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Rady Faculty of Health Sciences

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End of Term Final Report

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Project Title: Functional Disability to Evaluate the Risk of Arthritis in First-degree Relatives of Patients With Rheumatoid Arthritis

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Summary (250 words max single spaced):

Objective: The events that occur prior to the onset of rheumatoid arthritis (RA) continue to be delineated. We examined the relationship between self-reported joint symptoms, functional disability, and anticitrullinated protein antibody (ACPA) status in a cohort of first-degree relatives (FDR) of patients with RA who are at risk of future disease development.

Methods: We studied a cohort of 607 FDR of First Nations (FN) patients with RA who are at increased risk for future RA development, and analyzed data collected at their enrollment study visit. In parallel, we analyzed data from 279 FN participants with no family history of RA. A subset of FDR developed inflammatory arthritis and we analyzed longitudinal data in this group.

Results: The prevalence of joint symptoms and functional disability was higher in FDR compared to non-FDR (all $P < 0.001$). Difficulty walking (37.3% vs 18.0%) and modified Health Assessment Questionnaire (mHAQ) results were higher in ACPA-positive FDR compared to ACPA-negative FDR, and mHAQ was independently associated with ACPA seropositivity (OR 2.79, 95% CI 1.56 -5.00). Longitudinally, in individuals who developed ACPA-positive RA, ACPA level and mHAQ score were significantly associated ($R = 0.45$, $P < 0.001$) in the preclinical period.

Conclusion: Compared to population-based controls, FDR have a high burden of joint symptoms and functional disability. Functional disability was most closely associated with ACPA seropositivity in the FDR, suggesting a direct role for ACPA outside of the context of clinically detectable synovitis. HAQ appears to be particularly valuable in the assessment of individuals at risk for future RA development.

Student Signature

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Introduction

Rheumatoid arthritis (RA) is a chronic, progressive autoimmune disease that primarily targets the synovial joints. If not treated effectively early in the disease course, RA causes irreversible articular damage leading to disability and functional decline.[1] It is now well established that prior to the onset of clinically detectable RA (pre-clinical RA), most individuals develop autoantibodies to citrullinated proteins (ACPA), along with a spectrum of other autoantibodies.[2] Individuals with RA associated autoantibodies may exhibit symptoms that are typical of established disease, such as joint pain (arthralgia), tenderness and stiffness. Thus, a key clinical research strategy has emerged where individuals with RA-associated autoantibodies and suggestive symptoms such as arthralgia are recruited into structured cohorts and followed longitudinally for onset of clinically detectable synovitis. Much has been learned about the preclinical stages of RA from cohort studies of at-risk individuals who present with clinically suspect arthralgia, with or without detectable RA autoantibodies.[1,3-6] While these two key clinical domains are often the basis of referral to specialized centres, the relationship between the two domains remains poorly understood. Whether symptom onset is representative of a key biological event that stratifies individuals at the highest risk to develop inflammatory arthritis remains unknown. Furthermore, exactly how to evaluate individuals with pre-clinical RA symptoms has not been clearly defined.[7]

Based on a model where human ACPA are introduced into a mouse, it has been suggested that the ACPA may have a direct role in generating pain.[8] It is proposed that ACPA activate cellular mechanisms that drive chemokines to modulate nociception.[9] Moreover, other models have shown that immune complex-mediated activation of neurons facilitates mechanical hypersensitivity.[10] Indeed, it was recently shown in an at-risk FDR cohort that individuals with ACPA displayed enhanced symmetrical joint pain.[11] Therefore, if pathogenic ACPA lead to enhanced pain, it might provide a biological explanation for pre-clinical symptomatology in individuals who develop inflammatory arthritis.

To better understand the interplay between symptomatology and autoantibody development in pre-clinical RA, we undertook a prospective longitudinal study of unaffected First Nations (FN) first-degree relatives (FDR) of RA patients, a population with a strong predilection to develop RA due, in part, to high prevalence of HLA-DRB1 risk alleles. This genetic risk-variant has been shown to closely associate with the development of ACPA.[12,13] Given this, we sought to understand the interaction between self-reported symptomatology, functional disability and ACPA seropositivity, and our hypothesis was that these variables would be associative in individuals at high risk for RA development.

