Measurement and evaluation of isotypes of anti-citrullinated fibrinogen and anti-citrullinated alpha-enolase antibodies in juvenile idiopathic arthritis

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Abstract

Objective

Our objective was to evaluate sera from juvenile idiopathic arthritis (JIA) patients to investigate the presence of isotypes (IgA, IgG, IgM) of anti-citrullinated fibrinogen and anti-α-enolase antibodies and their association with rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) antibody isotypes.

Methods

Sera were obtained from 89 JIA patients and were measured for isotypes (IgA, IgM) of anti-citrullinated and native fibrinogen and anti-α-enolase antibodies by enzyme-linked immunosorbent assay. Results were compared to anti-CCP antibody isotypes and RF isotypes, in addition to previously measured IgG anti-citrullinated fibrinogen and α-enolase antibodies.

Results

IgA anti-citrullinated fibrinogen antibodies were positive in 20 JIA patients and IgM in 11 JIA patients. Two IgM RF-positive polyarthritis patients were positive for all 3 isotypes of anti-citrullinated fibrinogen antibodies. IgA anti-citrullinated α-enolase antibodies were positive in 7 JIA patients and IgM in 9 JIA patients. IgA and IgG anti-citrullinated fibrinogen antibodies were commonly found in JIA patients positive for IgG anti-CCP antibodies and IgM RF. IgG anti-CCP antibodies and IgM RF levels were significantly higher in JIA patients with 3 or more anti-citrullinated autoantibody isotypes present.

Conclusion

We have shown that isotypes of anti-citrullinated fibrinogen and α-enolase can be found in the serum of children with JIA of all onset types. Citrullinated autoantibody isotype diversity may indicate a more severe disease course in JIA patients.

Key words

juvenile idiopathic arthritis, anti-cyclic citrullinated peptide antibodies, rheumatoid factor, fibrinogen, alpha-enolase

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Introduction

Juvenile idiopathic arthritis (JIA) is a heterogeneous group of arthritides occurring in children less than 16 years of age and persists for at least six weeks with no known etiology (1). It is the most common chronic rheumatic disease of childhood and many therapies have been developed (2). If a more specific biomarker is found, it would help in developing treatment regimens. Of the seven JIA subtypes, IgM rheumatoid factor (RF)-positive polyarticular JIA most closely resembles adult rheumatoid arthritis (RA). As the name indicates, the presence of IgM RF is one of the factors used to define this subset of patients (1). Citrullination and the immune response to citrullinated proteins, and specifically IgG anti-cyclic citrullinated peptide (anti-CCP) antibodies have been a valuable diagnostic tool in adult RA, and were included as part of the 2010 classification criteria for RA (3, 4). Several studies have indicated the importance of the anti-CCP antibodies in the pathogenesis of JIA, particularly in IgM RF-positive polyarthritis (5). Anti-CCP antibodies were included in the American College of Rheumatology (ACR) features associated with poor prognosis and disease activity in JIA patients with arthritis of 5 or more joints (6). It is also realised that citrullinating enzymes and citrullinated proteins may have a role in the inflammatory process in the joints (4).

The role of IgG anti-CCP antibodies in RA and JIA has become better understood, with recent studies focusing on identification of targets for the citrulline modification and determining isotype usage of the anti-CCP antibody response (7-10). We previously evaluated anti-CCP antibody isotypes (IgA, IgG, IgM) (11), and more recently investigated the presence of anti-citrullinated fibrinogen and α-enolase antibodies as target proteins for citrullination in JIA (10). In the present study, we build on our previous work by studying the presence of isotypes of anti-citrullinated fibrinogen and α-enolase antibodies and try to determine if the presence of these antibodies confers a more severe disease course or measuring these antibodies would replace or complement measurement of anti-CCP antibodies.

