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Original Article

The Notch1/Jagged1 Pathway is Involved in the Development of Rheumatic Heart Disease

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ABSTRACT

Objective: To investigate the role of Notch1/Jagged1 in the development of rheumatic heart disease (RHD). **Methods:** 35 RHD patients who underwent mitral valve replacement, 35 healthy volunteers, and 10 dead persons by traffic accident were selected in the present study. The morphological changes of mitral valve in the RHD patients were observed. The mRNA and protein levels of Notch1/Jagged1 in the mitral valves were assayed using real-time PCR and western blot, respectively. The location and distribution of Notch1/Jagged1 proteins in the mitral valves were analyzed by immunohistochemistry. Flow cytometry (FCM) was used to measure the positive ratios of Notch 1/Jagged1 in isolated peripheral mononuclear cells. Results: Histomorphology data showed that the fusion of commissures, cusps, and chordae tendinea resulted in thickened, shortened and inflexible alterations of the mitral valves. Real-time PCR and western blot assays revealed that the mRNA and protein levels of Notch1/Jagged1 were significantly higher in the impaired mitral valves of the RHD patients compared to controls. Immunohistochemistry revealed that Notch1/Jagged1 proteins were mainly located in the cytoplasm of fibroblasts in the mitral valves. The positive ratio of Jagged1 in peripheral mononuclear cells in the RHD patients was significantly elevated compared to the controls. **Conclusion:** Notch1/Jagged1 expression in the damaged mitral valves of the RHD patients is significantly increased compared to controls. Meanwhile, the positive expression ratio of Jagged1 in the peripheral mononuclear cells of the RHD patients is markedly up regulated. In summary, the Notch1/Jagged1 pathway correlates with the development of RHD.

Key words: Rheumatic heart disease, Notch1, Jagged1, Mitral valve, Peripheral mononuclear cell.

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INTRODUCTION

The Notch signaling pathway is a highly conserved cell signaling system in biological evolution. It has been reported that this ancient signaling system plays critical roles in various types of proliferation including arterial and endothelial growth, and myocardial development.[1-3] Notch signaling pathway also widely affect cellular functions, including apoptosis,[4-5] differentiation[6-9] and developmental lineage choices.[10-11] In humans, there are 4 Notch receptors, including Notch1, 2, 3, 4 , as well as 5 Notch ligands (Jagged1 and 2, Deltal-like ligand 1, 3 and 4), have been found in different types of tissues and cells.[12] The binding of Jagged 1 or 2 will produce a series of proteolytic reactions. As a result, the cleaved Notch releases an intracellular domain of Notch (NICD), which translocates into the nucleus and functions as a transcriptional activator of downstream genes.[13-14]

Some studies have revealed that Notch signaling significantly promote T-cell development in the hematopoietic system,[6,15-16] and regulate differentiation and functions of Th1/Th2 whose out of balance is associated with permanent impairment of cardiac valves in peripheral blood cells.[17] More recent researches have demonstrated Notch signaling to be involved in tissue fibrosis and functions.[18-21] Furthermore, Notch signaling might be important to control the maintenance and commitment of a cardiac stem cell compartment to renew cardiac tissue.[22] One study has demonstrated that cardiac developmental defects emerged when Notch1 intracellular domain (NICD1) was over expressed, or Notch signaling in the embryonic cardiomyocyte compartment was selectively silenced.[23] To date, there are plenty of studies identified that perturbed Notch-Jagged signaling system accounts for some forms of congenital and adult cardiac disease (including aortic valve disease).[24-25] Endothelial deletion of Jagged1 leads to valve calcification, which is one of important evidences for Jagged 1 in valve morphogenesis.[26] Insufficiency of Notch1 receptor is also indicated in aortic valve disease in humans.

Valvular heart disease which originates from rheumatic heart disease (RHD) is one of main causes for heart failure in West China. Despite there are many studies to prevent and treat RHD, the morbidity and mortality are still very high in this region. As we know, the main alterations of valvular disease derives from RHD are immune and autoimmune responses. Actually, details of the mechanism to the pathological variations in RHD are unclear. To characterize the role of Notch signaling, which maybe the link between immune cell and cardiac valve, specifically in the development and evolution of RHD, in the present study herein, we investigated the expression of Notch1/Jagged1 in the mitral valves and peripheral mononuclear cells of the RHD patients.

