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Relationship of YWHAH Single Nucleotide Polymorphisms to Markers of Rheumatoid Arthritis Disease Severity

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Abstract

Introduction: Serum 14-3-3 eta provides diagnostic and prognostic information in Rheumatoid Arthritis (RA). It is coded by the *YWHAH* gene with single nucleotide polymorphisms (SNPs) linked to bipolar disorder and schizophrenia. It was hypothesized that 6 previously described *YWHAH* SNPs might relate to markers of RA disease severity such as seropositivity or erosive changes.

Methods: Association study performed at an academic hospital Rheumatology Clinic. Subjects studied were 18 years of age or older with RA. TaqMan analysis screened for *YWHAH* SNPs rs2246704, rs2853884, rs3747158, rs4820059, rs7291050, rs933226. Primary endpoint was presence of at least one copy of the *YWHAH* SNPs with the markers of RA disease severity.

Results: Seventy-four subjects were enrolled, with data available for 71. Mean age was 60.7 ± 11.7 years old with most of Caucasian ancestry. Female to male ratio was very close to 3:1. Minor allele frequencies for the 6 *YWHAH* SNPs studied were comparable to known frequencies. Erosive changes were more likely observed when at least one copy of rs4820059 was present (OR 3.85 [95% Confidence Interval (95% CI): 1.17, 12.67] (p = 0.022)). Extra-articular manifestations were less likely when at least one copy of rs933226 was present (OR 0.12 [95% CI: 0.02, 0.97] (p =0.027)). Seropositivity was not associated with either association.

Conclusions: Though a modest cohort, two *YWHAH* SNPs studied have an association with RA disease severity. It is unclear the exact functional impact that these SNPs have as they are intronic, but future studies should further explore the associations observed.

Introduction

Rheumatoid Arthritis (RA) is a chronic autoimmune disease with the cardinal manifestation of synovial inflammation. When left untreated it is a disease of substantial morbidity and disability. In a U.S. cohort, up to 35% of patients with RA experienced work disability after 10 years of disease activity [1]. Establishing a diagnosis early in the disease course, as well as obtaining accurate prognostic information about future disease activity, is critical to the effective management of RA [2]. Serologic tests such as the Rheumatoid Factor (RF) and anti-cyclic citrullinated peptide (CCP) antibody test assist in diagnosis and providing some prognostic information about RA, but these two tests along with the C-Reactive Protein, provide only 32% of total variance in predicting joint destruction [3]. Additional tests are thus needed to fill this gap.

Serum 14-3-3 eta, which was first described in 2007 as being elevated in arthritis, has been shown to be overexpressed in the serum of RA patients as compared to healthy individuals and those with other types of inflammatory arthritis [4,5]. The 14-3-3 family of proteins was named based on chromatographic elution properties and are ubiquitously expressed intracellular chaperon proteins [6] which bind to and regulate the biologic activity of a variety of other intracellular proteins [7]. Multiple studies have demonstrated that 14-3-3 eta provides additional information about RA to the clinician—it assists in making diagnosis, provides prognostic assessment of disease severity, and provides insight regarding response to therapy [6,8]. Thus, 14-3-3 eta serves as an important mechanistic biomarker informing the clinician about a patient's RA.

14-3-3 eta is the product of the *YWHAH* gene, located on chromosome 22q12.3 [9]. It is about 13 kilobases long, consisting of two exons separated by a single intron. This gene has been implicated in linkage studies to bipolar disorder and schizophrenia [9]. Several *YWHAH* gene variants have also been implicated in major mental illness susceptibility [9]. Six *YWHAH* single nucleotide polymorphisms (SNPs) were identified in a prior study: rs2246704, rs2853884, rs3747158, rs4820059, rs7291050, and rs933226 [9]. Grover, *et al*. demonstrated that rs2246704 was associated with bipolar disorder with an Odds Ratio (OR) of 1.31 (p=0.03) and psychotic BP with an OR of 1.66 (p=0.002). All six of these SNPs are intronic.

To date no studies have searched for a possible relationship between *YWHAH* SNPs and markers of RA disease severity. Considering the aforementioned diagnostic and prognostic properties of 14-3-3 eta, it was hypothesized that one or several of the previously

described 6 *YWHAH* SNPs would be related to RA disease severity.