Methods

Patient samples

Sera were obtained from the outpatient clinics at the Saint Louis University Medical Center and Cardinal Glennon Children's Medical Center following informed consent. Heparin-treated sera were frozen in 50µl aliquots and stored at -80°C until analysed. A total of 89 (74 female/15 male) JIA serum samples were collected, including 16 with IgM RF-positive polyarthritis, 36 with IgM RF-negative polyarthritis, 24 with oligoarthritis, and 13 with systemic-onset arthritis. All JIA patients in this study fulfilled the International League of Associations for Rheumatology (1).

Clinical data regarding signs of active disease (joint pain and swelling, limitations of range of motion, fever, rash, visceral involvement, and inflammatory markers) were collected from patient records of the Pediatric Rheumatology clinics. Active disease was defined as active synovitis and/or elevated acute phase parameters. Sixty-six (66%) patients had active disease at the time of sample collection. Radiological data were evaluated for signs of joint damage (defined as joint space narrowing and/or joint erosions) by musculoskeletal radiologists and reviewed by the Pediatric Rheumatologists. Radiographic damage was reported in 20 (21%) JIA patients.

For controls, sera from 3 patients (3 females) with psoriatic arthritis and 3 patients with enthesitis-related arthritis (ERA) (3 males) were collected (Table I). Sera were also collected from 10 healthy children (9 female/1 male) at the well-child clinic at Cardinal Glennon Children's Medical Center (Table I). The study was approved by the Institutional Review Board of the Saint Louis University Medical Center.

Laboratory evaluation

Erythrocyte sedimentation rate (ESR) was determined by modified Westergren technique and considered elevated at ≥15mm/hr. C-reactive protein (CRP) was determined by electroimmunoassay and a value of ≥0.8mg/dl was considered elevated. Initial determination of IgM RF positivity was performed by nephelometry or latex agglutination, which is how patients were classified

for seropositive JIA. The QUANTA Lite RF ELISA (INOVA Diagnostics, Inc., San Diego, CA) was used for detection of IgA and IgM RF according to manufacturer's instructions. The cut-off value for a positive IgA or IgM RF was ≥6 Units. A third generation anti-CCP antibody test, the QUANTA Lite CCP3 ELISA (INOVA Diagnostics, Inc.) was used for detection of IgG anti-CCP antibodies according to manufacturer's instructions. The cut-off for a positive result was ≥20 Units. IgA and IgM anti-CCP antibodies were measured by ELISA as previously described (11). Positive cut-off points were calculated at optical density (OD)≥0.16 for IgA anti-CCP antibodies and OD≥0.43 for IgM anti-CCP antibodies.

In vitro deimination of human fibrinogen

Deimination of fibrinogen was performed as previously described (10, 12, 13). Briefly, plasminogen-depleted human fibrinogen (95% pure, Calbiochem, Meudon France) was purified by affinity chromatography on a protein-G column (HiTrap protein G, GE Healthcare, Piscataway, New Jersey) according to the manufacturer's instructions. Deimination was the performed using rabbit skeletal muscle peptidyl arginine deiminase (PAD) (Sigma, St. Louis, Missouri, USA, 7 U/mg fibrinogen) in 0.1M Tris-HCl (pH 7.4), 10mM CaCl2, 5mM dithiothreitol for 2 hours at 37oC.

Citrullinated and native fibrinogen and α-enolase ELISAs

ELISAs with citrullinated and native forms of fibrinogen were performed as previously described (10, 12, 13). For isotyping, horseradish peroxidase (HRP)-labeled goat anti-human IgA, IgG, or IgM (Antibodies Incorporated, Davis, CA) diluted 1:15,000 was added to the wells and incubated for one hour at room temperature.

Citrullinated and native α-enolase ELISAs were performed as previously described (10, 14). Ninety-six well plates were incubated for one hour at room temperature with HRP-conjugated goat anti-human IgA, IgG, or IgM (Antibodies Incorporated) diluted 1:10,000 in radioimmunoassay buffer.

