The presence of *P2RX7* single nuclear polymorphism is associated with a gain of function in P2X7 receptor and inflammasome activation in SLE complicated with pericarditis

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Abstract

Objective

A case control study was conducted to evaluate the possible influence of P2RX7 single nuclear polymorphism and P2X7 receptor activity in the susceptibility of SLE with pericarditis in Chinese Han population.

Methods

We studied a cohort of SLE patients with (SLE+PCS) or without (SLE-PCS) history of pericarditis and demographic, therapeutic and clinical data were retrospectively collected. P2RX7 489 C>T (His155Tyr) genotype of each subject was analysed and classified as CC or C>T (CT and TT) variant. After isolation of peripheral blood mononuclear cells and macrophages from whole blood by centrifugation on Ficoll gradient, in vitro macrophage releases of IL-1β and IL-18 primed by LPS were evaluated by cytometric bead array. NLRP3 expression were evaluated by western blot after normalisation of house-keeping gene α-Tubulin. Finally, P2X7 receptor activity was analysed after stimulation with agonist ATP, by measuring permeability changes using ethidium bromide (EB) uptake fluorescent probe. The Hardy-Weinberg Equilibrium (HWE) analysis was used to detect the association of P2RX7 489C>T SNP with SLE complicated with pericarditis. Spearman linear regression analysis was performed to evaluate the association of macrophages uptake of EB and NLRP3 expression.

Results

In total 68 SLE+PCS patients and 72 SLE-PCS patients from the cohort were enrolled. No significant differences in demographic, disease activity and serological features were found between the two subgroups. The HWE analysis detected a significant positive association between SLE+PCS and the 489 C>T SNP (OR=0.65, 95%CI (0.46-0.92), p=0.03). No association was found in SLE-PCS patients carrying either genotype CC or C>T. It was shown that in vitro inflammasome-dependent IL-1β/IL-18 release from macrophages was higher in SLE+PCS patients compared to SLE-PCS, especially in those bearing the C>T variant genotype, and consequently the WB analysis ofNLRP3 expression in SLE+PCS patients bearing C>T genotype was significantly higher compared to the other genotype carriers (F=13.1, p<0.01). We also detected that macrophages of SLE+PCS patients carrying SNP 489C>T showed a higher EB uptake in response to ATP than subjects carrying wild type (CC). The Spearman linear regression analysis showed a significant association of macrophages EB uptake and NLRP3 expression as well as its dependent IL-1β and IL-18 in SLE+PCS subjects carrying SNP 489 C>T.

Conclusion

Our results suggest that 489 C>T polymorphism of the P2RX7 gene is associated with activation of inflammasome NLRP3 and an increased release of IL-1β and IL-18. The EB uptake increase in macrophages of LE+PCS subjects carrying 489C>T displays the functional upregulation of P2RX7, which may be involved in the pathogenesis of SLE complicated with pericarditis in the presence of P2RX7 SNP 489C>T.

Key words

systemic lupus erythematosus, pericarditis, P2X7 receptor, NLRP3 inflammasome, single nuclear polymorphism

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Introduction

Pericarditis is one of the typical SLE manifestations often associated with increased inflammatory indices including IL-1β (1) and could respond to colchicine, whose action may be mediated by effect on microtubules during P2X7 receptor assembly (2). Colchicine has been reported to be effective in treatment for pericarditis in SLE and recurrent pericarditis via IL-1β blockade (3) and to decrease the rate of recurrence of acute idiopathic pericarditis (4, 5).

The human P2X7 receptor is an ATP-ligand-gated cationic channel which has been shown to mediate the ATP-induced apoptotic death of dendritic cells, monocytes and is mainly expressed on macrophages. The receptor is encoded by a gene within human chromosome locus at 12q24 (6). Meanwhile, the role of ATP-gated transmembrane cation channel P2X7 receptor in autoimmunity has received particular attention due to its widespread involvement as a key regulatory element of IL-1β and IL-18 cytokines maturation.