METHODS

Participants

There were 35 RHD patients, including 14 male and 21 female, who underwent mitral valve replacements in the Affiliated Hospital of Luzhou Medical College (Luzhou, Sichuan Province, China) from August 2009 to March 2010 were selected for the present study. The age was 50.8±11.7 years. The RHD diagnosis was confirmed by echocardiography and biopsy tests. The exclusion criteria included congenital heart disease, senile valvular disease, syphilitic valvular disease, infective endocarditis, insufficiency of the papillary muscle, rupture of chordae tendineae and mucoid degeneration and other similar diseases. Only one patient had family history and her sister also died of RHD. All patients who were preoperative given the routine administration had grading II cardiac function (New York Heart Function Assessment). Meanwhile, 35 healthy volunteers (16 male and 19 female) who had no valvular disease, and 10 persons (6 male and 4 female) who died by traffic accidents served as controls. A positive diagnosis also was confirmed by echocardiography. Each patients and volunteers (including the dead persons by accidents) had a result of electrocardiogram (ECG) test or been found results in their physical examination file. In the present study herein, the RHD

patients, the healthy volunteers, and the relatives of the dead persons by accidents read, understood, and signed the informed consent. This study was also approved by the Committee of the Affiliated Hospital of Luzhou Medical College (Luzhou, China). The detail is listed in Table 1. It should be noted that both the healthy and dead controls had no heart disease or any complications. Echocardiography results showed that 35 (100%) RHD patients had mitral stenosis, 21 (60%) had mitral insufficiency, 13 (37.1%) had aortic stenosis/ insufficiency, and 27 (77.1%) had tricuspid insufficiency.

Histology and morphology of the mitral valves

The separated mitral valves, which acquired from RHD patients and dead persons timely, were cut into a size of 3.0×1.0 mm. After then the samples were stained by hematoxylin and eosin (H.E.) and observed under CX31 optical microscope (Olympus Co., Tokyo, Japan). The cardiac mitral valve tissues for real-time PCR, western blot, and immunohistochemistry assays were from the same valves that were used for the histology and morphology assays. The pathological changes of the mitral valves tissues were observed and assessed by two independent pathologists. Any discrepancy between the two reviewers was resolved by reexamination to achieve an agreement.

Real-time PCR assay of Notch1/Jagged1 mRNAs in the mitral valves

The total RNA was extracted from 50-100 mg of the separated mitral valves, in accordance with the manufacturer's instructions, respectively. After DNase Ⅰ treatment, 2 μg of RNA was reverse transcribed to cDNA using AMV reverse transcriptase. Standard reaction system consisted real-time PCR Master Mix SYBR Green I, forward primer, reverse primer, cDNA, and ddH_2O. The reaction conditions included denaturation at 95℃ for 4.5 min and annealing at 58℃ for 40 sec. The data were analyzed using IQ5 software of the Gene express module (Bio-Rad, CA, USA). There were three replicate reactions were performed and values were normalized to the housekeeping gene β-actin, *CT* values were determined by using the 7500 System SDS Software (version.1.2.3; Applied Biosystems, USA), and $\Delta\Delta CT$ values were computed using the housekeeping gene β-actin; *CT* values as internal controls. Expression ratios were finally calculated in accordance with $2^{-}\Delta\Delta CT$ method.[27]

Western blotting assay of Notch1/Jagged1 proteins in the mitral valves

The cardiac mitral valve tissues (about 50 mg) were lysed with 1mL of pre-cooled RIPA lysate. Subsequently, the cells were disrupted using ultrasound at 80 W and 5 sec each time. After the cells suspension was centrifuged, the supernatants were collected. The concentration of the total protein was quantified by the Bradford method. The proteins were separated on 12% SDS–PAGE, and then they were semidry-transferred onto a polyvinylidene fluoride (PVDF) transfer membrane which was then washed with tris buffered saline (TBS). Subsequently, goat anti-human polyclonal Jagged1 antibody (1:200, Abcam, USA) and anti-human Notch1 antibody (1:100, eBioscience, USA) were added and coincubated at 4°C overnight. Then HRP-conjugated rabbit anti- goat IgG (1:1000) (Zhongshan Golden Bridge Biotechnology Co., Beijing, China) was added and incubated at room temperature for another 2 h. The membrane was stained by enhanced chemiluminescence (ECL) reagent (Pierce, USA), and imaged on X-ray film (Fuji film, Tokyo, Japan) by autoradiography. Quantity one Imagine System and analysis software (Bio-Rad, CA, USA) were used to analyze the specific straps quantitatively.

