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Patients with down syndrome have increased prevalence of rheumatoid factor but not autoantibodies to anti-cyclic citrullinated peptide

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A B S T R A C T

The association between Down syndrome (DS), a genetic disorder resulting from trisomy of the 21st chromosome, and the autoantibodies of rheumatoid arthritis (RA) has been proposed but not unequivocally proven. The aim of this study was to determine whether adult patients with DS present higher levels of anti-cyclic citrullinated peptide (anti-CCP) antibodies and/or rheumatoid factor (RF) than the general population. Our results showed that none of the 68 patients with DS had anti-CCP antibodies, whereas among 204 age- and sex-matched controls these autoantibodies were present in one person. However, DS patients presented a higher number of RF positive cases than controls (11.7% to 3.2% respectively; Fisher's exact test, $p = .027$). The higher number of RF positive cases in the DS group without increase of anti-CCP antibodies may be indicative of immune disturbances in general rather than RA in these patients. Our study supports the view that RA does not occur with higher frequency in patients with DS than in the general population.

1. Introduction

Down syndrome (DS) is a genetic disorder that results from trisomy of the 21st chromosome [1]. Aside from impaired development of the nervous system and predisposition for a variety of disorders, such as obesity and periodontal disease, autoimmune diseases have also been shown to have higher prevalence among patients with DS [2–6]. The most common autoimmune diseases associated with DS are thyroid disease, celiac disease, and type 1 diabetes; the co-occurrence of rheumatoid arthritis (RA) has also been reported but not yet entirely validated [7–11].

Rheumatoid arthritis is a chronic autoimmune disease characterized by progressive inflammation, swelling, and pain in the joints, but is sometimes difficult to recognize at an early stage [12]. The prevalence of RA is estimated to range between 0.2%–1%, making it one of the most common autoimmune diseases [13]. It is characterized by the appearance of mainly two types of antibodies: disease-specific anti-cyclic citrullinated peptide (anti-CCP) antibodies and less-specific rheumatoid factor (RF). The latter reacts with the Fc region of IgG and is also detected in other diseases besides RA. Both antibodies may be detected by laboratory tests years before the onset of clinical manifestations of the disease [12].

The aim of the current study is to evaluate the presence of anti-CCP antibodies and RF in the sera of DS patients to determine whether they are more prone to RA compared to the general population.

Abbreviations: DS, Down syndrome; RA, Rheumatoid arthritis; RF, Rheumatoid factor; Anti-CCP, Anti-cyclic citrullinated peptide (antibodies)

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Table 1
Characteristics of the Down syndrome (DS) and control groups tested for IgG anti-CCP and IgM RF (DS patients with mosaicism - numbers in brackets).

Characteristics	Patients with DS	Controls for IgG anti-CCP	Controls for IgM RF
Studied (n)	68	204	125
Males (n)	43 (3)	129	75
Females (n)	25 (1)	75*	50**
Median age in years (IQR)	32.0 (24.8–42.0)	32.5 (24.8–41.0) ***	29.0 (24.0–40.0) ****

IQR – interquartile range.

* Non-significant difference between male/female ratios in DS and control group used for IgG anti-CCP studies ($P = 1.00$; Chi-square test).

** Non-significant difference between male/female ratios in DS and control group used for IgM RF studies ($P = .65$; Chi-square test).

*** Non-significant difference between age distribution in DS and control group used for IgG anti-CCP studies ($P = .79$; Mann-Whitney U test).

**** Non-significant difference between age distribution in DS and control group used for IgM RF studies ($P = .28$; Mann-Whitney U test).

2. Materials and methods

2.1. Patients and control group

This study included 68 adult patients (43 males) with DS (see Table 1 for detailed characteristics) and controls (described below). The patients with DS had been diagnosed in one of two medical genetic centers in Estonia and blood samples were obtained during site visits to patients' homes or nursing-homes or during their visits to the Medical Genetic Center of the University of Tartu. The diagnosis of DS was confirmed by cytogenetic analysis according to the International System for Human Cytogenetic Nomenclature as described elsewhere [14]. Of the 68 patients with DS, 64 had regular trisomy 21 and 4 had either translocation or mosaicism. None of the patients had been diagnosed with RA at the time of the study.

Two control groups were used. For IgG anti-CCP antibody studies we used a population of 204 persons (129 males) without any autoimmune or other immunological diseases, selected from the population sample collected by the Estonian Biobank (Estonian Genome Center, University of Tartu). This control group has been used for autoantibody prevalence studies among healthy persons [15]. These controls, selected for the present study, were matched for gender and age to the DS patients resulting in three controls per one DS patient (Table 1).

For IgM RF controls only 33 samples from the Estonian Biobank were available. Thus we supplemented this group with blood samples from 92 healthy volunteers (laboratory personnel, students; Table 1) to get a relevant age-and sex matched control group. Both EDTA-plasma and sera were used. All samples were stored at −20 °C or below.

The study was approved by the Ethics Review Committee on Human Research of the University of Tartu. All studied patients or their guardians and control persons have given informed consent for the study.

2.2. Antibody assays

Antibody testing for IgG anti-CCP was performed with ImmunoCAP EliA IgG (Thermo-Fisher Scientific, Phadia GmbH, Freiburg, Germany) on the ImmunoCap 100 instrument. The EliA CCP wells were coated with citrullinated synthetic peptides (second generation antigen). Values above 10 U/ml were considered positive, as recommended by the manufacturer. The EliA method was used to assay IgM RF with wells coated with aggregated rabbit IgG antigen in which the positive result was considered greater than or equal to 5.0 IU/ml. In RA the anti-CCP and RF tests perform on EliA platform with sensitivity of 80.3% and 58.0%, and at the specificity 97.0% and 91.6%, respectively (data of Thermo-Fisher Scientific, Phadia GmbH).