Methods

Cohort recruitment

Methods and protocols for patient recruitment into this study were described in our group's previously published work for the cohort of First Degree Relatives (FDR).[14] In brief, First Nations probands with RA who met the 2010 ACR/EULAR criteria[15] were asked to help recruit their eligible FDR for longitudinal follow up. Patients with RA (probands) and FDR were required to have at least 3 grandparents with First Nations ethnicity by self-report and be age 18 or older. The cohort has been described in previous publications, and participants were followed for an average of approximately 5 years.[5] Non-FDR were recruited from a separate study investigating environmental risk factors associated with RA autoantibodies.

Ethics

All study participants provided informed consent in accordance with the Declaration of Helsinki. The Biomedical Research Ethics Board of the University of Manitoba approved all aspects of the study (Board approval number HS14453). Specific community research agreements were put in place with the study communities, and we established an arthritis advisory board to provide First Nations peoples oversight for the study.

Participants entered the longitudinal study and underwent annual examination for the presence of clinical synovitis. At inception, all FDR were examined by a rheumatologist to confirm the absence of inflammatory arthritis and 44 tender and swollen joint counts were recorded using a homunculus. Clinical assessments for new symptoms that were triggered by participants occurred as soon as possible. If synovitis was detected in one or more joints, the participant was deemed to have progressed into inflammatory arthritis (IA). ACPA were detected using cyclic citrullinated peptide-2 or cyclic citrullinated peptide-3 ELISA using the manufacturer's cut-off. A second cohort recruited for an alternate but related study was used as a non-FDR comparator group. Of note, all non-FDR included in this study were recruited from rural locations.

Survey

Demographics, body mass index (BMI) and smoking history were recorded at the inception visit. A survey was completed by each participant which included a modified health assessment questionnaire (mHAQ), along with other questions on daily function. Three visual analog scales were completed on fatigue, patient global health and pain. An arthritis symptom survey was also used to identify participant experiences with symptoms that are commonly observed in RA, including joint pain, stiffness and swelling.

Statistical Analysis

Data from each participant's inception survey was extracted and loaded into R for the statistical analysis. Missing data was imputed using multiple imputation by chained equations (MICE).[16] We observed very few responses to the ordinal scale that were greater than 1; therefore, we converted answers to binary[17], using a logical cut-off of yes (some difficulty, much difficulty or unable to do) and no (performed without any difficulty). Inception data analysis are presented as either continuous (median with IQR) or as a proportion (percentage, %). Chi-square test was used to analyze binary outcomes, while Wilcoxon signed-rank test was used for continuous outcomes, and within each survey we corrected for multiple comparisons using the Benjamini-Hochberg method. Relationships between continuous variables were analyzed using Spearman's correlation. A correlation matrix was constructed to analyze the relationship within patient reported symptoms using Spearman's correlation corrected by Bonferroni. Logistic regression models were constructed to identify independent variables that associated with ACPA seropositivity. mHAQ, the variable of interest, and it's association with ACPA was controlled for sex, age and location (rural/urban). Our results suggested that functional disability, specifically difficulty walking on flat ground, was enriched in ACPA seropositive FDR. To better understand why some FDR report difficulty walking on flat ground, we extracted clinical joint exams from all individuals who reported difficulty with walking (n = 118) and a cohort of FDR without difficulty walking, matched by propensity score derived on age, sex, BMI and location (rural/urban).

Although ACPA seropositivity is the best predictor for the development of future RA, we have previously shown in our prospective cohort of at-risk FDR that individuals who are ACPA positive commonly seroconvert to ACPA negative when followed over time[5]. For our longitudinal analysis, we filtered out participants who only had a single visit or a single ACPA measurement (missing ACPA levels were excluded from the analysis and not imputed). We then surveyed longitudinal serological data and classified each participant as: (1) persistently ACPA negative or ACPA seroconverted, where the participant was ACPA positive, but on subsequent visits became ACPA negative and remained so until their last visit, (2) persistently ACPA positive, or (3) developed ACPA-positive RA. All graphs were generated in R using the package ggplot2. For all statistical tests, an adjusted p-value of < 0.05 was considered statistically significant.

Results

Self-reported joint symptoms and functional disability are more prevalent in FDR of RA patients compared to non-FDR.