Patient results from the duplicated wells were averaged and the OD from the blank wells containing phosphate buffered saline (PBS)/0.05% Tween was subtracted from the average. Serum was considered positive if the titer reached 2 SD above the mean for healthy controls. For anti-citrullinated fibrinogen antibodies, cut-off values were calculated at OD≥0.09 for IgA, OD≥0.59 for IgG, and OD≥1.7 for IgM. For anti-citrullinated α-enolase antibodies, cut-off values were calculated at OD≥0.20 for IgA, OD≥0.91 for IgG, and OD≥0.73 for IgM.

Statistical analyses

Statistical analysis was performed using the Statistical Package Social Science (SPSS) programme version 19.0 (Chicago, IL). Correlations were analysed using Spearman's rho correlation coefficient. Correlations were considered as either strong (>0.7), moderate (0.7–0.5), fair (0.49–80.3), or poor

Table I. Demographic and laboratory features of patients with JIA and Controls, and healthy children.

Parameters	JIA Group (n=89)	Healthy Group (n=10)	Psoriatic arthritis (n=3)	Enthesitis-related arthritis (n=3)
Age, mean±SD years (range)	11.3±5.9	14.0±5.9	17 (15-17)	16 (5-17)
Sex, no. of females/males	77/18	9/1	3/0	0/3
Disease Duration, mean±SD years (range)	3.9±4.4 (0-16)	NA	4 (0-8)	2.5 (1-7)
Tender/swollen joint count, mean±SD years	7±6 (0-24)	NA	2 (1-8)	2 (1-2)
No. of patients with joint damage (%)	20 (21.1)	NA	0	0
No. of patients with active disease (%)	66 (69.5)	NA	0	0
ESR, mean±SD mm/hr (% positive)	27±29 (50.5)	NA	8±4 (0)	7±2 (0)
CRP, mean±SD mg/dl (% positive)	1.6±2.0 (36.8)	NA	0.7±1.2 (0)	0.4±1.2 (0)
IgM RF, mean±SD Units (% positive)	20±35 (48.4	0	0	0
IgG anti-CCP antibodies, mean±SD Units (% positive)	25±73 (14.7)	0	0	0

Table II. Citrullinated antibody isotype concentration and positivity in JIA subtypes, SLE patients, and healthy children.

	IgA anti-citrullinated fibrinogen antibodies Mean±SD	IgM anti-citrullinated fibrinogen antibodies Mean±SD	IgA anti-citrullinated enolase antibodies Mean±SD	IgM anti-citrullinated α-enolase antibodies Mean±SD
IgM RF+ polyarthritis (n=16)	0.11±0.08	0.71±0.50	0.18±0.12	0.40±0.36
Positivity n (%)	6 (37.5%)	2 (12.5%)	1 (6.3%)	2 (12.5%)
IgM RF- polyarthritis (n=36)	0.10±0.09	0.81±0.68	0.15±0.11	0.47±0.32
Positivity n (%)	12 (33.3%)	3 (8.3%)	4 (11.1%)	4 (11.1%)
Oligoarthritis (n=24)	0.06±0.06	0.79±0.59	0.13±0.08	0.39±0.23
Positivity n (%)	1 (4.2%)	4 (16.7%)	1 (4.2%)	1 (4.2%)
Systemic-onset (n=13)	0.07±0.07	0.71±0.67	0.14±0.09	0.49±0.33
Positivity n (%)	1 (7.7%)	2 (15.4%)	1 (7.7%)	2 (15.4%)
Enthesitis (n=3)	0.13±0.07	0.71±0.30	0.14±0.06	0.29±0.08
Positivity n (%)	1 (33.3%)	0 (0%)	0 (0%)	0 (0%)
Psoriatic (n=3)	0.10±0.11	0.59±0.26	0.12±0.06	0.40±0.11
Positivity n (%)	1 (33.3%)	0 (0%)	0 (%)	0 (0%)
Healthy (n=10)	0.04±0.03	0.71±0.50	0.09±0.05	0.39±0.17
Positivity n (%)	0 (0%)	0 (0%)	1 (10.0%)	0 (0%)