Immunohistochemistry assay of Notch1/Jagged1 in the mitral valves

Specimens from 35 RHD patients and 10 died controls were used for this study. The tissue sections were suffered from degreasing and rehydration, and then processed using the streptavidin immunoperoxidase method. Briefly, the sections were submitted to antigen retrieval at 95°C and incubated in 10% normal serum, followed by an overnight incubation at 4°C with the diluted goat anti-human Notch1 (1:100, eBioscience, USA)/Jagged1 (1:200, Abcam, USA). After that, the samples were incubated with biotinylated anti-goat immunoglobulins for 15 min at 37°C, followed by streptavidin peroxidase complexes. After hematoxylin counterstaining, immunostaining was quantificationally scored by a CM-2000B imaging analysis system (Beijing University of Aeronautics and Astronautics, Beijing, China). Identification of immunohistochemistry results was according to the criteria proposed by Maruyama. The membrane, cytoplasm, and nucleus were observed. If gray yellow or claybank particles were more than 10% in the cytoplasm and nucleus, it was regarded as positive.

Flow cytometry assays of positive Notch1/Jagged1 expressions in peripheral mononuclear cells

Anticoagulant venous blood, 1 mL, was collected from each of RHD patients and healthy controls. Then the mononuclear cells were separated and harvested by a gradient separation method in accordance with the manufacturer's instructions of a human mononuclear cells separation medium kit (Lengton Biotech. Inc., Shanghai, China). The cells were resuspended and adjusted to a concentration of 1×106/mL. Notch1-PE and Jagged1-FITC, 100 μl respectively, added to co-culture with the treated cells for 10-15 min at room temperature, and without light. Then material was centrifuged and washed with PBS twice. The positive ratios of Notch1/Jagged1 in the cells were detected using a FACScan Flow Cytometer (Becton Dickinson, USA). The data were analyzed by Cell Quest 3.0 software (Becton Dickinson, USA).

Data presentation and statistic analysis

Data are presented as mean± standard deviation, and Student-Newman-Kenls (SNK) test was employed to compare the difference between groups using SPSS11.0 statistics software (SPSS Inc., Chicago, IL, USA). A p value of less than 0.05 was considered significant.

RESULTS

General information of participants

There were no significant differences in baseline values, including age, gender, and nationality among groups (Table 1). Meanwhile, no other complications including diabetes mellitus, hyperthyroidism, and immunologic diseases were found in these participants.

Morphological changes of mitral valve in RHD patients

Fusion of the mitral valve apparatus including commissures, cusps, and chordae tendinea resulted in the shortened, thickened (Maximum thickness=0.6 cm) aspects and inflexibility of these structures. Normal structures of mitral valves were severely destroyed and replaced by a mass of fibrous tissues, leaving cicatrization and hyalinosis. The new micro vessels arose in stenotic valves around the lymphocytes (Figure 1).

The mRNA and protein levels of Notch1/Jagged1 were upregulated in the mitral valves of the RHD patients

The results showed that both Notch1 and Jagged1 mRNAs in the mitral valves of the RHD patients were significantly upregulated compared to controls (Both P<0.01) (Figure 2a). Furthermore, western blot data also

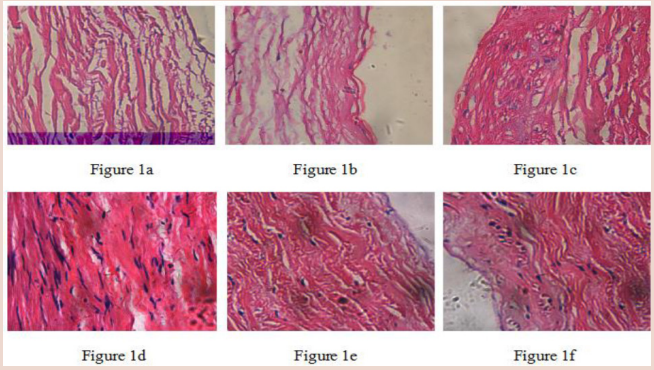

Figure 1: The morphological changes of mitral valves (HE staining ×400). a-c Normal structure of mitral valve; d-e, The mitral valve from the RHD patients is replaced by a mass of fibrous tissue. Meanwhile, newborn micro vessels are found around lymphocytes. (RHD: rheumatic heart disease)

