Electronic cigarettes and the risk of Rheumatoid Arthritis

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Abstract

Electronic cigarettes have become increasingly popular in the past decade, despite the fact that there has been little research done on health risks associated with their use. Research has shown that smoking traditional cigarettes in conjunction with genetic polymorphisms results in significantly higher risk for developing seropositive rheumatoid arthritis (RA). RA is classified into two subsets, seropositive and seronegative, with seropositive patients being defined based on the production of autoantibodies. The purpose of this study was to investigate the potential health risks for developing seropositive rheumatoid arthritis in electronic cigarette users. EDTA blood samples were obtained from traditional smokers, electronic cigarette users, and nonsmokers totaling 45 participants. DNA was extracted from EDTA tubes and used to genotype for the presence of risk alleles rs24766001 in *PTPN22* and rs6910071 in *HLA-DRB1* using TaqMan SNP Genotyping Assay. In addition, autoantibodies seen commonly in RA (ie. antibodies to citrullinated proteins and Rheumatoid factor) were measured in all samples using an Enzyme Linked Immunosorbent Assay (ELISA) or an agglutination test, respectively. Levels of the antibodies were compared across genotypes of each group using an ANOVA pair wise comparisons. The data was then used to assess a similar disease model between electronic cigarette users versus traditional cigarette users and the development of seropositive rheumatoid arthritis. It was expected that, similar to traditional cigarettes, electronic cigarettes will cause inflammation that interact with the genetic risk factors to produce antibodies to citrullinated proteins which modulate the disease mechanism of seropositive RA. These results would educate electronic cigarette users of potential health risk factors of seropositive RA associated with this form of smoking.

Introduction

Electronic cigarettes have become increasingly popular in the past decade, despite the fact that little research has been done on health risks associated with their use. A potential risk of smoking electronic cigarettes is the development of Rheumatoid Arthritis. Rheumatoid arthritis (RA) is an autoimmune inflammatory rheumatic disease that affects approximately 0.5–1 % of the population and causes chronic synovial inflammation eventually leading to joint destruction and disability. Traditional Cigarette smoking along with genetic risk factors in the PTPN22 and the HLA-DRB1 genes leads to inflammation and the production of citrullinated proteins. The production of these proteins is caused by the activation of one of four enzyme variants of peptidyl-arginine deiminases (PADs). These altered proteins cause the immune system to create ACPA and RF, which mediate the pathogenesis of RA.

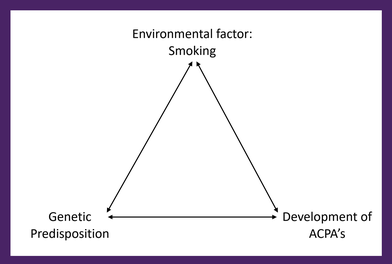


Figure 1. Disease risk model correlating Environmental and Genetic risk factors along with presence of antibodies for development of Rheumatoid Arthritis.

NOTE: Previous diagnosis of RA was done by testing for rheumatoid factor (RF) in patients, however that type of testing was often inaccurate. New research has found that Rheumatoid factor can often be elevated in multiple other autoimmune diseases or infections. This emerging and successful research on the development of ACPA's in relation to risk factor of developing RA provides much more specificity in the diagnosis of Rheumatoid Arthritis.

Results

An ANOVA was performed to analyze overall statistical significance of data (P-value 9.025e-08). T-test pairwise comparisons were then performed between each group to evaluate significant differences between groups. Each treatment group will be stratified by genotypes. The samples without the genetic polymorphism will be used as controls while the others will be grouped by presence of mutant type. Results as follows:

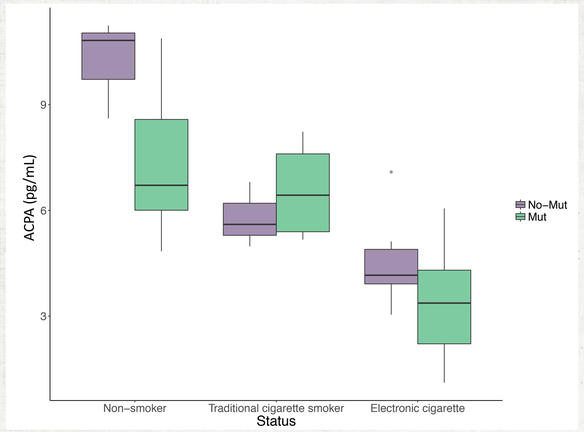


Fig. 2: ACPA Concentration (U/mL) subdivided into genotype across Non-Smokers, Tradtitional cigarettes, and Electronic Cigarettes.

