ABSTRACT

Title of Thesis: NEW METHODOLOGY TO IDENTIFY
                        POTENTIAL ENVIRONMENTAL
                        TRIGGERS FOR ANCA-ASSOCIATED
                        VASCULITIDES

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A series of rare autoimmune disorders that affect the blood vessels, vasculitis is chronic and potentially deadly. Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) comprise three diagnostic forms of this autoimmune disorder: granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA or Churg-Strauss syndrome). The limited resources available to vasculitis researchers have been mostly targeted toward treatment and relapse prediction with a small amount of research examining genetic and environmental etiologic factors. Therefore, additional research is needed to understand the role of gene-environment interactions in AAV etiology. This thesis reviews the current body of AAV literature with a focus on candidate genes, occupational and environmental exposures, and the hygiene hypothesis. It also designs an original survey and study methodology to further investigate these

etiologic factors. A better understanding of AAV etiology will lead to prevention and improved treatment of these costly diseases.

NEW METHODOLOGY TO IDENTIFY POTENTIAL ENVIRONMENTAL
TRIGGERS FOR ANCA-ASSOCIATED VASCULITIDES

by

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Dedication

This thesis is dedicated to my fellow vasculitis patients who fight this disease every day and to the medical staff at MedStar Georgetown University Hospital who saved my life.

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List of Abbreviations

AAV: ANCA-associated vasculitis

ANCA: Anti-neutrophil cytoplasmic antibodies

EGPA: Eosinophilic granulomatosis with polyangiitis

GPA: Granulomatosis polyangiitis

MPA: Microscopic polyangiitis

MPO-ANCA: Myeloperoxidase-ANCA

PR3-ANCA: Proteinase 3-ANCA

Chapter 1: Introduction

ANCA-Associated Vasculitides

Background

Research on vasculitides, a rare group of autoimmune disorders, has increased significantly in the last three decades (Pagnoux 2016, Koldingsnes and Nossent 2008). The label vasculitides encompasses more than 15 different types of vasculitis (Koldingsnes and Nossent 2008), diseases characterized by an overactive immune response that causes inflammation and subsequent swelling in blood vessel walls. Vasculitides are further categorized by the size of the blood vessels affected by the immune response and then by their pathology. Small-vessel vasculitides predominantly affect capillaries, venules, and arterioles and include two main classifications: anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides and non-ANCA small-vessel vasculitides (Calatroni et al. 2017). This paper focuses on ANCA-associated vasculitides (AAV).

Granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA or Churg-Strauss), the three diseases categorized as AAV, occur when B cells produce ANCA. These ANCA induce neutrophils to excrete proinflammatory cytokines, which in turn prime the neutrophils to bind to endothelial cells in blood vessel walls, causing inflammation and vascular damage (Pagnoux 2016, Calatroni et al. 2017).

AAV Epidemiology

This group of vasculitides is rare; studies have estimated a worldwide incidence of AAV ranging from 2 to 20 cases per million people per year and a worldwide prevalence of 23 to 184 cases per million people (Berti et al. 2017, Pagnoux 2016). These large ranges can be attributed to the difficulty of studying AAV as well as the influence of geographic patterns and AAV type. European studies estimate an incidence of 10–20 cases per million people per year (Chen and Kallenberg 2010), while a smaller-scale study in Olmsted County, Minnesota found an incidence of 33 cases per million per year with individual annual incidence rates of 13 cases per million, 16 cases per million, and 4 cases per million for GPA, MPA, and EGPA respectively (Berti et al. 2017). Furthermore, though these diseases usually manifest between ages 50 and 70 (Chen and Kallenberg 2010, Pagnoux 2016), onset can happen at any age. AAV incidence in children has been estimated at between 0.5 and 6.39 cases per million people per year (Calatroni et al. 2017). Current AAV incidence rates are higher than those of past decades, but this increase may be attributed to increased awareness and a better understanding of the diseases following the discovery of ANCA in 1985 (Koldingsnes and Nossent 2008).

Patient Demographics

While autoimmune disorders are more common in women (Ngo et al. 2014), AAV is distributed fairly evenly between the sexes, though some studies found greater incidence in men (Chen and Kallenberg 2010, Watts et al. 2015), and a study from China found an increased incidence in women (Chen et al. 2008). AAV also differs by race and ethnicity. In the United States, whites are significantly more likely

to have AAV when compared with African Americans (Koldingsnes and Nossent 2008). However, while a Chicago study evaluating AAV severity found similar numbers of cases for white and Hispanic patients, Hispanic patients had more damage and higher levels of disease activity at the time of diagnosis (Sreih et al. 2015). This difference may be attributable to health care availability, as language barriers and health care costs often prevent Hispanic Americans from seeking early treatment (Weinick et al. 2004).

Disease Pathology

Presentation

Each type of AAV presents slightly differently. Though joint pain, rashes, respiratory problems, neuropathy, and organ involvement may be hallmarks of any of the three, specific symptoms may be more likely depending on AAV type. MPA is often accompanied by renal disease, musculoskeletal problems, skin reactions, respiratory effects, and gastrointestinal involvement. GPA often first presents as sinus or ENT issues and constitutional symptoms and is then followed by renal, respiratory, musculoskeletal, and/or skin problems. Finally, EGPA has been linked to asthma and often presents with sinusitis, nasal polyps, lung infiltrates, and skin lesions (Calatroni et al. 2017).

Mechanisms

Despite some differences in manifestation, AAV are often studied together because of their similar pathology. AAV is not fully understood; many treatments are based on the treatments used for other autoimmune disorders without a complete

understanding of the mechanisms of action or the future implications of these treatments. However, the presence of ANCA, which was historically considered a diagnostic marker of AAV, is now understood to play a pathogenic role in AAV development. An unknown triggering event induces the production of ANCA, which in turn prime neutrophils and monocytes to begin an immunoresponse, including inflammation and vascular necrosis. Underregulated B and T cells incorrectly continue the immunoresponse, which further damages blood vessels and significantly reduces the oxygen flow to organs (Jarrot and Kaplanski 2016).

Although ANCA play similar roles in the overactive immune response, there are two main forms of these autoantibodies: ANCA with cytoplasmic immunofluorescence patterns (c-ANCA) and ANCA with perinuclear immunofluorescence patterns (p-ANCA). C-ANCA normally responds to proteinase 3 and may therefore be referred to as PR3, while p-ANCA responds to myeloperoxidase and is therefore often referred to as MPO. PR3 are detected in 60–80 percent of GPA cases, while MPO are present in 80–90 percent of MPA cases and 35–40 percent of EGPA cases (Calatroni et al. 2017). As a result of the importance of ANCA in AAV pathogenesis as well as new data from cluster analyses, some researchers support a reclassification of AAV by phenotype (by ANCA type and organ involvement) rather than by symptom (GPA vs. MPA vs. EGPA) (Watts et al. 2015).

Relapse and Remission

There are two stages of AAV treatment: remission induction, which lasts about 3–6 months, and maintenance, which lasts about 12–24 months, though both

stages may last longer depending on practitioner discretion. Remission induction

involves the use of powerful immunosuppressants like rituximab or

cyclophosphamide, as well as glucocoritcoids (Mahr et al. 2013, Comarmond and

Cacoub 2014). The maintenance stage of treatment aims to prevent or limit relapses

in AAV patients. Relapses or "flares" are common in AAV with GPA, in which 50

percent of cases relapse, having the highest risk of relapse (Comarmond and Cacoub

2014, Mahr et al., 2013, Walsh et al. 2012). Unfortunately, the reason for the relapse

or disease flare is usually unknown (Johns Hopkins Vasculitis Center 2018).

Therefore, maintenance treatment using immunosuppressant drugs such as

methotrexate, azathioprine, or rituximab is necessary (Comarmond and Cacoub,

2014).

Genetic Etiology

Geographic Patterns

Studies have shown that different ANCA types and AAV diagnoses vary in

prevalence by region. MPO-ANCA is significantly more prevalent in southern

Europe, Asia, and some parts of the Pacific, but PR3-ANCA is more prevalent in

North American and northwestern Europe (Renauer et al. 2016). Accordingly, there is

a higher incidence of MPA in southern Europe and Asia and a higher incidence of

GPA in northern Europe and Australia (Berti et al. 2017). These differences have

been interpreted as support for both genetic and environmental etiologic factors; some

researchers assert that AAV cases in similar regions may share common genetic

backgrounds, while others claim that environmental triggers could be distributed similarly to disease cases (Chen and Kallenberg 2010).

Candidate Genes

Candidate genes for AAV differ by study; however, the histocompatibility complex HLA, which is implicated in other autoimmune disorders, is consistently identified as a potential genetic factor for AAV (Pagnoux 2016, Calatroni et al. 2017). A 2007 review study compared potential genetic polymorphisms across multiple vasculitis types, but found that most polymorphisms either had not been studied in AAV, or that no association had been found (Brogan 2007).

Evidence for Gene-Environment Interaction

Despite these genetic factors, AAV is not directly heritable. Although a family may have multiple autoimmune disorders, a Swedish study found that close relatives of GPA patients have a relative risk of disease of only 1.56, which, given the low prevalence of disease, is a fairly small risk (Knight et al. 2008). To the author's knowledge, there are no twin studies on AAV, though one twin case report in Italy found two male, monozygotic twin children developed renal limited AAV within three years of one another, which the authors took as support for a genetic cause of AAV (Giani et al. 2002).

Geographic AAV patterns, genetic studies, and limited familial clustering of AAV highlight potential genetic components of AAV etiology. However, while studies have identified multiple candidate genes, no single definitive genetic marker for AAV has been found. Therefore, it is likely that AAV involves a complex genetic

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inheritance that may rely on gene-environment interactions (Knight et al. 2008).

Despite evidence for the role of environmental exposures in AAV etiology, the

interaction between genetic predisposition and environmental triggers is not well

understood (Renauer et al. 2016). Therefore, as explained by Watts et al. 2015,

identifying environmental triggers of AAV is necessary for developing etiologic

solutions based on gene-environment interactions (Watts et al. 2015).