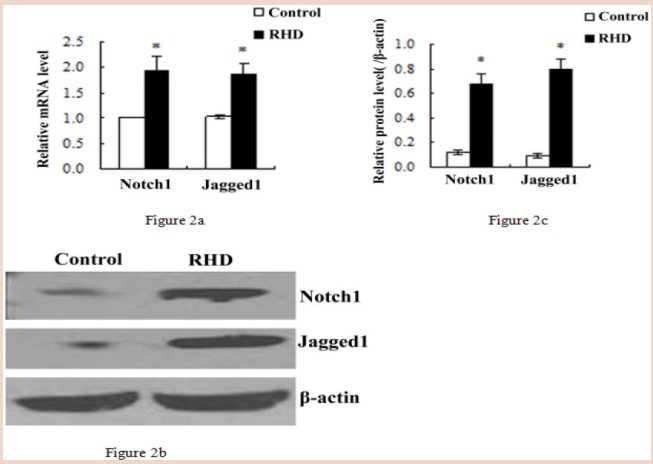

Figure 2: The mRNA and protein expression of Notch1/Jagged1 in the mitral valves. (a), QRT-PCR assay of Notch1/Jagged1 mRNAs in the mitral valves. *P<0.01 vs. Control; (b), Western blotting assay of Notch1/Jagged1 proteins in the mitral valves. *P<0.01 vs. Control; (c), The protein expression of Notch1/Jagged1 in the mitral valves. *P<0.01 vs. Control. (QRT-PCR: quantitative real-time polymerase chain reaction)

demonstrated that the protein expression of Notch1 and Jagged1 in the mitral valves of the RHD patients were significantly higher than those in the control. The differences were significant (P<0.01) (Figure 2b).

Immunohistochemistry assay of Notch1/Jagged1 in the mitral valves

To identify the location and distribution of Notch1/Jagged1 in the mitral valves, we used an immunohistochemistry assay in this part of the experiment. The result revealed that the amount of fibroblasts was significantly increased and their arrangement became disordered, compared to the normal control (Figure 3). Gray yellow or clay bank particles, which represented positive staining of Notch1/Jagged1, were found located in the cytoplasm of fibroblast. Positive cells spread all over the field or distributed in the focal field (Figure 3b and 3e). The average integral optical density (IOD) of Notch1 in the mitral valve in the RHD patients was markedly elevated compared with that in the control (0.4231±0.0539

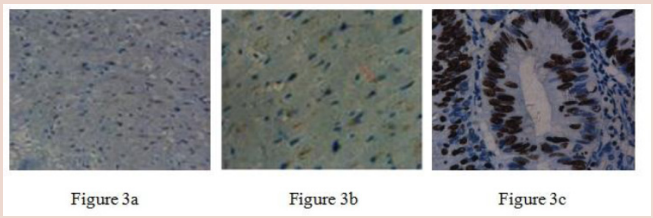

Figure 3: Immunohistochemistry assays of Notch1/Jagged1 in the mitral valves (Streptavidin-perosidase ×400). Compared with normal control (a), more fibroblasts are presented in RHD mitral valve and there was lots of clay bank particles (Shown by red arrow) which shows that Jagged1 expresses in the cytoplasm of fibroblast (b). Abundant of similar particles are found in positive control (colon cancer cells) (c).

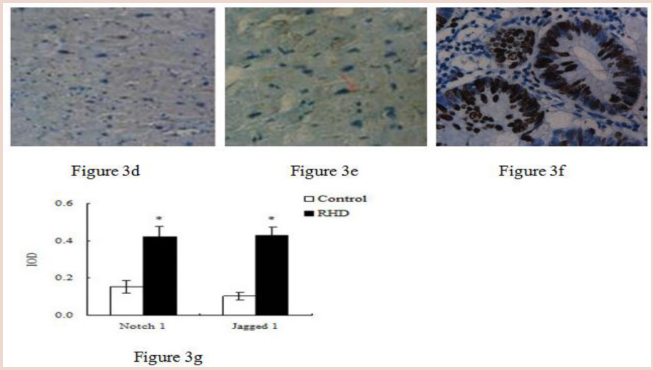

Figure 3b: Immunohistochemistry assays of Notch1/Jagged1 in the mitral valves (Streptavidin-perosidase ×400). D to F display a similar situation: many claybank particles (Shown by red arrow) (Notch 1) emerge in the cytoplasm of the RHD patients (e), similar to positive control (Colorectal cancer) (f), but are negative in normal control (d). *P<0.05 vs. Control. (IOD: integral optical density; RHD: rheumatic heart disease).

vs. 0.1528±0.0331, P<0.05). Similarly, the IOD of Jagged1 was significantly higher than the control (0.4305±0.0421 vs. 0.1027±0.0203, P<0.05) (Figure 3g).

