Factor and Linkage Analysis of Cardiovascular Risk Traits in the Norfolk Isolate

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Running title: Norfolk Island Factor and Linkage Analysis

Keywords: Norfolk Island, population isolate, factor analysis, linkage analysis, 5q35
Abstract

Objective(s):
An individual’s risk of developing cardiovascular disease (CVD) is influenced by genetic factors. This study focussed on mapping genetic loci for CVD-risk traits in a unique population isolate derived from Norfolk Island.

Methods:
This investigation focussed on 377 individuals descended from the population founders. Principal components factor analysis was used to extract orthogonal factors from 11 cardiovascular risk traits. Multipoint variance component methods were used to assess genome-wide linkage using SOLAR to the derived factors.

Results:
A total of 4 principal components (factors) accounting for 83% of the total variance were derived. Factor 1 was loaded with body size indicators, factor 2 with body size, cholesterol and triglyceride levels, factor 3 with LDL and total cholesterol levels and factor 4 with the blood pressures. Suggestive linkage for factor 2 ($h^2=0.36$) was identified on chromosome 5q35 (LOD=1.81; p=0.0014). While peak regions on chromosomes 10p11.2 (LOD=1.24; p=0.0054) and 12q13 (LOD=1.43; p=0.0054) were observed to segregate with factors 1 ($h^2=0.27$) and 3 ($h^2=0.43$), respectively.

Conclusion(s):
In summary, this study investigated a number of CVD risk traits in a unique isolated population. Our findings support the clustering of CVD risk traits and the results of prior genome scan studies of quantitative CVD risk traits.
Cardiovascular disease is a leading cause of morbidity and mortality world-wide. The World Health Organisation reported a total of 16.7 million deaths globally in 2002 to be a direct result of CVD [1]. An individual’s risk of developing CVD is influenced by multiple environmental and genetic factors. Major risk factors include tobacco use, physical inactivity, unhealthy diet, obesity, dyslipidemia, hypertension, diabetes mellitus and the metabolic syndrome. Combinations of these risk factors along with a positive family history significantly increase the likelihood of disease and its related effects on morbidity and mortality [2]. Although genes for rare mendelian forms of familial diabetes mellitus, dyslipidemia, hypertension and obesity have been identified, the type and number of genes underlying common cardiovascular disease-related phenotypes are yet to be fully elucidated [3-7].

Genome-wide studies of cardiovascular-related phenotypes report linkage to various chromosomal regions indicating that this disorder is genetically heterogeneous. To simplify dimensions of CVD risk, multivariate data reduction techniques such as principal component factor analysis (PCFA) have been employed to extract uncorrelated factors from numerous inter-correlated phenotypes [8,9]. This method has been used to identify loci linked to the clustering of CVD indicators particularly those comprising the metabolic syndrome [7,10-12] Of particular relevance is the use of a genetic isolate derived from Kosrae [10]. Isolated populations, like that of Kosrae, offer several advantages in gene mapping studies compared to outbred populations. Extreme geographical and cultural isolation reduce the effects of non-genetic variables by promoting a uniform lifestyle [13]. Genetic heterogeneity may also be reduced if the isolate is derived from a limited number of ancestors and has undergone population bottlenecks and endogamy during population expansion.

In the present study we tested the descendents of HMS Bounty mutineers and Tahitian population founders derived from the Norfolk genetic isolate [14]. Prior demographic analysis indicates this population possesses unique characteristics which may facilitate gene mapping studies of complex multifactorial diseases such as CVD [8]. Quantitative epidemiological data was available for related individuals on body size, blood pressure and lipoprotein and lipid levels, all factors which contribute to the risk
of CVD. To examine the relationship of these indicators, principal component analysis was performed to extract orthogonal factors. Linear scores were calculated for each individual and used to determine the heritability of each factor in the population cohort. Quantitative trait loci segregating with individual factors were then identified by multipoint variance components linkage analysis using a genome-wide panel of STRs.
Methods

Sample Ascertainment

Norfolk is a small, isolated volcanic island situated in the South Pacific Ocean, approximately 1,700 kilometres northeast of Sydney. In 2001, the Island’s permanent population totalled 1,574 individuals of whom 756 claimed to be of Pitcairn decent [15]. The population supports itself from local produce, however as a result of both isolation and small land mass the population is highly dependent on imports of primary produce and manufactured goods [15]. The Islanders live a relatively homogeneous lifestyle due to their isolation, strict quarantine and immigration laws and community centred culture. Furthermore, a large proportion of the adult population are descended from 9 Isle of Mann HMS Bounty mutineers, including acting lieutenant Fletcher Christian and 12 Tahitian women who relocated to Norfolk Island from Pitcairn Island in the 1850s [14].