In contrast to other P2X receptors, the P2X7 receptor C-terminal intracellular chain is about 240 amino acids longer and is essential for its function. P2X7 receptor is activated by high concentrations of and prolonged exposure to ATP. Once activated, it could undergo the formation of a much larger pore than any other P2X channel that allows the passage of large cations, including fluorescent dyes such as EB (6, 7). The functional consequences of this pore have been established for the P2X7 receptors in the immune system. Pore formation is necessary to trigger the innate immune response induced by ATP, a danger-associated molecular pattern (DAMP) that signals tissue damage and could induce NLRP3 inflammasome activation and consequently caspase-1 maturation (therefore pro-IL-1β cleavage and IL-1β release by immune cells (8, 9). Stimulation of the P2X7 receptor results in CD62L shedding, which is associated with human SLE, so the functional polymorphisms of this gene may affect SLE susceptibility. Several SNPs in the *P2RX7* gene that affect the function of this receptor have been described. For instance, whereas the 489 C>T (His155Tyr) SNPs could induce a gain of function (GOF) of the P2X7 receptors (10-12), the 853 G>A (Arg276His), 946 G>A(Arg307Gln), 1068 G>A (Ala348Thr), 1096 C>G (Thr357Ser) and 1513 A>C(Glu496Ala) SNPs could induce a loss of function (LOF) of the gene (13, 14).

The more relevant SNPs of *P2RX7* described correspond to the 946 G>A (Arg307Gln) and 1096 C>G (Thr357Ser) affect the binding site to ATP, whereas the 1513 A>C (Glu496Ala) affects the carboxyl terminal tail and leads to different receptor function, as assessed by ATP-induced Ca^{2+} dependent EB influx and IL-1β release. To date, hyperfunctional P2X7 receptor variants have been related to pro-inflammatory cytokine release, while the functional consequences of some *P2RX7* SNPs (1068 G>A (Ala348Thr) and 1513A>C (Glu496Ala)) were studied in recombinant cells *in vitro* and in a Chinese SLE cohort of large samples (15). The cohort study showed that 1068 G>A (Ala348Thr) was associated with SLE in particular carriers of A allele and AA/AG/(AG+AA) genotypes were at lower risk of SLE. In our study, we systemically assessed one candidate SNP 489 C>T which was selected based on previous published studies and had shown a possible association between SNPs and pericarditis in SLE patients carrying 489 C>T. It has been found that in SLE+PCS patients the presence of the SNP 489 C>T was associated with a prompted expression of NLRP3, suggesting a possible involvement of P2X7 receptor in the pathogenesis of SLE with pericarditis. Therefore, we carried out the case-control association study to test the hypothesis that 489 C>T SNPs of the *P2RX7* gene could be involved in the pathogenesis of activation of inflammasome in SLE patients with pericarditis.

Materials and methods

Study participants

This is a cross-sectional study that included 140 patients with 68 patients with history of pericarditis and 72 patients without pericarditis as a control group. This cohort was set up from

September 2014 to March 2017, and individuals included in this cohort have been followed up ever since. The diagnosis of pericarditis was confirmed by an independent ultrasound specialist. Their age ranged from 18 to 57 years and all fulfilled ≥4 American College of Rheumatology (ACR) classification criteria for SLE. Disease activity was determined using the SLEDAI-2k scale by an independent nurse. SLE patients undergoing or had a biologic therapy (belimumab or rituximab) were not excluded in the present study. All participants' serum T-SPOT test were negative (ruled out latent tuberculosis infections), serum albumin greater than 30 g/L (ruled out hypoalbuminaemia). None of the patients had a history of carcinoma, acute heart attack or chest surgery in the past 6 months. Patients were classified according to *P2RX7* genotype sequencing and divided into wild type (CC) or C>T carrier (CT/TT). The TongJi Medical ethnics committee approved the study and written informed consent was obtained from each patient.

Isolation of peripheral blood mononuclear cells (PBMC) and macrophages

Firstly, PBMCs were isolated from 15 ml of venous blood from each subject by a density gradient centrifugation. Blood was diluted 1:2 with PBS pH 7.3 and layered over 10ml of Ficoll-Histopaque (Sigma) and centrifuged at 2500 rpm for 20 minutes at 4°C. Cells were washed two times with PBS and suspended at $2x10^6$/ml in RPMI-1640 supplemented with 10% fetal bovine serum, 2mM L-glutamine, and 50U/ml penicillin and 50μg/ml streptomycin. Secondly, in order to enrich for macrophages, cells were allowed to adhere for 1h at 37°C and 5% CO_2, then washed two times until later use as indicated (16).

Genomic DNA isolation

Genomic DNA was extracted from PBMC using a WIZARD genomic DNA kit (Promega, Madison, USA) according to the manufacturer's protocol. DNA quality was determined by spectrophotometry at 260nm (BioTek, USA) and agarose gel electrophoresis. DNA was then stored at -20 °C until Sequencing analysis.