Positive ratio of Jagged1 was elevated in the peripheral mononuclear cells of the RHD patients

In order to assess the role of Notch1/Jagged1 in the development of RHD comprehensively, we employed FCM to assay positive ratios of Notch1/Jagged1 in peripheral mononuclear cells. The FCM results showed that the positive ratio of Jagged1 in the peripheral mononuclear cells of the RHD patients was strikingly higher than that observed for the controls (healthy volunteer) ((17.63±5.28)% vs. (3.17±1.23)%, P<0.01) (Figure 4b). However, there was no significant difference in the positive expression rate of Notch1 between the RHD patients and the controls ((2.50±1.26)% vs. (2.07± 0.93)%, P=0.11) (Figure 4a).

DISCUSSION

Rheumatic heart disease (RHD) is mainly caused by rheumatic fever, and the most common damage is mitral stenosis, usually accompanied by mitral insufficiency or aortic stenosis and/or insufficiency. During the last few years, there are remarkable changes in the diagnosis, evaluation

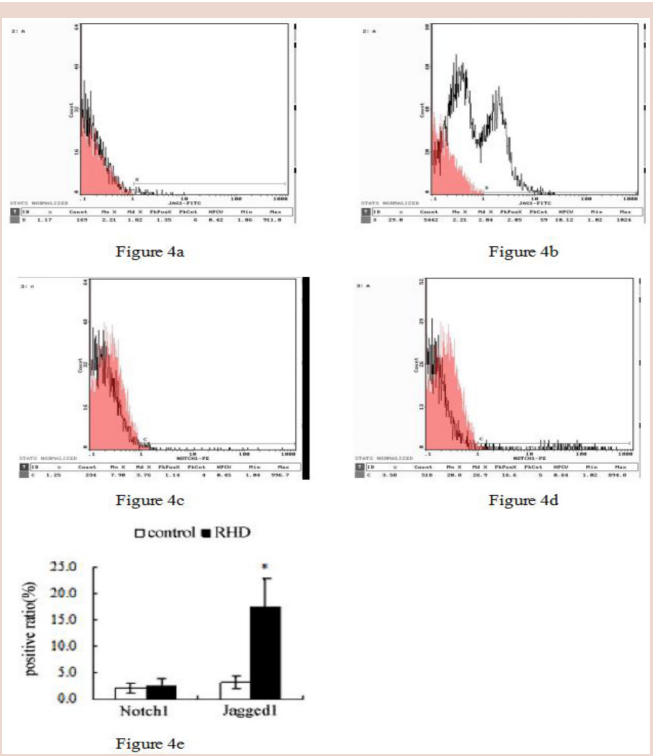

Figure 4: Positive expression ratios of Notch1/Jagged1 in the peripheral blood mononuclear cells. (a), Notch1-RHD; (b), Notch1-Control; (c), Jagged1-Control; (d), Jagged1-RHD; (e), Positive expression ratios of Notch1/Jagged1.*P<0.01 versus Control.

and management of RHD, but high morbidity and mortality still exists, especially in west China.

The conspicuous characteristic of RHD is that it is closely associated with group A streptococci infection. In some degrees, it is deemed as an autoimmune disease. There have antigen driven immune responses in cardiac lesions of RHD patients after a common initial bacterial challenge.[28] The autoantibodies, cytokines, activated T lymphocytes (CD4+/CD8+ T cells), mononuclear cell and other important molecules participant in this process. Recent studies demonstrated that the persisting penetration of T lymphocytes implies the damage of the valve is continuous and developing. More advanced studies revealed that the penetrating T lymphocytes are directly involved in the occurrence and development of RHD, especially CD4+ T cells which can differentiate into Th1 and Th2.[29-31] Actually, the balance between Th1/Th2 is of great importance to autoimmune diseases. Roberts *et al*. bound several antibodies to valvar endothelium and found a plenty of CD4+ T cells' infiltration around the complex.[32] Similarly, Ellis showed that clonal T cells from the RHD patients could react with streptococci M6 protein, cardiac myosin, valvular protein and laminin.[33]

Notch signaling is a key pathway in evolution, which can modulate cellular and tissue differentiation accurately.[34] A Notch signaling pathway is comprised of Notch receptors such as Notch1, 2, 3 and 4, Notch ligands including Jagged1, Jagged2, Delta1, Delta3 and Delta4, intracellular DNA binding proteins, and Notch modulators. These receptors and ligands form the DSL (Delta/Serrate/Lag-2) family in which the members are trans membrane proteins and hydrolyzed by interaction. After that, they penetrate into the nucleus and control the target genes.