Collection and phenotypic characterisation of the Norfolk Island cohort has been previously described in detail [8,16]. Briefly, ethical clearance was granted prior to the commencement of the study by the Griffith University Human Research Ethics Committee. Study participants over 18 years of age were recruited via local media announcements. All participants signed informed consent statements prior to inclusion in the study. A detailed questionnaire was used to obtain specific information from study participants including ancestry, lifestyle habits and extensive medical history. Participants were extensively phenotyped for anthropometric measures, blood pressure, lipids, lipoproteins and blood chemistry. DNA was isolated from lymphocytes using a standard salting out procedure[17].

Pedigree Structure

A total of 377 individuals were determined to have familial links to the Tahitian (Polynesian) and HMS Bounty mutineer founders and were thus the focus of this investigation. These related individuals make up part of the current Norfolk pedigree (n=6379) that extends through 11 generations to the original population founders [8]. To alleviate analysis burden imparted by the presence of multiple inbreeding and marriage loops in early generations and the large volume of missing data, the pedigree was trimmed (n=978) using a peeling algorithm in the pedigree database management system PEDSYS [18]. A total of 285 genotyped and phenotyped individuals
comprised the trimmed pedigree structure; the remaining 92 individuals became disjoint and though not included in the linkage analysis results, were retained for principal component analysis and covariate screening.

**Genome Wide Scan**

Samples were genotyped at the Australian Genome Research Facility (AGRF) using the Applied Biosystems (AB) PRISM Human Linkage Mapping Set version 2.5. The linkage mapping set comprised of 382 highly polymorphic dinucleotide microsatellite markers, spaced at an average distance of 10cM throughout the human genome. Markers were individually amplified by PCR using fluorescently labelled primer pairs. Markers were then pooled into panels for capillary separation on the AB3730 DNA Analyser. Genotyping results were analyzed using AB GeneMapper software version 4.0.

**Statistical Methods**

Data was screened using SPSS version 14.0. Measurements greater than or equal to 4 standard deviations from the mean were assessed and any data entry errors or extreme outliers were excluded. Factors with a high kurtosis were log transformed. Only subjects with measurements for all phenotypes were included in the factor analysis. Differences in the sex-specific means of the quantitative phenotypes were investigated by one way analysis of variance (ANOVA).

Principal components factor analysis was used to extract orthogonal factors from cardiovascular and obesity related measurements. Obesity related traits included body mass index (BMI) calculated as kg/m², hip circumference, waist circumference, percentage body fat and weight. Cardiovascular related traits included blood pressures (systolic and diastolic), lipids (total cholesterol and triglycerides) and lipoprotein levels (HDL and LDL). The initial factor solution (factor 1) explained the maximum variance, while successive components explained progressively smaller portions of the total variance. Factors were simplified by orthogonal rotation (varimax). This minimised the number of variables with high loadings on each factor. Principal components with eigenvalues greater or equal to 1 were retained (principal components with variances less than 1 contain less information than one of the original variables and hence are not worth retaining). Relationships between factors
are explained by factor loadings, values greater than or equal to 0.4 were used to indicate meaningful correlations between the factor and the variable.

A regression method was used to estimate factor score coefficients for the retained principal components and formed the basis of linkage phenotypes for each individual. Factor scores had a mean of 0 and a standard deviation of 1, skewness and kurtosis were less than 0.6, satisfying the assumption of normality. The 4 factor scores were screened for the covariate effects of age, sex, age$^2$ and their interactions, prior to calculating heritability estimates using SOLAR.