Methods

This cross sectional/association study was performed at a single academic medical center Rheumatology Clinic. Subjects studied were 18 years of age or older with a diagnosis of RA. Rheumatoid Arthritis was defined by either the 1987 or 2010 American College of Rheumatology (ACR) Classification Criteria, depending on year diagnosed. Seronegative RA subjects had either extra-articular manifestations typical for RA, radiographic or magnetic resonance imaging (MRI) of the hands which demonstrated synovial inflammation or erosions consistent with a polyarticular symmetric small joint arthritis favoring RA, or were diagnosed by another rheumatologist with RA but received routine care at this hospital. Data regarding markers of RA disease severity, specifically serologic status, erosive changes on X-Ray or MRI, deformities noted clinically, and extra-articular manifestations were collected over a 2 ½ year period. Seropositivity was defined as having any one or a combination of Rheumatoid Factor level (measured by nephelometry), anti-CCP antibody test, or the 14-3-3 eta test above the manufacturer cut-off for a positive test. Erosive status was defined as marginal resorption of bone involving the carpal bones, metacarpophalangeal (MCP) joints, or proximal interphalangeal (PIP) joints as documented by a board-certified, hospital employee Radiologist on either X-Ray or MRI. Synovial enhancement of these joints on MRI (in the absence of erosive changes) was made solely by a board-certified, hospital employee Radiologist. Deformities were observed and recorded clinically. These could include but were not limited to fixed or reducible (able to clinically restore the joint to an anatomic neutral position) volar subluxation of the phalanges at the MCPs, Swan-neck or boutonniere deformities or the phalanges, 'Z' deformity of the thumb, or volar elevation of the distal ulna in relationship to the radius ("piano key" deformity). Finally, extra-articular manifestations were observed clinically or documented radiographically and included but were not limited to nodulosis on extensor surfaces of the fingers or elbows, interstitial lung disease on imaging (either Chest X-Ray or Computed Tomography imaging), or scleritis/episcleritis (either documented in the past by a previous Rheumatologist or eye specialist or observed by the primary investigator).

The performance of six TaqMan SNP genotyping assays was evaluated across 16 control DNA samples of known allele calls, in addition to the sponsor samples themselves. The six *YWHAH* SNPs studied were rs2246704, rs2853884, rs3747158, rs4820059, rs7291050, rs933226. TaqMan genotyping analysis was conducted consistent with industry standards. A panel of 16 previously genotyped HapMap samples from the Coriell collection was identified; these HapMap samples have known genotypes for each of the six SNPs [10]. One evaluation run was carried out, comprised of the 16 Coriell controls and one serum specimen from each of the subjects enrolled. The evaluation run showed excellent allelic discrimination for five of the six assays, with good segregation of clusters for all samples. One assay (corresponding to variant rs3747158) had more dispersed clustering for several specimens. This led to three subject provided specimens having no call made for this assay, due to lower confidence. The observed genotypes for the 16 Coriell samples were 100% concordant with the expected known genotypes, with one exception. Based on the 1000 Genomes Project, the expected result for sample HG03926 at rs933226 was C:C. However, the observed genotype call was C:T cross all three replicates with tight and clean allelic clusters. Multiple Coriell controls representing the same allele state were specifically selected for this reason as errors in 1000 Genomes Project have been encountered before. The observed calls for four other Coriell controls in the same cluster are concordant with the expected known data (C:T). It was therefore concluded that the expected C:C call was incorrect and thus excluded from the data analysis.

The primary endpoint of this study was to identify a relationship between any of the six *YWHAH* SNPs with the known markers of RA disease severity of the subjects enrolled. Assuming a difference in proportions of 0.33, with a 2 tailed alpha set at 0.05, enrollment of 70 subjects was calculated to yield a power of 0.84. Chi squared testing was used for statistical analysis. This study was approved by the hospital Institutional Review Board before data collection began and consent was obtained from all subjects prior to enrollment. Two serum samples were obtained from each subject, one for the Taqman SNP genotyping and one for 14-3-3 eta level testing. 14-3-3 eta levels were measured at the time of enrollment to assist with studying whether 14-3-3 positivity or negativity affected the relationship of YWHAH SNPs to the primary endpoint.