Notch1 presents in most tissues including brain, heart, liver, and thymus gland. Jagged1 is abundantly expressed in T cells and antigen-presenting cells. The Notch- Jagged signaling pathway is activated by the Jagged1 which combined to the Notch intracellular domain, which exists in the surface of cells nearby, mediating the target genes by a series of transcription factors such as Hes-1, pre-Tα, Nrarp, Nur77, and others. This signaling pathway plays a crucial role in the development of the heart valve. In rat embryonic heart, it is essential to activate the Notch signaling pathway, which can induce epithelial cells to be the mesenchymal transition (EMT) that forms valve eventually.[35] Many studies have demonstrated that mutations of Notch genes were associated with congenital heart diseases, such as bicuspid aortic valve, calcification of valve, and ventricular septal defects.[36] Timmerman *et al*.[37] found that it would generate a hypertrophic valve when the Notch signaling pathway was continuously activated by Notch ICD gene. Meanwhile, a study also showed that mutation of the Jagged1 gene exists in 94% of Alagille syndrome patients whom commonly had pulmonary stenosis.[38]

In our study herein, the expressions of Notch1 and Jagged1 in the damaged valves of the RHD patients were significantly higher than those in the normal controls. Also, we found that there was significant proliferation of connective tissue and a mass of lymphocytes infiltration in the damaged valves. Thus, we speculated that the high expression of Notch1/Jagged1 might induce the differentiation and activation of T cells, thereby aggravating the impairment of the valves and promoting excessive proliferation of fibroblasts, which led to thickening and inflexibility of the valves.

It was found that Notch1 played an essential role in lymphoid stem cells' differentiation into T cells, and Jagged1 could restrain thymocytes transformation to B cells.[39-40] Furthermore, Notch1/Jagged1 had a great influence on the development of CD4+ and CD8+ T cells.[41] Additionally, Notch-Delta also could impel CD4+ T cells to transform into Th1 cells, while the balance between Th1 and Th2 is a critical factor for lesions of heart valves.[17] It was also found that blockage of Noctch1 signaling of peripheral blood mononuclear cells (PBMCs) could regulate the immune balance of Th1/Th2.[42] In the early study, researchers reported that PBMCs from rheumatic fever (RF) patients react with cell wall and membrane streptococcal antigens, which similar to the action of T-cell lines derived from heart valve specimens,[43] and that from RHD patients display a vigorous recall response to previous streptococcal infection.[44-45] The advanced studies demonstrated that peripheral and heart-infiltrating mononuclear cells produced a series of cytokines which are the mediators of myocardium and valvular inflammation and drive the autoimmune response and further lead to permanent and progressive valvular damage.[46-49]

In the present study, we found that it was Jagged1 but not Notch1 in the PBMCs of the RHD patients was significantly higher than that found in the healthy controls. It is well known that Jagged1 is one kind of ligands that can link the receptors of Notch, not only Notch1, but also Notch2 or 3. Thus, we thought that in the development of RHD, Jagged1 was more susceptible to be activated than Notch1.

