Published in final edited form as:

Hum Genet. 2008 December ; 124(5): 543–552. doi:10.1007/s00439-008-0580-y.

Linkage Mapping of CVD Risk Traits in the Isolated Norfolk Island Population

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Abstract

To understand the underlying genetic architecture of cardiovascular disease (CVD) risk traits, we undertook a genome-wide linkage scan to identify CVD quantitative trait loci (QTLs) in 377 individuals from the Norfolk Island population. The central aim of this research focused on the utilization of a genetically and geographically isolated population of individuals from Norfolk Island for the purposes of variance component linkage analysis to identify QTLs involved in CVD risk traits. Substantial evidence supports the involvement of traits such as systolic and diastolic blood pressures (SBP and DBP), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), body mass index (BMI) and triglycerides (TG) as important risk factors for CVD pathogenesis. In addition to the environmental influences of poor diet, reduced physical activity, increasing age, cigarette smoking and alcohol consumption, many studies have illustrated a strong involvement of genetic components in the CVD phenotype through family and twin studies. We undertook a genome scan using 400 markers spaced approximately 10cM in 600 individuals from Norfolk Island. Genotype data was analyzed using the variance components methods of SOLAR. Our results gave a peak LOD score of 2.01 localizing to chromosome 1p36 for systolic blood pressure and replicated previously implicated loci for other CVD relevant QTLs.

Keywords

Cardiovascular disease; QTL mapping; genetic isolate; genome scan; heritability; variance components analysis

Introduction

Cardiovascular disease (CVD) and its associated risk traits such as obesity, hypertension, diabetes and cholesterol imbalance together hold the unenviable title of the leading cause of death in many western populations such as the United States (Rosamond et al. 2008), as well as Australia, where CVD accounts for 38% of all deaths (AIHW 2004; NHFA 2005). Several factors are generally thought to be responsible for mortality associated with CVD, including reduced physical activity in both social and work life, cigarette smoking, together with poor diet choices which are generally high in saturated fat and sodium while lacking essential

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nutrients and fiber. However, in addition to lifestyle choices, it is understood that CVD pathogenesis is heavily influenced by genetic components as well as environmental exposures. Family history has been shown to be one of the most important risk factors for CVD development, emphasizing the role of genetic predisposition.

Studies focused on dissecting the genetics of CVD risk traits have established a strong familial component for the vast majority of CVD phenotypes evidenced by heritability (h^2) estimates ranging from 0.45 to 0.75 for body mass index (BMI), the obesity indicator (Bell et al. 2005; Liu et al. 2003; Maes et al. 1997; Sorensen et al. 1989). Additionally, early twin studies by McIlhenny *et al.* (1975) illustrated a strong genetic component acting on both systolic and diastolic blood pressures (SBP and DBP), where h^2 values of 0.78 and 0.61, respectively were found (McIlhenny et al. 1975). Heritability estimates calculated on multiple other CVD-related risk traits, such as leptin (Comuzzie et al. 1997), triglycerides (TG) (McQueen et al. 2003; Shearman et al. 2000), high- and low-density lipoprotein cholesterol (HDL-C, LDL-C) (Pollin et al. 2004), as well as diabetes indicator, blood glucose levels (Panhuysen et al. 2003) continually evidence a significant genetic contribution in the development of these CVD related traits. With the availability of such compelling evidence highlighting the genetic influence acting on CVD risk traits, it should not be surprising that complex disease gene mapping studies have been directed towards identifying and collecting extended families or even isolated populations. To compliment the isolated population approach it was imperative that statistical analysis programs were established with the explicit aim of tracking genetic aberrations as they segregate through affected family cohorts. To this end, the isolated population strategy has met with success in multiple populations groups, including the Sardinian isolate, where Angius *et al.* (2002) and later Falchi *et al.* (2004) identified loci for essential hypertension (2p24-p25) and LDL-C (2q21-q24), respectively (Angius et al. 2002b; Falchi et al. 2004). The 2005 hypertension study conducted by Kamide and colleagues examined polymorphisms in 14 genes under the 2p24 peak in a Japanese case/control population totaling 1880 individuals (Kamide et al. 2005). These authors reported contrasting gender affects since female participants displayed an association between a single nucleotide polymorphism (SNP) in the *HPCAL1* (hippocalcin-like 1) gene, while two SNPs in the *GREB1* (gene regulated by estrogen in breast cancer 1) gene showed association to essential hypertension in males (Kamide et al. 2005). Although, an earlier three-stage linkage study conducted on three independent hypertensive Chinese family cohorts showed no evidence of the hypertension linkage signal at one of the peak microsatellite markers, D2S168, reported in the Sardinian isolate (Angius et al. 2002b; Zhu et al. 2001). However, the Chinese study implicated a marker (D2S142) telomeric to the Sardinian hypertension peak as being both linked to and in association with essential hypertension (Zhu et al. 2001). Interestingly, this markers happens to be the same one as residing under the peak LOD score in the LDL-C linkage scan reported by Falchi *et al.* (2004), evidencing the heterogenic characteristics of CVD risk traits.

