3.6 Normal 7.2 Normal 3 Normal 12 Normal 12 Normal 12 Normal 12 Normal 14 Normal 6 Normal 6.8 Normal 6.8 Normal 5.6 IM 2 IM 4 IM 0.25 IM 1.25 IM 5.2 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 6 IM 5.6 IM 6.2 IM 6.2 IM </td <td>Normal</td> <td>4.8</td>	Normal	4.8
Normal 6.8 Normal 7.2 Normal 3 Normal 12 Normal 12 Normal 12 Normal 12 Normal 12 Normal 14 Normal 11.4 Normal 6 Normal 0.4 Normal 0.4 Normal 5.6 M 2 IM 4 IM 0.25 IM 5.2 IM 5.2 IM 5.2 IM 5.2 IM 5.2 IM 6 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 6 IM 6 IM 6 IM 6.2 IM 6.2 IM 6.2 IM 6.2 IM 5.2 IM 6.	Normal	5.6
Normal 3.6 Normal 7.2 Normal 3 Normal 12 Normal 12 Normal 14 Normal 14 Normal 6 Normal 10.4 Normal 6.8 Normal 5.6 IM 2 IM 4 IM 0.25 IM 1.25 IM 5.2 IM 5.2 IM 5.2 IM 5.2 IM 5.2 IM 5.6 IM 5.6 IM 5.2 IM 5.2 IM 6 IM 5.6 IM 5.6 IM 6.2 IM 6.2 IM 6.2 IM 5.2 IM 6.4 IM 5.2 IM	Normal	6.8
Normal 7.2 Normal 12 Normal 12 Normal 12 Normal 14 Normal 6 Normal 10.4 Normal 6.8 Normal 5.6 IM 2 IM 4 IM 0.25 IM 5.22 IM 5.25 IM 5.25 IM 5.25 IM 5.25 IM 5.6 IM 6 IM 6.2 IM 6 IM 6.2 IM 6.2 IM 6.2 IM 5.2 IM 6.4 IM 5.6 IM	Normal	3.6
Normal 3 Normal 12 Normal 11.4 Normal 6 Normal 10.4 Normal 6.8 Normal 5.6 M 2 IM 4 IM 0.25 IM 1.25 IM 5.2 IM 5.4 IM 5.4 IM 5.2 IM 5.4 IM 5.6 IM 6 IM 6 IM 6.2 IM 6 IM 5.2 IM 6.4 IM 5.6 IM 5.6 IM 5.6 <td>Normal</td> <td>7.2</td>	Normal	7.2
Normal 12 Normal 11.4 Normal 10.4 Normal 6.8 Normal 5.6 IM 2 IM 4 IM 0.25 IM 1.25 IM 5.2 IM 5.25 IM 5.2 IM 5.4 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 6 IM 6.4 IM 6 IM 5.2 IM 6.4 IM 5.6 IM 5.6 IM 5.6 <td>Normal</td> <td>3</td>	Normal	3
Normal 12 Normal 11.4 Normal 6 Normal 10.4 Normal 6.8 Normal 5.6 IM 2 IM 4 IM 0.25 IM 1.25 IM 5.2 IM 5.25 IM 5.25 IM 5.2 IM 5.2 IM 5.2 IM 5.2 IM 5.2 IM 5.6 IM 6 IM 6.2 IM 6.2 IM 6.2 IM 6.4 IM 5.6 IM 5.6 IM 6 IM 6 <td>Normal</td> <td>12</td>	Normal	12
Normal 11.4 Normal 10.4 Normal 6.8 Normal 5.6 IM 2 IM 4 IM 0.25 IM 1.25 IM 5.2 IM 5.2 IM 5.25 IM 7 IM 5.2 IM 5.2 IM 7 IM 5.2 IM 7 IM 5.2 IM 6 IM 5.4 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 6 IM 6.2 IM 6.4 IM 5.2 IM 5.2 IM 6.4 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 5.6	Normal	12