We used survey data generated from two distinct FN cohorts, one cohort of RA proband FDR (n = 607) and another non-FDR cohort (n = 279) to identify specific symptoms enriched in those at future risk of RA development. There were no significant differences in sex between the two cohorts, but the FDR cohort was slightly older (35.1 vs 31.8 years, $p = 0.003$, Table 1). We found that all self-reported joint symptoms were significantly increased in FDRs (Figure 1A). A wide range of functional disability based on the mHAQ questionnaire also revealed clear differences between the two cohorts (Figure 1B). Overall, the mean mHAQ score was higher in the FDR cohort (0.22 vs 0.09, $p < 0.0001$, Figure 1C), suggesting higher global functional disability. Given the inherent sex bias of RA, we stratified our analysis based on sex, to better determine if our observations were consistent across both males and female sex. Indeed, we observed substantial differences in functional disability in FDR compared to non-FDR when we restricted the analysis to either males or females alone. Using a correlation matrix (Figure 1D), we found that the self-reported joint symptoms correlated only modestly with the measures of functional disability, despite a high degree of correlation internally within each of these types of measures.

ACPA positive FDR report increased functional disability compared to ACPA negative FDR.

ACPA seropositivity remains the most important predictor of imminent inflammatory arthritis[5,18] and, based on animal models and in vitro studies, may itself lead to pain.[9,10] Given that our at-risk cohort of FDR reported a high prevalence of joint symptoms and functional disability as a group, we next sought to determine if ACPA seropositivity, a marker of enhanced risk for imminent RA, was associated with any specific participant reported experience. In total, 51 (8.4%) participants in our cohort were ACPA seropositive at their inception visit and characteristics were similar between the two groups. Interestingly, we found that self-reported joint symptoms were not more prevalent in ACPA seropositive FDR compared to seronegative. In contrast to joint symptoms, we observed clear differences in functional disability in the ACPA seropositive FDRs. Indeed, all the responses to the mHAQ questions were higher in seropositive individuals, with difficulty walking on flat ground (37.3% vs 18.0%, $p = 0.01$) reaching statistical significance (Figure 2A). Mean mHAQ score was also higher in ACPA seropositive individuals (0.38 vs 0.21, $p = 0.03$, Figure 2B). We used logistic regression to assess if mHAQ score was associated with ACPA seropositivity, and found that after controlling for baseline variables, mHAQ was independently associated with ACPA seropositivity (OR = 2.79, 1.56 to 5.00, Figure 2C).

Given that difficulty walking on flat ground is more prevalent in ACPA seropositive FDR, we next sought to understand clinical factors that associate with functional disability. Based on clinical exam, FDR who reported difficulty walking had increased joint tenderness to the lower extremity (42.4% vs 21.2%, $p = 0.02$, Figure 3A, B). Analysis of the remaining joint locations suggested diffuse joint tenderness elicited on clinical exam, including in the upper extremity, amongst those who endorsed difficulty walking (Figure 3C). There was a strong association between total tender joints and mHAQ score ($R = 0.47$, Figure 3 D), indicating that joint pain may be one of many variables that leads to reduced functional capacity.

Functional disability at inception is associated with longitudinal risk of arthritis in FDR.
To gain further insight into the relevance of functional disability in assessing those at-risk to develop RA, we next explored longitudinal outcomes in our FDR cohort. In total, we were able to classify 325 individuals based on their serological and clinical status with sufficient longitudinal follow-up (Table 2). Median follow-up time was 61 (IQR 54) months. 19 (5.8%) individuals developed ACPA-positive RA, 22 (6.8%) were persistently ACPA seropositive, 59 (18.2%) had transient ACPA that seroconverted after follow-up and 225 (69%) were persistently ACPA negative (Table 2), totalling 284 FDR (87.4%) that were longitudinally classified as ACPA negative. Inception mHAQ trended higher in FDR with persistent ACPA positive (0.22) and ACPA-positive RA (0.26) compared to ACPA negative (0.19). There were also trends towards increased difficulty with several specific activities of daily living in individuals who were ACPA positive or developed ACPA positive RA. For example, the prevalence of difficulty lifting a glass increased in a stepwise manner across our outcomes: 6.3% ACPA negative, 13.6% ACPA positive and 15.8% ACPA positive RA ($p = 0.06$, Figure 4A). A similar trend was observed in those with difficulty walking on flat ground, which was more prevalent in persistent ACPA positive individuals compared to ACPA negative (36.4% vs 15.5%, $p = 0.02$). Consistent with our previous analysis, self-reported joint symptoms were not associated with longitudinal outcomes.