Both trait and genetic heterogeneity are major confounding factors which limit the success of disease gene mapping strategies (Lander and Schork 1994; Schork 1992). Hence, researchers have sought to exploit the inherent characteristics of isolated populations such as limited number of initial founders and shared environment to reduce or control to some extent the variance attributable to these confounding factors. This study implemented the variance-components linkage genome scan strategy identify quantitative trait loci (QTLs) influencing CVD risk traits in the genetically and geographically isolated population of individuals from Norfolk Island, located off the Eastern seaboard of Australia. Interest in the island of Norfolk, which is situated between Australia and New Zealand a top of a volcanic ridge, stems from its unique history with origins as a British convict settlement in the late 18th century before Earl Grey and Queen Victoria decided to gift the island to the descendents of the 'Bounty' mutineers and their Tahitian wives who were heading toward an unknown future on Pitcairn Island with a population growth rate overtaking available food and water supplies (Edgecombe 1999;

Hoare 1999; Shapiro 1968). The initial relocation of the Pitcairn population (n=193) arrived on Norfolk on June 8, 1856 a date now celebrated as Norfolk's national day or 'Bounty Day' commemorating the new settlement.

Census data compiled in 2001 suggest that the Norfolk population totaled 1,574 residents, 309 of which were under the age of 15 (Mathews 2001). Our study commenced in early 2000, where we collected phenotypic measures, extensive individual and family medical history as well as blood samples from 600 Norfolk Island participants with the specific aim of evaluating disease prevalence as well as undertaking genetic mapping strategies (Bellis et al. 2008; Bellis et al. 2005). One characteristic of isolated populations valuable to geneticist researchers is the propensity of these populations to maintain complete genealogical records (Angius et al. 2002a; Angius et al. 2001; Escamilla 2001; Peltonen 2000). The ancestral origins of the Norfolk Island are well documented to have originated from divergent founding paternal and maternal lineages, European and Tahitian, respectively. Furthermore, exhaustive genealogical documents indicate that the population grew from a limited number of initial founders (nine males, twelve females) and in relative isolation in the early generations of population expansion (Shapiro 1968). Evidence of the Island's strict immigration laws are obvious by the limited numbers of surnames, resulting in the world's only telephone directory which includes nicknames to differentiate between individuals with the same name (Squires 2006).

Materials and Methods

Sample Ascertainment and Phenotyping

Collection and initial analysis of the Norfolk population has previously been described in detail (Bellis et al. 2005). Phenotypic demographics including height, weight and full blood screen data were compiled and stored in the purpose-built Norfolk Island database at time of sample donation and questionnaire completion. Traits included for linkage analysis were SBP, DBP, BMI, TC, HDL-C, LDL-C, and TG. Informed consent was obtained from participants prior to inclusion in the study. Additionally, prior to collection of samples or individual phenotyping the Griffith University Human Research Ethics Committee granted ethical clearance.