Continuous variables are expressed as mean±SD. OD: optical density; n: number of patients; RF: rheumatoid factor; SD: standard deviation. Cut points for positive values: IgA anti-citrullinated fibrinogen antibodies (OD≥0.09), IgM anti-citrullinated fibrinogen antibodies (OD≥1.7), IgA anti-citrullinated α-enolase antibodies (OD≥0.20), IgM anti-citrullinated α-enolase antibodies (OD≥0.73).

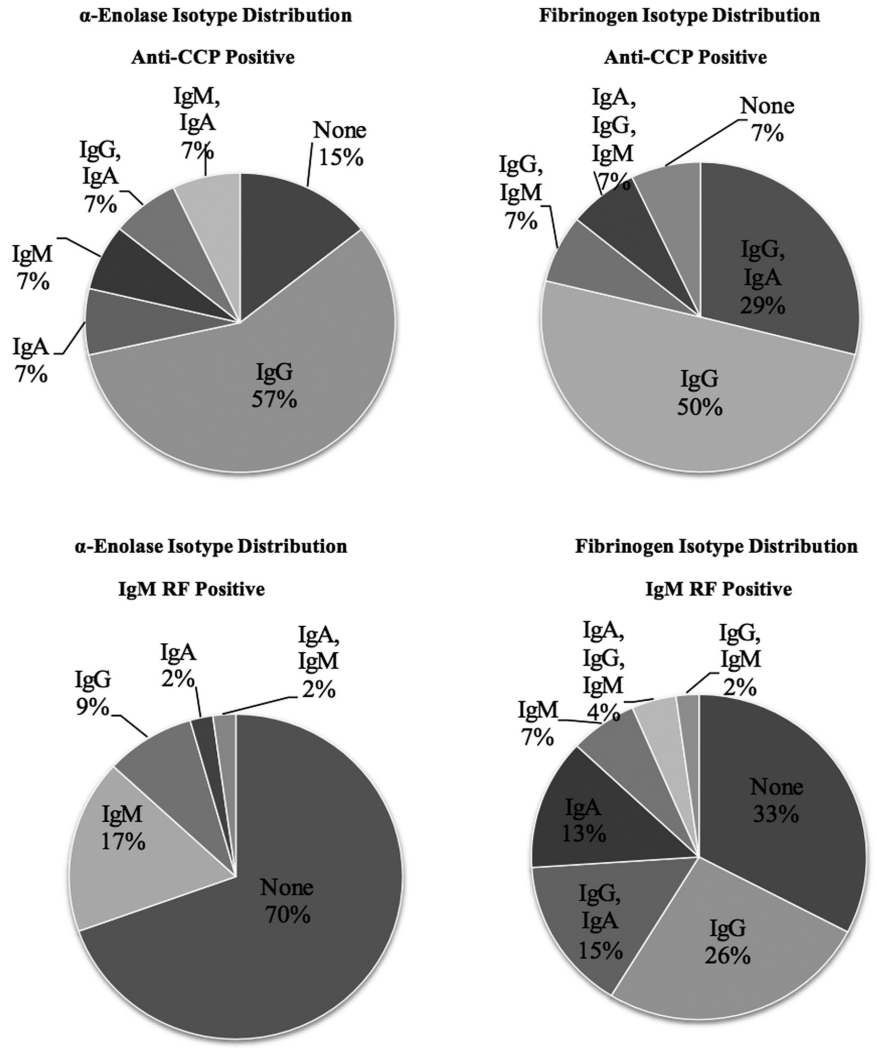

Fig. 1. Distribution of anti-citrullinated fibrinogen and α-enolase antibody isotypes in JIA patients positive for IgG anti-CCP antibodies (n=14) and IgM RF (n=46) by ELISA. CCP, cyclic citrullinated peptide; RF, rheumatoid factor.

