**The Relationship Between the Risk of Smoking-Related Disease, Inflammatory Response and Single-Nucleotide Polymorphisms**

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**Abstract**

It is common knowledge that smoking cigarettes causes a plethora of health problems, such as cardiovascular disease, pulmonary disease and a variety of cancers, collectively called smoking-related diseases (SRDs)1. Systemic inflammation (SI) plays a key role in the development and progression of many SRDs, leading researchers to explore the relationship between cigarette smoking and inflammatory biomarkers2,3,4. Notable inflammatory biomarkers include elevated levels of immune response cells in the CD8dim lymphocyte group as well as certain phenotypes of monocytes and neutrophils5. While the risks of smoking cigarettes are well known and studied, there is only ongoing research to refer to when assessing the likelihood of developing a SRD. The risk of developing a SRD is not based on cigarette smoking alone, which explains why only some cigarette smokers develop a SRD. Genetic predisposition appears to be a promising lead as to explaining this phenomenon. Single-nucleotide polymorphisms (SNPs) are small variants in DNA, varying by a single nucleotide that can be used as biomarkers for disease6. A variety of SNPs have been identified as being associated with cigarette smoking and SRDs. Two telling SNPs associated with smoking and SI are CRP rs1800947, GZB rs81929175.

**Introduction**

Smoking refers to the act of inhaling the smoke of burning plant material, particularly tobacco, which is most frequently consumed in the form of cigarettes. Cigarettes are readily available to adults for purchase in numerous settings, like convenience and grocery stores. There are approximately 328,400,00 people living in the United States7 and 34,000,00 of those people smoke cigarettes8. Even though the risks of using tobacco is commonly known and the practice is considered to be objectionable by many, there are plenty of people who still partake. For some cigarette smokers, a SRD is in their future. For others, they will never develop a SRD despite their tobacco consumption. A good place to start looking for answers is in our genes and our immune response.

**Recent Progress**

 A 2017 Swedish study found that cigarette smokers have increased levels of immune response cells, such as total circulating white blood cells (WBCs), lymphocytes, monocytes, neutrophils and basophils, as well an increase in C-Reactive Protein (CRP) levels. Elevated level of these cells and proteins are also seen in immunocompromised individuals, such as those with cancer of the head and/or neck, Human Immunodeficiency Virus (HIV) and Lupus erythematosus. However, the development and severity of SRD as well as the extent of activation in the immune system seems to be at least somewhat governed by SNPs. Seeing that systemic inflammation is essential to understanding SRDs, this study explores SNPs in genes that deal with cell death, such as Perforin (PRF) and Granzyme B (GZB). Four different SNPs, CRP rs1800947, GZB rs8192917, PRF rs10999426 and PRF rs3758562 were studied to examine the relationships between cell death, systemic inflammation and concentrations and type of immune response cells. Those with CRP rs1800947 has the highest CRP levels, associated with the progression of cancer and those with GZB rs8192917 have the increased levels of CD8dim lymphocytes, which is associated with an immunocompromised state like that seen in HIV patients. Over-expression of GZB in CD8 T-lymphocytes is a biomarker for systemic lupus erythematosus. The CRP rs1800947 CC genotype had the highest levels off CRP, suggesting that individuals with this SNP have an increased risk and severity of systemic inflammation in relation to cigarette smoking. Increased levels of low-expressed CD8 (CD8dim) in T-lymphocytes were associated with the Granzyme (GZB) rs8192917 AA genotype. High levels of CD8dim are associated with autoimmune disease. In contrast to GZB rs8192917 and CRP rs1800947, both PRF SNPs seemed to be consistent across smokers and non-smokers5.

**Discussion**

The results of the primary study were quite telling, especially when put into context with other relevant research. This study focuses more on the relationship between SNPs and the biomarkers of SI rather than the relationship between SNPs and the biomarkers of SRD. This approach is quite refreshing as it brings new considerations and data to the subject which has mainly been explored through a different, but comparable, lens. This property makes this study particularly unique. This information on SNPs and their related biomarkers could be used to assess the risk of developing a SRD and/or immune disease. It is possible that SNPs, such as GZB rs8192917 and CRP rs1800947, could be identified via genetic testing to help smokers make informed decisions regarding their smoking habits. There is a possibility that knowing this information could encourage people to quit smoking, especially if they are genetically predisposed to SRDs or SI. However, to truly understand the relationship between cigarette smoking, SRD, SI and SNPs, all must be analyzed in context to each other. Given the novelty of this particular study, it would be beneficial to follow-up with additional research that explores not only the relationship between SNPs and the biomarkers of SI, but also the relationships between SNPs and the biomarkers of SRD, as well any possible correlation between the biomarkers of SRD and the biomarkers of SI.