Genotyping

DNA was extracted from whole blood samples using the salting-out method (Miller et al. 1988). All genotyping undertaken for this genome scan was performed at the Australian Genome Research Facility (AGRF), Melbourne. All primer sets were optimised by PCR. The genome scan included 400 microsatellite (mid-density) markers (ABI Prism Linkage Mapping Set, Version 2.5 [MD-10, LMSV2.5]) with an average spacing of 9.2cM (maximum gap between markers 26.11cM) and an average heterozygosity of 0.79. The loci were selected from the Génethon human linkage map (Dib et al. 1996; Gyapay et al. 1994; Weissenbach et al. 1992), based on chromosomal locations and heterozygosity. The map positions were generated from the same CEPH genotype data used for the 1996 Génethon map (Dib et al. 1996). Markers were genotyped in 28 multiplexed panels. All PCRs were performed under standard conditions in a total volume of 6µL, using a PTC-225 DNA Engine Tetra (MJ Research Inc, Waltham, MA, USA). Primers were labeled with fluorescent dyes (FAM, HEX, and NED) [Applied Biosystems LMSV2.5]. PCR products were then pooled into multiplex panels of 10-20 markers and electrophoresed on a 3730 DNA Analyzer (Applied Biosystems). Genescan software (Applied Biosystems) assigns tracking for each sample lane. Files are then imported into Genotyper (Applied Biosystems) software that interprets the electropherogram and assigns genotypes.

Statistical Analysis

Pedigree Confirmation

Generally, the AGRF employs pedigree-checking procedures to detect Mendelian inconsistencies using PedManager (version 0.9) (<http://www-genome.wi.mit.edu/>) however, considering the complexity of the Norfolk Island pedigree and relationships between individuals were yet to be confirmed, alternate data cleaning strategies were required. The data were checked for Mendelian inheritance errors by use of the PEDSYS (Dyke 1996) program INFER and Simwalk2 (Sobel et al. 2002) to eliminate typing errors. The Pedigree Relationship Statistical Test (PREST) (McPeck and Sun 2000) was employed to detect relationship misspecification. This program detects pedigree errors by determining whether the pattern of allele-sharing in the marker data fits the expected pattern of allele-sharing based on proposed relationships. After correction (occurrences of nonpaternity were resolved by changing the pedigree structure), PREST was re-run to confirm that family relationship re-assignments were consistent with estimated IBD patterns. Loki (Heath 1997) was employed to compute matrices of empirical estimates of IBD allele sharing at points throughout the genome for every relative pair. The IBD matrices are required for our linkage analysis. PREST, Simwalk2 (Sobel and Lange 1996; Weeks et al. 1995) and Loki require chromosomal maps of genotype markers for all 22 autosomes. This analysis used the sex-averaged chromosomal maps, which were obtained from the Marshfield Centre for Medical Genetics.

To reduce the computation burden of QTL and IBD estimation analysis using complex extended pedigrees such as the Norfolk Island pedigree, the program PEDTRIM within the PEDSYS suite was developed to remove individuals who do not contribute information for genetic analysis. The program iteratively removes from a pedigree all individuals whose phenotypic or genotypic data are missing or known to be of poor quality, if they have (a) no offspring or (b) no parents and a single offspring (Dyke 1996).

Heritability Estimation and QTL Mapping

Heritability for each trait was estimated using genetic variance component modeling as implemented in SOLAR software, version 4.0.6 (Almasy and Blangero 1998). Levels of kurtosis were surveyed and appropriate data transformations undertaken prior to analysis. Multipoint linkage analysis as implemented in SOLAR software, version 4.0.6 (Almasy and Blangero 1998), was performed to detect and localise quantitative trait loci influencing variation in CVD-risk traits. This approach has been described in detail (Amos 1994; Goldgar 1990). To account for the possible introduction of false-positive results, we applied our modified version of the method present by Feingold *et al* (1993) to resultant *P*-values for each LOD score (Feingold et al. 1993) and found that by accounting for the finite number of markers genotyped as well as the Norfolk Island pedigree complexity, our threshold evidencing genome-wide suggestive linkage became $\text{LOD} = 1.675$ ($\alpha=0.05$). Furthermore, simulations estimating the power of the Norfolk Island pedigree were calculated based on the revised LOD score evidencing suggestive linkage while factoring in previously calculated heritability estimates for each trait.

Results

Pedigree Confirmation

The Norfolk Island genealogy dates back approximately ten generations to the initial founders and contains 6379 individual entries linked together within 2185 nuclear families. The complexity of the island's heritage is evident considering 5750 individuals reside within a single multifamily pedigree exhibiting 1661 marriages and 1233 founders. It was hypothesized that the collected sample population ($n=600$) were located within the last five generations of the

genealogy. It was imperative that our analyses determine the exact relationship status of the sample population, thus enabling accurate calculation of IBD matrices and heritability estimation. Our preliminary heritability calculations presented previously (Bellis et al. 2005) were based purely on available genealogical information thus introducing the possibility of biased estimates due to incorrect pedigree structure.

