pylori, influenza viruses, cytomegalovirus, Epstein-Barr virus, and herpes simplex virus 1) and CVD [Mendall et al., 1995; Fraser et al., 2003; Guan et al., 2012; Sorlie et al., 2000; Simanek et al., 2011; Ibrahim et al., 2005]. In addition, pathogen burden (i.e., the number of different infections in a given individual) has been implicated in the development of atherosclerosis, suggesting that cumulative infectious dose may increase CVD risk [Espinola-Klein et al., 2002; Rupprecht et al., 2001; Rosenfeld and Campbell 2011]. Individuals vary in their susceptibility to infectious diseases, which is attributable to various factors such as sex, age, nutritional status, socioeconomic status, and stress, etc. [Armstrong et al., 2001; Dowd and Aiello, 2009], and host genetic variation also plays a role [International HIV Controllers Study, 2010; McLaren 2012; Timmann et al., 2012; Maran et al., 2012].

Participants in the Genetics of Coronary Artery Disease in Alaska Natives (GOCADAN) study were previously shown to have moderate to high seroprevalence rates for five pathogens (*Helicobacter pylori*, cytomegalovirus, herpes simplex virus 1, herpes simplex virus 2, and *Chlamydophila pneumoniae*) that are suspected to play a role in CVD [Zhu et al., 2006]. In this population, pathogen burden was positively correlated with C-reactive protein (CRP), which is a marker of chronic inflammation and is considered to be a CVD risk factor, and CRP in turn was positively correlated with CVD [Howard et al., 2008; Howard et al., 2010]. Here we report seroprevalence rates for a larger number of GOCADAN participants, as a follow-up to the results presented by Zhu et al., [2006], and we determine the genetic contribution to differences in antibody level and conduct genome-wide linkage analysis in order to localize genetic factors influencing these traits.

Methods

Study population

Participants included 801 family members (Table I) who participated in the GOCADAN study, which was designed to identify genetic risk factors for CVD [Howard et al., 2005; Ebbesson et al., 2006]. The study population resides in 8 villages and the city of Nome in the Norton Sound region of Alaska, and is predominantly Inupiaq. The majority of individuals (678 out of 801) belong to a single, large, complex pedigree that was broken into smaller pedigrees in order to facilitate analysis. Phenotyped individuals in this sample belong to 128 pedigrees, the largest of which includes 162 family members, representing up to 5 generations. Most phenotyped individuals are members of extended pedigrees. Participants were recruited during the years 2000–2004, ranged from 18–91 years of age (with an average age of 45 years), and consisted of 463 women and 338 men. This study also utilized archived samples that were collected from the same participants 15 to 20 years earlier (referred to here as the “baseline” visit) by the Centers for Disease Control (CDC), prior to this study. The baseline age of participants ranges from >1 year to 74 years old. Permissions to conduct the study were granted by the Norton Sound Health Corporation and the institutional review boards of all participating institutions. Signed statements of consent were obtained from all study participants.

Serology

Serum samples were collected from participants during 2000 to 2003 and stored at −80°C. Archived serum samples were obtained from the CDC in Alaska, which were collected 15–20 years earlier, prior to the initiation of the GOCADAN study. Both the archived (or “baseline”) and “follow-up” serum samples were thawed immediately before testing, and assays were run at the same time for both sets of samples. Not all study participants were phenotyped for antibody levels against all pathogens for both visits, and the exact sample sizes (ranging from 495 to 782) are given in Table II. Commercially available ELISA assays

were used to quantify IgG antibody levels against the following pathogens: *Helicobacter pylori* and cytomegalovirus (CMV) (Wampole, Cranbury NJ); and herpes simplex virus 1 (HSV1) and herpes simplex virus 2 (HSV2) (Focus, Cypress, CA). An IgG titer ≤0.9 was considered seronegative, 0.9 to 1.1 indeterminate, and ≥ 1.1 seropositive. IgG antibodies to *Chlamydophila pneumoniae* were quantified using microimmunofluorescence. The antigen used in this test consisted of elementary bodies (EBs) of the Finnish strain Kajaani 6 (Laboratory for Chlamydia and Respiratory Bacterial Infections, National Public Health Institute, Finland) and an IgG titer ≥1:32 was considered seropositive for *C. pneumoniae*, as previously described [Zhu et al., 2006; Wang, 2000]. Seroconversion was calculated as the proportion of seronegative individuals at the baseline clinic visit who were seropositive at the follow-up clinic visit. Seroreversion (i.e., a negative seroprevalence result at the second

the follow-up visit were fairly low for *C. pneumoniae* and HSV-2 (16% and 17%, respectively) and quite high for HSV-1, *H. pylori* and CMV (seroconversion rates ranged from 40% to 67%), reflecting the higher seroprevalence of these pathogens (Table I). As primary infection with many of these pathogens occurs in childhood, it is not surprising that some individuals seroconverted during the 15–20 year time period given that a number of individuals were children at the time that the baseline samples were collected by the CDC.