 Three common features of cigarette smoke are well-documented across research on this topic: exposure to cigarette smoke usually results in SI, it can change the function of a variety of molecular and cellular components and increases the overall circulating amount of immune response cells9,10,11,12,13,14. Other related studies present conflicting information regarding whether cigarette smoke incites or suppresses inflammatory response, or possibly both simultaneously. This discrepancy appears to based in what type of lymphocyte is being observed. In the primary study, the CD8+ subgroup is the subject of analysis. The CD8+ T-cells serve to destroy compromised cells by releasing cytokines, cytotoxic granules such as perforin and granzymes as well as utilizing Fas/FasL interactions19. Other sources support that not only the CD8+ subgroup is present in elevated levels, but also other T-cells, such as Th17, Treg and Memory T-cells9. As previously mentioned, increased levels of T-cells are indicative of an immunocompromised state and SI. Participants with the SNP GZB rs8192917 had the highest levels of CD8dim, which suggests they are predisposed to SRD or an immunocompromised state.

 Specialized T-cells, such as those belonging to the CD8+ subgroup, have Toll-Like Receptors (TLRs). TLRs serve to recognize the difference between our own cells and foreign threats20. When TLRs respond to a threat, it leads to inflammation. Another study presents evidence that cigarette smoke increases the responsiveness of these receptors, which could lead to a response that plays into SI. Not only are CD8+ T-cells levels elevated, but their receptors are more responsive. This detail may be important in understanding the relationship between SI and the CD8+ subgroup. If further research into this topic is performed, this factor should be considered. Comparatively, in another study, Natural Killer (NK) cells, which are another type of lymphocyte with TLRs, produce conflicting data as to whether cigarette smoke suppressed or incites SI10. Given this information, TLRs may not be single and final answer to this question. It may lie in another function that differs between groups and subgroups, such the need, or lack of, activation to kill other cells.

 An increase in immune response cells was observed in smokers in the primary study. This is consistent with results from other studies, which suggests that cigarette smoke does indeed stimulate the immune system into action, causing immune cells to be produced17. The degree of activation seems to be related to increased CRP levels. Increased CRP is an excellent indicator of inflamed, immunocompromised state, making this connection quite significant18. CRP is a protein that is produced by the liver to accompany inflammation. To put it simply, where there are high CRP levels, there is inflammation. Another study reports that CRP levels are elevated in smokers, regardless of how much or how often they smoke21. This supporting detail ties together the relationship between inflammation, SNP CRP rs1800947, high CRP levels and SRD.

 To summarize the information presented above, there appears to be two important SNPs related to SI and cigarette smoking: GZB rs8192917 and CRP rs1800947. These SNPs at least somewhat govern in what way and how much the immune system responds to cigarette smoke. Cigarette smoke is associated many different diseases, including immune disease. This information could be useful to assess the risk of developing immune issues in cigarette smokers. It should also be noted that different groups and subgroups of lymphocytes react differently in the presence of cigarette smoke. CRP levels are also important, as high levels are directly tied to inflammation. More research should be done regarding as to what differences are present between different types and subgroups of lymphocytes and what causes some to suppress or incite SI. More research should also be performed on other CRP and GZB SNPs, as those seem promising.

 Regarding error analysis, this study presents two issues: that there is a very large ratio of women to men and that SNPs vary between population15,16. In the primary study, there were 14 men and 56 women. These numbers reflect the smoking culture of Sweden, where women smoke more frequently then men. This may be an issue, because there is evidence that women are at a higher risk for SRD then men16. If this is the case, there is reason to believe that the results of this study cannot be applied to generally. Furthermore, SNPs vary from population to population. This could also present an issue, as this was performed with a relatively small group of individuals, all of which are Swedish15. To fix these issues, a larger group of individuals from multiple regions of the country should participate, as well as more men so that these findings could be applied more generally.

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