Potential Environmental Etiology

Inhaled Irritants

The respiratory tract involvement in AAV led researchers to hypothesize that

inhaled irritants from air pollution or occupational exposures may contribute to the

causal pathway of this group of vasculitides (Koldingsnes and Nossent 2008). Most

studies evaluating the effects of inhaled irritants on AAV development highlight the

potential causal role of silica, a mineral found in soil, sand, and rock. Although the

exact mechanisms of action for silica dust are not fully understood, researchers

hypothesize that silica's ability to stimulate lymphocytes, monocytes, and

macrophages, as well as neutrophil-mediated immune response may trigger ANCA

activation (Brogan 2007, Chen and Kallenberg 2010).

Studies have identified construction work (Brogan 2007), farming, mill and

textile work (Chen and Kallenberg 2010), and even being a first responder (Webber et

al. 2015) as having both a likelihood of silica exposure and an increased risk of AAV

and other autoimmune disorders. However, although Chen and Kallenberg reported

that as many as 22–46 percent of AAV cases may be attributable to silica exposure,

most cases associated with silica exposure involve MPO-ANCA, meaning c-ANCA may have a different cause (Chen and Kallenberg 2010).

Medications

Certain medications have also been implicated in AAV etiology. Studies have identified the thyroid medication propylthiouracil (Watts et al. 2015, Calatroni et al. 2017, Chen and Kallenberg 2010), the blood pressure medication hydralazine, the antibiotic minocycline (Watts et al. 2015, Chen and Kallenberg 2010), and anti-inflammatory/immunosuppressive drugs such as penicillamine (Calatroni et al. 2017, Chen and Kallenberg 2010), dapsone (Calatroni et al. 2017) and hydralazine (Chen and Kallengberg 2010) as being associated with AAV. However, these drugs treat conditions that may already be associated with or even contribute directly to AAV, therefore giving the impression that the drugs induced AAV, despite the patient's potential medical predisposition.

Furthermore, drug-induced AAV has been found to be more mechanistically heterogeneous, with increased activity of other, rarer neutrophil antigens besides ANCA (Watts et al. 2015). Additionally, some drug-induced AAV has been found to subside after stopping use of the drug (Chen and Kallenberg 2010), meaning drug use may not contribute significantly to an etiologic solution.

Infections

In addition to inhaled pollutants, some cases of AAV are thought to have been triggered by bacterial or viral infections (Calatroni et al. 2017, Pagnoux 2016, Watts et al. 2015). The most likely culprit suggested by the current body of literature is bacterial infection by *Staphylococcus aureus*, which is particularly indicated in cases

of GPA. *S. aureus* peptides are homologous with PR3, indicating a potential mechanism for the development of PR3-ANCA (Chen and Kallenberg 2010). However, like silica dust exposure, *S. aureus* infection may only contribute to one type of AAV, and is unlikely to be present in all cases.

Chemical Exposures

Finally, there is some evidence that exposure to organic solvents may be causally linked to AAV. Floreani et al. suggested that exposure to a variety of solvents including dry cleaning chemicals, paint thinners, nail polish removers, and cleaning supplies is associated with a number of autoimmune disorders including systemic vasculitis (Floreani et al. 2016). A Swedish study on GPA occurrence and relative risks reported a potential link between organic solvents and GPA (Knight et al. 2008). However, exposure to organic solvents is understudied with regard to AAV.

The Hygiene Hypothesis

Overview

In addition to the various genetic and environmental factors implicated in AAV etiologic research, the hygiene hypothesis, popularized in the field of immunology, may also help explain the development of this group of diseases. Though each iteration of the hygiene hypothesis attempts to explain the recent increase of atopic disease, allergy, and autoimmunity in wealthier countries, its definition varies slightly by author. Some researchers frame it as a tradeoff between infant mortality and allergy, suggesting that lower rates of childhood infections lead to the development of inflammatory diseases (Wills-Karp et al. 2001, Strachan 2000).

Others go further, proposing that the reduced exposure to microorganisms and antigens impairs the development of an effective immune system and increases later responses to potential triggers (Sironi and Clerici 2010, Versini et al. 2015, Liu and Murphy 2003). Generally, the hygiene hypothesis states that because high GDP countries often have more sanitization and public health infrastructure, exposures to pathogenic infections and microbiota have decreased, which in turn leads to incomplete development of the immune system. This incomplete development of the immune system leaves children in wealthier countries more likely to generate an inflammatory response to environmental triggers, thereby producing atopic, allergic, and autoimmune disease.

In 1989, David Strachan published research demonstrating that lower rates of hay fever in British children were associated with having older siblings; he suggested this meant that infections and lower standards of hygiene were protective for allergic diseases (Strachan 1989). Although Strachan is given credit for coining the hygiene hypothesis, other earlier studies already had begun to notice this potential relationship between microbial exposure and increased prevalence of chronic inflammatory diseases. For example, in 1966, Leibowitz et al. published a study on Israelis with multiple sclerosis (MS). They found that MS was positively associated with higher levels of sanitation in patients' childhood homes (Leibowitz et al. 1966). By 1970, Greenwood reported lower prevalence of autoimmune disease in Nigeria and attributed this to parasitic infections in childhood (Greenwood and Cantab 1968, Greenwood et al. 1970, Versini et al. 2015).

This body of literature aligns with recent studies reporting increases in inflammatory diseases (Rook 2012) such as allergies (Strachan 2000), asthma (Okada et al. 2010), and autoimmune disorders including inflammatory bowel disease, ulcerative colitis, Crohn's disease, type-1 diabetes, MS (Versini et al. 2015), and systemic lupus erythematosus (Mu et al. 2015), particularly in industrialized countries like the United States (Brady 2013). Though it is difficult to determine whether increased awareness of these diseases on the part of doctors contributed to the rise in their diagnoses, it is worth investigating the hygiene hypothesis and its potential explanation for immune system development.

Despite these historical studies and recent increases in atopic disease, allergy, and autoimmunity, Weber et al. warns researchers not to confuse the hygiene hypothesis with general cleanliness. They contend that the hypothesis must be carefully defined via specific immunological mechanisms (Weber et al. 2015). These mechanisms likely include improper immunoregulation attributable to an imbalance of helper T cells (Schaub et al. 2006, Okada et al. 2010, Wills-Karp et al. 2001), the production of proinflammatory cytokines (Schuijs et al. 2015), reduced function of regulatory T cells (Wills-Karp et al. 2001), and limited Toll-like receptor (TLR) priming of dendritic cells (Sironi and Clerici 2010). Proponents of the hygiene hypothesis say these mechanisms are influenced by viral and bacterial infections, environmental exposure to microbes, or a combination of the two (Schaub et al. 2006). These exposures contribute to the gene-environment interactions at play in the hygiene hypothesis (Wills-Karp et al. 2001, Rook 2012, Schaub et al. 2006). Given the relatively short timeframe of these increases in atopic, allergic, and autoimmune

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disease, it is unlikely that the increase could be solely due to significant genetic changes (Versini et al. 2015). However, it is possible that genetic polymorphisms in genes related to the control of infections could, in combination with environmental triggers, allow for the development of such diseases (Okada et al. 2010).

Normally, microbial exposures like viruses, bacteria, and parasites, in combination with exposure variables and genetic variation, elicit a controlled immune response. However, if these immune responses are not properly modulated or if genetic components predispose individuals to disease, the original microbial exposure triggers a cascade effect resulting in allergy, asthma, or autoimmunity (Liu and Murphy 2003). One potential contributor to improper immune system regulation is the imbalance of helper T cells. There are two types of helper T cells, Th1 and Th2, distinguishable by the responses they trigger. Th1 produces proinflammatory cytokines like interleukin 2 (IL-2), while Th2 produces interleukin 3 (IL-3) and interleukin 4 (IL-4), among others. IL-2 promotes the development of T regulatory cells, and the cytokines produced by Th2 lead to the formation of immunoglobin E (IgE), both of which are crucial to a responsive immune defense (Okada et al. 2010, Schaub et al. 2006).

However, if there is not a balance between Th1 and Th2 cells, the immune response could lead to excessive inflammation and damage. Left unbalanced, IL-2 from Th1 can trigger organ-specific autoimmune disease (Wills-Karp et al. 2001), and IgE from Th2-produced IL-3 and IL-4 can promote hypersensitivity and allergic response (Schaub et al. 2006). Therefore, in terms of the hygiene hypothesis, it is suggested that if the Th2 skew seen at birth in infants does not correct itself through

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environmental exposures children will overproduce IgE, resulting in an atopic or allergic response (Wills-Karp et al. 2001, Gale 2002). Since the hygiene hypothesis was originally applied to atopy and allergy, the Th1/Th2 balance is rarely used to explain the increase in autoimmunity. Nevertheless, it stands to reason that if an imbalance of Th1 is connected to autoimmunity and babies are born with the Th2 skew (Wills-Karp 2001, Gale 2002), then an excess of environmental stimulation to create Th1 response could shift the Th1/Th2 balance in favor of Th1 and increase the likelihood of an autoimmune disorder.

Still, some researchers contest that using the Th1/Th2 balance as the mechanism for the hygiene hypothesis is overly simplistic. Sheikh et al. found no inverse relationship between atopy and autoimmunity, and Weiss highlighted the unexplained rise in Th1-mediated autoimmune disorders. Both articles dismissed the hygiene hypothesis (Sheikh et al. 2003, Weiss 2008). However, Sironi and Clerici use the shortcomings in the Th1/Th2 explanation, particularly the fact that Th1 cytokines have been found in cases of atopic disease, to argue for the consideration of other potential mechanisms (Sironi and Clerici 2010).

Instead of relying on the Th1/Th2 balance, some proponents of the hygiene hypothesis use regulatory T cells (T_{regs}) and TLR to explain the inflammation in atopic, allergic, and autoimmune disease. CD4+ T_{reg} cells produce the anti-inflammatory cytokine interleukin 10 (IL-10), meaning that T_{reg} cells can provide negative feedback to an immune response (Wills-Karp et al. 2001, Sakaguchi et al. 2009). When activated, the TLR proteins prime dendritic cells, which in turn release T_{reg} cells and reduce inflammation (Sironi and Clerici 2010). A 2014 study found that

cord blood from Karelian (ethnic Finns living in Russian) babies born in low income families with lower levels of sanitization had more innate TLR responses when compared to Finnish babies, thereby suggesting a role for the hygiene hypothesis in immunoregulation (Kallionpaa et al. 2014). Reduced inflammation as a result of proper immunoregulation would likely clearly point to fewer chronic inflammatory diseases such as atopy, allergy, and autoimmunity.