PREST analysis produced an inferred Norfolk Island pedigree structure comprised of 6537 individuals, inflated slightly from the original 6379 since PREST automatically includes missing parents when only one parent is known, thus, maintaining important sib linkages. Additionally, PREST results enabled identification of sample number errors/contamination events. In such instances genotypes were blanked prior to QTL analysis.

Removal of uninformative individuals using the PEDTRIM program produced a pedigree structure of 1078 individuals, of which our study population had sampled 377 individuals, these individuals formed the basis of the CVD QTL mapping in SOLAR.

Table 1 illustrates the relationships present in the 1078 member pedigree as well as the phenotyped individuals. Deeper investigation revealed a pedigree composition of 272 marriages with 682 individuals connected in one complex ten-generation pedigree possessing 249 married-in spouses.

To define the genetic basis of CVD within the Norfolk Island population, we performed a genome-wide scan to localise genomic regions influencing quantitative risk traits associated with CVD. Genotype data (n=400 dinucleotide microsatellites) were available from 600 individuals who participated in the Norfolk Island study.

Epidemiological data pertaining to the sampled population who possessed an ancestral link to initial founders (total n=377, 171 males, 206 females) are listed in Table 2. Demographic analysis suggested there was little variation between genders when considering participants' age (48.9 ± 16.9 and 49.5 ± 16.4 years, respectively) but differed significantly with respect to BMI (men, 27.4 ± 3.6 ; women 25.4 ± 4.7 kg/m², $P < 0.05$). In fact, significant differences between males and females were identified for all CVD-risk traits analyzed, with the exception of age.

Interestingly, an independent samples t-test suggested that the male study population appears to possess an increased risk of developing CVD since they presented with elevated levels of obesity, cholesterol, triglycerides and systolic blood pressure when compared with their female counterparts (Table 2). Additionally, considered cardioprotective, HDL-C levels for males were significantly lower than females while the inverse was the case for LDL-C (CVD-risk factor).

CVD-risk Trait QTL Mapping

Due to high levels of kurtosis in some traits it was necessary to transform the distribution of some traits, therefore, for consistency all quantitative trait data were subjected to an inverse normal transformation. Additionally all traits were adjusted for the effects of covariates such as sex and age (including age $\times$ sex and age² $\times$ sex).

Presented in Table 3 are five genomic regions corresponding LOD scores above 1.675 (evidence of suggestive linkage, $\alpha=0.05$) identified in the Norfolk Island genome-wide scan for CVD risk traits. Calculations assessing the inherent power of the complex Norfolk Island pedigree revealed that detection of a QTL accounting for approximately 30% of the variation would be detected with 50% power.

Variance components multipoint linkage analysis results from the 10cM genome-wide scan revealed the highest LOD score of 2.01 on chromosome 1p36 at marker D1S2697 (37.05cM) for SBP (Figure 1). Our suggestive linkage result further strengthens claims that a hypertension-related gene may reside at the 1p36 locus. This region of chromosome 1 continually provides researchers with positive linkage signals for CVD-related traits, especially hypertension, obesity, and diabetes (Angius et al. 2002b; Benjafeld et al. 2005; Cheng et al. 2001; Glenn et al. 2000; Hoffmann et al. 2007; Liu et al. 2004; Nakayama et al. 2004; Pan et al. 2000; Pausova et al. 2005).

Suggestive linkage for SBP was also found on chromosome 8 at marker D8S505 (LOD = 1.82, 60.87cM). Bell *et al* (2006) undertook novel interaction analysis between loci related to hypertension with results suggesting the D8S505 marker is involved in an epistatic relationship with the D15S128 marker, located at 15q11.2 (Bell et al. 2006). QTL mapping revealed the largest LOD score for HDL-C was a 6cM region on chromosome 18q22 between markers D18S462 and D18S1161 (LOD = 1.83), with weaker evidence on chromosomes 20p13, 7q11 and 7q21.

Multiple QTLs for total cholesterol were detected on chromosome 2 in the Norfolk Island population with the strongest signal localising to the distal end of the chromosome close to marker D2S396 (LOD = 1.80, 232.9cM) and a weaker peak proximally located at marker D2S112 (LOD < 1.5, 141.62cM).