Direct sequencing and analysis

The SNP 489C>T (rs208294) of each subject was determined in genomic DNA samples. A SureSelect Human All Exon 50Mb Kit was used for in-solution enrichment of the coding exons sequence (rs208294), according to the manufacturer's protocol. Adapter sequences for the Illumina HiSeq 2000 were ligated, and the enriched DNA samples were subjected to standard sample preparation. An Illumina CASAVA v. 1.8 pipeline was used to produce sequence reads. A Burrows-Wheeler Aligner algorithm was used to align sequence reads to the human reference genome and variants were analysed using GATK software. The sequencing was performed by an independent technician in a laboratory of medicine at TongJi Hospital.

Macrophages release of IL-1β and IL-18 determined by cytometric bead array

Macrophages ($2x10^6$) resuspended in 500μl of RPMI 1640 culture medium were seeded in 24-well tissue culture plates. Macrophages were prepared to adhere to the plastic plate for 2h at 37°C. Then, non-adherent cells were removed, and plates washed 2 times with PBS. Cells were primed for 3h at 37°Cwith or without 100ng/mL LPS. Then, the culture medium was replaced and cells were stimulated with 5mM ATP at 37°C for 1h. Finally, cell-free supernatants were collected and stored at -20°C until analysed for IL-1β and IL-18 by cytometric bead array.

Uptake of ethidium bromide in freshly prepared macrophages

Macrophages ($3x10^6$) were incubated at 37°C for 5 min, in a solution containing: 145 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$ and 10 mM Na-HEPES, pH 7.4 (normal extracellular solution).10 μM of EB and 100 μmol/L of ATP were then added and cells were kept under the same conditions for an additional period of 10 min. Total plated cells were counted with phase contrast, and permeabilised cells were counted with fluorescence assay. The excitation/emission wavelength used for EB was 520/640 nm, respectively. Macrophages from a healthy donor were treated with 50 μM colchicine as the negative control and washed twice with extracellular solution.

Inflammasome activation of macrophages determined by western blot

Macrophages ($3x10^6$) were incubated within 5mM APT for 30 min prior to inflammasome stimulation. Then cells were primed in the presence of 100 ng/mL LPS for 3h, washed 2 times in PBS, lysed in SDS sample buffer. Finally, cell-free supernatants were collected and stored at -20°C until analysed. Totally 40μl/lane of concentrated protein samples were loaded onto a 12.5% SDS gel at 200v for 1h. Membranes were then blocked for 1 h with 3% bovine albumin in PBS, pH 7.4, and incubated 2h with a commercial rabbit anti-human NLPR3 primary polyclonal antibody (ab98151, Abcam, US, 1:2000 dilution). Subsequently, membranes were stripped and reprobed with a primary antibody against α-tubulin (A5060, Sigma Aldrich, 1:2000 dilution) to assure equal sample loading. Finally, membranes were incubated with a goat anti-rabbit secondary antibody (ab6721, Abcam, US, 1:5000 dilution), conjugated to horseradish peroxidase for 30 min. Labelled bands were visualised by the enhanced chemiluminescence method (46641, Pierce™ ECL Thermo Scientific) and images were captured and quantified by using FluorChem 8000 Imaging System and AlphaEaseFC software with α-tubulin used for normalisation.

Calculation of SLEDAI-2k score

The app Calculate by QxMD was downloaded from Apple Store then registered, and an independent nurse completed the questionnaire with each subject at each visit. SLEDAI-2K was developed as a modification of SLEDAI in order to include persistent, active disease in those descriptors that had previously only considered new or recurrent occurrences. Specifically, SLEDAI-2K allows persistent active

disease in alopecia, mucous membrane ulcers, rash, and proteinuria to be scored. SLEDAI-2K was validated against the original SLEDAI as a predictor for mortality and as a measure of global disease activity. It was also found to be equivalent to SLEDAI in describing changes in disease activity from one visit to the next (17).

Statistical analysis

SNPs analysis of the genotype and allele frequencies with comparisons between the two groups was performed using chi-square test by SPSS 10.1 software. Parametric analysis was performed using one-way analysis of variance (ANOVA). We then performed the Hardy-Weinberg Equilibrium test (http://www.oege.org/software/hwe-mr-calc.shtml) online to analyse the distribution of genotype. The Kruskal-Wallis analysis was used when data were not normally distributed. The Spearman linear correlation test was performed to test the relative value of inflammasome expression, cytokines and macrophages permeability. The *p*-value was calculated based on two-sided tests and 0.05 was defined as the criteria of significance.

Table I. Anthropometric and biochemical parameters of the SLE cohort population.