Genotypic data was analysed for discrepancies, including mendelian inheritance violations using the PEDSYS program INFER, while the program PREST was used to verify the pedigree structure [18]. Markers for discrepant individuals were either corrected or excluded from the analysis. Multipoint variance component linkage methods were used to assess linkage between the 382 autosomal markers and quantitative phenotypes using the statistical program SOLAR [19]. Additionally, each quantitative phenotype was simulated under the null hypothesis of no linkage. In this process, a fully-informative marker, unlinked to the trait was simulated and trait linkage was tested at that marker 10,000 times for each quantitative linkage phenotype. This information was used to calculate empirical P values for peak LOD scores.
Results

Table 1 displays the population and sex-specific means and standard deviations of the original measurements used in the PCFA for related Norfolk Island Individuals. The cohort used in this study consisted of 171 men and 206 women. The mean age of both male and female were 49.4 and 49.2 years, respectively, with little deviation in the variance between genders (p>0.05). The remaining 11 quantitative phenotypes indicated significant (p<0.05) sex-specific differences in trait variance. Males were observed to have significantly higher values pertaining to hip and waist circumference, BMI, weight, total triglycerides, total cholesterol, LDL and diastolic and systolic blood pressures compared to females. Females had significantly higher values of percentage body fat and HDL levels than men.

Analysis of BMI revealed Norfolk Islanders, particularly males were on average slightly overweight. The accepted ranges for BMI were as follows; values of 25-29kg/m² were classified as overweight, 30-34kg/m² as obese and 35 or above morbidly obese. Of particular interest to CVD, trait break down of BMI indicated that 39.0 percent of adults were overweight, 15.9 percent were obese and 3.2 percent were morbidly obese. Women had a higher percentage of body fat than men, however this difference is expected.

Waist circumference is an indicator of abdominal obesity, which according to the World Health Organisation (WHO) increases the risk of CVD in males and females with measurements exceeding 101cm and 89cm respectively [1]. The average waist circumference of males (94.6cm) and females (81.6cm) was within the recommended range. According to World Health Organisation guidelines 24.6 percent of males and 22.8 of females had values of waist circumference exceeding the recommended range and therefore at an increased risk of developing CVD.

The average systolic (127.4) and diastolic (75.7) blood pressure, a strong indicator of hypertension and CVD, was within expected normal limits. Analysis of blood pressure revealed 27.1 percent of related individuals to be hypertensive based on a systolic value ≥ 140mmHg and/or a diastolic reading ≥ 90 mmHg.
Lipid analysis was compared to published WHO European guidelines [1]. Mean total cholesterol (5.6mmol/L) exceeded the WHO European recommendation of less than 5.0mmol/L. Mean total triglycerides (2.0mmol/L) exceeded the recommended less than 1.7mmol/L. Mean LDL Cholesterol levels (2.8mmol/L) were in the healthy range of less than 3.0mmol/L. While HDL cholesterol levels in men (1.3mmol/L) and women (1.5mmol/L) were within the recommended limits (equal or greater than 1.0mmol/L in men and 1.2mmol/L in females).

Table 1. Phenotypic Characteristics of Participants (Mean +/- Standard Deviation)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Both Sexes (N = 377)</th>
<th>Males (N = 171)</th>
<th>Females (N = 206)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>49.2 ± 16.6</td>
<td>49.4 ± 17.0</td>
<td>49.2 ± 16.8</td>
</tr>
<tr>
<td>Hip circumference(cm)</td>
<td>102.0 ± 9.6</td>
<td>103.0 ± 7.1</td>
<td>101.0 ± 10.9</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.3 ± 4.6</td>
<td>27.6 ± 4.0</td>
<td>25.4 ± 4.7</td>
</tr>
<tr>
<td>Percentage body fat (%)</td>
<td>30.4 ± 8.6</td>
<td>24.5 ± 6.6</td>
<td>35.3 ± 6.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.0 ± 16.2</td>
<td>85.9 ± 14.4</td>
<td>68.4 ± 12.9</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>87.1 ± 14.3</td>
<td>94.6 ± 12.7</td>
<td>81.6 ± 12.1</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.4 ± 0.4</td>
<td>1.3 ± 0.3</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>Total triglycerides (mmol/L)</td>
<td>2.0 ± 1.2</td>
<td>2.3 ± 1.3</td>
<td>1.7 ± 1.0</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.6 ± 1.1</td>
<td>5.8 ± 1.1</td>
<td>5.5 ± 1.1</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>2.8 ± 1.0</td>
<td>3.0 ± 1.0</td>
<td>2.7 ± 0.9</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>127.4 ± 22.3</td>
<td>133.3 ± 19.2</td>
<td>121.8 ± 22.2</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>75.7 ± 12.6</td>
<td>78.6 ± 12.5</td>
<td>72.3 ± 11.5</td>
</tr>
</tbody>
</table>