CONCLUSION

It suggested that there was a close correlation between Notch1/Jagged1 pathways and the development of RHD. Actually, a larger sample is needed to clarify the role of Notch1/Jagged1 in the development of RHD more deeply. In our future work, we would like to explore the relationships more particularly between other Notch receptors and ligands to illustrate the more details about how the Notch signaling pathway works in RHD, which may give beneficial clinic significance for the therapy of RHD.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Gridley T. Notch signaling in the vasculature. Curr Top Dev Biol. 2010;92:277-309.
2. Benedito R, Roca C, Sorensen I, *et al.* The notch ligands Dll4 and Jagged1 have opposing effects on angiogenesis. Cell. 2009;137(6):1124-35.
3. Jundt F, Anagnostopoulos I, Forster R, *et al.* Activated Notch1 signaling promotes tumor cell proliferation and survival in Hodgkin and anaplastic large cell lymphoma. Blood. 2002;99(9):3398-403.
4. Deftos ML, He YW, Ojala EW, *et al.* Correlating notch signaling with thymocyte maturation. Immunity. 1998;9(6):777-86.
5. Sade H, Krishna S, Sarin A. The anti-apoptotic effect of Notch-1 requires p56lck-dependent, Akt/PKB-mediated signaling in T cells. The Journal of biological chemistry. 2004;279(4):2937-44.
6. Hue S, Kared H, Mehwish Y, *et al.* Notch activation on effector T cells increases their sensitivity to Treg cellmediated suppression through upregulation of TGF-betaRII expression. Eur J Immunol. 2012;42(7):1796-803.
7. Artavanis TS, R MD, Lake RJ. Notch signaling: cell fate control and signal integration in development. Science. 1999;284(5415):770-6.
8. Huppert SS, Le A, Schroeter EH, *et al.* Embryonic lethality in mice homozygous for a processing-deficient allele of Notch1. Nature. 2000;405(6789):966-70.
9. Chang L, Noseda M, Higginson M, *et al.* Differentiation of vascular smooth muscle cells from local precursors during embryonic and adult arteriogenesis requires Notch signaling. Proc Natl Acad Sci USA. 2012;109:6993-8.
10. Martinez AA, Zecchini V, Brennan K. CSL-independent Notch signalling: a checkpoint in cell fate decisions during development. Current opinion in genetics and development. 2002;12(5):524-33.
11. Rangarajan A, Talora C, Okuyama R, *et al.* Notch signaling is a direct determinant of keratinocyte growth arrest and entry into differentiation. The EMBO journal. 2001;20(13):3427-36.
12. Hansson EM, Lendahl U, Chapman G. Notch signaling in development and disease. Seminars in cancer biology. 2004;14(5):320-8.
13. Iso T, Kedes L, Hamamori Y. HES and HERP families: multiple effectors of the Notch signaling pathway. Journal of cellular physiology. 2003;194(5):237-55.
14. Stoop JN, Van DM RG, Baan CC, *et al.* Regulatory T cells contribute to the impaired immune response in patients with chronic hepatitis B virus infection. Hepatology. 2005;41(4):771-8.
15. Radtke F, Wilson A, MacDonald HR. Notch signaling in T- and B-cell development. Current opinion in immunology. 2004;16(2):174-9.
16. Radtke F, Wilson A, Mancini SJ, *et al.* Journal title??? Nature immunology. 2004;5:247-53.
17. Osborne BA, Minter LM. Notch signalling during peripheral T-cell activation and differentiation. Nature reviews Immunology. 2007;7(1):64-75.
18. Fan YH, Dong H, Pan Q, *et al.* Notch signaling may negatively regulate neonatal rat cardiac fibroblast-myofibroblast transformation. Physiol Res. 2011;60(5):739-48.
19. Kennard S, Liu H, Lilly B. Transforming growth factor-beta (TGF- 1) down-regulates Notch-3 in fibroblasts to promote smooth muscle gene expression. J Biol Chem. 2008;283:1324-33.
20. Liu T, Hu B, Choi YY, *et al.* Notch1 signaling in FIZZ1 induction of myofibroblast differentiation. Am J Pathol. 2009;174(5):1745-55.
21. Mann J, Oakley F, Akiboye F, *et al.* Regulation of myofibroblast trans differentiation by DNA methylation and MeCP2: implications for wound healing and fibrogenesis. Cell Death Differ. 2007;14(2):275-85.
22. Nemir M, Pedrazzini T. Functional role of Notch signaling in the developing and postnatal heart. Journal of molecular and cellular cardiology. 2008;45(4):495-504.
23. Paschalis K, Catarina C, Ekaterina S, *et al.* Distinct Roles for Cell Autonomous Notch Signaling in Cardiomyocytes of the Embryonic and Adult Heart. Circ Res. 2010;106(3):559-72.
24. High FA, Jain R, Stoller JZ, *et al.* Murine Jagged1/Notch signaling in the second heart field orchestrates Fgf8 expression and tissue-tissue interactions during outflow tract development. J Clin Invest. 2009;119(7):1986-96.