Nevertheless, in spite of these potential mechanistic explanations for the hygiene hypothesis, detractors argue that most of the evidence in favor of the hygiene hypothesis is a result of epidemiological studies, and therefore are unable to demonstrate causality (Macpherson and Harris 2004). Furthermore, some argue that the lungs of children with asthma and atopy-implicated respiratory diseases are primed or underdeveloped before birth, potentially as a result of maternal vitamin D deficiencies (Weiss 2008). However, as prenatal and maternal exposure are also implicated in the hygiene hypothesis (Ege et al. 2006), this research does not rule it out.

Atopy, Allergy, and Autoimmunity

Given the potential role of the hygiene hypothesis in explaining the development of atopic, allergic, and autoimmune disease, many researchers suggest it be used to develop immunotherapeutic tools including vaccines and bacterial extracts (Schaub et al. 2006, Rook 2012, Okada et al. 2010). However, the largest and most important implication of the hygiene hypothesis is its contribution to etiologic research and preventative care. In order to identify potential etiology, it is important

to understand how direct and indirect exposure factors can induce the mechanistic responses explained in the previous section.

Exposures related to the hygiene hypothesis are most likely to have a protective effect when they occur prenatally or within the first three years of life (von Mutius 2010, Schaub et al. 2006). Direct factors that have been associated with the development of chronic inflammatory disease include bacterial and parasitic infections. Indirect factors include socioeconomic status, chronic exposure to farms, daycare attendance, family size and composition, pet ownership, and mode of delivery. It is unclear exactly how these direct and indirect factors regulate the immune system, but they have been implicated in epidemiological and experimental studies and should be examined further.

Although bacterial infections are known to cause a defensive immune response in humans, studies supporting the hygiene hypothesis suggest childhood bacterial infections may also prime the immune system to avoid the disordered inflammatory responses found in atopic, allergic, and autoimmune disease. A cohort study in Munich found that a higher concentration of endotoxins—molecules produced by Gram-negative bacteria—in the home during the first year of a child's life was associated with lower incidence of asthma, atopic sensitization, eczema, and hay fever (Weber et al. 2015). A 2002 study including data from Germany, Austria, and Switzerland also identified endotoxin exposure as protective, reporting that endotoxin levels found in children's bedding were inversely associated with hay fever, atopic asthma, and atopic sensitization (Braun-Fahrlander et al. 2002). The inverse relationship between childhood endotoxin exposure and atopic disease has

been attributed to endotoxin priming of the immune system that limits release of proinflammatory cytokines (Braun-Farhlander et al. 2002, Schuijs et al. 2015).

Similarly, parasitic infections influence immunoregulation and promote tolerance of antigens. While parasitic infections can be deadly, studies suggest that early exposure to parasites like helminths can prevent atopic and allergic responses later in life. A study in Caracas, Venezuela found that when children were treated with anti-helminth medication, they had increased allergic hypersensitivity as indicated by a skin prick test and increased allergen-specific IgE antibodies in their serum (Lynch et al. 1993). This suggests that, though helminth infection can cause intestinal damage, exposure to these parasites can lead to a better-regulated immune system. However, the exact mechanism of helminth influence on the immune system is not well understood. Helminths are generally thought to affect different levels of the immune system to create a modulatory network (Versini et al. 2015, Maizels 2016). Gale suggests that helminths activate T_{reg} cells and induce the release of IL-10, thereby reducing an inflammatory response (Gale 2002).

In addition to these microbial infections, indirect factors related to the hygiene hypothesis have played a demonstrable role in the development of chronic inflammatory diseases. These factors are likely linked to microbial exposure, but the mechanisms are not always clear. Like Strachan 1989 and the studies that came before, studies on the indirect factors supporting the hygiene hypothesis are often epidemiological rather than experimental. Nevertheless, they highlight behaviors, lifestyles, and social dynamics that may influence the development of atopic, allergic, and autoimmune disease. Additionally, pinpointing indirect factors of disease

development can lead to the identification of new mechanisms at play in the hygiene hypothesis. Mechanisms that vary widely across differences in these implicated factors are more likely to contribute to disease development as defined in the hygiene hypothesis (Strachan 2000).

As with most health issues, socioeconomic status (SES) likely plays a role in the development of chronic inflammatory diseases. However, unlike with other health issues, SES has been positively associated with the development of these diseases. The hygiene hypothesis explains this by relating increased SES with reduced microbial exposure and a correspondingly underregulated immune system. A German study conducted after the country's reunification found that people in what had been West Germany were more likely to have bronchial hyperresponsiveness and atopic sensitization when compared to people living in the former-East Germany (Nowak et al. 1996). More recently, a 2013 study in Brazil used questionnaires, serum IgE levels, and skin prick tests to determine reactivity phenotypes for children. The authors found that children with more highly educated mothers lived in cleaner environments and had lower rates of pathogenic infections. These children were also found to have the most reactive phenotype and a higher likelihood of atopy (Figueiredo et al. 2013).

In addition to lower SES, chronic prenatal and early exposure to farms is thought to be protective against the development of atopic and allergic disease. Studies have shown that living on a farm as a child is protective against allergic rhinitis (Kipelainen et al. 2000), hay fever (von Ehrenstein et al. 2000) and asthma, independent of other factors (Kipelainen et al. 2000, von Ehrenstein et al. 2000). This

protective effect also applies to children's prenatal farm exposure due to maternal activity during pregnancy (Ege et al. 2006).

Notably, a recent study comparing asthma risk between Amish and Hutterite farm children demonstrates that all farming exposure is not equally protective. Amish children live on less industrialized farms and have significantly lower rates of asthma when compared with Hutterite children (Stein et al. 2016). This difference means that the variation of endotoxin exposure levels due to farming practices and type of livestock is likely the direct factor determining the effects of farm exposure (Schuijs et al. 2015, Stein et al. 2016, Ege et al. 2006, von Ehrenstein et al. 2000, Remes et al. 2003).

The effects of family size and composition can be traced back to the Strachan's work beginning the hygiene hypothesis (Strachan 1989). Since then, studies have continued to demonstrate the protective effects of older siblings in the development of atopic and allergic disease (Strachan 1995, Strachan et al. 1996, Bodner et al. 1998). Additionally, it has been suggested that this protective sibling effect may be more pronounced when the father also has atopic disease (Mattes et al. 1998). There is also some evidence that day care attendance within the first six months of life can reduce the risk of developing asthma, though the underlying mechanisms for this are likely similar to those for family size and composition (Ball et al. 2000).

Like family size and composition, pet ownership in early life can also affect the development of atopic and allergic disease. A recent meta-analysis of studies on pet exposure and the risk of atopic dermatitis found that pet ownership, particularly

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dog ownership in early childhood was protective (Pelucchi et al. 2013). These results echoed a 1999 experimental study on allergic rhinitis, asthma, and allergy, though the 1999 study also found a slight protective effect for cat ownership (Hesselmar et al. 1999).

Finally, in addition to the above social and behavioral factors contributing to the development of chronic inflammatory disease, mode of delivery may also have significant effects on the immune system. Neu and Rushing report that the increase of autoimmune and allergic diseases in the United States parallel the increase in caesarean sections (C-sections). They suggest that mode of birth—C-section vs. vaginal—affects which microbes colonize the gut, which in turn can affect the development of the immune system. Although gut microbiomes usually become more uniform after the first year of life regardless of mode of birth, the emphasis of the hygiene hypothesis on exposures during or before the first year of life qualifies mode of birth as a factor of interest (Neu and Rushing 2011). However, additional studies are required to better define the interplay between mode of birth and the hygiene hypothesis.

AAV in the Context of the Hygiene Hypothesis

Although some hygiene hypothesis research acknowledges the potential role of early microbial exposures and immune system modulation in the development of autoimmune diseases, AAV have not been evaluated with regard to this hypothesis. ANCA were only discovered in 1982, meaning that they are only beginning to be understood as a pathologic mechanism (Kallenberg 2011). This recent discovery of ANCA pathogenesis combined with the low prevalence of AAV has led to the

underrepresentation of AAV in etiologic research. Nevertheless, the disease development factors identified in research on the hygiene hypothesis may be applicable to AAV.

The recent increase in diagnosed autoimmune disorders, though perhaps attributable in part to the increased awareness of these diseases, has been associated with the hygiene hypothesis (Versini et al. 2015, Brady 2013). Therefore, it is plausible that this hypothesis could also play a role in the development of AAV. Beginning with the Th1/Th2 mechanism implicated in some hygiene hypothesis research, AAV as an autoimmune disorder would likely fall on the Th1-skewed side of the spectrum. External exposures like bacteria, viruses, and other microbes that can trigger a Th1 response may be implicated, either in priming the immune system for a more measured response or in causing AAV by triggering an overactive response (Liu and Murphy 2003, Johnson et al. 2003). As in the above explanation of the Th1/Th2 balance, there is contradictory information that calls into question the applicability of this potential mechanism. Additionally, if the hygiene hypothesis relies heavily on a Th2 skew, as suggested by some studies, organ-specific Th1-mediated autoimmune disorders similar to AAV may not be pertinent (Wills-Karp et al. 2001, Weiss 2008).

However, because there are some similarities between asthma and bronchial hyperreactivity-related disease and AAV, it is possible that the hygiene hypothesis' relevance to asthma development provides enough of a connection to implicate the hypothesis in AAV etiologic research. As explained in the environmental etiology section above, inhaled irritants are thought to play a role in AAV etiology. As asthma may also result from inhaled irritants, it follows that the two may have some overlap

in etiologic factors. Liu and Murphy state that childhood autoimmune disorders could have similar causes as asthma (Liu and Murphy 2003), but because AAV tends to present in 50- to 70-year-old adults, this relationship could be less applicable to AAV generally. Nevertheless, this potential relationship between asthma and AAV development may be relevant for EGPA specifically, given that EGPA is more likely to develop in patients with asthma (Calatroni et al. 2017). Furthermore, the eosinophils found in EGPA patients may align more with a Th2 immune response (Liu and Murphy 2003), meaning that EGPA could be implicated in the Th2 skew found in hygiene hypothesis literature.