Next, we isolated longitudinal data from 19 ACPA positive RA individuals who were followed into arthritis onset (progressor). We plotted mHAQ scores over sequential visits as individuals progressed and found that mHAQ was stable longitudinally, up until the point of progression into inflammatory arthritis (Figure 4B). Interestingly, we found that this pattern was symmetric with longitudinal ACPA levels (Figure 4B). Indeed, in our 19 Progressors, there was a positive correlation ($R = 0.43$, $p < 0.001$, Figure 4C) between mHAQ and ACPA as individuals were followed into disease onset.

Discussion
Accumulating evidence from both prospective and retrospective cohort studies of individuals who ultimately developed RA have provided important insights into the biological and immunological events that precede RA onset.[1-3,5,19-21] It is now unequivocal that specific autoantibodies directed towards post-translationally modified proteins are detectable during the preclinical phase for most patients who develop seropositive RA.[2,3] These autoantibodies, particularly ACPA, remain the single most important predictor of future RA development in otherwise unaffected individuals.[5,22,23] It is also well established that a spectrum of newly developed and evolving symptoms that include arthralgia, muscle weakness, paresthesia, and fatigue is often reported in the months immediately preceding the onset of clinically detectable joint inflammation.[24] The best described of these is what has been termed clinically suspect arthralgia (CSA), a poorly defined

symptom complex that incorporates elements of pain, stiffness, and subjective swelling in specific joints and joint areas.[25,26] To date, the relationship between ACPA and self-reported symptoms such as CSA is incompletely understood, particularly since most published data have been from cohorts of individuals who were included based on exhibiting either RA autoantibodies, CSA, or both. In the current study we prospectively evaluated this relationship in cohorts of individuals who had varying degrees of risk for future RA development based on family history, patterns of ACPA evolution over time, and ultimately the development of inflammatory arthritis. Our results indicate that the symptoms of joint pain, stiffness, and subjective joint swelling were common and non-specific, but that self-reported measures of functional limitations, such as those identified using the mHAQ questionnaire, were more specific and predictive of ACPA persistence and future RA. These results have implications for the design of future studies of the preclinical phase of RA.

A direct association between ACPA and joint pain, outside the known context of seropositive inflammatory arthritis, is supported by animal models demonstrating that human ACPA uniquely induce pain behaviour in mice. This process is proposed to be mediated by the nociceptive effects of CXCL1, which is produced by osteoclasts that are directly activated by ACPA. Thus, aspects of preclinical joint symptomatology[26-28], may be directly mediated by the ACPA, despite the absence of detectable synovial inflammation. In the current study, we attempted to address this hypothesis in a longitudinal cohort of at-risk FDR of RA patients that was not selected on the basis of either the detection of ACPA or the presence of joint symptoms. We showed that ACPA positivity, rather than being associated with a higher prevalence of joint symptoms, is more closely related to specific functional limitations such as difficulty walking on flat ground. These self-reported functional parameters performed better as prognostic indicators of future inflammatory arthritis and ACPA persistence than did direct questioning of the presence of joint symptoms. These findings are also consistent with an emerging hypothesis that synovitis in RA may be detectable earliest in the feet[29] and is strongly associated progression to RA in ACPA positive individuals. Our data also suggests that self-reported functional disability may be indicative of subclinical joint inflammation, which is detectable as joint tenderness on clinical exam. Given the well-recognized risk of RA associated with ACPA seropositivity, our data promotes the use of functional questionnaires to assess RA risk, above other tools such as self-reported joint symptoms.