Results

Seventy-four subjects were enrolled, with data available for 71 as blood was not obtained from 2 subjects and one subject enrolled twice. Demographics of this cohort are shown in Table 1. Mean age was 60.7 ± 11.7 years old. The majority (over 70%) of subjects were of Caucasian ancestry. Female to male ratio of subjects was very close to 3:1. Seropositivity was observed in 50 (70.4%) of the subjects. Twenty-three subjects (32.4%) had deforming disease, 55 (77.5%) had erosive changes documented on imaging, and 15 (21.1%) had extra-articular manifestations.

Age (Mean ± standard deviation)	**60.7 ± 11.7 years old**
Female : Male	53 : 18
Ethnicity	
Caucasian	51 (71.8%)
African American	15 (21.1%)

Age (Mean ± standard deviation)	60.7 ± 11.7 years old
Hispanic	3 (4.2%)
Asian	2 (2.8%)
Seropositivity*	50 (70.4%)
RF and/or anti-CCP antibody test	49 (69.0%)
14-3-3 eta test	29 (40.8%)
Deforming	23 (32.4%)
Erosive	55 (77.5%)
Extra-articular manifestations	15 (21.1%)
Scleritis	1
Nodulosis	8
Pulmonary (ILDz or pleurisy)	6

* - had any of the 3 serum biomarkers detected
Key: RF – Rheumatoid Factor; anti-CCP IgG – anti-cyclic citrullinated peptide IgG; ILDz – interstitial lung disease
Table 1: Demographics and RA findings (n = 71)

The distributions of the ancestral allele, SNP, and minor allele frequency (MAF) for this cohort and as compared with that available at dbSNP are shown in Table 2.[D] Hardy-Weinberg equilibrium satisfied for all SNPs. MAF for each of the 6 *YWHAH* SNPs observed in this cohort were comparable to those in the dbSNP.

	Ancestral allele	SNP	Minor allele frequency (dbSNP)	Minor allele frequency (cohort) ('n')
rs2246704	C	T	37.2% (C)	45.1% (C) (32)
rs2853884	T	C	20.4% (C)	25.4% (C) (18)
rs3747158	A	G	37.9% (A)	41.2% (A) (29)
rs4820059	G	A	22.5% (A)	32.4% (A) (23)
rs7291050	A	G	14.8% (G)	21.1% (G) (15)
rs933226	T	C	10.8% (C)	15.5% (C) (11)

dbSNP accessed 9 March 2017[11]
Table 2: Minor Allele Frequencies

For the primary endpoint, SNPs that reached statistical significance are noted in Table 3. Erosive changes were more likely to be observed when at least one copy of rs4820059 was present with an OR of 3.85 [95% Confidence Interval (95% CI): 1.17, 12.67] (p = 0.022). This relationship was powered at 0.74. The presence of at least one copy of rs4820059 increased the presence of erosive changes from 64.5% to 87.5%. Extra-articular manifestations were *less* likely when rs933226 was present with an OR of 0.12 [95% CI: 0.02, 0.97] (p =0.027) and was powered at 0.82. The presence of at least one copy of rs933226 decreased chance of developing extra-articular manifestations from 28.6% to 4.5%. Accounting for 14-3-3 eta positivity and seropositivity attributable to either RF or anti-CCP IgG testing, there were no statistically significant trends, with OR and 95% CI shown in Table 3.

SNP	Clinical Manifestation	OR [95% CI]	Power
rs4820059	Erosive disease	3.85 [1.17, 12.67] (p = 0.022)	0.74
(accounting for 14-3-3 eta positivity)		2.10 [0.79, 5.57]	
(accounting for seropositivity)		1.90 [0.69, 5.24]	
rs933226	Extra-articular manifestations	0.12 [0.02, 0.97] (p = 0.027)	0.82
(accounting for 14-3-3 eta positivity)		0.53 [0.18, 1.52]	
(accounting for seropositivity)		0.70 [0.24, 2.04]	

Key: SNP – Single Nucleotide Polymorphism; OR – Odds Ratio; 95% CI – 95% Confidence Interval
Table 3: Primary Outcome