Another possible link between the hygiene hypothesis and AAV development is the geographic patterns found for each. A 2010 review article reported north-south and west-east disease gradients that aligned with the hygiene hypothesis. In North America, Europe, and China, allergic and autoimmune diseases were more prevalent in the north, while infectious diseases were more prevalent in the south. In Europe, there was also a higher prevalence of autoimmune disease in the west and a higher prevalence of infectious disease in the east (Okada et al. 2010). Notably, similar gradients are found for AAV. As explained in the genetic etiology section above, MPO-ANCA is more prevalent in southern Europe, while PR3-ANCA is more prevalent in northwest Europe. While, these patterns have mostly been attributed to genetics, it is possible that environmental factors could be affecting gene expression. Taken as support of the hygiene hypothesis, these gradients could imply an association between MPO-ANCA-associated vasculitis (MPA) and infection and an

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association between PR3-ANCA-associated vasculitis (GPA) and a lack of immune system priming.

As a result of the limited information on AAV etiology, it is difficult to gauge the relevance of the hygiene hypothesis to the development of these diseases. Future studies are required to further examine this potential relationship. In addition to identifying any associations between hygiene hypothesis factors and AAV development, these studies should focus on examining the timing of the exposure to these factors. Infections have been shown to trigger autoimmune disorders, so the theory of immunoregulation by these same infections is only plausible if the timing of exposure determines the result (Versini et al. 2015).

Chapter 2: Study Methodology and Novel Survey Design

Research Question

Although the etiology of vasculitis is presumed to have both genetic and environmental components, further research is needed to clarify this interaction. The goal of this study is to address the research question: Which environmental triggers could make genetically predisposed individuals more susceptible to ANCA-associated vasculitis?

Study Design

This study will be a matched case-control design in which there are two controls for each case. Cases have a doctor's diagnosis of AAV (GPA, MPA, EGPA, or an unspecified AAV), and controls are the AAV-free biological siblings and first-cousins of cases. Retrospective data will be collected via a 45- to 60-minute online survey. In keeping with the inclusion criteria of a current National Institute of Environmental Health Sciences study on siblings and systemic rheumatic disorders, sibling pairs should be full biological siblings who are the same sex and within five years of age (National Institute of Environmental Health Sciences 2016). First-cousin controls should also be biologically related to cases, of the same sex, and within five years of age of cases. Ideally, matched sibling and cousin controls will reduce genetic variation and eliminate the need for costly genotyping.

Participants must have been born in the United States. They also must have access to a computer and be computer literate (i.e. they are able to send emails and fill

out the online survey). Given the age distribution of AAV, study participants must be at least 18 years of age in order to provide more occupational and environmental exposure data and avoid confounding by any factors that may affect earlier disease onset.

Participant Recruitment

Given the exploratory nature of this study and the lack of comprehensive exposure information for cases and controls, it is not possible to conduct sample size calculations for this study (Dupont 1988). Since AAV is rare and this study's inclusion criteria are somewhat stringent, the goal is to recruit 500 cases and 1,000 corresponding controls. In order to increase the power of the study, any cases and their corresponding controls in addition to this 500-case goal will also be included. Before recruitment of any participants begins, the author will obtain approval from the University of Maryland Institutional Review Board (IRB). The author has already completed her Collaborative Institutional Training Initiative (CITI) certification for social and behavioral research.

Study recruitment will be conducted in two parts with the potential for a third round, depending on physician support of this project. First, cases will be contacted through three Facebook support groups: Vasculitis Foundation Discussion Forum, ANCA Vasculitis, and Let's Talk Vasculitis. As of June 2018, approximately 258 people with vasculitis have expressed interest in participating in the survey (~200 from Vasculitis Foundation Discussion Forum, ~18 from ANCA Vasculitis, and 40 from Let's Talk Vasculitis). However, all interested participants may not meet the inclusion criteria.

Ideally more participants would hear about the survey via word-of-mouth. Additionally, recruitment could be expanded to other smaller Facebook support groups if necessary. Although it is not possible to verify the Facebook-identified cases via medical records, all report a doctor's diagnosis of ANCA-associated vasculitis. Since the survey requires a sibling and first-cousin control for each case, it would be difficult for potential participants to inaccurately represent their diagnosis and identify controls who would support their claim.

In order to complement the data from self-reported cases, study recruitment will also include potential identification through existing cohorts of vasculitis patients at the National Institutes of Health's Vasculitis Translational Research Program (VTRP) and Medstar Georgetown University Hospital. In accordance with HIPAA, doctors will send letters and the study recruitment flyer directly to patients to maintain the privacy of their medical diagnoses. Mail is preferable over email for the original recruitment due to the age range of the cohort and the repute of mail from these institutions. Recruitment flyers will include the secure study email address so that potential participants can immediately get more information, provide informed consent, and answer screener questions. Anonymized data from the study will be shared with all participating doctors in order to help inform their future research.

Finally, contacts of the author have provided information on vasculitis researchers at The Johns Hopkins University School of Medicine and University of Pennsylvania School of Medicine who may be open to assisting with recruitment. If identifying cases through Facebook support groups, the VTRP, and Medstar Georgetown University Hospital does not procure a minimum of 500 cases, the

author will contact these additional researchers to request support in the recruitment process.

As cases are recruited through the Facebook groups and patient cohorts, those interested will be asked to email a secure University of Maryland email address to obtain access to the online survey. Upon emailing the study address, participant cases will be sent the link to the REDCap survey explained in the following section. The email with the survey link will also ask that the participant cases contact their own sibling and first-cousin controls. The email will include a flyer that cases can share with their potential controls to provide information on the study as well as the study contact information. If controls are interested in taking the survey, they can email the study address for a survey link. They will be asked to list the name of their relative with vasculitis who has already consented to and taken the survey so that the surveys can be linked.

Once the case participant and their controls have taken the survey, all three will be entered in a raffle to win a Visa gift card (amount to be determined). The raffle will occur at the end of data collection and the winner will be contacted via the email they used to take the survey.

Survey Development

Topics to Assess

Chapter 1 reviewed the current literature on AAV etiology. In the creation of the study methodology and development of the survey, the author selected what appear to be the most relevant environmental exposures and factors in AAV etiology.

The topics selected for inclusion in this survey (Appendix A) were demographic information, disease diagnosis and symptoms, health behaviors and characteristics, current occupation and work history, exposure from hobbies and living environment, and early childhood exposure. Participants will also have a chance to add their own thoughts on the survey and the potential causes of vasculitis.

Information on demographics and disease will be used to describe the population and further categorize the groups used for analysis. Most of the exposure questions are designed to collect information on the topics explored in Chapter 1. Questions on occupation, hobby, and home exposure assess potential exposures to inhaled irritants and solvents. Early childhood exposure questions will help evaluate the validity of the hygiene hypothesis for AAV development. Some of the factors implicated in the earlier literature review are not assessed in the survey. Medication use and infection were deemed too difficult to accurately assess without undue burden on participants. Overall, because this is a pilot study relying on sparse epidemiologic research, many of the survey questions are limited in scope. The purpose of the survey is to identify areas for future etiologic research. Further information about the rationale for each of the 43 questions can be found in Appendix B.

Sourcing Validated Questions

In order to evaluate the potential associations between these exposures and characteristics and the development of AAV, the author developed a comprehensive, 43-question survey with validated questions sourced from established research projects (Appendix A). Generic demographic questions were taken from national surveys including National Health and Nutrition Examination Survey (CDC n.d.) and

the Behavioral Risk Factor Surveillance System (CDC 2018). Specific exposure questions were accessed via the following documented research protocols: American Thoracic Society's Epidemiology Standardization Project (Ferris 1978), the Birmingham Vasculitis Activity Score (Birmingham Vasculitis Activity Score, n.d.), and the Early Life Exposures Assessment Tool (Walker and Bennett n.d.).

These research protocols have been carefully developed and tested. Benjamin Ferris, MD developed the Epidemiology Standardization Project to ensure that data on respiratory symptoms was collected uniformly (Ferris 1978). The Birmingham Vasculitis Activity Score (BVAS) identifies nine categories of vasculitis symptoms and lists clinical manifestations of these symptoms under each. Although it is used only by medical professionals, BVAS was used in the AAV etiologic survey created in this thesis to help participants with vasculitis comprehensively report their symptoms. The Early Life Exposures Assessment Tool (ELEAT) was developed by University of California, Davis researchers Cheryl Walker, MD and Deborah Bennett, PhD using funding from Autism Speaks. ELEAT focuses on prenatal and childhood exposures relevant to autism spectrum disorder (ASD), but the questions are more widely applicable (Walker and Bennett n.d.).

After reviewing the aforementioned surveys in full, the author used the database PhenX Toolkit (RTI International 2018) to search for additional questions on exposures relevant to AAV etiology. Questions found using PhenX Toolkit were from the 2001 Parkinson's Disease, Environment, and Genes (PEG) Study (Ritz 2001), the 1996 Long Island Breast Cancer Study (NIH 1996), and CDC's Pregnancy Risk Assessment Monitoring System (PRAMS) Phase 7 (CDC 2017). If questions on

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AAV-relevant topics were not available from any of these sources, original questions were created modeled on the wording of questions from these validated sources.

Analysis and Expected Results

Demographics and Disease

The analysis of the survey responses will be completed using SAS statistical software. Demographic information will be used to calculate descriptive statistics. The information provided by all participants in the demographics section and by cases in the disease section could be used to further divide analysis. For example, disease type (GPA, MPA, EGPA) or severity (BVAS score) could be used to divide cases and evaluate associations accordingly (i.e. determining whether an exposure could have an effect on disease severity). However, given the small sample size of this study, further division of participants would significantly reduce the statistical power.

In order to better describe the sample and to prepare for exposure analysis, it may be necessary to control for race and education. However, given the geographic ranges of AAV and the fact that this study will be based in the United States, it is likely that the majority of participants will be white, so controlling for race may not be informative. Finally, participant addresses should be geocoded and mapped using GIS software (see Appendix B, 13 for more information).