Variables	SLE+PCS n=68	SLE-PCS n=72	*p*-value
Female/Male	11/57	12/60	0.94
Age (years)	36.7 ± 6.7	39.2 ± 10.2	0.83
Low levels of C3 or C4 n (%)	26 (38.2%)	31 (43.1%)	0.56
SLEDAI-2K	12.6 ± 4.6	13.1 ± 6.7	0.41
Body-mass index	23.5 ± 4.2	24.6 ± 3.5	0.39
CRP (mg/dl)	5.81 C 2.41	6.45 ± 2.70	0.15
ESR (mm/h)	32.4 ± 7.8	26.6 ± 9.6	0.24
PLT ($\times 10^9$/L)	96.9 ± 26.7	113.7 ± 68.4	0.43
RBC ($\times 10^{12}$/L)	3.17 ± 0.56	3.09 ± 0.54	0.77
WBC ($\times 10^9$/L)	4.35 ± 1.22	3.94 ± 0.86	0.35
Anti-dsDNA (+), n (%)	15 (22.1%)	10 (13.9%)	0.21
Anti-ACA (+), n (%)	7 (10.3%)	8 (11.1%)	0.88
Organ involvement			
Kidney, n (%)	15 (22.1%)	18 (25.0%)	0.68
Musculoskeletal, n (%)	23 (33.8%)	30 (41.7%)	0.34
Muscutanoues, n (%)	20 (29.4%)	21 (29.2%)	0.97
Neuropathy, n (%)	2 (2.9%)	0 (0%)	0.14
Respiratory, n (%)	3 (4.4%)	2 (2.8%)	0.60
Current medication			
Proton pump inhibitor, n (%)	54 (79.4%)	52 (72.2%)	0.32
NSAID, n (%)	5 (7.4%)	8 (11.1%)	0.44
Methotrexate, n (%)	15 (22.1%)	20 (27.8%)	0.43
Cyclophosphamide, n (%)	9 (13.2%)	15 (20.8%)	0.23
Tacrolimus, n (%)	8 (11.8%)	11 (15.3%)	0.54
Azathioprine, n (%)	7 (10.3%)	15 (20.8%)	0.08
Mycophenolate mofetil, n (%)	19 (27.9%)	21 (29.2%)	0.87
Hydroxychloroquine, n (%)	56 (82.3%)	60 (83.3%)	0.88

Results

Demographic and clinical data analysis

A total of 140 participants, among which totally 68 patients with history of pericarditis and 72 patients without history of pericarditis enrolled in the study. Demographic and disease-related characteristics of the participants are shown in Table I. One-way ANOVA for continuous variables (data expressed as arithmetic mean ± standard deviation SD). There were no differences between patients and controls with regard to body mass index (BMI), ration of auto-antibodies and complement 3 or 4.The biomedical parameters of subjects have been presented in Table I. More than 97% of them take low to medium equivalent dose (2.5–15 mg/day) of prednisone, or a combination with or without immunomodulatory therapy. Patients were found to be 17.8% (25/140) positive for anti-DNA, and 10.7% (15/140) expressed some of the extractible nuclear antibodies (ENA) at the time of the study.

Genotype distribution in SLE cohort

After genotyping, we identified the three possible genotypes SNP among SLE cohort: the wild-type homozygous CC as well as the heterozygous CT and homozygous TT, which presented the 489 C>T variant. As expected, the 489 C>T variants were present in SLE+PCS as well as in SLE-PCS patients. Specifically, genotype frequency of 489 C>T is 51.47% in SLE+PCS patients, while only 32.88% in SLE-PCS patients carried the genotype (χ^2=5.02, p=0.025). We then performed the Hardy-Weinberg Equilibrium test (http://www.oege.org/software/hwe-mr-calc.shtml) and consequently a significant difference was also obtained for the 489 C>T variant minor allelic frequency (MAF) 0.35 in SLE+PCS population and MAF=0.22 in SLE-PCS population. Homozygous genotype frequency (TT) was slightly higher in SLE+PCS group (17.65%) compared to SLE-PCS (10.96%), however, this difference was not statistically significant (χ^2=2.54, p=0.110).