The highest QTL peak revealed for LDL-C was found localized to chromosome 7q22 (LOD = 1.62, 104.86cM), centrometric to the HDL-C peak reported above. Chromosome 12q13 also revealed the presence of a weaker QTL peak at marker D12S85. Similar to LDL-C, evidence of a multiple QTLs involved in TG variation was found on chromosomes 1p36 (LOD = 1.51, 45.33cM) and 16p12 (LOD < 1.5).

Discussion

As a complex disease, CVD is characterized by convincing evidence of a genetic component, strong environmental effects, undefined mode of inheritance, and potentially multiple genes of varying effect acting independently as well as interactively. The arduous task of identifying CVD-related susceptibility genes is thus complicated by the fact that the interaction of these factors, results in a variety of disease phenotypes. This complex situation may account for why, despite the number of genome-wide scans focused on CVD risk factors during the past decade, the success in terms of the identification of a major susceptibility gene to date has been limited.

It is known that the power and the robustness of the linkage results rely critically on the study sample sizes used, among several other factors such as heritability and relationship structure of the study population. Compared with the commonly used sibling-pair approach (Haseman and Elston 1972), the variance component method used in the present study provided a more powerful test, which can be expanded to handle pedigrees of arbitrary size and complexity (Almasy and Blangero 1998; Williams and Blangero 1999).

The use of isolated populations for disease gene mapping purposes has included populations as diverse as the phenotypes investigated. European researchers have focused their efforts on historically and culturally distinct populations from Scandinavia including Finland (Peltonen et al. 1999; Varilo et al. 2000; Varilo et al. 2003; Wessman et al. 2002) and Iceland (Gulcher and Stefansson 1998; Helgason et al. 2003), Mediterranean regions of Italy, especially Sardinia (Angius et al. 2002a; Angius et al. 2001; Angius et al. 2002b; Eaves et al. 2000; Falchi et al. 2004; Pugliatti et al. 2003; Tenesa et al. 2004; Zavattari et al. 2000) and Corsica, France (Latini et al. 2004). North American isolated population researchers have mainly been concerned with large extended pedigrees, for example, the Hutterites (Abney et al. 2000, 2001; Abney et al.

2002; Newman et al. 2003; Newman et al. 2004; Ober et al. 2001; Weiss et al. 2006), while several remote Polynesian populations are presently involved in disease gene mapping studies (Bonnen et al. 2006; Han et al. 2002; Kayser et al. 2003; Murray-McIntosh et al. 1998; Redd and Stoneking 1999; Shmulewitz et al. 2001; Tsai et al. 2004; Wijsman et al. 2003).

This study applied the variance component method of QTL mapping in an isolated population possessing a complex family pedigree. However, prior to running analyses aimed at identifying genomic loci involved in CVD-related risk trait variation, it was imperative to establish the exact pedigree structure of the Norfolk Island population. To achieve this, genotyping results from the genome scan data were applied to pedigree confirmation routines using recently developed statistical programs to infer kin relationships. Furthermore, pedigree refinement analyses or trimming enabled the exclusion of uninformative individuals prior to our QTL analysis thus reducing the computational burden of calculating IBD matrices for individuals who ultimately add little to the overall linkage signal but significantly increase the calculation time. As a means of controlling for the potential of false-positive results, simulations were run to predict the LOD score associated with suggestive linkage. These simulations were specific for both the Norfolk Island pedigree structure and marker density employed for the genome-wide scan and are based on a modified version of the methods presented by Feingold *et al.* (1993). Suggestive linkage occurred at a LOD = 1.675. We report five genomic regions in four CVD risk traits where LOD score exceeded this level.

Our results detailed several interesting loci possibly involved in the variation of particular CVD-risk traits, specifically systolic blood pressure, cholesterol and HDL (as well as BMI, LDL-C and TG, to a lesser extent). The region on chromosome 1p36 which produced the peak LOD score of 2.01 for systolic blood pressure in this study continually presents researches with interesting results when CVD traits are analysed (Benjafield et al. 2005; Cheng et al. 2001; Liu et al. 2004; Nakayama et al. 2004; Pausova et al. 2005; Speirs et al. 2005). Furthermore, Angius *et al.* (2002) employed a two-stage genotyping process of QTL fine-mapping in a Sardinian genetic isolate that identified adjacent markers on chromosome 1p36 for essential hypertension (Angius et al. 2002b). While Nakayama *et al.* (2004) applied a different strategy of case-control association using STR markers in a Japanese population, the results again implicated the involvement of markers in the 1p36 region as being associated with essential hypertension (Nakayama et al. 2004). Asian populations appear to possess similar genetic structure to Caucasians in this region of chromosome 1 considering another study investigating essential hypertension in Chinese families produced a peak LOD score in the flanking 1p31 locus (Ge et al. 2005).