Environmental Exposures

In order to evaluate whether the assessed environmental exposures are potential risk factors for the development of AAV, SAS software will be used to calculate the adjusted odds ratios using conditional logistic regression. Multiple

conditional logistic regression may be used if data demonstrate that more than one exposure lead to statistically significant increased odds of developing AAV. While all exposures assessed in the survey have been associated with AAV development in the existing literature, the author expects significant ORs for occupations associated with dust exposure (i.e. construction and landscaping). A significant positive association between the diseases assessed in the survey and the development of AAV would be useful in identifying exposures that may affect the etiology of both diseases.

As with any retrospective study, recall bias and limited temporality are limitations for this study. However, by asking specific questions about the timing of the vasculitis diagnosis and listing options for the questions to assist participants in answering accurately, the author has sought to mitigate these limitations. Finally, environmental exposures will be further elucidated by controlling for genetic clustering of vasculitis and other autoimmune disorders. Additional information can be found in Appendix B.

Hygiene Hypothesis

As with the environmental exposure assessment, hygiene hypothesis data should be evaluated using adjusted odds ratios from conditional logistic regression. Multiple conditional logistic regression should also be run on related factors such as number of siblings and birth order to create a more comprehensive model. However, because the hygiene hypothesis has not been applied to AAV, future studies will be needed to identify the exposures associated with any factors found to be significant.

The principal issue in applying the hygiene hypothesis to AAV etiology is that timing is difficult to take into account. As explained previously, a major sticking

point in the hygiene hypothesis is the difficulty of distinguishing between triggering

and protective infections. This issue is even more apparent in AAV given the lack of

information on latency periods. Finally, although the geographic gradients associated

with the hygiene hypothesis give reason to suspect that GPA may be particularly

associated with hygiene hypothesis-related exposures, because AAV pathology is not

well understood, mechanisms to explain this association are not available. Therefore,

current AAV studies, including this thesis, are limited to epidemiologic methods.

Conclusion

In cases of chronic disease, prevention is often the only way to reduce disease

prevalence. Understanding the etiology of diseases like ANCA-associated vasculitis

empowers patients to take precautions and allows medical professionals to give more

specific advice. The study introduced in this thesis is preliminary; the data collected

from the proposed survey will only suggest associations and areas for future research.

Nevertheless, the study is necessary to both AAV prognosis and patient peace-of-

mind. Finally, although the author has previously been skeptical of the idea of a

vasculitis "cure," as put forth by the Vasculitis Foundation, determining the etiology

of AAV will allow for a better understanding of its pathogenesis, which in turn could

lead to earlier AAV identification, potentially while the disease is still reversible.

Appendices

APPENDIX A: AAV Survey for Cases and Controls

Thank you for participating in this study on the potential triggers for ANCA-associated vasculitis. Your responses and the responses of your cousins and siblings will help us learn more about the potential causes of ANCA-associated vasculitis. By comparing your answers to those of your family members, we will hopefully be able to identify patterns that tell us more about this disease. Please answer each question as honestly and accurately as possible. The survey should take about 45 to 60 minutes, but may be completed more quickly, depending on your answers.

Thank you for your support! We look forward to sharing the anonymized results with you.

BACKGROUND
The following section contains questions that give us more information about you and your participation in this study.

1) What is your full name?

2) Please provide an email address at which you can be reached. This will be used to follow up with you if necessary and also to award the raffle winner their prize.

3) What is your birthdate?
- MM/DD/YYYY

4) What is your sex?
- Male
- Female
- Other

5)
5a) Are you a vasculitis patient, a sibling of a vasculitis patient, or a cousin?
- Patient (Proceed to 5b)
- Sibling (Proceed to 5c)
- Cousin (Proceed to 5c)

5b) Which form of vasculitis do you have? (Proceed to 6)
- Granulomatosis with polyangiitis (GPA/Wegener's)
- Microscopic polyangiitis (MPA)
- Eosinophilic granulomatosis with polyangiitis (EGPA/Churg-Strauss)
- Other (Please specify)

5c) What is the name of your relative with vasculitis participating in this study?

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6) In what country were you born?
  a) Born in one of the 50 U.S. States or Washington, D.C.
  b) Born elsewhere

DEMOGRAPHIC INFORMATION
The following questions ask about different demographic information including your race, income, and education level. This information will help us understand more about the vasculitis community when compared with those without vasculitis.

7) In what state do you currently live?

8) What is the zip code where you currently live?

9) Are you Hispanic, Latino/a, or of Spanish origin?

10) Which one or more of the following would you say is your race?
- White
- Black or African American
- American Indian or Alaska Native
- Asian
  - Asian Indian
  - Chinese
  - Filipino
  - Japanese
  - Korean
  - Vietnamese
  - Other Asian
- Pacific Islander
  - Native Hawaiian
  - Guamanian or Chamorro
  - Samoan
  - Other Pacific Islander

11) What is the highest grade or year of school you completed?
- Never attended school or only attended kindergarten
- Grades 1-8 (Elementary)
- Grades 9-11 (Some high school)
- Grade 12 or GED (high school graduate)
- College 1 yr to 3 yrs (some college or technical school)
- College 4 yrs or more (college graduate)
- Other (Please specify)

12) This question is about your combined family income. Household income means your income plus the income of your family members that live in and/or contribute to your household. Is your annual **household** income from all sources for the past year:

- Less than $25,000
- Less than $35,000 ($25,000 to $34,999.99)
- Less than $50,000 ($35,000 to $49,999.99)
- Less than $75,000 ($50,000 to 74,999.99)
- $75,000 or more

13) Where are your current and previous residences? Please list.
  - Address (Street, City, State, ZIP code if available, Country)
    - Dates lived in location (Year to Year)

VASCULITIS DIAGNOSIS AND SYMPTOMS
This section of the survey will only be available to those who selected responded "Patient" to question 5a. The controls will be sent directly to the Health Behaviors and Characteristics section below.
Now we will ask you some questions about your condition.

14) When were you diagnosed?
  - Month, Year

15)
  15a) Do you have c-ANCA (ex. PR3)?
    - Yes
    - No
    - Don't know

  15b) Do you have p-ANCA (ex. MPO)?
    - Yes
    - No
    - Don't know

16) Please select the symptoms that you experienced at the time of your diagnosis. Please only select the symptoms that occurred **before** your diagnosis. The symptoms are divided into categories, but please select all that apply.
On the REDCap version of the survey each category currently in bold is left as a title and the symptoms are all displayed as a checklist.

General symptoms
- Muscle pain
- Joint pain or arthritis
- Fever
- Noticeable weight loss

Skin symptoms
- Infarct (as reported to you by a doctor)
- Purpura (red and/or purple rash)
- Skin ulcer or sore

- Gangrene (as reported to you by a doctor)
- Other skin symptoms (please specify)

Mouth, eye, or genital symptoms
- Mouth ulcers or sores
- Genital ulcers or sores
- Eye bulging
- Eye swelling or inflammation
- Conjunctivitis (pink eye) or eyelid inflammation
- Blurred vision
- Sudden loss of vision
- Bleeding or blood clot in your eye

Ear, nose, or throat symptoms
- Bloody nose/crusts inside the nose/sores in the nose
- Tenderness or pain in your sinuses
- Narrowing of your windpipe/trachea or tightness in your throat
- Hearing loss

Lung symptoms
- Wheeze
- Fluid in your lungs (as reported to you by a doctor)
- Bleeding in your lungs
- Respiratory failure that required you to use artificial respiration

Cardiovascular symptoms
- Medical staff unable to find your pulse
- Heart murmur (as reported to you by a doctor)
- History of one or more heart attacks
- Heart failure
- Other heart problems or symptoms (please specify)

Abdominal symptoms
- Abdominal pain
- Bloody diarrhea

Kidney symptoms
- Hypertension (high blood pressure)
- Protein in your urine (as reported to you by a doctor)
- Blood in your urine (as reported to you by a doctor)
- Elevated creatinine levels (as reported to you by a doctor)

Nervous system symptoms
- Headache
- Meningitis (as reported to you by a doctor)
- Disorientation or confusion

- Seizures
- Stroke
- Spinal cord lesion (as reported to you by a doctor)
- Neuropathy or numbness

17) How long after your initial symptoms did you go to a doctor?
- _____ months

18) How long after your initial symptoms were you diagnosed?
- _____ months

19) Are you in remission?
- Yes
- No

20) Have you had any disease flares?
- Yes
  - o Please list when each flare occurred after your initial diagnosis. If you can't remember the exact time of the flare, please provide an estimate.
    - Month, Year
- No

HEALTH BEHAVIORS AND CHARACTERISTICS

The following questions ask about your health and health behaviors. These questions cover smoking, allergies, mental health, and illness (other than vasculitis). This section starts with question 21. If you are not a vasculitis patient, you'll have skipped questions 14 through 20.