Increased IL-1β and IL-18 release from primed macrophages in SLE cohort

The hyperfunction of the P2X7 receptors induces the release of the naturally occurring antagonist of IL-1β (18). The 489 C>T SNPs upregulated P2X7 receptors function (9). So, we explored the possible effect of the polymorphism of the gene *P2RX7* on release of pro-inflammatory cytokines. All subjects were classified according to the presence of 489 C>T polymorphism as carrier C>T (CT or TT) or wild-type (CC). The analysis showed a significant correlation of the 489 C>T polymorphism with IL-1β (Fig. 1A) and IL-18 (Fig. 1B) released by macrophages among SLE cohort. Interestingly, we found that releases of IL-1β and IL-18 were significantly higher in SLE+PCS patients bearing the 489 C>T variant compared to the wild type (CC) or all the other genotype carriers in SLE-PCS patients.

Table II. Assessment of frequency distribution of *P2RX7* genotype polymorphism in the SLE cohort population.

Genotype	SLE+PCS (n=68, %)	SLE-PCS (n=73, %)	OR (95%CI)	*p*-value	χ^2
CC	33 (48.53%)	49 (67.12%)	Reference		
CT	23 (33.82%)	16 (21.92%)	0.468 (0.473-1.006)	0.053	3.73
TT	12 (17.65%)	8 (10.96%)	0.449 (0.162-1.238)	0.110	2.54
C>T	35 (51.47%)	24 (32.88%)	0.657 (0.232-0.938)	0.025	5.02

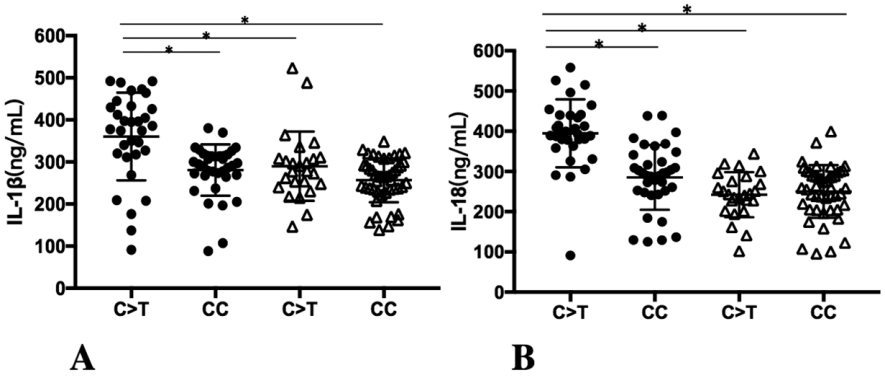

Fig. 1. Comparison of levels of IL-1 β and IL-18 released from primed macrophages in SLE cohort bearing variant functional polymorphism of *P2RX7*.
Graphs represent arithmetic mean ± SD. Statistical analysis was performed using one-way ANOVA test followed by Turkey *post hoc* test, n=3 experiments each in triplicate.
**p*<0.05 was considered as significant. Horizontal brackets show statistically significant differences between the indicated groups. • represents SLE patients with pericarditis (SLE+PCS); while Δ represents SLE patients without pericarditis (SLE-PCS).

Expression of NLRP3 on macrophages of SLE cohort by quantitative western blot

In this part, we continued to evaluate if the presence of the489 C>T polymorphism in SLE+PCS patients has effect on inflammasome activation which is associated with L-1β and IL-18. All subjects were classified according to the presence of 489 C>T polymorphism as carrier (CT+TT-genotype) or wild-type (CC-genotype). The typical western blot gels (Fig. 2A) and summarised data (Fig. 2B) showing the NLRP3 expression of each patient. Results are a representative of at least three independent experiments. NLRP3 expression was denoted by relative grey value after normalised by α-Tubulin.

Correlation of NLRP3 with proinflammatory cytokines and SLEDAI-2K in SLE+PCS patients

NLRP3 inflammasome was found to be activated or highly expressed on macrophages cells in autoimmune diseases. Besides, one of the most potent activators of the NLRP3 inflammasome is extracellular ATP acting at the P2X7 receptor. As we expected, after priming effect of LPS treatment on macrophages, as a consequence of increased NLRP3 expression, IL-1β (Fig. 1A) and IL-18 (Fig. 1B) increased in SLE+PCS cohort carrying 489 C>T genotype and as expected they have a significant positive correlation of with NLRP3 expression (Fig. 3A-B), however in SLE-PCS (data not shown) such correlation was not observed. Unexpectedly, the SLEDAI-2K score (Fig. 3C) which represents disease activity showed no association with NLRP3 expression (p=0.422).