Discussion

This research confirms that there is a high level of seroprevalence and long-term antibody persistence among the study participants. Our results are similar to those of an earlier investigation of the same five infectious pathogens in a smaller sub-set of 610 participants [Zhu et al., 2006], with only slight differences due to our exclusion of indeterminate values (i.e., samples with antibody titers falling within the 0.9 to 1.1 range) and an increased sample size used here. Both studies report a substantial level of chronic infection in this Alaska Native population, which suffers from CVD. Chronic infection, such as seen in this population, may lead to prolonged systemic and/or localized inflammation caused by the host's immune system that may ultimately result in atherosclerosis and other ageing-related diseases, as suggested by other studies [Mendall et al., 1995; Fraser et al., 2003; Guan et al., 2012; Sorlie et al., 2000; Simanek et al., 2011; Ibrahim et al., 2005; Espinola-Klein et al., 2002; Rupprecht et al., 2001; Rosenfeld and Campbell, 2011]. Indeed, previous research involving GOCADAN study participants demonstrated a significant, positive correlation between pathogen burden and CRP, a risk factor for CVD [Howard et al., 2008].

The heritability estimates presented here show that individual genetic differences do contribute to the majority of these serological phenotypes in this sample of Alaska Natives, suggesting that pathogen exposure alone is not enough to determine infection status, at least if one assumes that genetic factors do not primarily modify behavior that influences exposure risk, frequency and intensity. Many of these pathogens are common and are transmitted via the oral/respiratory route, suggesting that every individual is exposed repeatedly throughout life. For this reason, a seronegative status is informative for genetics investigations, as this status is unlikely to simply reflect the absence of exposure. HSV-2 is an exception. It is the only primarily sexually-transmitted pathogen examined here, and exposure to it therefore may be limited. This may also explain the small heritability estimates for this pathogen, as seronegative individuals may provide little information on host genetic effects. In an attempt to address this, we analyzed the quantitative antibody levels of HSV-2 seropositive individuals only (n=254 for baseline visit, and n=354 for follow-up visit), as these individuals must have been exposed (assuming absence of false positives). The resulting heritability estimates were 0.00 (p=0.5) for both visits. This may indicate that the low heritability levels obtained with HSV-2 cannot be explained entirely by non-exposure, although the reduced sample size complicates interpretation. The biological interpretation of our results is complicated by the nature of serological phenotypes. The presence of antibodies indicates a past or present infection, but it is not clear whether, for example, a high antibody titer represents success in warding off infection, or whether the immune system was less efficient at dealing with the invading pathogen, or if it is related to the timing of infection (that the infection was more recent).

Significant heritability estimates justify further investigation in order to localize underlying genetic factors that may influence these traits. Our genome-wide linkage analysis for *C. pneumoniae* IgG antibody titer trait gave a significant LOD score of 3.13 at 15q22.31. Several potential candidate genes lie within this large region (the 1-LOD linkage region spans 15q22.1 – 15q26.2), including *SMAD6* (located at 15q22.31), which is involved in endocytosis and negative regulation of apoptosis by inhibiting signaling by the TGF- superfamily [Lee et al., 2011; Park, 2005]. *C. pneumoniae*, a common cause of acute respiratory infection, is an obligate intracellular bacterium that induces apoptosis resistance in host cells in order to prevent eradication of infected epithelial cells during the early stage of infection [Rajalingam et al., 2001; Fischer et al., 2001]. Other genes in this region that may be involved in genetic control of antibody levels to *C. pneumoniae* include: SEMA7A (at 15q24.1), which stimulates cytokine production, chemotaxis, and superoxide release by monocytes; IL-16 (at 15q25.1), a pro-inflammatory cytokine that influences CD4+ T

lymphocytes, eosinophils, and monocytes and upregulates IL-2 receptors; and AEN (apoptosis-enhancing nuclease, at 15q26.1). We did not find significant evidence of linkage, however, for the remaining four pathogens. This may have to do with antibody titers being an imprecise measure of current or past infection, as they are not a direct measure of the pathogens themselves. It is also possible that the size of the effect is small and/or that our modest sample size (by genome wide analysis standards) makes us unable to detect potential susceptibility loci for these other pathogens at this time. However, a comparison of the genomic regions of interest identified in our study with previously published genome-wide association studies (Supplemental Table III) points to a number of genes involved in immune response (including *FREM2*, which may be involved in antibody response to infection with CMV), coronary heart disease and other age-related diseases (e.g., type 2 diabetes and Alzheimer's disease) that are potentially of interest.