We observed large differences in self-reported symptoms, both joint-specific and functional, between FDR and non-FDR. We also found that there was only a modest correlation between joint symptoms and functional disability, suggesting that these domains may capture unique aspects of pre-clinical symptomatology. Indeed, others have reported high rates of regional pain in FDR of RA patients.[7,30] FDR may have heightened self-awareness of RA symptoms due to their exposure to family members who have arthritis. Further, the FDR cohort was slightly older than non-FDR, which may explain increased self-reported pain. The psychological impact of the perceived increased risk of RA development in FDR[31], and how this modulates the pain experience, requires further study. Recently, results from an at-risk cohort of FDR suggested that ACPA is associated with heightened symmetrical joint pain using the Symptoms in Persons At Risk or Rheumatoid Arthritis (SPARRA) questionnaire.[11] Notably, we did not find an association between ACPA and joint pain by self-report in this study. It is important to note the differences between this cohort study and our own. The prevalence of joint pain amongst FDR in our cohort is greater than 50%, while in the SPARRA study only 17.9% of FDR reported joint pain. Our cohort is entirely First Nations, compared to the SPARRA cohort which is more than 90% Caucasian. Further, most of

our cohort are active cigarette smokers, which has complex interaction with chronic pain.[32] Unfortunately, this study did not report associations between functional status and seropositivity. Contrasting our results with this recent, highly relevant study is of great importance to improving our understanding of pre-clinical symptomatology across different at-risk populations.

While our patient reported survey provided a robust dataset of pre-clinical RA symptoms, there are important limitations to consider. Most of the studied variables collected are by self-report, which introduces recall bias.[33] Joint tenderness, though more prevalent in those with difficulty walking, is a non-specific measure of subclinical joint activity. Though firm conclusions cannot be drawn to this association, there are clearly individuals who experience difficulty walking without objective joint tenderness on examination. This suggests that there are other potential unmeasured variables that lead to difficulty walking, including non-joint related pain and muscle weakness. Imputation of data can lead to misleading results and bias, particularly if data are not missing at random.[34] It is important to note that individuals without any survey data were excluded from our analysis. Finally, the relatively low number of Progressors in our cohort (n = 19) reduces the statistical power and generalizability of our observations.

In conclusion, we present evidence that functional disability is associated with inception and longitudinal outcomes in an at-risk FDR cohort. Further, we provide the first human evidence that ACPA is associated with symptomatology in the absence of inflammatory arthritis. Joint symptoms and tenderness on clinical exam, despite being highly prevalent in FDR, were not associated with ACPA seropositivity or the development of inflammatory arthritis. Overall, these data provide clear evidence that functional disability, specifically difficulty walking, is representative of pre-clinical RA symptomatology and may provide important insights into individuals at the highest risk to develop clinical synovitis.

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Table 1. Inception demographic of two recruited cohorts.

	FN FDR (n = 607)	**FN non-FDR (n = 279)**
Sex (Female), %	60.1	52.4
Age	35.1 (20.9)	31.8 (17.6)
Smoker, %	85.8	88.5%
Pack Years	3.5 (11.8)	5 (11.8)
BMI	28.7 (9.1)	30.4 (9.8)

Continuous variables are reported as median (IQR), while proportional variables are reported as %. Age was significantly higher in the FDR cohort (p = 0.003). BMI: Body mass index.

Table 2. Inception demographics of FDR cohort with longitudinal follow up.

	Longitudinal Outcome		
	ACPA negative (n = 284)	**Persistent ACPA positive (n = 22)**	**ACPA positive RA (n = 19)**
Age	34.4 (20.8)	33.3 (20.2)	27.5 (11.2)
Sex (Female), %	66.2	68.2	73.7
Rural, %	65.1	59.1	63.2
Months follow-up	61 (57)	52.5 (31.4)	87 (63.5)
Smoker, %	83.5	86.4	94.7
Pack Years	3.5 (10.8)	3.0 (6.6)	3.0 (10.9)
BMI	29.6 (9.3)	28.5 (9.4)	25.6 (11.3)
ACPA +, %	6.0	31.8	57.9
mHAQ	0.19 (0.36)	0.22 (0.35)	0.26 (0.37)

Continuous variables are reported as median (IQR), while proportional variables are reported as %. BMI: Body mass index. ACPA: Anti-citrullinated protein antibody. mHAQ: modified Health Assessment Questionnaire.