Quantification of EB uptake and regression study of macrophages permeabilisation in SLE+PCS patients

EB was induced by ATP in refreshisolated macrophages. The results permeabilisation of the cationic dyes EB in macrophages expressing P2X7 receptors increased. We performed EB uptake experiments to assess the parameters that may contribute permeability of macrophages in SLE+PCS patients, using freshly obtained or overnight cultured macrophages in SLE+PCS (n=20). The ANOVA analysis (Fig. 4A) showed that in SLE+PCS patients bearing C>T the uptake of EB of macrophages is higher than the others. Correlation analysis was performed and found that permeability of macrophages was associated with IL-1β (r^2=0.809, p<0.01) and IL-18 (r^2=0.856, p<0.001). About 63% of freshly harvested resident macrophages took up EB after treatment with 5 mM ATP. The result showed that in SLE+PCS cohort after challenging with ATP macrophages permeabilisation is positively associated with IL-1β (Fig. 4B), IL-18 (Fig. 4C) and NLRP3 expression (Fig. 4D), so we have made the hypothesis that after the P2X7 receptor activation, the pore of the membrane lead to "leakage" of the proinflammatory cytokines.

Discussion

The human P2X7 receptor is a double transmembrane receptor which has a ubiquitous distribution and is most exclusively expressed on immune cells. In macrophages and similar myeloid cells primed by lipopolysaccharide (LPS), activation of P2X7 receptor by extracellular ATP opens a cation channel/pore allowing massive K^+ efflux associated with processing and secretion of pro-inflammatory cytokines including IL-1β and IL-18. In a rat model of brain injury, data was shown that P2X7 receptor blockade prevents NLRP3 inflammasome activation and the P2X7 is upstream of NLRP3 activation (19). A *P2RX7* genotype 489C>T containing the His155 to Thr SNP or a truncated P2X7 splice variant that increases the activity of this receptor have been reported. It may produce allosteric changes in homomeric formations of P2X7 receptor subunits and pore protein-folding structure. It has been reported that 489C>T polymorphism leads to a gain of function ofP2X7receptor, which affects macrophages from RA patients with the 489C>T genotype, showed higher responses to ATP (11).

After whole sequencing three possible genotypes were identified in the study cohort: the wild-type homozygous CC as well as the heterozygous CT and homozygous TT, which presented the 489 C>T variant. We first performed the Hardy-Weinberg Equilibrium test and

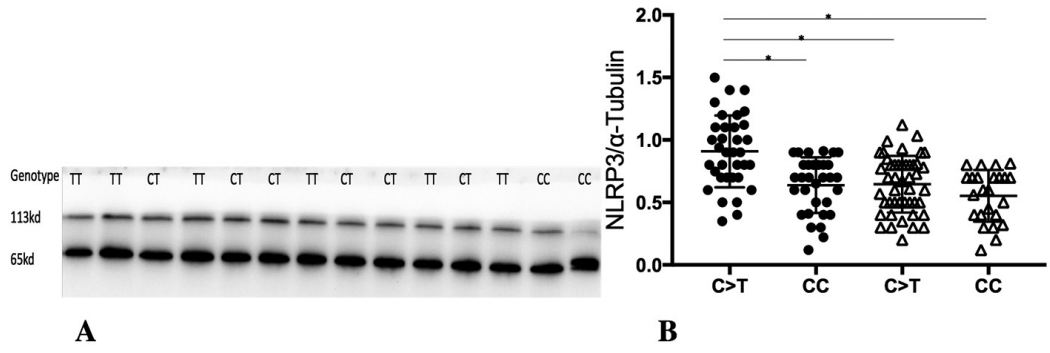

Fig. 2.
A: Upper Lane: Wb detection of different samples of NLRP3 (113kd). Lower lanes: Wb detection of housekeeping gene α-Tubulin (65kd).
B: • represents SLE patients with pericarditis (SLE+PCS) and Δ represents SLE patients without pericarditis (SLE-PCS).