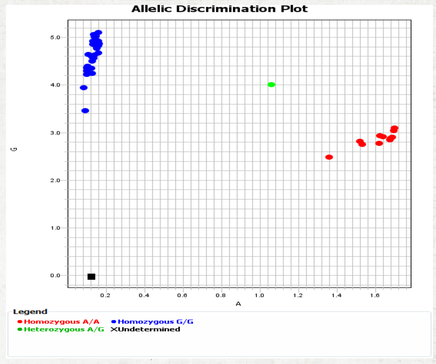


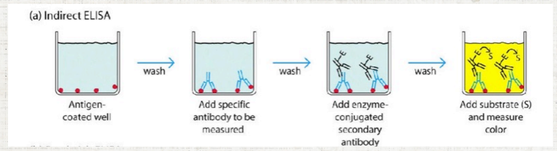
Fig 3: Allelic Discrimination Plot. Each participants genotype plotted with Allele 1 A/A (wild type) vs Allele 2 G/G (mutation).

Table 1	ACPA Concentration and genotype	
Status	Mean ACPA (pg/mL)	P-Value
Non-Smoker	8.056	*******
Traditional Smoker	6.236	0.0120
Electronic Cigarette users	3.783	6.67e-09

In table 1 our control for analysis is the Non-smoker group. An ANOVA was preformed between traditional Smokers and Non-Smokers and also Electronic Cigarette users and Non-smokers. Both resulted in P- Values that were reported statically significant ($p<0.05$).

Materials and Methods

Study Population
The participants were found using flyers posted around the Weber State University campus, local businesses, personal contacts, classroom announcements, as well as on social media. Volunteers were asked to complete a survey upon arrival. Following the survey, blood samples were collected from each of the participants. Samples were stored in cryo-tubes at -80 degrees Celsius until testing was completed. A signed informed consent document was obtained at time of collection. Included researchers received IRB approval for this study.



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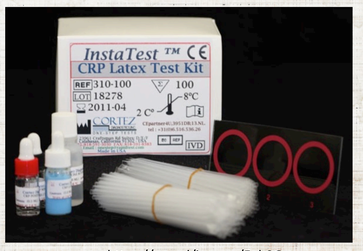
Elisa testing
The presence of ACPA's was quantified using an ELISA (Enzyme linked immunosorbent assay) kit. The presence of antibodies to citrullinated protein antigens was quantified based off of color (concentration of antibody) present.

Latex Agglutination testing
RF factor was tested using a Latex agglutination Kit. A drop of antigen was mixed with patient serum and when antibodies (RF Factor) exist, visible agglutination occurs.



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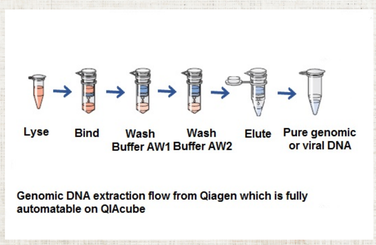

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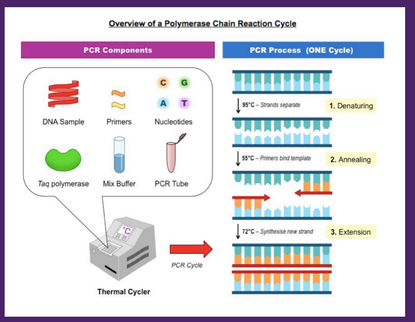
DNA extraction
Using the whole blood, the DNA was extracted from the red blood cells via a Qiagen DNA Extraction Kit. DNA was stored at -80 Celsius until genotyping was performed.

Genotyping
DNA samples were tested for each SNP (rs2476601 and rs6910071) using a TaqMan assay. This qPCR assay was run on a Quantstudio real-time PCR instrument in the MLS department. TaqMan™ uses real-time PCR (polymerase chain reaction) to amplify the target region of DNA. The Taqman uses allele specific probes to genotype each SNP. It was expected that the samples that express the mutations will have higher concentrations of ACPA's and RF factor present.



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Discussion

- Electronic Cigarette users displayed statistically significant lower levels of ACPA's meaning that there could be a mechanism for lowering levels of these antibodies.
- Although presence of ACPA concentrations varied between samples, all were below the reference value for Rheumatoid Arthritis (Negative < 30 U/ml)
- Environmental factors could also be contributing to the ACPA levels in the participants. In Northern Utah during the Fall/Winter season air quality is lower due to excess pollution and inversion effect.
- There was one sample that tested positive for RF that showed low/ normal levels for ACPA
  - Validates argument that RF does not have high specificity for RA. This person could be positive for some other autoimmune disease.

Limitations

- Small sample size
- Most samples came from young donors (around 20 years of age)
- Uneven sample groups
- One run of ACPA ELIZA
- Location- Inversion leads to lowered air quality
- Could a different location have different results
- Custom TaqMan[tm] Assay was non functioning
  - DNA did not replicate in order to quantify it.

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