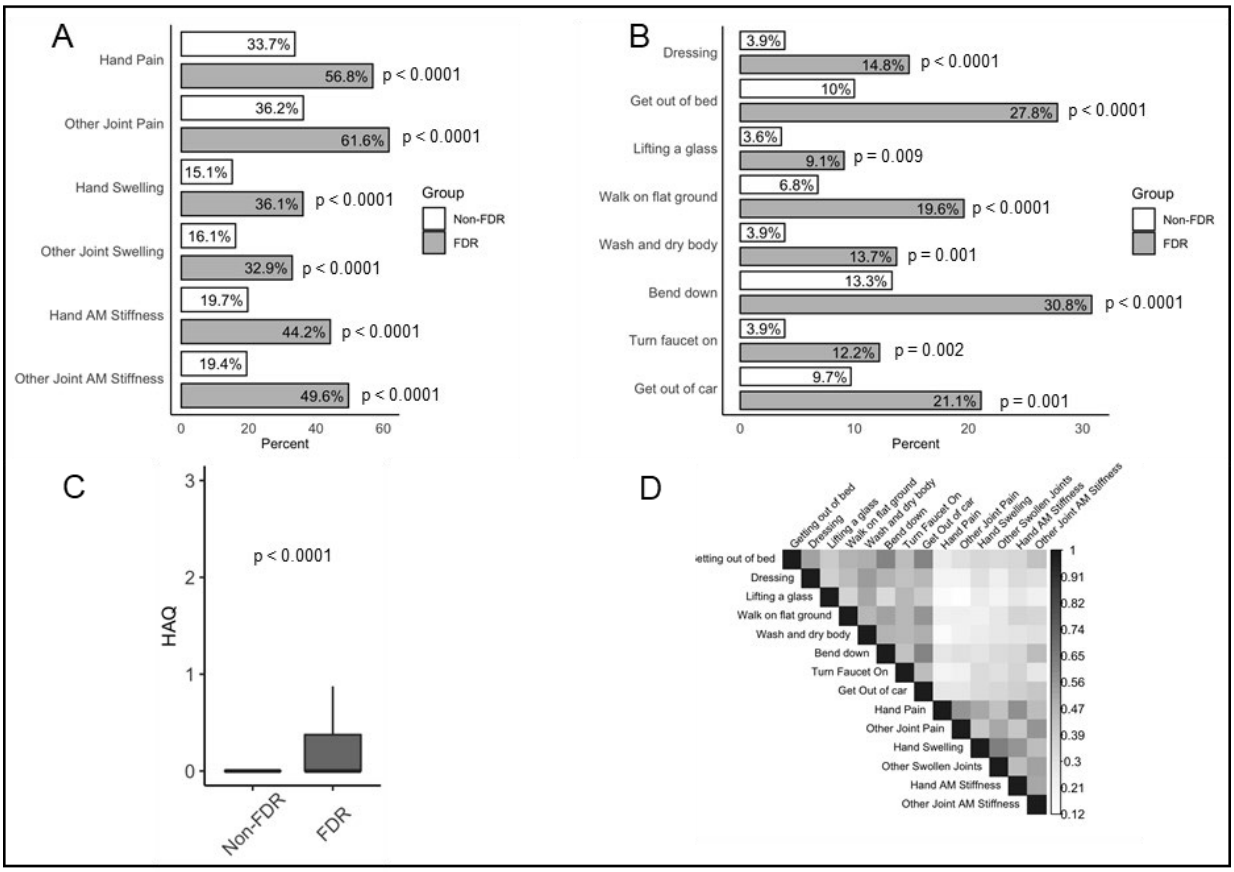

Figure 1: Differences in survey responses between FDR and non-FDR cohorts. (A) Joint symptom survey affirmative responses by self-report. Questions focused primarily on hand, or non-hand (Other) symptomatology. (B) Functional survey responses by self-report, % represents prevalence of difficulty with tasks listed above. (C) mHAQ scores (D) Correlation matrix of questions from all responses in joint symptom and functional surveys (FDR and non-FDR cohort), coloured by Spearman correlation coefficient. FDR: First degree relative. HAQ: health assessment questionnaire. mHAQ: modified health assessment questionnaire.