The characteristics of the derived principal components are detailed in Table 2. The PCFA extracted 4 factors, which explain nearly 83% of the total variation of the 11 original quantitative traits. Factor 1 has high loadings of traits that reflect body size, particularly adiposity (hip circumference, BMI, Percentage body fat, weight and waist circumference) and explains the largest portion of the total variance (44%). Factor 1 is a strong indicator of atherosclerosis. Factor 2 is loaded predominantly with HDL, total triglycerides, weight and waist circumference are associated with obesity and atherogenic dyslipidemia. Comparably, factor 3 is loaded with LDL and total cholesterol, which is a strong indicator of ischemic stroke and heart attack risk, while factor 4 contains high loadings of blood pressure which reflects the risk of essential hypertension.
Table 2. Coefficients and variances of factors satisfying the eigenvalue \( \geq 1 \) criterion

<table>
<thead>
<tr>
<th>Variables</th>
<th>Factor 1</th>
<th>Factor 2</th>
<th>Factor 3</th>
<th>Factor 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hip circumference</td>
<td>0.871</td>
<td>0.264</td>
<td>0.079</td>
<td>0.218</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.829</td>
<td>0.393</td>
<td>0.091</td>
<td>0.273</td>
</tr>
<tr>
<td>Percentage body fat</td>
<td>0.782</td>
<td>-0.290</td>
<td>0.089</td>
<td>-0.126</td>
</tr>
<tr>
<td>Weight</td>
<td>0.618</td>
<td>0.565</td>
<td>-0.005</td>
<td>0.311</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>0.613</td>
<td>0.535</td>
<td>0.128</td>
<td>0.358</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>-0.049</td>
<td>-0.882</td>
<td>0.059</td>
<td>0.034</td>
</tr>
<tr>
<td>Total triglycerides*</td>
<td>0.165</td>
<td>0.641</td>
<td>0.342</td>
<td>0.207</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.116</td>
<td>0.045</td>
<td>0.958</td>
<td>0.206</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>0.051</td>
<td>0.074</td>
<td>0.949</td>
<td>0.118</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.130</td>
<td>0.140</td>
<td>0.150</td>
<td>0.889</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.153</td>
<td>0.051</td>
<td>0.189</td>
<td>0.878</td>
</tr>
</tbody>
</table>

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Eigenvalue</td>
<td>4.84</td>
<td>1.88</td>
<td>1.29</td>
<td>1.09</td>
</tr>
<tr>
<td>Total Variance (%)</td>
<td>43.97</td>
<td>17.06</td>
<td>11.72</td>
<td>9.91</td>
</tr>
<tr>
<td>Accumulative Variance (%)</td>
<td>43.97</td>
<td>61.04</td>
<td>72.76</td>
<td>82.67</td>
</tr>
</tbody>
</table>

*Log transformed. Factor loadings in bold type are \( >0.4 \).

Three of the four factors were significantly heritable after covariate correction. The heritabilities of factors 1 to 3 were \( 0.27 \pm 0.13 \) (p=0.02), \( 0.36 \pm 0.17 \) (p=0.01) and \( 0.43 \pm 0.12 \) (p=0.002). Factor 4 was not significantly heritable (p>0.05). The genome scan revealed several linkage peaks yielding LOD scores >1. Table 3 details the PCFA phenotypes and markers that produced maximum LOD scores in regions throughout the genome. The highest LOD score detected in this study was linked to factor 2. Suggestive linkage for this phenotype was observed on chromosome 5q35 with a maximum LOD score of 1.81 for marker D5S400 (p=0.0014) (Figure 1). The 1-LOD support interval extends from 174 to 193cM. The factor 2 phenotype was observed to segregate with additional regions on chromosomes 1p, 4q, 12q, 14q, 15q, 17q and 18q (p<0.05) producing LOD scores >1. Analysis of factor 1 and factor 3 phenotypes identified peak linkage signals on chromosomes 10p11.2 (LOD=1.24; p=0.0054) and 12q13 (LOD=1.43; p=0.0054), respectively (Figure 2 and 3). Additional linkage peaks for factor 1 were localised to chromosomes 12q and 20p, while only one other peak was observed on chromosome 21q for factor 3.
### Table 3. Summary of PCFA genome scan results*

