A population-based study of polymorphisms in genes related to sex hormones and abdominal aortic aneurysm.

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Abstract

Male gender and family history are risk factor for abdominal aortic aneurysm (AAA). We hypothesised that genes involved in sex hormones might be important in AAA. We investigated the association of aortic diameter with single nucleotide polymorphisms (SNPs) in genes determining circulating sex hormones and their action. We genotyped 74 tagging SNPs across 4 genes (*steroid 5α reductase, subfamily A, polypeptide 1 (SRD5A1)*, *cytochrome P450, family 19, subfamily A, polypeptide 1 (CYP19A1)*, *androgen receptor (AR)* and *estrogen receptor 2 (ESR2)*) related to sex hormone production and action in 1711 men, 640 of whom had an AAA. One genotype was also assessed in an independent cohort of 782 men, of whom 513 had large AAAs. Associations were assessed adjusting for other risk factors for AAA. One SNP in *CYP19A1* was strongly associated with aortic diameter. Subjects who had the rare homozygote genotype (TT) for CYP19A1 g.49412370C>T (SNP ID rs1961177), had increased aortic diameter (coefficient 5.058, standard error 1.394, p=0.0003, under a recessive model). This SNP was not associated with aortic diameter in an independent cohort which included patients with larger AAAs. Our findings do not support an important role of genetic polymorphisms in genes determining sex hormones in aortic dilatation in men. The association of one SNP in CYPA9A1 with small but not large AAA may suggest differences between AAA formation and progression. This SNP warrants further investigation in another large population including patients with small AAAs.

Key words: Aorta; aneurysm; sex hormones.

Introduction

Abdominal aortic aneurysm (AAA) primarily affects men with population screening studies demonstrating an increased prevalence of approximately 5-fold compared to women[1]. Currently the reasons for the male propensity for AAA are not known but maybe linked to sex hormones, since in experimental models androgens have been positively linked to aortic dilatation[2]. The production, metabolism and response to sex hormones is controlled by an array of enzymes and receptors, including steroid 5α reductase, aromatase (estrogen synthetase), androgen and estrogen receptors[3,4]. Steroid 5α reductase enzymes are responsible for the conversion of testosterone to the more potent androgen dihydrotestosterone[3]. Aromatase (estrogen synthetase) is a cytochrome P450 enzyme that converts androgen precursor steroids to estrogens[4]. The responses to circulating sex hormones are determined by the androgen and estrogen receptors. Genetic factors appear to play an important role in the production, metabolism and response to male sex hormones and therefore maybe relevance to AAA, which has been shown to have inherited risk factors[5,6]. We selected 4 genes important in the production, metabolism and response to sex hormones in which to examine the association of genetic polymorphisms with aortic dilatation. We hypothesised that single nucleotide polymorphisms (SNPs) in the steroid *5α reductase, subfamily A, polypeptide 1 (SRD5A1)*, *cytochrome P450, family 19, subfamily A, polypeptide 1 (CYP19A1)*, *androgen receptor (AR)* and *estrogen receptor 2 (ESR2)* genes were associated with aortic dilatation and examined this within sub-set of the Health In Men Study (HIMS). We attempted to replicate any positive associations in an independent cohort from New Zealand.

Methods

Study design and subjects: HIMS consists of a cohort of men who originally participated in a trial of screening for AAA and has been previously described in detail[7,8]. For the current study genotyping was undertaken in all men with AAAs from whom DNA was available (n=640) and 1071 randomly selected age-matched men without an AAA as controls. Any SNPs found to be

significantly associated with aortic diameter were further assessed in an independent cohort of subjects from New Zealand[9]. These subjects included 513 men with large AAAs (80% had undergone aortic repair) and 269 healthy elderly men from the same region of Otago. All subjects included in both cohorts had undergone abdominal ultrasound. Ultrasound reproducibility was assessed during subject recruitment and 95% confidence intervals were <3mm[9]. The definitions of clinical risk factors such as hypertension, dyslipidemia, diabetes, coronary heart disease (CHD) and waist to hip ratio were as previously described[7].

Genotyping: The Haploview software package was used to define the linkage disequilibrium blocks and to choose tagging SNPs within the location of *SRD5A1* (n=14), *CYP19A1* (n=39), *AR* (n=5) and *ESR2* (n=16) genes using HapMap Phase II data utilising a pairwise approach (minor allele frequency >5% and r^2 >0.8)[10]. Regions analysed included the entire gene, plus additional sequences 10 kb upstream and downstream of the gene. With this approach 100% of the variation in the genes was captured. Genotyping on the HIMS subjects was carried out using the Illumina Golden Gate® assay on an Illumina BeadLab System at University of Western Australia. Genotype calls were made using Bead Studio Genotyping Module software package Version 3.1 (Illumina, Inc., San Diego, CA). Mono-allelic SNPs and those with genotyping efficiency <15% were excluded. As a result findings for SRD5A1 g.10386C>G (SNP ID rs4702379), SRD5A1 g.720102C>T (SNP ID rs6872996), CYP19A1 g.1667A>C (SNP ID rs2445768), CYP19A1 g.16698C>T (SNP ID rs8025374), AR g.98970A>G (SNP ID rs2361634) and ESR2 g.63762130G>T (SNP ID rs1152583) were excluded. Genotyping efficiency for the other 68 SNPs was between 97 and 100%. Genotyping in the New Zealand cohort was carried out using polymerase chain reaction as previously described[9].