Based on our results and those of others, chromosome 1p36 presents a promising region for traits involved in CVD risk. In a whole-genome scan, Stone *et al.* (2002) reported suggestive linkage to severe obesity in females at 1p36 (Stone et al. 2002). A another study in Pima Indians, Norman *et al.* (1998) reported linkage to percentage body fat at 1p31-21, a region adjacent to 1p36 (Norman et al. 1998). The chromosome 1p36 contains an important candidate gene *TNFA2*, the allelic polymorphisms of which were associated with obesity, leptin levels, and insulin resistance (Fernandez-Real et al. 2000).

Chromosome 8p12 recorded a less interesting LOD score of 1.82 in the Norfolk Island population, again for systolic blood pressure. This region is flanked by results reported by researchers investigating essential hypertension in the Sardinian isolate (Falchi et al. 2004).

The peak LOD score for TC was recorded on chromosome 2p36 at marker D2S206 (LOD=1.80, 240.79cM), replicating previous results for TC by Bosse *et al.* (2004) where a peak LOD score of 2.04 was recorded at 241.2cM for individuals enrolled in the Québec Family Study (Bosse et al. 2004). Furthermore, this region of chromosome 2 has been implicated by two other

genome-wide scans. Firstly, in the study by Tang *et al.* (2003) where multiple CVD-related risk traits were subjected to data reduction through factor analysis and secondly, the sib-pair analysis of Harrap *et al.* (2002) both provided evidence of linkage which overlapped the locus identified in the present Norfolk study (Harrap *et al.* 2002; Tang *et al.* 2003). Lastly, this region is in close proximity to regions linked to various obesity traits, HDL-C, and type 2 diabetes (Arya *et al.* 2002; Elbein and Hasstedt 2002; Wiltshire *et al.* 2001; Wu *et al.* 2002). Hager *et al.* (1998) and Deng *et al.* (2002) observed modest linkage for a QTL influencing obesity and percent fat mass at marker D2S206 which is the same marker reported here (Deng *et al.* 2002; Hager *et al.* 1998).

The search for possible candidates in the peak LOD region reveals genes including thyroid hormone receptor interactor 12 (TRIP12, 2q36.1), 5-hydroxytryptamine (5-HT, serotonin) receptor 2B (HTR2B, 2q36.3-q37.1), and insulin receptor substrate 1 (IRS1, 2q36). Thyroid hormones, mediating via thyroid hormone receptors, exert pleiotropic effects on many physiological aspects, such as metabolism of lipids, carbohydrates and proteins (Utiger 2001). The second candidate, HTR2B, is one of several mediators of the neurotransmitter, serotonin. Serotonin plays an important role in the regulation of appetite. In rats, stimulation of the HTR2B receptors leads to hyperphagia or hypophagia depending on the level of basal feeding (De Vry and Schreiber 2000). Polymorphisms in the genes encoding 5-HT 2A and 2C receptors have been associated with obesity and type 2 diabetes.

In addition to the 2p36 locus, Bosse *et al.* (2004) indicated the presence of an interesting region on chromosome 12q14.1 for involvement in HDL-C, however, linkage analysis in the Norfolk Island population failed to support these findings.

Although the variance components linkage analysis undertaken on the isolated Norfolk Island population recorded only one LOD score above 2.00 (for SBP), it is evident from the literature that this study has successfully identified regions previously implicated by other researchers, thus suggesting that the Norfolk Island isolated population model for disease gene mapping may have the potential to identify stronger linkage signals following collection of additional samples.