(<0.3). Patient groups were compared using the Independent samples *t*-test and the χ^2 test for proportions. For tables with cells with small frequencies, Fisher's exact test was used. A *p*-value of ≤0.05 was considered statistically significant.

Results

Serum reactivity to native and citrullinated autoantibody isotypes

Twenty (22.5%) JIA patients were positive for IgA anti-citrullinated fibrinogen antibodies and 11/89 (12.4%) JIA patients were positive for IgM anti-citrullinated fibrinogen antibodies (Table II). Polyarticular JIA patients, both IgM RF-negative and IgM RF-positive, demonstrated the highest level of reactivity with IgA citrullinated fibrinogen. Levels of IgA anti-citrullinated fibrinogen antibodies were significantly higher in IgM RF-positive polyarthritis patients (OD=0.11±0.08) compared to healthy children (OD=0.04±0.03; *p*=0.02). IgA anti-citrullinated fibrinogen antibody levels were also raised in IgM RF-negative polyarthritis patients compared to healthy children, but did not reach statistical significance. No significant differences were noted in levels of IgM anti-citrullinated fibrinogen antibodies between JIA subtypes and healthy children. One psoriatic arthritis and one ERA were positive for IgA anti-citrullinated fibrinogen antibodies. None of the healthy children were positive for IgA or IgM anti-citrullinated fibrinogen antibodies.

When evaluating combinations of positivity among the anti-citrullinated fibrinogen isotypes, it was noted that 18 JIA patients were positive for IgG only, 8 for IgA only, and 6 for IgM only. Ten JIA patients were positive for both IgG and IgA anti-citrullinated fibrinogen antibodies, two for both IgG and IgM, and one was positive for both IgA and IgM. Two IgM RF-positive polyarthritis patients were positive for all 3 isotypes of anti-citrullinated fibrinogen antibodies.

Seven (7.9%) JIA patients were positive for IgA anti-citrullinated α-enolase antibodies and 9/89 (10.1%) JIA patients were positive for IgM anti-citrullinated α-enolase antibodies. As with the citrullinated fibrinogen isotypes, IgM RF-positive (OD=0.18±0.12) and IgM RF-negative polyarthritis (OD=0.15±0.11) patients had higher levels of IgA anti-citrullinated α-enolase antibodies compared to controls (OD=0.09±0.05; $p<0.05$). No significant differences in levels of IgM anti-citrullinated α-enolase antibodies were noted between JIA subtypes and healthy children. None of the psoriatic arthritis or ERA patients were positive for IgM anti-citrullinated α-enolase antibodies. None of the healthy children were positive for IgM anti-citrullinated α-enolase antibodies, while one healthy child was positive for IgA anti-citrullinated α-enolase antibodies.

Combinations of positivity for anti-citrullinated α-enolase antibody isotypes were less diverse than for anti-citrullinated fibrinogen antibody isotypes. Eight JIA patients were positive for IgM only, 7 for IgG only, and 4 for IgA only. Two JIA patients were positive for both IgG and IgA anti-citrullinated α-enolase antibodies and one JIA patient was positive for both IgA and IgM anti-citrullinated α-enolase antibodies. One IgM RF-positive polyarthritis patient was positive for 7 autoantibodies, including IgA, IgG, and IgM anti-CCP antibodies, IgA and IgM RF, IgG anti-citrullinated fibrinogen antibodies, and IgG anti-citrullinated α-enolase antibodies.

Serological correlations

IgA anti-citrullinated α-enolase antibodies demonstrated a significant correlation with IgA anti-citrullinated fibrinogen antibodies (r=0.31, $p\leq0.001$),

IgG anti-citrullinated α-enolase antibodies (r=0.32, $p \leq 0.001$), IgA anti-CCP antibodies (r=0.33, $p \leq 0.001$), IgG anti-CCP antibodies (r=0.30, $p \leq 0.001$), and IgA RF (r=0.33, $p=0.001$). IgM anti-citrullinated α-enolase antibodies correlated significantly with IgM anti-citrullinated fibrinogen antibodies (r=0.34, $p \leq 0.001$).