Normal 6 Normal 10.4 Normal 6.8 Normal 5.6 IM 2 IM 4 IM 0.25 IM 1.25 IM 5.2 IM 5.25 IM 7 IM 5.2 IM 5.2 IM 7 IM 5.25 IM 7 IM 5.2 IM 6 IM 7.6 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 6 IM 6.2 IM 6.2 IM 6.4 IM 5.2 IM 5.4 IM 5.6 IM 5.6 IM 5.6 IM 5.6	Normal	11.4
Normal 10.4 Normal 5.6 IM 2 IM 4 IM 0.25 IM 1.25 IM 5.2 IM 5.25 IM 7 IM 5.2 IM 5.25 IM 7 IM 5.2 IM 5.2 IM 5.2 IM 5.2 IM 5.2 IM 5.2 IM 6 IM 5.6 IM 5.6 IM 6.2 IM 6.2 IM 6.2 IM 6.2 IM 6.2 IM 5.2 IM 6.4 IM 5.2 IM 5.2 IM 6.4 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 5.6	Normal	6
Normal 6.8 Normal 5.6 IM 2 IM 4 IM 0.25 IM 1.25 IM 5.2 IM 5.25 IM 7 IM 5.2 IM 5.2 IM 7 IM 5.2 IM 5.2 IM 5.2 IM 5.2 IM 5.2 IM 6 IM 5.6 IM 5.6 IM 6.2 IM 6.2 IM 6.2 IM 6.2 IM 5.2 IM 6.4 IM 5.2 IM 6.4 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 5.6	Normal	10.4
Normal 5.6 IM 2 IM 4 IM 0.25 IM 1.25 IM 5.2 IM 5.25 IM 7 IM 5.2 IM 5.25 IM 7 IM 5.2 IM 6 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 6 IM 6.2 IM 6.2 IM 6.2 IM 6.4 IM 5.2 IM 6.4 IM 5.6 IM 5.4	Normal	6.8
IM 2 IM 4 IM 0.25 IM 1.25 IM 5.2 IM 5.25 IM 7 IM 5.2 IM 6 IM 7.6 IM 5.6 IM 5.6 IM 6.4 IM 6.2 IM 6.4 IM 6.4 IM 5.2 IM 6.4 IM 5.4 IM 6.3 IM 5.4 IM 6.4 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 6.4 IM 0 IM 0	Normal	5.6
IM 4 IM 0.25 IM 1.25 IM 5.2 IM 7 IM 5.25 IM 7 IM 5.2 IM 7 IM 6 IM 5.6 IM 5.6 IM 5.6 IM 6.2 IM 6.2 IM 6.2 IM 6.2 IM 6.2 IM 6.4 IM 5.2 IM 6.4 IM 5.2 IM 6.4 IM 5.4 IM 5.6 IM 6.4 IM 0 IM	IM	2
IM 0.25 IM 1.25 IM 5.2 IM 7 IM 5.2 IM 7 IM 5.2 IM 7 IM 5.2 IM 6 IM 5.6 IM 5.6 IM 6 IM 6.2 IM 6.2 IM 6.2 IM 6.2 IM 6.2 IM 6.4 IM 5.2 IM 6.4 IM 5.2 IM 6.4 IM 5.6 IM 6.4 IM 0 IM 0 IM 0	IM	4
IM 1.25 IM 5.2 IM 7 IM 5.2 IM 6 IM 7.6 IM 5.6 IM 5.6 IM 5.6 IM 6.4 IM 6.2 IM 6.4 IM 5.2 IM 6.4 IM 5.6 IM 6.4 IM 0 IM 0	IM	0.25
IM 5.2 IM 5.25 IM 7 IM 5.2 IM 6 IM 7.6 IM 5.6 IM 5.6 IM 6.2 IM 6.2 IM 6.2 IM 6.2 IM 5.2 IM 6.4 IM 5.2 IM 6.4 IM 5.2 IM 6.4 IM 5.2 IM 6.4 IM 5.4 IM 6.8 IM 5.6 IM 5.6 IM 5.6 IM 6.4 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 6.4 IM 0 IM 0 IM 0	IM	1.25