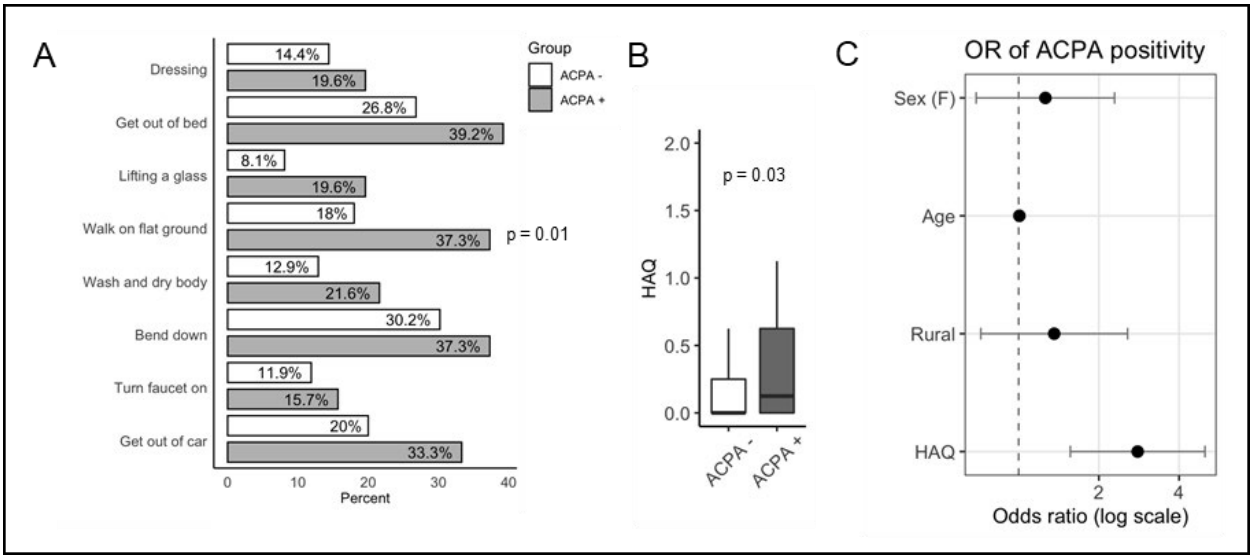

Figure 2: Functional differences between ACPA positive FDR and ACPA negative FDR. (A) Functional survey responses by self-report, % represents prevalence of difficulty with tasks listed above. (B) mHAQ scores and total number of tender joints on clinical exam (C) Forest plot of odds ratio (OR) for mHAQ (OR 2.70, 1.56 to 5.00) after controlling for age, sex and community (rural vs urban). X-axis scale is logarithmic. FDR: First degree relative. ACPA: anti-citrullinated protein antibodies. mHAQ: modified health assessment questionnaire.