<table>
<thead>
<tr>
<th>Factor</th>
<th>Chromosome</th>
<th>cM</th>
<th>Nearest Marker (cM)</th>
<th>LOD</th>
<th>Empirical P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>66</td>
<td>D10S208 (61.1)</td>
<td>1.24</td>
<td>0.0054</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>73</td>
<td>D12S83 (75.1)</td>
<td>1.14</td>
<td>0.0071</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>23</td>
<td>D20S115 (25.1)</td>
<td>1.10</td>
<td>0.0081</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>46</td>
<td>D1S234 (45.44)</td>
<td>1.16</td>
<td>0.0082</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>207</td>
<td>D4S426 (206.5)</td>
<td>1.01</td>
<td>0.0138</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>181</td>
<td>D5S400 (180.4)</td>
<td>1.81</td>
<td>0.0014</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>162</td>
<td>D12S1659 (162.3)</td>
<td>1.08</td>
<td>0.0105</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>109</td>
<td>D14S65 (108.6)</td>
<td>1.05</td>
<td>0.0118</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>15</td>
<td>D15S1002 (15.5)</td>
<td>1.14</td>
<td>0.0088</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>76</td>
<td>D17S1868 (75.9)</td>
<td>1.29</td>
<td>0.0053</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>82</td>
<td>D18S64 (83.8)</td>
<td>1.25</td>
<td>0.0061</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>65</td>
<td>D12S368 (67.5)</td>
<td>1.43</td>
<td>0.0035</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>17</td>
<td>D21S1256 (13.2)</td>
<td>1.03</td>
<td>0.0126</td>
</tr>
</tbody>
</table>

*Only chromosomal regions producing LOD Scores ≥1 are displayed

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**Figure 1.** Multipoint linkage results for factor 2 on chromosome 5.
Figure 2. Multipoint linkage analysis for factor 1 on chromosome 10.

Figure 3. Multipoint linkage results for factor 3 on chromosome 12.
Discussion

Isolates, such as Norfolk are unique populations to study complex multi-factorial disorders. The combination of geographical and cultural isolation leads to individuals sharing a common environment, minimising differences in lifestyle factors such as diet, exercise and sanitation compared to outbred populations. This has been observed particularly in Amish and Hutterite populations [20,21]. The homogeneous environment shared by individuals is of great significance in studies of complex disorders, particularly those of cardiovascular origin where there appears to be a threshold effect influenced by lifestyle factors. Norfolk is also of interest in genetic studies as a large number of individuals in this population can trace their heritage back to a small number of families derived from the original Bounty mutineer and Polynesian founders. The limited number of ancestors minimizes genetic heterogeneity. This can reduce the number of susceptibility genes underlying disease. As a result of the expected reduction in genetic and non-genetic variables, population isolates with known founder effect have been exploited in numerous gene mapping studies of complex disorders [20-26].

This current study focussed on a large complex family from the Norfolk Isolate to dissect the genetic and environmental variables underlying CVD risk. We performed PCFA with orthogonal rotation to reduce 11 inter-correlated variables into groups of independent (un-correlated) factors. This data reduction method identified factors that explained 83% of the variation in the original quantitative traits. PCFA identified four distinct components underlying CVD risk in this family. The factor accounting for the largest portion of variation was strongly loaded by factors relating to obesity, which has long been known to be an independent predictor of CVD-related morbidity and mortality [27]. The second largest component, factor 2, reflected traits of obesity loaded with HDL and triglyceride levels. Increased waist circumference, elevated total triglycerides and reduced HDL cholesterol are 3 indicators of metabolic syndrome and a strong predictor of CVD risk [28]. Factor 3 was composed of serum LDL and total cholesterol levels, which when elevated promote arteriosclerosis, increasing the risk of chronic heart disease (CHD), ischemic stroke and heart attack [29]. Lastly, factor 4 reflected the risk of essential hypertension.
Previous factor analysis studies have focused primarily on the metabolic syndrome, a major risk factor for CVD characterised by the clustering of traits of insulin resistance, hypertension, dyslipidemia and obesity [7,10-12,26,30]. Unfortunately, as values of serum levels of fasting glucose and fasting insulin were not available for our study participants, insulin resistance could not be directly assessed in this study. However, our findings are consistent with other factor analysis studies in that obesity and lipid levels displayed high loadings in the derived components [7-11,31]. In particular, the high loadings of weight and waist circumference in both the first and second components suggest a close relationship between traits of obesity and CVD risk in Norfolk Islanders. This is supported by demographic studies, which report a higher prevalence of obesity and dyslipidemia in individuals of Polynesian ancestry [32,33].