Statistical analysis: Hardy-Weinberg equilibrium was tested on a contingency table of observed verses predicted phenotype frequencies using a modified Markov-chain random-walk algorithm. To test our hypotheses we investigated the association of genotyped SNPs and aortic diameter using linear regression adjusting for other risk factors for AAA (age, smoking, CHD, dyslipidemia,

hypertension, diabetes, waist to hip ratio). Each of the bi-allelic SNPs was coded into three genotype classes and analysed under codominant models (0=major allele homozygote, 1=heterozygote, 2=minor allele homozygote). Any significant codominant models were explored further (dominant and recessive models) to determine the best fitting model using Akaiki information criteria. Haplotype frequencies were estimated from unphased genotype data using an expectation maximisation algorithm under the assumption of Hardy-Weinberg equilibrium. Haplotypes were associated with aortic diameter using a generalized linear recessive model based on the initial genotyping results. Computations were undertaken using SimHap v1.0.2 (http://www.genepi.org.au/simhap.html) and SSPS 14.0 (SPSS Inc., Chicago, IL., USA). Genotypes associated with aortic diameter within the HIMS at a Bonferroni corrected p value of <0.0007 (based on the 74 SNPs assessed) were examined in a second independent cohort.

Results

Association of genotypes with aortic diameter in the HIMS subjects

Genotyping was carried out in 1711 HIMS subjects of whom 640 (37%) had an AAA (Table 1). 68 of the 74 (92%) SNPs passed quality assessments, were in Hardy Weinberg equilibrium in controls and therefore were assessed for association with aortic diameter. One SNP in CYP19A1 (CYP19A1 g.49412370C>T; SNP ID rs1961177) was independently associated with aortic diameter in HIMS subjects (Supplemental Table). Median aortic diameters were 24.2 (inter-quartile range 21.1-31.3), 24.5 (inter-quartile range 21.1-32.7) and 30.6mm (21.9-40.0) for men with CC (n=1317), TC (n=329) and TT (n=32) genotypes. CYP19A1 g.49412370C>T (SNP ID rs1961177) was independently associated with aortic diameter under a recessive model (coefficient 5.058, standard error 1.394, p=0.0003), including Bonferroni correction for multiple testing.

Haplotype analysis in the HIMS subjects

A total of 14 linkage dysequilibrium blocks were identified within the CYP19A1 gene using HapMap Phase II data (Fig. 1). We assessed the association of haplotype combination from these

different blocks with aortic diameter in HIMS subjects. Four haplotypes were demonstrated to be significantly associated with aortic diameter, after adjusting for other risk factors and multiple testing (Table 2). In particular the relative common haplotype CTT defined by the g.49386747C>G (SNP ID rs17523922), g.49394252C>T (SNP ID rs3751591), and g.49412370C>T (SNP ID rs1961177) which was present in 10% of men, p=0.0001 (Table 2).

Genotyping in the New Zealand men

We further examined g.49412370C>T (SNP ID rs1961177) in an independent cohort of 782 men from Otago, 513 (66%) of whom had an AAA. The patients with AAA in the New Zealand sample had a mean diameter of just under 6cm, compared to <4cm for the Western Australian men (Tables 1). CYP19A1 g.49412370C>T (SNP ID rs1961177) was not associated with aortic diameter in the New Zealand subjects (coefficient 0.076, standard error 0.284, p=0.788).

Discussion

To our knowledge this is the first published report examining the association of polymorphisms in genes determining circulating sex hormones and aortic diameter. Since male gender is an important risk factor for AAA we hypothesised that polymorphisms in four genes important in the production and action of sex hormones would be associated with aortic dilatation. One SNP within intron 1 of *CYP19A1* was strongly associated with aortic diameter within the HIMS cohort which included patients with small AAAs. The importance of this genetic locus in aortic dilatation was further supported by further analysis which identified a haplotype including this SNP as highly associated with aortic diameter in HIMS subjects. This genetic variation within *CYP19A1* was not however associated with aortic diameter in the New Zealand subjects where patients had much larger AAAs. Given the large number of SNPs examined and the lack of replication our findings do not support a consistent association between polymorphisms in these genes and aortic diameter in men. Our findings do not however rule out a role of the one SNP highlighted in this study in early stage AAA formation where different mechanisms may be involved compared to later stage aneurysm

progression. Also our study did not examine all genes involved sex hormone production and functions such as that encoding estrogen receptor 1. The assessment of the SNP in CYP19A1 in another population screened for AAA in which aortic diameter and risk factors have been carefully assessed would be worthwhile when such a group becomes available.