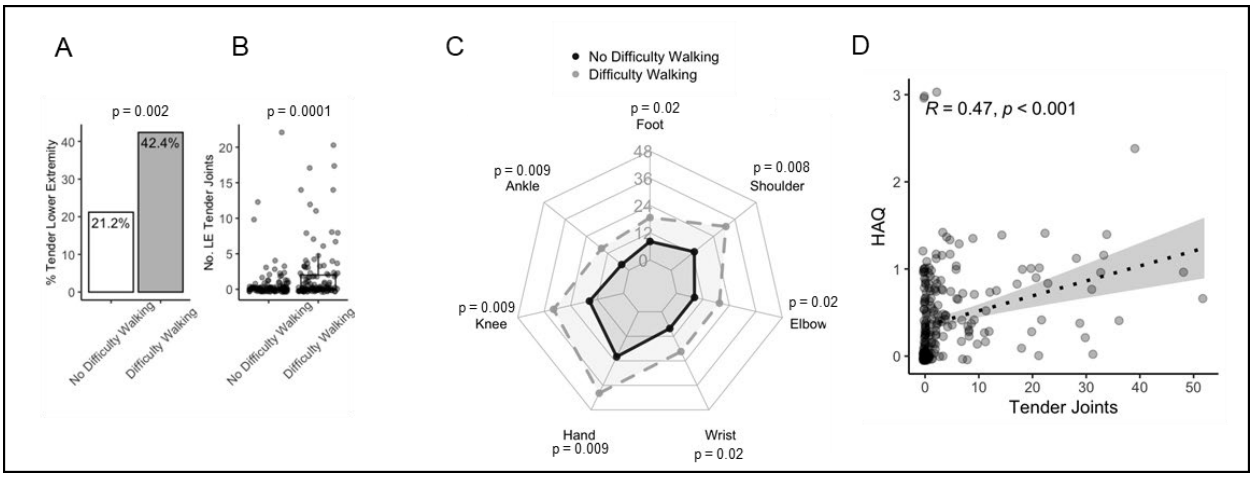

Figure 3: Individuals with self-reported difficulty walking display diffuse joint tenderness on clinical exam. (A) Presence of lower extremity (LE) tender joints on clinical exam expressed by percentage (%) (B) Number of LE tender joints on clinical exam (C) Radar plot of prevalence of joints tenderness split by anatomical location. % is annotated on graph. (D) Spearman correlation between tender joints and mHAQ in FDR. FDR: First degree relative. mHAQ: modified health assessment questionnaire.

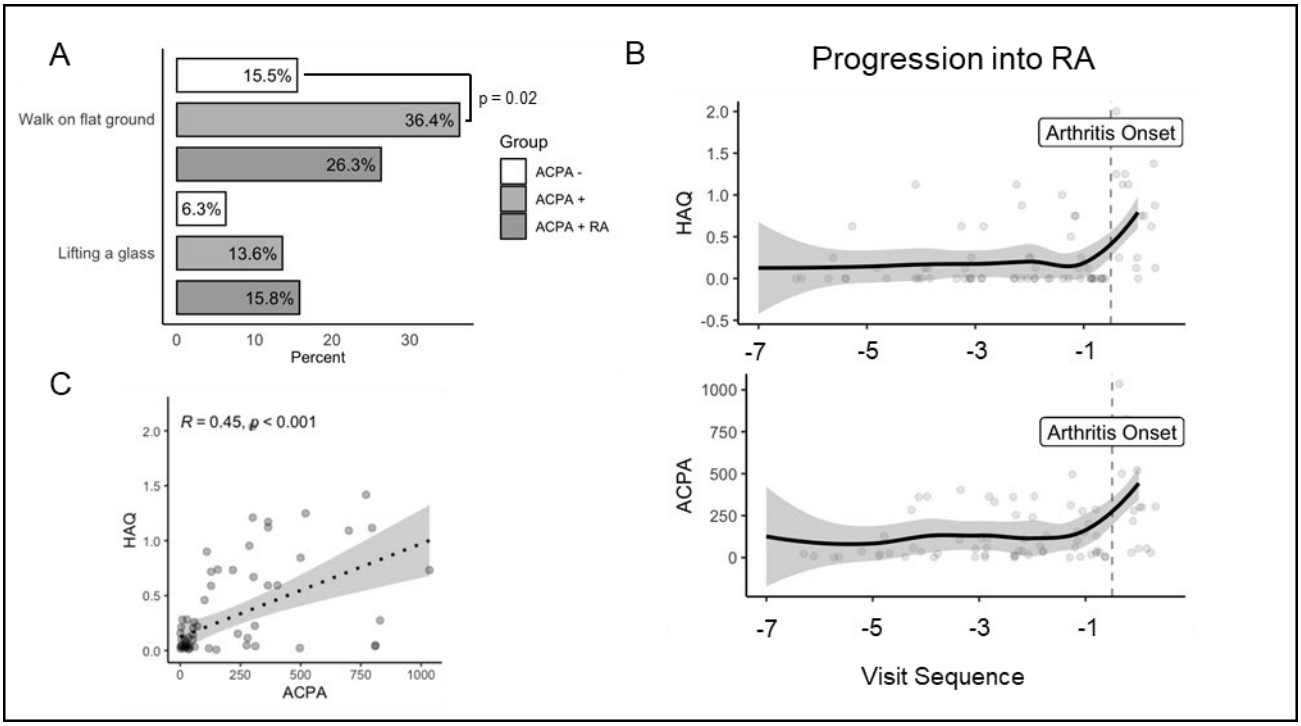

Figure 4: Longitudinal associations between functional disability and persistent ACPA seropositivity of the development of ACPA+ RA. (A) Functional survey responses by self-report, % represents prevalence of difficulty with tasks listed above at inception. (B) Longitudinal HAQ or ACPA level (AU) over sequential visits for individuals who progressed into IA (n = 19). (C) Spearman correlation between ACPA level and HAQ in Progressors into the development of ACPA+ RA. ACPA: anti-citrullinated protein antibodies.