Relationship between citrullinated autoantibody isotypes and IgG anti-CCP antibodies and IgM RF

Anti-citrullinated fibrinogen and anti-α-enolase antibody isotypes were frequently positive in JIA patients who were also positive for IgG anti-CCP antibodies and IgM RF (Fig. 1). The most common anti-citrullinated fibrinogen isotypes in IgG anti-CCP antibody positive JIA patients were IgG alone or the combination of IgG and IgA. Similarly, in JIA patients positive for IgM RF by ELISA, IgA and IgG alone or the combination of IgA and IgG anti-citrullinated fibrinogen antibodies were frequently positive. IgG anti-citrullinated α-enolase antibodies occurred more frequently than the IgA or IgM isotypes in JIA patients positive for IgG anti-CCP antibodies. IgM was the most common citrullinated α-enolase antibody isotype present in JIA patients positive for IgM RF by ELISA. JIA patients' positive for IgG anti-CCP antibodies demonstrated significantly increased levels of IgA anti-citrullinated α-enolase antibodies, IgG anti-citrullinated fibrinogen antibodies, IgM and IgA anti-CCP antibodies, and IgA and IgM RF (Table III).

A total of 13 JIA patients had 3 or more anti-citrullinated fibrinogen, α-enolase, or CCP antibody isotypes present, including 4 with IgM RF-positive polyarthritis, 8 with IgM RF-negative polyarthritis, and one with systemic-onset disease. JIA patients with 3 or more isotypes demonstrated significantly higher levels of IgG anti-CCP3 (90±113 U) antibodies and IgM RF (43±33U) compared to those with less than 3 isotypes present (15±59 U and 16±34 U, respectively; $p<0.05$) (Fig. 2).

Discussion

Anti-CCP antibodies are highly specific for RA and their presence clinically is

Table III. Differences in citrullinated autoantibody and serological marker levels in JIA patients positive for IgG anti-CCP antibodies antibodies compared to JIA patients negative for IgG anti-CCP

Variable	JIA IgG CCP Positive n=14	JIA IgG CCP Negative n=81	*p*-value
IgA anti-citrullinated α-enolase antibodies (OD)	0.22 ± 0.13	0.13 ± 0.08	0.024*
IgG anti-citrullinated α-enolase antibodies (OD)	0.72 ± 0.71	0.46 ± 0.47	0.085
IgM anti-citrullinated α-enolase antibodies (OD)	0.35 ± 0.34	0.45 ± 0.29	0.272
IgA anti-citrullinated fibrinogen antibodies (OD)	0.12 ± 0.09	0.08 ± 0.08	0.129
IgG anti-citrullinated fibrinogen antibodies (OD)	1.83 ± 1.17	0.52 ± 0.58	0.001*
IgM anti-citrullinated fibrinogen antibodies (OD)	0.67 ± 0.78	0.78 ± 0.61	0.566
IgA anti-CCP antibodies (OD)	0.38 ± 0.62	0.06 ± 0.10	0.001*
IgM anti-CCP antibodies (OD)	0.95 ± 1.3	0.35 ± 0.54	0.004*
IgA RF (U)	17 ± 20	2 ± 12	0.018*
IgM RF (U)	66 ± 56	12 ± 21	0.004*
CRP (mg/dl)	1.2 ± 2.0	1.6 ± 2.0	0.534
ESR (mm/hr)	25 ± 23	28 ± 31	0.809