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Conflict of interest statement

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Legend to the figures

Figure 1: Schematic diagram of linkage disequilibrium blocks within CYP19A1 gene. Black triangles represent linkage disequilibrium (LD) blocks; deep red represents strong LD; red to white represents moderate to no LD among the single nucleotide polymorphisms.

Table 1: Comparison of subjects with and without AAA undergoing genotyping.

Subjects	**HIMS**			**New Zealand**		
Characteristic	**AAA**	**No AAA**	**P value**	**AAA**	**No AAA**	**P value**
Number	640	1071		513	269	
Aortic diameter (mm)	36.1±6.7	22.2±3.0	<0.001	59.5±16.8	20.4±0.2	<0.001
Age (years)	73.3±4.3	73.2±4.3	0.68	72.3±8.0	67.9±6.5	<0.001
Hypertension	345 (54%)	444 (41%)	<0.001	265 (52%)	67 (25%)	<0.001
Diabetes mellitus	67 (10%)	80 (8%)	0.05	40 (8%)	8 (3%)	0.01
Dyslipidemia	285 (45%)	375 (35%)	<0.001	198 (39%)	71 (26%)	0.001
Ever smoker	551 (86%)	672 (66%)	<0.001	444 (87%)	140 (52%)	<0.001
CHD	257 (40%)	228 (21%)	<0.001	205 (40%)	0 (0%)	<0.001

Nominal variables are presented as numbers and compared with chi squared. Continuous variables are presented as mean ± standard deviation and compared with Mann Whitney U test. CHD= Coronary heart disease.

Table 2: The association of CYP19A1 haplotypes with aortic diameter.

SNP ID	LD Block	Haplotype	Frequency	Copy	Coefficient	SE	P	Mean aortic diameter
rs12439137	3	AAT	0.0547	0	-	-	-	27.0
rs700518	4			1				
rs727479	5			2	7.7420	2.2060	**0.0005**	34.7
rs700518	4	ATT	0.0522	0	-	-	-	27.0
rs727479	5			1				
rs10459592	6			2	7.1140	2.1210	0.0008	34.1
rs17703883	4	TTT	0.0905	0	-	-	-	27.0
rs727479	5			1				
rs10459592	6			2	5.6600	1.6350	**0.0006**	32.7
rs727479	5	TTG	0.0527	0	-	-	-	27.0
rs10459592	6			1				
rs767199	7			2	7.1140	2.1210	0.0008	34.1
rs7172156	8	GGG	0.0444	0	-	-	-	27.0
rs3889391	9			1				
rs7181886	10			2	5.8540	2.2110	0.0082	32.9
rs7172156	8	GGT	0.0844	0	-	-	-	27.0
rs3889391	9			1				
rs2470157	10			2	4.3070	1.8120	0.0176	31.3
rs12050772	8	TGG	0.0455	0	-	-	-	27.0
rs3889391	9			1				

rs7181886	10			2	5.8540	2.2110	0.0082	32.9
rs3889391	9	GGC	0.0455	0	-	-	-	27.0
rs7181886	10			1				
rs17523880	11			2	5.8540	2.2110	0.0082	32.9
rs7181886	10	GCC	0.0460	0	-	-	-	27.0
rs17523880	11			1				
rs17523922	12			2	4.6400	2.1250	0.0291	31.6
rs2470152	11	CCC	0.0967	0	-	-	-	27.0
rs17523922	12			1				
rs3751591	13			2	6.2440	1.7150	**0.0003**	33.2
rs17523922	12	CCT	0.1052	0	-	-	-	27.0
rs3751591	13			1				
rs2445762	14			2	4.7700	1.7200	0.0056	31.8
rs17523922	12	CTT	0.1003	0	-	-	-	27.0
rs3751591	13			1				
rs1961177	14			2	6.5240	1.6720	**0.0001**	33.5

Shown are haplotype frequencies, coefficients and p values for the association of presented haplotypes with aortic diameter (mm) using a recessive model and adjusting for other risk factors outlined in the methods. The estimated mean aortic diameters and p values are shown for men with 0 or 1 copies compared to those with 2 copies of each haplotype. Shown in bold are the p values below the pre-specified level for significance, taking into account multiple testing. SNP= Single nucleotide polymorphism; LD= Linkage dysequilibrium.