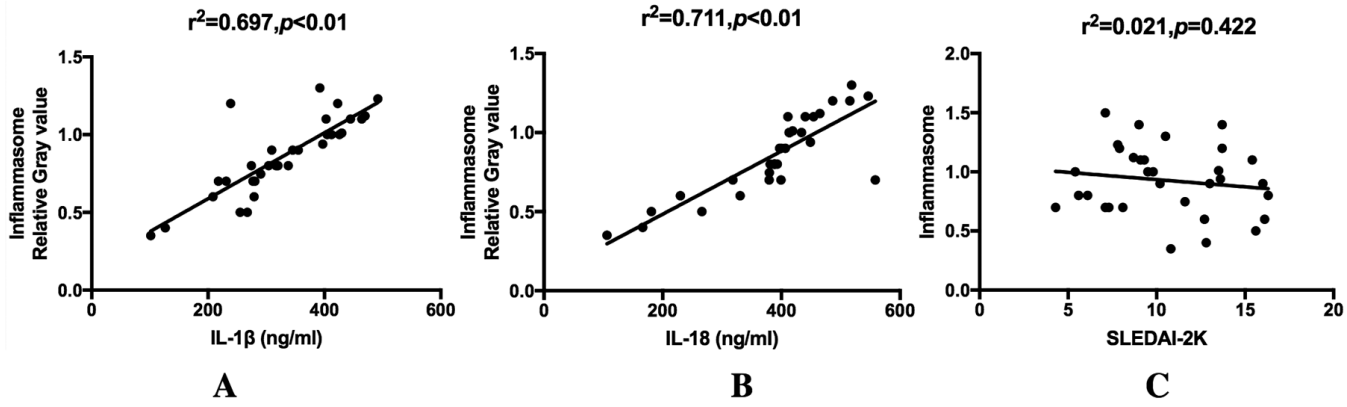

Fig. 3. IL-1β (**A**) and IL-18 (**B**) is associated with NLRP3 expression in macrophages of SLE+PCS patients. Inflammasome was calculted with relative grey valute after normalisation with α-Tublin. Analysis was performed using Spearman correlation and $p<0.05$ was considered significant.

found a Minor Allele Frequency (MAF) and found allele SNP 489 C>T had a MAF of 0.35 in SLE+PCS and 0.22 in SLE-PCS patients. As expected, a Chinese SLE study (15) and our previous study showed that C>T variants were present in SLE patients as well as in healthy controls. We then performed the Chi-square test to compare the difference of 489 C>T variant distribution in the SLE cohort. Specifically, 51.4% of SLE+PCS patients carried the 489 C>T variant (CT/TT) whereas 32.8% of SLE-PCS subjects did. Thereafter, we detected a significantly positive association between pericarditis in SLE and SNP of 489 C>T (OR=0.3916, p=0.0045). Then, we explored the possible effect of the 489 C> T polymorphism of the gene *P2RX7* on the levels of NLRP3 inflammasome and its relevant cytokines released from macrophages and further investigated the cellular permeability in SLE+PCS patients.

IL-1β and IL-18 as downstream cytokines of inflammasome activation are pivotal mediators of the host response to infections, tissue injury and immunologic challenges. The role of the P2X7 receptor as a susceptibility factor in pathogenesis of pericarditis complicated with SLE has not been studied previously. The reported prevalence of pericarditis detected by echocardiographic studies, ranges from 11% to 54%, this variability is partially attributable to the methods applied to document pericardial disease and whether symptomatic or asymptomatic cases are included. In our SLE cohort, the prevalence of pericarditis is 37.3% and our results show that ATP could induce the release of IL-1β and IL-18 of macrophages in SLE+PCS patients significantly higher than that in SLE-PCS patients. In addition, the enhanced release of IL-1β and IL-18 observed in SLE+PCS individuals was associated to the presence of the 489 C>T SNP.

The NLRP3 inflammasome is a multiprotein complex, which was formed by NOD-like receptors (NLRs) family members, adaptor ASC (apoptosis-associated speck-like protein containing a CARD) and Caspase-1. We have found increased expression of NLRP3 on macrophages of SLE+PCS patients carrying 489 C>T polymorphism, on the contrary, in SLE-PCS patients no significant difference about inflammasome expression and downstream pro-cytokines IL-1β/IL-18 were found in carriers of various genotypes (CC/CT/TT). So, we continued to explore the possible effect of the *P2RX7* 489 C>T polymorphism and the inflammasome expression. Although the activity of P2X7 receptor was regarded as involved in the pathogenesis of autoimmune diseases, including SLE and RA (11), however, variable of activity of this receptor has been detected in different studies of SLE and the presence of SNP may account for the lost or gain of function of the receptor. In our study, we found that the expression of NLRP3 expression is strongly associated SNP 489 C>T in SLE+PCS subjects, where T allele and C>T genotypes were at higher risky correlation with SLE complication of pericarditis.