IM 5.25 IM 7 IM 5.2 IM 6 IM 7.6 IM 5.6 IM 5.6 IM 6 IM 6.2 IM 6.2 IM 6.2 IM 6.4 IM 5.2 IM 6.4 IM 5.6 IM 6.4 IM </td <td>IM</td> <td>5.2</td>	IM	5.2
IM 7 IM 5.2 IM 6 IM 7.6 IM 5.6 IM 5.6 IM 6 IM 6 IM 6.2 IM 6.2 IM 6.4 IM 5.2 IM 6.4 IM 5.6 IM 6.4 IM 0 IM 0	IM	5.25
IM 5.2 IM 6 IM 7.6 IM 5.6 IM 5.6 IM 6 IM 6.2 IM 5.2 IM 6.4 IM 6 IM 5.2 IM 6.4 IM 5.2 IM 6.8 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 6.4 IM 0 IM 0	IM	7
IM 6 IM 7.6 IM 5.6 IM 3.6 IM 6 IM 6.2 IM 5.2 IM 6.4 IM 5.2 IM 6 IM 5.2 IM 6 IM 5.2 IM 5.2 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 6.4 IM 0 IM 0	IM	5.2
IM 7.6 IM 5.6 IM 3.6 IM 6 IM 6.2 IM 5.2 IM 6.4 IM 6 IM 5.2 IM 6.4 IM 5.2 IM 6 IM 5.2 IM 5.2 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 5.6 IM 6.4 IM 0 IM 0	IM	6
IM 5.6 IM 5.6 IM 3.6 IM 6 IM 6.2 IM 5.2 IM 6.4 IM 3.4 IM 6 IM 5.2 IM 6 IM 5.6 IM 6.4 IM 0 IM 0	IM	7.6
IM 5.6 IM 3.6 IM 6 IM 6.2 IM 5.2 IM 6.4 IM 3.4 IM 6 IM 5.2 IM 6 IM 5.6 IM 0 IM 0	IM	5.6
IM 3.6 IM 6 IM 6.2 IM 5.2 IM 6.4 IM 3.4 IM 6 IM 5.2 IM 5.2 IM 5.6 IM 5.6 IM 6.4 IM 0 IM 0	IM	5.6
IM 6 IM 6.2 IM 5.2 IM 6.4 IM 3.4 IM 6 IM 5.2 IM 5.2 IM 5.6 IM 5.6 IM 6.4 IM 6.8 IM 5.6 IM 6.4 IM 0 IM 0	IM	3.6
IM 6.2 IM 5.2 IM 6.4 IM 3.4 IM 6 IM 5.2 IM 2.2 IM 6.8 IM 5.6 IM 7.6 IM 6.4 IM 0 IM 0	IM	6
IM 5.2 IM 6.4 IM 3.4 IM 6 IM 5.2 IM 2.2 IM 6.8 IM 5.6 IM 7.6 IM 6.4 IM 0 IM 0	IM	6.2
IM 6.4 IM 3.4 IM 6 IM 5.2 IM 2.2 IM 6.8 IM 5.6 IM 7.6 IM 6.4 IM 0 IM 0	IM	5.2
IM 3.4 IM 6 IM 5.2 IM 2.2 IM 6.8 IM 5.6 IM 7.6 IM 0 IM 0	IM	6.4
IM 6 IM 5.2 IM 2.2 IM 6.8 IM 5.6 IM 7.6 IM 6.4 IM 0 IM 0	IM	3.4
IM 5.2 IM 2.2 IM 6.8 IM 5.6 IM 7.6 IM 6.4 IM 0 IM 0	IM	6
IM 2.2 IM 6.8 IM 5.6 IM 7.6 IM 6.4 IM 0 IM 0	IM	5.2
IM 6.8 IM 5.6 IM 7.6 IM 6.4 IM 0 IM 0	IM	2.2
IM 5.6 IM 7.6 IM 6.4 IM 0 IM 0	IM	6.8
IM 7.6 IM 6.4 IM 0 IM 0	IM	5.6
IM 6.4 IM 0 IM 0	IM	7.6
IM 0 IM 0	IM	6.4
Ŭ Ő	IM	0
	IM	õ
		-

Histological categories	Immunostaining score
IM	0.4
IM	0
IM	2.4
IM	0.4
IM	1.2
IM	0
IM	0.2
IM	4.8
IM	1.2
IM	5.6
IM	2.4
IM	2
IM	0.8
IM	2.8
IM	3.4
IM	1.2
IM	4
IM	4.4
IM	4.4
IM	5.2
IEN	0
IEN	0
IEN	0