21)
    21a) Have you ever smoked cigarettes?
    - Yes
        - Do you now smoke cigarettes (as of 1 month ago)?
            - Yes
                - How many cigarettes do you smoke per day now?
            - No
                - If you have stopped smoking cigarettes completely, how old were you when you stopped?
        - How old were you when you first started regular cigarette smoking? ___ age in years
        - On average over the entire time you smoked, how many cigarettes did you smoke per day?
        - Do or did you inhale the cigarette smoke?
            - Not at all
            - Slightly
            - Moderately

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- Deeply
- No

21b) Have you ever smoked a pipe regularly?
- Yes
    - Do you now smoke a pipe (as of 1 month ago)?
        - Yes
            - How much pipe tobacco are you smoking now? _____ oz per week (a standard pouch of tobacco contains 1.5 oz)
        - No
            - If you have stopped smoking a pipe completely, how old were you when you stopped?
    - How old were you when you started to smoke a pipe regularly? ___ age in years
    - On average over the entire time you smoked a pipe, how much pipe tobacco did you smoke per week? _____ oz per week (a standard pouch of tobacco contains 1.5 oz)
    - Do or did you inhale the pipe smoke?
        - Not at all
        - Slightly
        - Moderately
        - Deeply
- No

21c) Have you ever smoked cigars regularly?
- Yes
    - Do you now smoke cigars (as of 1 month ago)?
        - Yes
            - How many cigars do you smoke per week now?
        - No
            - If you have stopped smoking cigars completely, how old were you when you stopped?
    - How old were you when you first started smoking cigars regularly? ___ age in years
    - On average over the entire time you smoked cigars, how many cigars did you smoke per week?
    - Do or did you inhale the cigar smoke?
        - Not at all
        - Slightly
        - Moderately
        - Deeply
- No

21d) Have you ever smoked cigarillos?
- Yes

- Do you now smoke cigarillos (as of 1 month ago)?
  - Yes
    - How many cigarillos do you smoke per week now?
  - No
    - If you have stopped smoking cigarillos completely, how old were you when you stopped?
- How old were you when you first started smoking cigarillos? ___ age in years
- On average over the entire time you smoked, how many cigarillos did you smoke per week?
- Do or did you inhale the cigarillo smoke?
  - Not at all
  - Slightly
  - Moderately
  - Deeply
- No

21e) Have you ever smoked E-cigarettes (also called vaping)?
- Yes
  - Do you now smoke E-cigarettes (as of 1 month ago)?
    - Yes
      - How many times per day do you use your E-cigarette?
    - No
      - If you have stopped smoking E-cigarettes completely, how old were you when you stopped?
  - How old were you when you first started smoking E-cigarettes? ___ age in years
  - On average over the entire time you smoked E-cigarettes, how many minutes did you smoke per day?
  - Do or did you inhale the E-cigarette smoke?
    - Not at all
    - Slightly
    - Moderately
    - Deeply
- No

22) Has a doctor, nurse, or other health professional ever told you that you had any of the following?
  22a) Ever told you have asthma?
  - Yes
    - How old were you when you were told you have asthma?
    - If you have vasculitis, were you diagnosed with asthma **before** you began having vasculitis symptoms?
      - Yes
      - No

- I do not have vasculitis.
  - Do you still have asthma?
    - Yes
    - No
- No

22b) Ever told you have a depressive disorder (including depression, major depression, dysthymia, or minor depression)?
- Yes
  - How old were you when you were told you have a depressive disorder?
  - If you have vasculitis, were you diagnosed with a depressive disorder **before** you began having vasculitis symptoms?
    - Yes
    - No
    - I do not have vasculitis.
- No

22c) Ever told you have an anxiety or stress-related disorder (Obsessive Compulsive Disorder, generalized anxiety, PTSD, etc.)?
- Yes
  - How old were you when you were told you have an anxiety or stress-related disorder?
  - If you have vasculitis, were you diagnosed with an anxiety or stress-related disorder **before** you began having vasculitis symptoms?
    - Yes
    - No
    - I do not have vasculitis.
- No

22d) Ever told you have allergies to the environment?
- Yes (Select all that apply)
  - Pollen
    - How old were you when you were told you have this allergy?
    - If you have vasculitis, were you diagnosed with this allergy **before** you began having vasculitis symptoms?
      - Yes
      - No
      - I do not have vasculitis.
    - Do you still have this allergy?
  - Mold spores
    - How old were you when you were told you have this allergy?
    - If you have vasculitis, were you diagnosed with this allergy **before** you began having vasculitis symptoms?
      - Yes
      - No

- I do not have vasculitis.
    - Do you still have this allergy?
- Dust mites
    - How old were you when you were told you have this allergy?
    - If you have vasculitis, were you diagnosed with this allergy **before** you began having vasculitis symptoms?
        - Yes
        - No
        - I do not have vasculitis.
    - Do you still have this allergy?
- Animal dander/fur
    - How old were you when you were told you have this allergy?
    - If you have vasculitis, were you diagnosed with this allergy **before** you began having vasculitis symptoms?
        - Yes
        - No
        - I do not have vasculitis.
    - Do you still have this allergy?
- Other (Please specify)
    - How old were you when you were told you have this allergy?
    - If you have vasculitis, were you diagnosed with this allergy **before** you began having vasculitis symptoms?
        - Yes
        - No
        - I do not have vasculitis.
    - Do you still have this allergy?
- No

22e) Ever told you have allergies to food?
- Yes
    - Cow's milk
        - How old were you when you were told you have this allergy?
        - If you have vasculitis, were you diagnosed with this allergy **before** you began having vasculitis symptoms?
            - Yes
            - No
            - I do not have vasculitis.
        - Do you still have this allergy?
    - Soy
        - How old were you when you were told you have this allergy?

- If you have vasculitis, were you diagnosed with this allergy **before** you began having vasculitis symptoms?
  - Yes
  - No
  - I do not have vasculitis.
- Do you still have this allergy?
- Eggs
  - How old were you when you were told you have this allergy?
  - If you have vasculitis, were you diagnosed with this allergy **before** you began having vasculitis symptoms?
    - Yes
    - No
    - I do not have vasculitis.
  - Do you still have this allergy?
- Wheat
  - How old were you when you were told you have this allergy?
  - If you have vasculitis, were you diagnosed with this allergy **before** you began having vasculitis symptoms?
    - Yes
    - No
    - I do not have vasculitis.
  - Do you still have this allergy?
- Peanuts
  - How old were you when you were told you have this allergy?
  - If you have vasculitis, were you diagnosed with this allergy **before** you began having vasculitis symptoms?
    - Yes
    - No
    - I do not have vasculitis.
  - Do you still have this allergy?
- Tree nuts
  - How old were you when you were told you have this allergy?
  - If you have vasculitis, were you diagnosed with this allergy **before** you began having vasculitis symptoms?
    - Yes
    - No
    - I do not have vasculitis.
  - Do you still have this allergy?
- Shellfish
  - How old were you when you were told you have this allergy?

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- If you have vasculitis, were you diagnosed with this allergy **before** you began having vasculitis symptoms?
  - Yes
  - No
  - I do not have vasculitis.
- Do you still have this allergy?
- Other (Please specify)
  - How old were you when you were told you have this allergy?
  - If you have vasculitis, were you diagnosed with this allergy **before** you began having vasculitis symptoms?
    - Yes
    - No
    - I do not have vasculitis.
  - Do you still have this allergy?
- No

22f) Ever told you have allergies to medication?
- Yes
  - Penicillin
    - How old were you when you were told you have this allergy?
    - If you have vasculitis, were you diagnosed with this allergy **before** you began having vasculitis symptoms?
      - Yes
      - No
      - I do not have vasculitis.
    - Do you still have this allergy?
  - Sulfa drugs
    - How old were you when you were told you have this allergy?
    - If you have vasculitis, were you diagnosed with this allergy **before** you began having vasculitis symptoms?
      - Yes
      - No
      - I do not have vasculitis.
    - Do you still have this allergy?
  - Insulin
    - How old were you when you were told you have this allergy?
    - If you have vasculitis, were you diagnosed with this allergy **before** you began having vasculitis symptoms?
      - Yes
      - No
      - I do not have vasculitis.
    - Do you still have this allergy?

- X-ray contrast/Iodine
    - How old were you when you were told you have this allergy?
    - If you have vasculitis, were you diagnosed with this allergy **before** you began having vasculitis symptoms?
        - Yes
        - No
        - I do not have vasculitis.
    - Do you still have this allergy?
- Other (Please specify)
    - How old were you when you were told you have this allergy?
    - If you have vasculitis, were you diagnosed with this allergy **before** you began having vasculitis symptoms?
        - Yes
        - No
        - I do not have vasculitis.
    - Do you still have this allergy?
- No

22g) Ever told you have allergies to chemicals?
- Yes
    - Detergents
        - How old were you when you were told you have this allergy?
        - If you have vasculitis, were you diagnosed with this allergy **before** you began having vasculitis symptoms?
            - Yes
            - No
            - I do not have vasculitis.
        - Do you still have this allergy?
    - Other (please specify)
        - How old were you when you were told you have this allergy?
        - If you have vasculitis, were you diagnosed with this allergy **before** you began having vasculitis symptoms?
            - Yes
            - No
            - I do not have vasculitis.
        - Do you still have this allergy?
- No

22h) Ever told you have a clotting disorder?
- Yes
    - Clotting Disorder
        - How old were you when you were told you have a clotting disorder?

- If you have vasculitis, were you diagnosed with this clotting disorder **before** you began having vasculitis symptoms?
  - Yes
  - No
  - I do not have vasculitis.
- Deep Vein Thrombosis
  - How old were you when you were told you had deep vein thrombosis?
  - If you have vasculitis, were you diagnosed with deep vein thrombosis **before** you began having vasculitis symptoms?
    - Yes
    - No
    - I do not have vasculitis.
- Pulmonary Embolism
  - How old were you when you were told you had a pulmonary embolism?
  - If you have vasculitis, were you diagnosed with a pulmonary embolism **before** you began having vasculitis symptoms?
    - Yes
    - No
    - I do not have vasculitis.
- Unknown Clotting Disorder
  - How old were you when you were told you have an unknown clotting disorder?
  - If you have vasculitis, were you diagnosed with this clotting disorder **before** you began having vasculitis symptoms?
    - Yes
    - No
    - I do not have vasculitis.
- No
22i) Ever told you have hypertension/high blood pressure
- Yes
  - How old were you when you were first told you had high blood pressure?
  - If you have vasculitis, were you diagnosed with high blood pressure **before** you began having vasculitis symptoms?
    - Yes
    - No
    - I do not have vasculitis.
  - Do you still have high blood pressure?
- No

22j) Ever told you had a thyroid disorder? (Hypothyroidism, Hyperthyroidism, Graves Disease, etc.)

- Yes
  - How old were you when you were told you had a thyroid disorder?
  - If you have vasculitis, were you diagnosed with a thyroid disorder **before** you began having vasculitis symptoms?
    - Yes
    - No
    - I do not have vasculitis.
- No

22k) Ever told you have eczema?

- Yes
  - How old were you when you were first told you had eczema?
  - If you have vasculitis, were you diagnosed with eczema **before** you began having vasculitis symptoms?
    - Yes
    - No
    - I do not have vasculitis.
- No

22l) Ever told you had hay fever?

- Yes
  - How old were you when you were first told you had hay fever?
  - If you have vasculitis, were you diagnosed with hay fever **before** you began having vasculitis symptoms?
    - Yes
    - No
    - I do not have vasculitis.
- No

22m) Ever told you had an autoimmune disorder **other than** vasculitis? (Including but not limited to lupus, multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, type 1 diabetes mellitus, psoriasis, etc.)