Discussion

Serum 14-3-3 eta is a relatively new biomarker for RA as it is overexpressed in the blood of RA patients as compared to healthy subjects or other types of inflammatory arthritis [5]. Several well designed studies have demonstrated that serum 14-3-3 eta provides insight about RA, not only assisting in the diagnosis, but also in providing important prognostic information about a patient's disease as well as response to therapy [6,8]. Understanding more about this protein is important to understanding RA. 14-3-3 eta is the product of the YWHAH gene. This gene has been implicated in other studies to be related to bipolar disorder and schizo-

phrenia [9]. Six SNPs were identified in a previous study of subjects with mental health illness, and thus it was postulated that potentially these SNPs might be related to disease manifestations in RA. Determining the impact that various SNPs might have on protein expression in RA as well as disease phenotypes (such as seropositive vs. seronegative status) shed important insight on disease pathophysiology. For example, patients with anti-cyclic citrullinated peptide positive RA differ from seronegative ones in genetics and environmental risk factors [12]. The presence of anti-cyclic citrullinated peptide autoantibodies were associated with genetic interaction between the shared epitope (SE) and PTPN22 risk allele, and this highlighted how points out how T cell activation plays a central pathogenic role in the development of anti-cyclic citrullinated peptide positive RA [12]. A meta-analysis in 2010 confirmed that the STAT4 rs7574865 polymorphism is associated with RA susceptibility in different ethnic groups with a prevalence that was ethnicity dependent [13]. No association between STAT4 rs7574865 and the presence of rheumatoid factor or anti-cyclic citrullinated peptide autoantibodies was observed in another study [14]. While this study of the 14-3-3 eta protein was modest in size, it suggests an association between two of the six SNPs studied, rs4820059 and rs933226, with erosive changes and extra-articular manifestations, respectively. These associations were independent of 14-3-3 eta positivity or seropositivity in general.

This study has several strengths. It is the first study to assess a possible relationship between *YWHAH* SNPs and markers of RA disease severity. Second, the SNPs studied in this trial appear to be important in several illnesses, and at least regarding their relationship in RA, should be the focus of a larger trial in the future. Third, the MAF for the SNPs in this cohort were comparable to those published elsewhere, suggesting that the study cohort has a genetic background similar to that observed elsewhere. Fourth, the relation of the *YWHAH* SNPs to markers of RA disease severity appears to be independent of 14-3-3 eta positivity or seropositivity in general, suggestive of a unique association between the SNP and RA disease severity. Again, this trial is a preliminary assessment and future trials should validate whether or not this observation would hold up in a larger trial.

This study does have several limitations. First, this was a single center study with modest sample size. As noted, one of the associations (rs4820059) was minimally underpowered at 0.74, with only one association (rs933226) adequately powered with the cohort recruited. Second, in this study there was no adjustment for multiple testing, specifically no Bonferroni correction was applied. The reason for not doing so was to explore as many possible relationships between the candidate SNPs and markers of RA disease severity. When the study was designed, a concern existed that if an overly conservative correction such as a Bonferroni correction were to have been strictly applied; there potentially would have been no positive results. It was hoped that by preliminarily identifying a relationship between the SNPs and markers of RA disease severity, future trials would be able to build on this information to confirm or refute the findings. Third, the study design permits associations to be made but cannot demonstrate casuality. It would be prudent for a future study testing the associations observed to include cell studies that test the effect that the SNP might have on transcription, translation, and ultimately protein function. It is still unknown what impact the 6 *YWHAH* intronic SNPs might have, especially as the effect that intronic SNPs have on protein translation is an evolving area of genetics.

In conclusion, based on the diagnostic and prognostic importance of the serum 14-3-3 eta test in RA, this study was designed to look at 6 previously described *YWHAH* SNPs that have known associations with other illnesses, studying them for a possible relationship to markers of RA disease severity. Two SNPs, rs4820059 and rs933226, emerged as having an association with erosive changes and (inversely with) extra-articular manifestations. Limitations in the study design and modest cohort size limit broad application of this data, however future studies should be conducted to further explore the relationship of the *YWHAH* SNPs noted in this study.

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