We detected a substantial genetic component in 3 of the 4 factors. The fourth factor, which incorporated a blood pressure component, was not significantly heritable. A total of 40 individuals were using anti-hypertensive medications at the time of collection. They were included in this analysis and since medication obviously affects blood pressure measurements, this may have impacted on heritability estimates for this quantitative trait.

The genetic predisposition of these phenotypes was investigated by means of non-parametric variance component linkage analysis. The most significant linkage signal in this study was observed on chromosome 5q35. This locus was linked to the second component (factor 2) comprising weight, waist circumference, HDL and total triglyceride levels. Linkage analysis in founder isolates of Kosraen and Amish origin have reported this locus to segregate with serum leptin levels and BMI, respectively [26,34]. Additional family studies have reported segregation with traits of BMI, HDL levels, LDL levels, serum leptin levels, lean mass, body fat measures and type II diabetes mellitus within a 10cM interval of D5S400 [12,35-41]. Linkage results support this chromosome 5 locus to be strongly influenced by traits of obesity. Interestingly, the 5q34-qter chromosomal region contains numerous obesity gene candidates. One such gene, the Beta-2-Adrenergic Receptor (ADRB2; OMIM 109690) spanning 5q32-34, a member of the G protein-coupled receptor superfamily, functions as a major lypolytic receptor in human adipocytes [42]. Variations of this
gene has been reportedly associated with nocturnal asthma, arteriosclerosis, hypertension, obesity, metabolic syndrome and type 2 diabetes [43-48]. However, despite the number of positive associations there appears to be inconsistencies between studies, for instance candidate gene study conducted in 7,808 middle-aged white subjects was unable to demonstrate any consistent associations between ADRB2 variants and obesity, hypertension or type 2 diabetes [49].

In addition to the 5q35 locus, we identified peak LOD score linked to Factor 1 and Factor 3 on chromosomes 10p11.2 near marker D10S208 and 12q13 near marker D12S368, respectively. Though not significant at the genome-wide threshold for linkage, these two regions have been reported to segregate with CVD-related risk traits. The chromosome 10 marker, D10S208 has been reported to be linked with obesity in 2 large studies in European and African Americans [50,51]. The second strongest signal in this study (LOD=1.43; p=0.0035) observed on chromosome 12q13. The Factor segregating with this locus was loaded with LDL and total cholesterol and is in the same vicinity of a region reported to segregate with LDL-cholesterol and LDL-apoB levels [52]. In addition to these peak regions for the 3 heritable factors, linkage (LOD>1; p<0.05) was identified on chromosomes 1, 4, 12, 14, 15, 17, 18, 20 and 21. Though these genetic loci were not significant at the genome-wide threshold for linkage, their presence supports the underlying heterogeneous nature of cardiovascular-related phenotypes.

In conclusion, factor analysis reduced 11 interrelated CVD risk traits to 4 newly defined factors. As these factors are uncorrelated, each one can be interpreted to represent a distinct phenotype underlying CVD risk in Norfolk Islanders. These factors are influenced by a strong genetic component in this family. Linkage analysis identified a suggestive locus underlying CVD risk on chromosome 5, which has been reported to segregate with similar phenotypes. We also identified two other peak regions on chromosome 10 and 12 for factors 1 and 3 that might be important as they support findings of previous studies. Our findings support the clustering of CVD risk factors and suggest the involvement of multiple genetic loci.
Acknowledgements

This research was supported by funding by the National Health and Medical Research Council (NHMRC) of Australia, the Medical Bioinformatics Genomics Proteomics Program (MGBPP) and the National Heart Foundation (NHF). Genotyping was performed by the Australian Genome Research Facility (AGRF). The authors would specifically like to thank Paul Jardine (GU) for computational support and Dr. Thomas Dyer and Dr. Jac Charlesworth (SFRB) for providing statistical expertise and assistance. Lastly, our appreciation to the Norfolk Islander’s who volunteered for this study.
References