- Yes (please specify)
  - How old were you when you were first told you had this autoimmune disorder?
  - If you have vasculitis, were you diagnosed with this autoimmune disorder **before** you began having vasculitis symptoms?
    - Yes
    - No
    - I do not have vasculitis.
- No

OCCUPATION

The following questions ask you about your job history and any exposures you may have experienced in the workplace.

23) Which of the following **best** describes your current occupation? After selecting the category, please specify your job title in the provided box.

      Agriculture/Farming
      Construction
      Food Preparation/Food Server/Waiter or Waitress
      Health Care Worker/Doctor/Nurse
      Janitor/Housekeeper
      Laboratory Technician/Scientist
      Landscaping
      Manufacturing/Factory
      Military
      Public Safety (Police, Firefighter, Security Guard)
      Repair Services/Mechanic/Plumber
      Retail/Sales/Cashier
      Student
      Teacher/Teacher's Aid
      Transport equipment operatives/Drivers
      Warehouse/Stockroom
      Work in an office environment
      Not Employed
      Other (Please specify)

24) What was your occupation before your current position? After selecting the category, please specify your job title in the provided box.

      Agriculture/Farming
      Construction
      Food Preparation/Food Server/Waiter or Waitress
      Health Care Worker/Doctor/Nurse
      Janitor/Housekeeper
      Laboratory Technician/Scientist
      Landscaping
      Manufacturing/Factory
      Military
      Public Safety (Police, Firefighter, Security Guard)
      Repair Services/Mechanic/Plumber
      Retail/Sales/Cashier
      Student
      Teacher/Teacher's Aid
      Transport equipment operatives/Drivers
      Warehouse/Stockroom
      Work in an office environment
      Not Employed

Other (Please specify)

25) Please select the categories for all occupations you have held.
Agriculture/Farming
Construction
Food Preparation/Food Server/Waiter or Waitress
Health Care Worker/Doctor/Nurse
Janitor/Housekeeper
Laboratory Technician/Scientist
Landscaping
Manufacturing/Factory
Military
Public Safety (Police, Firefighter, Security Guard)
Repair Services/Mechanic/Plumber
Retail/Sales/Cashier
Student
Teacher/Teacher's Aid
Transport equipment operatives/Drivers
Warehouse/Stockroom
Work in an office environment
Never Employed
Other (Please specify)

26) Have you ever worked for a year or more in any dusty job?
- Yes
  ▪ If yes, which industry? (Please specify)
  ▪ What year did you begin working in this industry?
  ▪ How many years total did you work in this industry?
  ▪ Was dust exposure:
    o Mild
    o Moderate
    o Severe
- No

27) Have you ever been exposed to gas or chemical fumes in your work?
- Yes
  ▪ If yes, which industry? (Please specify)
  ▪ What year did you begin working in this industry?
  ▪ How many years total did you work in this industry?
  ▪ Was gas or fume exposure:
    o Mild
    o Moderate
    o Severe
- No

28) In work **or** daily life, were you regularly exposed to any of the following?

- Asbestos
- Volatile chemicals or solvents
- Coal or stone dusts
- Coal tar/pitch/asphalt
- Diesel engine exhaust
- Dyes
- Formaldehyde
- Gasoline or gasoline exhaust
- Paints
- Pesticides/herbicides (such as weed killer/Roundup, permethrin, chlorpyrifos, etc.)
- Textile fibers/dusts
- Wood dust
- Varnish/lacquer

HOBBIES

The following question asks whether you participate in any of the listed hobbies and how often you take part in these hobbies.

29) Have you ever participated for 6 months or longer in any of the following hobbies?
- Hobbies using glues
    - In what year were you first involved in this hobby?
    - In what year were you last involved?
        - Box for year or allow to check "still involved"
    - On average, about how many hours per month have you used glues for this hobby?
- Hobbies involving soldering, such as jewelry making or stained glass
    - In what year were you first involved in this hobby?
    - In what year were you last involved?
        - Box for year or allow to check "still involved"
    - On average, about how many hours per month have you soldered?
- Developing photographs
    - In what year were you first involved in this hobby?
    - In what year were you last involved?
        - Box for year or allow to check "still involved"
    - On average, about how many hours per month have you participated in this hobby?
- Oil painting
    - In what year were you first involved in this hobby?
    - In what year were you last involved?
        - Box for year or allow to check "still involved"
    - On average, about how many hours per month have you participated in this hobby?
- Woodworking or refinishing furniture
    - In what year were you first involved in this hobby?

- In what year were you last involved?
  - Box for year or allow to check "still involved"
- On average, about how many hours per month have you participated in this hobby?
- Ceramics or pottery making
  - In what year were you first involved in this hobby?
  - In what year were you last involved?
    - Box for year or allow to check "still involved"
  - On average, about how many hours per month have you participated in this hobby?
- Leather crafting
  - In what year were you first involved in this hobby?
  - In what year were you last involved?
    - Box for year or allow to check "still involved"
  - On average, about how many hours per month have you participated in this hobby?
- Other activities involving the use of chemicals (Please specify)
  - In what year were you first involved in this hobby?
  - In what year were you last involved?
    - Box for year or allow to check "still involved"
  - On average, about how many hours per month have you participated in this hobby?
- Gardening
  - In what year were you first involved in this hobby?
  - In what year were you last involved?
    - Box for year or allow to check "still involved"
  - On average, about how many hours per month have you participated in this hobby?

OTHER EXPOSURES
The following questions ask about exposures in or around your home.

30) In the last ten years were there any renovations to your home, such as adding a room, putting up or taking down a wall, replacing windows, or refinishing floors?
- Yes
  - Were these renovations before you began having vasculitis symptoms?
    - Yes
    - No
    - I do not have vasculitis
- No

31) In the last ten years was any pesticide fogger / fumigator used **inside** your home?
- Yes
  - Was this use of pesticides before you began having vasculitis symptoms?
    - Yes

- No
- I do not have vasculitis
- No

32) In the last ten years did professionals (exterminators, landscape/ garden service) apply any pesticides to kill bugs **outside** your home?
- Yes
  - o Was this use of pesticides before you began having vasculitis symptoms?
    - Yes
    - No
    - I do not have vasculitis
- No

EARLY CHILDHOOD EXPOSURES
The following questions ask about your early life as an infant and young child.

33) How were you delivered?
- Vaginally
- Cesarean delivery (C-section)
- Don't know

34) Were you born early?
- Yes, I was very premature (born between 23 and 28 weeks of pregnancy)
- Yes, I was somewhat premature (born between 29 and 36 weeks of pregnancy)
- No, I was not born early (born between 37 and 40+ weeks of pregnancy)
- Don't know

35) In your family are you the:
- First child
- Second child
- Third child
- Other (Please specify)

36) How many siblings (full or half) do you have that lived in the same house as you growing up?
- Total number of siblings: _____
- Number of older brothers: _____
- Number of younger brothers: _____
- Number of older sisters: _____
- Number of younger sisters: _____

37) The next two questions are about the home or homes you lived in during your **first year of life**.

37a) Was this home within ¼ mile (~400 meters) of an agricultural field or golf course?
- Yes
- No

37b) Was this home a farm?
- Yes
  - o What kind of farm?
    - ▪ Livestock
      - o What kind of livestock? (Please specify)
      - o During your first three years of life, did you have regular contact with the farm animals?
      - o Did your mother have regular contact with the farm animals while she was pregnant with you?
    - ▪ Crops
    - ▪ Livestock and crops
      - o What kind of livestock? (Please specify)
      - o During your first three years of life, did you have regular contact with the farm animals?
      - o Did your mother have regular contact with the farm animals while she was pregnant with you?
- No

38) Did your family have a dog, cat, or bird living in your home from three months prior to your mother becoming pregnant with you through your first year after birth?
- No
- Cat
- Dog
- Bird
- Cat + dog
- Cat + bird
- Dog + bird
- Cat + dog + bird

39) Did you attend daycare in your first three years of life?
- Yes
- No

ADDITIONAL INFORMATION
The following section is the final part of this questionnaire. These questions ask about diseases in other family members and give you a chance to offer your own thoughts and feedback.

40) Does anyone else in your family have vasculitis?
In this question, "family" refers to your biological relatives, or those related to you by blood. This may include maternal and paternal biological grandparents, birth parents, biological siblings, the biological siblings of your birth parents (i.e. aunts and uncles),

the children of your birth parents' siblings (your cousins), but **<u>does not</u>** include people who married into your family (ex. in-laws) or someone who was adopted but is not genetically related to you.
- Yes
    - If yes, what is their relationship to you (ex. mother, father, grandparent, paternal uncle, maternal uncle, maternal cousin, etc.)?
- No

41) Does anyone else in your family have an autoimmune disorder other than vasculitis (not including allergies)? Again, as in the above question, here "family" refers only to people genetically related to you. Autoimmune disorders include illnesses such as lupus, multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, type 1 diabetes mellitus, psoriasis, etc.
- Yes
    - If yes, what is their relationship to you (ex. mother, father, grandparent, paternal uncle, maternal uncle, maternal cousin, etc.)?
- No

42) What do you think caused your/your relative's vasculitis?

43) Do you have any other comments on your survey responses or suggestions on how to improve this study?

This concludes the survey. Thank you for your participation and support of the vasculitis community!

APPENDIX B: Survey Key

<u>BACKGROUND</u>
1) The name of the participant will be used to match the cases to the controls. It will be matched using the controls' responses to question 5c.

2) The email address will allow for follow up should it be necessary. It will also be used to contact the participant who wins the raffle prize. Additionally, many potential participants have asked whether the results of this study will be made available to them. This email address can be used to distribute a plain language guide.

3) The formatting of this question was sourced from NHANES 2005–2006 (CDC n.d.). It will be used to calculate participant ages and to ensure that all controls are within five years of age of the cases.

4) This question is from BRFSS 2018 (CDC 2018). It will be used to examine the sex ratio in the surveyed population, as well as to ensure that all controls are the same sex as their matched case.

5) These questions determine whether the participant answering the survey is a case or control. The branching patterns for the rest of the survey will be based off of the answers to these questions. 5a determines case or control status, 5b follows up with cases to ensure that they have AAV and not a different form of vasculitis, and 5c asks controls to provide the name of the relative who invited them to the study so that their answers can be matched to their case.