CCP: cyclic citrullinated peptide; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; JIA: juvenile idiopathic arthritis; OD: optical density; RF: rheumatoid factor; U: units. Cut-off points for positive values: IgA anti-citrullinated α-enolase antibodies (OD≥0.20), IgG anti-citrullinated α-enolase antibodies (OD≥0.91), IgM anti-citrullinated α-enolase antibodies (OD≥0.73), IgA anti-citrullinated fibrinogen antibodies (OD≥0.09), IgG anti-citrullinated fibrinogen antibodies (OD≥0.59), IgM anti-citrullinated fibrinogen antibodies (OD≥1.7), IgA anti-CCP antibodies (OD≥0.16), IgM anti-CCP antibodies (OD≥0.43), IgG anti-CCP antibodies, (≥20U), IgA RF (≥6U), IgM RF (≥6U), CRP (≥0.8mg/dl), ESR (≥15mm/hr). *p*-values were considered statistically significant at <0.05. *indicates a statistically significant difference.

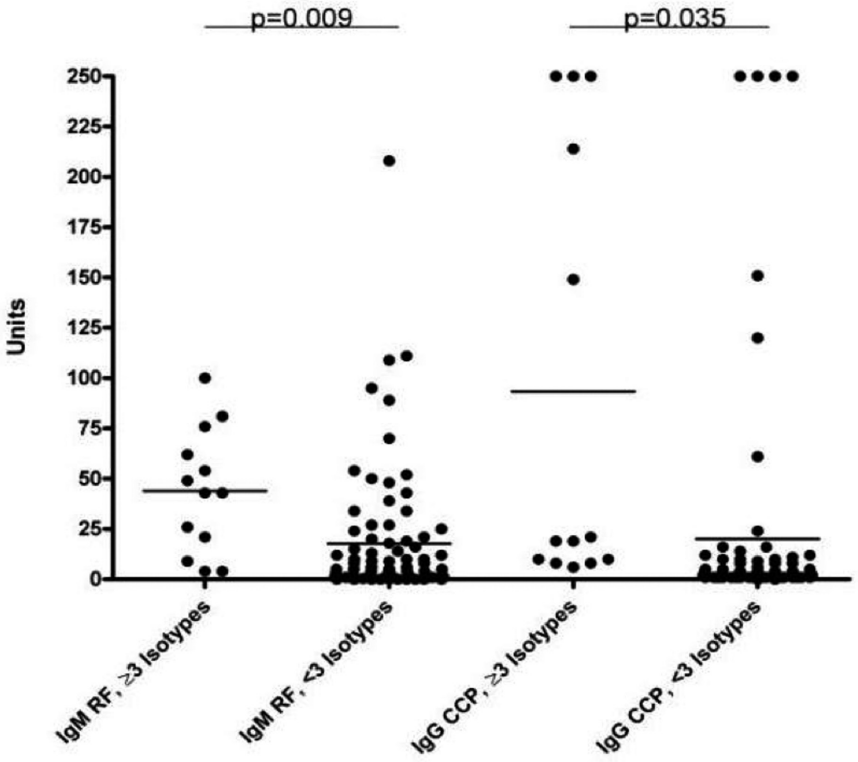

Fig. 2. IgM RF and IgG anti-CCP antibody levels based on the presence of ≥3 anti-citrullinated fibrinogen, α-enolase, CCP (IgA and IgM) antibody isotypes (n=13) or <3 anti-citrullinated fibrinogen, α-enolase, CCP (IgA and IgM) antibody isotypes (n=82). Bars indicate the median levels of IgG anti-CCP antibodies and IgM RF in each group. *p*-values were considered statistically significant at <0.05. CCP:cyclic citrullinated peptide; RF: rheumatoid factor.