25. Garg V, Muth AN, Ransom JF, *et al.* Mutations in NOTCH1 cause aortic valve disease. Nature. 2005;437(7056):270-4.
26. Hofmann JJ, Briot A, Enciso J, *et al.* Hofmann, Anais Briot, *et al.* Endothelial deletion of murine Jag1 leads to valve calcification and congenital heart defects associated with Alagille syndrome. Developmen. 2012;139:4449-60.
27. Yu R, Chen HP, Yan XF. Application of real-time fluorescence quantitative PCR accompanied with comparison of Delta CT for diagnosis of Down's syndrome from a single cell. Zhonghua yi xue yi chuan xue za zhi. 2007;24(2):200-2.
28. Guilherme L, Dulphy N, Douay C, *et al.* Molecular evidence for antigen-driven immune responses in cardiac lesions of rheumatic heart disease patients. Int Immunol. 2000;12(7):1063-74.
29. Guilherme L, Weidebach W, Kiss MH, *et al.* Association of human leukocyte class II antigens with rheumatic fever or rheumatic heart disease in a Brazilian population. Circulation. 1991;83(6):1995-8.
30. Kemeny E, Grieve T, Marcus R, *et al.* Identification of mononuclear cells and T cell subsets in rheumatic valvulitis. Clinical immunology and immunopathology. 1898;52(2):225-37.
31. Raizada V, Williams RCJ, Chopra P, *et al.* Tissue distribution of lymphocytes in rheumatic heart valves as defined by monoclonal anti-T cell antibodies. The American journal of medicine. 1983;74(1):90-6.
32. Roberts S, Kosanke S, Terrence DS, *et al.* Pathogenic mechanisms in rheumatic carditis: focus on valvular endothelium. The Journal of infectious diseases. 2001;183(3):507-11.
33. Ellis NM, Li Y, Hildebrand W, *et al.* T cell mimicry and epitope specificity of cross-reactive T cell clones from rheumatic heart disease. Journal of immunology. 2005;175(8):5448-56.
34. Radtke F, Fasnacht N, Macdonald HR. Notch signaling in the immune system. Immunity. 2010;32(1):14-27.
35. Fischer A, Steidl C, Wagner TU, *et al.* Combined loss of Hey1 and HeyL causes congenital heart defects because of impaired epithelial to mesenchymal transition. Circ Res. 2007;100:856-63.
36. Garg V, Muth AN, Ransom JF, *et al.* Mutations in NOTCH1 cause aortic valve disease. Nature. 2005;437(7056):270-4.
37. Timmerman LA, Grego BJ, Raya A, *et al.* Notch promotes epithelial-mesenchymal transition during cardiac development and oncogenic transformation. Genes and development. 2004;18(1):99-115.
38. Warthen DM, Moore EC, Kamath BM, *et al.* Jagged1 (JAG1) mutations in Alagille syndrome: increasing the mutation detection rate. Human mutation. 2006;27(5):436-43.
39. Han H, Tanigaki K, Yamamoto N, *et al.* Inducible gene knockout of transcription factor recombination signal binding protein-J reveals its essential role in T versus B lineage decision. International immunology. 2002;14(6):637-45.
40. Kawamata S, Du C, Li K, *et al.* Overexpression of the Notch target genes Hes *in vivo* induces lymphoid and myeloid alterations. Oncogene. 2002;21(24):3855-63.
41. Robey E, Chang D, Itano A, *et al.* An activated form of Notch influences the choice between CD4 and CD8 T cell lineages. Cell. 1996;87(3):483-92.
42. Pei J, Tang Z, Zang G, *et al.* Blockage of Notch1 signaling modulates the T-helper (Th)1/Th2 cell balance in chronic hepatitis B patients. Hepatol Res. 2010;40(8):799-805.
43. Yoshinaga M, Figueroa F, Wahid MR, *et al.* Antigenic specificity of lymphocytes isolated from valvular specimens of rheumatic fever patients. J Autoimmun. 1995;8(4):601-13.
44. Guilherme L, Oshiro SE, Faé KC, *et al.* T-cell reactivity against streptococcal antigens innthe periphery mirrors reactivity of heart-infiltrating Tlymphocytes in rheumatic heart disease patients. Infect Immun. 2001;69(9):5345-51.
45. Cunningham MW. Pathogenesis of group A streptococcal infections. Clin Microbiol Rev. 2000;13(3):470-511.
46. Guilherme L, Cury P, Demarchi LM, *et al.* Rheumatic heart disease: proin- flammatory cytokines play a role in the progression and maintenance of valvular lesions. Am J Pathol. 2004;165(5):1583-91.
47. Kikly K, Liu L, Na S, *et al.* The IL-23/Th17 axis: therapeutic targets for autoimmune inflammation. Curr OpinImmunol. 2006;18(6):670-5.
48. Guilherme L, Ramasawmy R, Kalil J. Rheumatic fever and rheumatic heart disease: genetics and pathogenesis. Scand J Immunol. 2007;66(2-3):199-207.
49. Guilherme L, Kalil J. Heart Disease: Molecules Involved in Valve Tissue Inflammation Leading to the Autoimmune Process and Anti-S. pyogenes Vaccine. Front Immunol. 2013;30:352-8.

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