Conclusions

The Norfolk Island population pedigree structure was confirmed through the analysis of 400 polymorphic STR sites across the entire genome. Genome-wide scan results were also applied to variance component linkage analysis which revealed several informative loci which support previous results in both isolated and general populations. Increasing marker density in these CVD-related risk QTLs regions would serve as a follow-up strategy to narrow the LOD peaks and allow prediction of possible candidate genes for functional analyses.

Acknowledgements

An Australian National Health and Medical Research Council (NHMRC) Medical Genomics, Bioinformatics and Proteomics Program (MGBPP) grant plus an Australian National Heart Foundation (NHF) grant supported this research. The SOLAR statistical genetics computer package is supported by a grant from the US National Institute of Mental Health (MH059490). The supercomputing facilities used for this work at the AT&T Genetics Computing center were supported in part by a gift from the AT&T Foundation. We would like to acknowledge the help of Paul Jardine and the Research Computing Services team at Griffith University for providing support on Griffith's Sun Solaris HPC cluster. Lastly, this study would not have commenced without the participation of Norfolk Island population, we thank them for their involvement in this and future research.

Abbreviations List

BMI, body mass index

cM, centimorgan
 CVD, cardiovascular disease
 IBD, identity-by-descent
 DBP, diastolic blood pressure
 HDL-C, high-density lipoprotein-cholesterol
 LOD, logarithm of the odds ratio
 LDL-C, low-density lipoprotein-cholesterol
 QTL, quantitative trait loci
 SBP, systolic blood pressure
 TC, total cholesterol
 TG, triglyceride

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Figure 1.
LOD Trace on Chromosome 1 for Systolic Blood Pressure.

Table 1
Number of Relative Pairs within Norfolk Island Pedigree

Pairwise Relationship(s)	Number of Pairs	
	Cleaned pedigree	Phenotyped subset
Pedigree members	1078	377
Founders	587	124
Non-founders	491	253
Relative pairs		
Parent-offspring	982	142
Siblings	270	80
Grandparent-grandchild	908	19
Avuncular	612	120
Half Siblings	50	20
3 rd degree	1677	283
4 th degree	1317	315
5 th degree	952	404
6 th degree	648	372
7 th degree	286	239
8 th degree	50	50
Unrelated	225,349	27,670

Relative pairs in phenotyped subset column were included where phenotypic and genotypic data for each member of the pair was used in the linkage analysis.

Table 2
CVD-Related Risk Trait Heritability Analysis and Gender Comparison

Characteristic	All	Men	Women	$h^{2\ddagger}$
Number	377	171	206	NA
Age (years)	49.2 ± 16.6	48.9 ± 16.9	49.5 ± 16.4	NA
BMI (kg/m)	26.3 ± 4.3	27.4 ± 3.6	25.4 ± 4.7 [*]	0.295
TC (mmol/L)	5.6 ± 1.1	5.8 ± 1.1	5.5 ± 1.1 [*]	0.405
TG (mmol/L)	2.0 ± 1.2	2.3 ± 1.3	1.7 ± 1.1 ^{**}	0.236
HDL-C (mmol/L)	1.4 ± 0.4	1.3 ± 0.3	1.5 ± 0.4 ^{**}	0.449
LDL-C (mmol/L)	2.8 ± 1.0	3.0 ± 0.9	2.7 ± 1.0 [*]	0.419
SBP (mmHg)	127.4 ± 22.3	134.0 ± 19.6	121.8 ± 22.9 ^{**}	0.314
DBP (mmHg)	75.9 ± 13.3	79.3 ± 12.6	73.0 ± 13.2	0.169 [§]

NA: Not applicable

[‡] Adjusted for sex-specific age and age²; All $P < 0.05$ (all traits except Age underwent inverse normal transformation [as implemented in SOLAR 4.0.6] for analysis and back transformed for presentation)

^{*} $P < 0.05$, men vs. women

^{**} $P < 0.0001$, men vs. women

[§] Not significant $P > 0.05$

Table 3

Preliminary Linkage Analysis results for several CVD-related risk traits.

Trait	Loci	Marker	LOD	Reference(s)
TC	2q37.1	D2S206	1.80	(Bosse et al. 2004)
HDL-C	18q22.3	D18S1161	1.83	Novel region
	20p12.3	D20S117	1.69	Novel region
SBP	1p36.22	D1S2667	2.01	(Nakayama et al. 2004; Pan et al. 2000)
	8p12	D8S505	1.82	(Ringel et al. 2000)