indicative of progression to erosive disease (15). Further studies have shown that anti-CCP antibodies are highly polyclonal in terms of epitope specificity and isotype usage ((16, 17). Longitudinal studies in RA patients have documented epitope spreading and all three isotypes IgG, IgA, and IgM have been detected (18). Their role in JIA remains undefined, but are commonly found in IgM RF-positive polyarticular JIA patients, the subtype most closely resembling adult RA (19-21). Anti-CCP antibody isotype usage has been studied in both RA and JIA, with more diverse isotype usage reflecting aggressive disease with a risk for future radiographic progression (7, 8, 11). Further, studies in RA and JIA have identified target proteins for citrullination, including fibrinogen and α-enolase (9, 10). A recent study showed that anti-homocitrullinated fibrinogen antibodies were specific for RA (22). They correlated in adults with RA with anti-CCP antibodies and concluded they may bind to the same shared epitope (22). In this study, we investigated the presence of isotypes of anti-citrullinated fibrinogen and α-enolase antibodies in JIA patients to further define their presence and whether they confer a more severe disease course.

The most commonly observed citrullinated isotypes were IgG and IgA anti-citrullinated fibrinogen antibodies (32% and 23%, respectively), and both were significantly elevated in IgM RF-positive polyarthritis patients compared to healthy children (10). In IgG anti-CCP antibody positive JIA patients, the most common pattern of citrullinated fibrinogen positivity was IgG alone (50%) or the combination of IgG and IgA isotypes (29%). In our previous study, we showed that IgG anti-citrullinated fibrinogen antibodies correlated significantly with IgG anti-CCP antibodies, IgM RF, and IgA RF [10]. IgG anti-citrullinated fibrinogen antibodies also demonstrated a relatively high sensitivity for IgM RF-positive polyarthritis (81.3%) (10).

Anti-citrullinated α-enolase antibody isotypes were less prevalent and diverse in JIA patients compared to anti-citrullinated fibrinogen antibody isotypes. However, several significant correlations were made with disease markers associated with joint damage and worsened disease. In a study of adult RA patients, 46% reacted with IgG citrullinated α-enolase in sera and α-enolase was also detected in the joint (19). No data is currently available on IgA and IgM anti-citrullinated α-enolase antibodies in adult RA, but our previous study confirms that IgG anti-citrullinated α-enolase antibodies are less prevalent in JIA than what has been reported in RA (10, 23).

The presence of 3 or more anti-citrullinated fibrinogen or α-enolase antibody isotypes was seen in 12 polyarthritis patients, and showed a significant association with higher levels of IgG anti-CCP antibodies and IgM RF ($p<0.05$). None of the oligoarthritis, disease controls, or normal healthy patients showed more than one antibody isotype. Additionally, IgA anti-citrullinated α-enolase antibodies, IgG anti-citrullinated fibrinogen antibodies, and IgA and IgM anti-CCP antibodies were significantly elevated in JIA patients who were also positive for IgG anti-CCP antibodies. Both IgG anti-CCP antibodies and IgM RF are known to be associated with radiographic progression (15, 16), indicating that isotype diversity may play a role in the disease course in JIA patients. The clinical relevance of the presence of the production of IgA and/or IgM anti-citrullinated fibrinogen and anti-enolase antibodies is being evaluated longitudinally to see if these patients have more long term severe disease.

We have shown that isotypes of anti-citrullinated fibrinogen and α-enolase can be found in the serum of children with various subtypes of JIA. While no direct correlation was made between the anti-citrullinated antibody isotypes and joint damage, data suggested that the presence of anti-citrullinated fibrinogen and α-enolase antibody isotypes may be associated with a more severe disease course, including radiographic progression. We have now demonstrated the presence of anti-citrullinated fibrinogen and α-enolase antibody isotypes in various subtypes of JIA. This adds to our previous findings that showed JIA patient sera strongly reacted to anti-citrullinated fibrinogen antibodies, mainly in IgM-RF-positive polyarticular patients (10) and, also, in our recent studies that demonstrated antibodies to anti-citrullinated type II collagen, and anti-citrullinated vimentin antibodies in JIA patients (24). We have now demonstrated that multiple citrullinated epitopes are present in the sera of patients with JIA. A higher percentage of antibodies have reacted with citrullinated fibrinogen and citrullinated type II collagen (10, 24). The studies indicate more widespread the reactivity the more severe disease.

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