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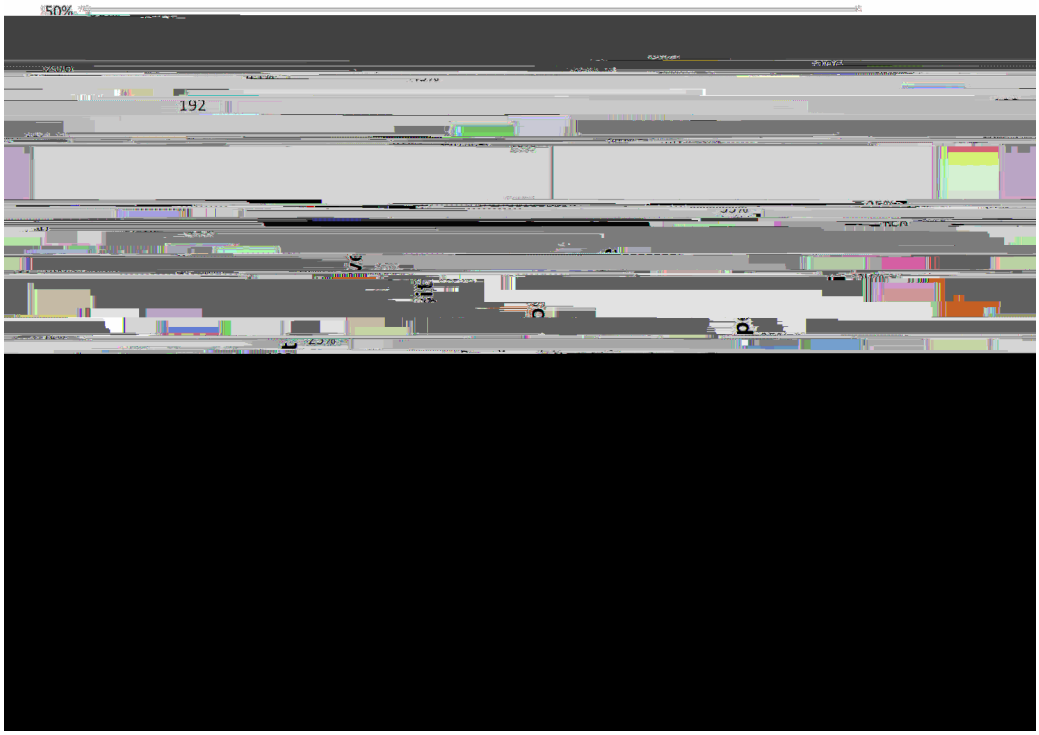

Figure 2.
Number of seropositive reactions to infectious pathogens for 467 participants in the GOCADAN study for both baseline and follow-up visits.

Figure 3.
Chromosome 15 linkage results for *C. pneumonia9-[rom9998 0 Tdbpr94 fo8 0 Td(819mT0np.m900 Q 1 0 0np.moli*

Table I

Information on pedigree relationships for study participants

Table II

Seroprevalence and seroreversion estimates for pathogens examined in this study.

	Number of individuals		Seroprevalence			
Pathogen	**Baseline visit**	**Follow-up visit**	**Baseline visit**	**Follow-up visit**	**Frequency of seroconversion***	**Frequency of seroreversion****
C. pneumoniae	495	484	39.6%	44.0%	15.6%	10.8%
H. pylori	781	766	76.8%	77.5%	50.0%	6.0%
CMV	782	767	86.7%	94.1%	67.1%	0.9%
HSV-1	764	750	86.6%	90.5%	39.2%	1.1%
HSV-2	764	749	33.2%	47.3%	17.1%	4.9%

* Seroconversion rate was computed only on individuals who had information from both clinic visits. It was defined here as the proportion of baseline seronegative individuals who are seropositive at the follow-up investigation. Changes to and from seroindeterminate status are ignored in this calculation.

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Table III

Heritability estimates with standard error for IgG antibody level traits.

	Heritability estimate of quantitative antibody traits (p-value)	
Pathogen	**Baseline visit**	**Follow-up visit**
C. pneumoniae	0.47 ± 0.11 (5.9×10^{-6})	0.61 ± 0.11 (3.1×10^{-9})
H. pylori	0.33 ± 0.11 (5.9×10^{-4})	0.13 ± 0.11 (0.12)
CMV	0.42 ± 0.13 (3.4×10^{-4})	0.50 ± 0.12 (2.2×10^{-6})
HSV1	0.59 ± 0.09 (2.1×10^{-13})	0.55 ± 0.10 (5.5×10^{-10})
HSV2	0.18 ± 0.10 (0.02)	0.14 ± 0.09 (0.05)
Pathogen burden*	0.18 ± 0.15 (0.10)	0.14 ± 0.14 (0.14)

* Number of seropositive reactions to pathogens examined in this study

Table IV

Maximum LOD scores for significant (LOD ≥ 2.86) and suggestive (LOD ≥ 2.00) results from multipoint linkage analysis of IgG antibody level traits.

Pathogen (visit)*	Closest Marker	cM location	Chromosomal location	Maximum LOD score
C. pneumoniae (f)	D1S450	21	1p36.22	2.22
	D15S153	62	15q22.31	3.13
	D19S220	62	19q13.13	2.76
H. pylori (b)	D8S264			