2.3. Statistical analyses

The R 3.1.2 language and environment (Free Software Foundation, Boston, MA) and GraphPad Prism 5 (GraphPad software, La Jolla, CA) software were used for statistical analysis and figure preparation, respectively. P values below 0.05 were considered statistically relevant.

3. Results

All subjects in the DS group tested negative for IgG anti-CCP antibodies, and there was one positive case in the control group (no significant difference between groups, $p > .05$). However, median levels of IgG anti-CCP antibody were significantly lower in DS patients (1.90 U/ml) than in controls (2.2 U/ml) (Mann-Whitney's U test, $p = .002$; Fig. 1A).

Eight of the DS patients (11.7%, CI 95%: 5.6–22.4) tested positive for IgM RF compared to four positive cases among the controls (3.2%, 95% CI: 1.0–8.5). This represents a statistically relevant difference between the two groups (Fisher's exact test, $p = .027$). The median level of IgM RF was 0.85 IU/ml (range 0.0–67.0) in the DS group and 1.0 IU/ml (range 0.0–43.0) in the control group (no significant difference; Mann-Whitney's U test, $p = .274$; Fig. 1B). The control person with IgG anti-CCP was IgM RF negative. There were no differences in the prevalence of any antibodies between the DS patients with regular trisomy 21 and with translocation/mosaicism (data not shown).

4. Discussion

We found that IgG anti-CCP antibodies were absent in all studied adult patients with DS. However, a statistically relevant increase in the frequency of IgM RF antibodies was observed in DS compared to controls. Previous studies on anti-CCP antibody occurrence among patients with DS have shown variable results. In 2007, Nisihara et al. [16] described a high prevalence (52.3%) of anti-CCP antibodies among 86 DS patients – a result which does not align with the findings of the present study. On the other hand, in a more recent study Berthelot et al. [17] observed a much lower prevalence of anti-CCP in DS patients, reporting

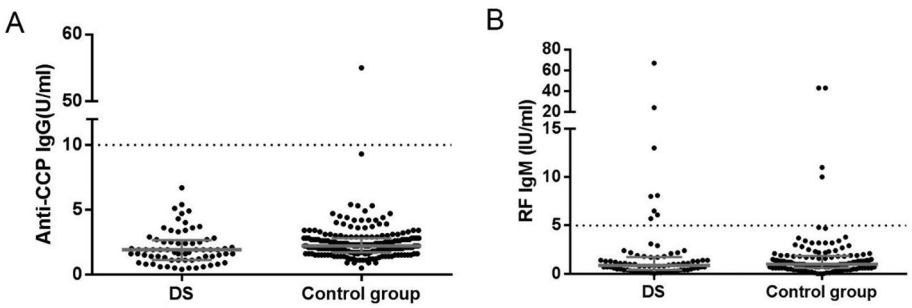

Fig. 1. IgG anti-CCP (A) and IgM RF levels (B) of the individuals in the DS and control group. The grey solid lines represent median and interquartile ranges and the dotted lines the cutoff value for both groups.

only 9.9% of positive cases among 121 subjects. Finally, Fisher et al. observed no anti-CCP positivity among 104 patients with DS, which is in good concordance with our results [18].

We found that IgM RF is more common in patients with DS than in age-matched individuals of the general population. This is in agreement with the results of Nisihara et al. (2007) who described 42 (28%) RF positive cases in 150 DS children [16]. A possible explanation for higher prevalence of IgM RF among DS might be the premature senescence of the immune system due to immune dysregulation as suggested by Utiyama et al. [20].

Possible explanations for the variability in the aforementioned anti-CCP results may include methods used for antibody assay and/or the sample group of patients. As it turns out, different research groups have used dissimilar assays. Nisihara et al. used the second generation enzyme-linked immunosorbent assay kit (INOVA Diagnostics, San Diego, CA, USA) to determine anti-CCP antibodies [16] and got higher test positivity compared to Berthelot et al. [17] and Fisher et al. [18] who used an anti-CCP2 assay (Eurodiagnostica Immunoscan CCPlus, Antony, France). We, however, used the ImmunoCAP assay of Phadia GmbH (Freiburg, Germany). Compared with the other anti-CCP assays, ImmunoCAP has similar or higher sensitivity and specificity [19].

The strength of this study is that both antibody assays, commonly used in RA, were performed by the same platform (ImmunoCAP) using the IgM RF and second generation IgG anti-CCP antibodies, which have been shown to offer results that are more credible compared to earlier versions of these assays [19,21]. Since blood samples were collected at various locations, this study offers useful material for future studies into autoimmune diseases in Estonia. Another strength of the present study is that we were able to assemble an age- and gender-matched control group sized 3:1 for the anti-CCP analyses. Unfortunately, we were unable to do so in IgM RF studies due to shortage of serum samples after performing ant-CCP assays, which we consider the main weakness of our study. Despite this limitation we were still able to match groups by age and gender.

5. Conclusion

Here we report that DS patients show an absence of RA-specific anti-CCP antibodies and an increased prevalence of RF. The results of this serological study seem to refute statements that RA is more common among DS patients than in the general population. The co-existence of RF antibodies without the clinical manifestations of RA is a matter which ought to be examined more thoroughly in the future, taking into account the peculiarities of humoral immunity development in DS.

Conflicts of interests

All authors stated that there are no conflicts of interest regarding the publication of this article.

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