6) This question is from NHANES 1999-2000 (CDC n.d.). It is a screener question, as one of the inclusion criteria for participation in this study is that participants must have been born in the United States.

<u>DEMOGRAPHIC INFORMATION</u>
7) This question is from BRFSS 2018 (CDC 2018). It will be used to quickly determine the distribution of people in the study. It will also be useful in verifying the answers to question 13 and will make coding residences easier.

8) This question is from BRFSS 2018 (CDC 2018). It will be used to quickly determine the distribution of people in the study. It will also be useful in verifying the answers to question 13 and will make coding residences easier.

9) This question is from BRFSS 2018 (CDC 2018). It is separate from the following question on race because Hispanic/Latino/a as a category refers to an ethnicity, rather than a race. People may identify as Hispanic/Latino/a and also select any of the races given in question 10. It will be used to provide demographic information on the surveyed population.

10) This question is from BRFSS 2018 (CDC 2018). It will be used both to describe the surveyed population and, depending on the variation in reported race, to control for race when examining potential associations between exposure and AAV development.

11) This question is from BRFSS 2018 (CDC 2018). It gives demographic information used to better describe the population, and it may be used as a control when examining potential associations between exposure and AAV development.

12) This question was adapted from BRFSS 2018 (CDC 2018) with additional wording from NHANES 2015-2015 (CDC n.d.). It will be used to describe the population and can also be used to determine whether AAV may vary by socioeconomic status. Additionally, given the high cost of AAV treatment, the income data for cases could be used to begin a commentary on Medicaid for patients with AAV.

13) This residence question was found using PhenX Toolkit. It is from the 2001 Parkinson's Disease, Environment, and Genes Study (PEG) (Ritz 2001). It will be used to geocode participants' residences to look for clusters. As a result of the date of birth information collected in question 3, it may be possible to color code residences by age (ex. residence before age 5). This would allow the author to examine location while also beginning to consider time as a factor in AAV development.

<u>VASCULITIS DIAGNOSIS AND SYMPTOMS</u>
14) This question allows the author to establish temporality between diagnosis and exposures/illness assessed later in the survey. It can also be used to establish cases' residential location at the time of diagnosis. Additionally, it can be used to evaluate whether most of the participants are recently diagnosed, or whether they have had the disease for an extended period of time.

15) This question will be used to identify disease phenotype, which may be a more accurate way to categorize disease. However, not all patients will know this information, so most of the analysis will rely on cases' self-identified disease type.

16) This list of nine categories and accompanying symptoms has been adapted from BVAS (Birmingham Vasculitis Activity Score n.d.). The author used more accessible language than the clinical terms used in BVAS. This will allow for the better description of the disease and may potentially allow cases' BVAS scores to be calculated, which could add a dimension of disease severity to the analysis.

17) This question, in combination with question 18, can assess whether there is a gap between the report of symptoms to a medical professional and the receipt of a diagnosis.

18) This question, in combination with question 17, can assess whether there is a gap between the report of symptoms to a medical professional and the receipt of a diagnosis.

19) This question helps assess the state of the cases' disease currently. It may also inform the author's assessment of disease severity.

20) This question collects information on the timing of disease flares in order to better understand the disease and allow for the association of a flare with a triggering exposure.

HEALTH BEHAVIORS AND CHARACTERISTICS

21) 21a through 21e assess smoking behavior. These questions are based off the format established in Ferris 1978 This will allow the author to gather very specific information on products used and frequency of smoking and can be used to evaluate a potential association between smoking and AAV.

22) This multiple part question collects limited information on the medical history of the cases and controls. The format and content of these questions are based on BRFSS 2018 (CDC 2018) and ELEAT (Walker and Bennett n.d.). An additional question to assess the temporality of these medical issues compared to the vasculitis diagnosis of cases has been added. These questions will be used to determine whether AAV is associated with any other medical issues. Asthma is included due to similar risks from inhaled exposures and its association with EGPA. Depressive and anxiety disorders are included because a recent study found that stress disorders were associated with the development of autoimmune diseases, including vasculitis (Song et al. 2018). Allergies, hay fever, and eczema are included because of the potential link between AAV and the hygiene hypothesis. Clotting disorders and hypertension are included given the cardiovascular nature of AAV. Thyroid disorders are included because of the influence of the thyroid on the immune system. Finally, autoimmune disorders are included due to their demonstrated familial clustering.

OCCUPATION

23) This question asks about current employment. The list of occupations has been adapted from ELEAT (Walker and Bennett n.d.) with additions from Ferris 1978. It can be used both to look for an association between job category and AAV development and to infer potential exposure by job category (ex. construction and silica dust). This question is also important because many vasculitis patients go on disability or stop working after their diagnosis. The data collected in this question may speak to this pattern.

24) This question asks about the job held before current employment. It can be used both to look for an association between job category and AAV development and to infer potential exposure by job category (ex. construction and silica dust). It is also useful to establish whether the participant has spent a number of years in the same

industry, and to identify which industry a participant worked in if they are no longer working.

25) This question will all the author to create a general work history. As in questions 23 and 24, it can be used both to look for an association between job category and AAV development and to infer potential exposure by job category (ex. construction and silica dust).

26) This question from Ferris 1978 is a crude measure of exposure. Unlike the occupation category where exposure must be assumed (ex. silica dust and construction), this question allows a more specific reporting of the exposure. The author is particularly interested in this question, given the documented association between silica dust exposure and AAV development.

27) This question from Ferris 1978 is a crude measure of exposure. Unlike the occupation category where exposure must be assumed (ex. silica dust and construction), this question allows a more specific reporting of the exposure. Gas and chemical fumes are inhaled irritants, and could also be a result of exposure to solvents, both of which are implicated in AAV research.

28) This question is adapted from ELEAT (Walker and Bennett n.d.) as another general measure of exposure. Notably, this question also asks about daily life in addition to work exposures.

HOBBIES
29) This question was adapted from the Long Island Breast Cancer Study (NIH 1996). It assesses chemical and physical exposures that occur during hobbies. It also assesses the frequency and duration of these potential exposures. Most of the exposures from these hobbies would be inhaled. As with the above questions on occupation type, exposure must be inferred. However, given the constraints of a retrospective study, that is likely the most accurate way to assess these exposures.

OTHER EXPOSURES
30) This question from ELEAT (Walker and Bennett n.d.) is a proxy for inhaled exposures from renovation. The time period is set in the last ten years to reduce recall bias. If the participant responds affirmatively, they are also asked about their vasculitis status and whether symptoms of their vasculitis (if they have it) developed before the renovations. This question on timing establishes basic temporality.

31) This question from ELEAT (Walker and Bennett n.d.) is a proxy for pesticide exposure. It can be assumed that individuals exposed to pesticides sprayed inside their homes will have been exposed to higher levels of these chemicals. The time period is set in the last ten years to reduce recall bias. If the participant responds affirmatively, they are also asked about their vasculitis status and whether symptoms of their vasculitis (if they have it) developed before the renovations. This question on timing establishes basic temporality.

32) This question from ELEAT (Walker and Bennett n.d.) is a proxy for pesticide exposure. It can be assumed that individuals exposed to pesticides sprayed outside their homes will have been exposed to lower levels of these chemicals. The time period is set in the last ten years to reduce recall bias. If the participant responds affirmatively, they are also asked about their vasculitis status and whether symptoms of their vasculitis (if they have it) developed before the renovations. This question on timing establishes basic temporality.

EARLY CHILDHOOD EXPOSURES
The following questions are all potentially relevant to the hygiene hypothesis.
33) This question is adapted from CDC's Pregnancy Risk Assessment Monitoring System (CDC 2017). It will be used to determine whether there is an association between mode of birth and development of AAV.

34) This is an original question created to assess timing of birth. Like mode of birth in question 33, timing of birth is thought to be associated with the development of the gut microbiome. Three options for timing of birth in addition to the "Don't know" option are included to distinguish between the premature birth that often requires NICU care and premature birth that may just mean more limited development (New York State Department of Health 2009).

35) This question was adapted from ELEAT (Walker and Bennett n.d.) and establishes birth order. Birth order has been implicated in the hygiene hypothesis as a mitigating factor for the development of allergic and atopic disease.

36) This is an original question that relates to question 35. It assesses number and sex of older and younger biologic (full or half) siblings that lived in the participants' childhood home. Number and sex of siblings has been implicated in the hygiene hypothesis. This question can also be used to calculate total number of siblings.

37) 37a and 37b ask about residence in the first year of life. 37a was adapted from ELEAT (Walker and Bennett n.d.) and can be used to assess inhaled exposures, including exposures to pesticides. This question does not include parks because parks likely use lower concentrations of pesticides (if any). Additionally, living close to a park is also potentially a confounder for SES, which is independently associated with the development of atopic, allergic, and autoimmune diseases. 37b was adapted from the PEG study (Ritz 2001), but additional questions regarding livestock type, contact with animals, maternal exposures, and crops were added by the author. These factors have been shown to alter the protect effects of the farm on the development of allergic disease and asthma.

38) This question is adapted from Ferris 1978 and ELEAT (Walker and Bennett n.d.). Exposure to pets, particularly dogs, at a young age has been shown to be protective against the development of atopic disease.

39) This question is adapted from Ferris 1978. Like sibling size, daycare attendance at a young age has been shown to be protective against the development of atopic disease.

ADDITIONAL INFORMATION

40) This question asks participants to identify any blood relatives with vasculitis. This will provide information about familial clustering, which can be used as a control to further reduce the influence of genetics in this study. However, if a case identifies a sibling or parent with vasculitis, this might point to an environmental exposure in or around their home and merits further investigation.

41) This question also assesses familial clustering of autoimmune disorders. Like question 40, this could be used to control for genetics in order to better isolate environmental factors. However, if a case identifies a sibling or parent with an autoimmune disorder, this might point to an environmental exposure in or around their home and merits further investigation.

42) This question allows participants to add their own thoughts about AAV etiology. It could provide new lines of research, and it is also likely to make cases feel more respected.

43) This question allows participants to clarify any of their answers and to give feedback on the survey.

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