These results suggest that underlying mechanisms might exist to modulate the activity of P2X7 receptor. In this regard, we consider that it would be interesting to assess the intracellular signal pathways induced through P2X7 recep-

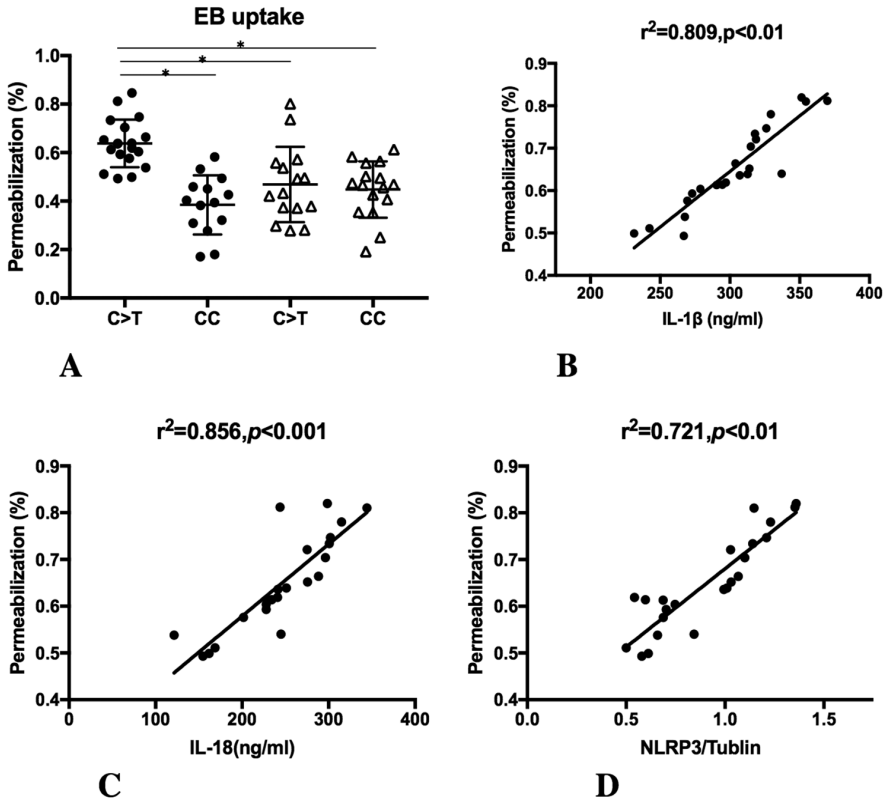

Fig. 4. One-way ANOVA followed by Tukey post hoc test (n=20, each experiment in triplicate) was shown in **A** and Δ represents SLE patients without pericarditis (SLE-PCS).
Spearman correlation analysis was performed and $p<0.05$ was considered significant.
A-**D**: • represents SLE patients with pericarditis (SLE+PCS).

tor in immune cells from SLE patients, mainly because different abnormalities have been described in other receptors of these cells. In our study it has been detected of an enhanced uptake of EB in response to ATP from macrophages of SLE+PCS patients. When SLE subjects were classified according to the *P2RX7* genotype, 489 C>T carriers showed a higher incidence of complication of pericarditis, with an increased induction of pore formation was observed in macrophages uptake of EB dye at 520/640nm wavelength fluorescence.

Because colchicine is known to affect adhesion and migration of immune cells (20), it was utilised as inhibitor of P2X7 receptor to study inflammasome expression analysis as the negative control. The anti-inflammatory effect of colchicine is notable and it underlies effectiveness as anti-gout acute attack, recurrent pericarditis (21) and familial Mediterranean fever (22). The mechanism was regarded as to block microtubule rearrangement induced by ATP via P2X7 receptors, consequently reducing inflammasome formation and IL-1β/IL-18 release. Our study displayed about 63% increases of freshly harvested resident macrophages took up EB (P2X7 receptor pore formation) after treatment with 5mM ATP. The pore formation has been linked to recruitment of the inflammasome culminating with IL-1β release. Conversely, in Silva's research colchicine-pretreated macrophages cultures could be observed approximately 58.5% reduction of the cation dye intake and down regulation of P2X7 receptor (23).

In the study we have made a preliminary finding that there might be an association between GOF of *P2RX7* 489 C>T SNP variants and inflammasome activation in SLE+PCS patients. Portale's and our study showed that the 489C>T SNP might not be associated to susceptibility to SLE indifferent ethnical population (24). However, it was implied that the presence of the 489 C>T SNP variant in *P2RX7* genotype resulted in GOF of P2X7 receptor that observed an increase of EB uptake by macrophages compared with the wild type genotype carrier (CC). Finally, it would be promising to explore other mechanisms that could regulate function of P2X7 receptor, including its internalisation or the presence of micro RNAs that regulate expression of this receptor to further study the pathogenesis of the pericarditis in SLE.

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