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Genome-Wide Linkage Analysis of Hemodynamic Parameters Under Mental and Physical Stress in Extended Omani Arab Pedigrees: The Oman Family Study

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Background: We performed a genome-wide scan in a homogeneous Arab population to identify genomic regions linked to blood pressure (BP) and its intermediate phenotypes during mental and physical stress tests. *Methods:* The Oman Family Study subjects (N = 1277) were recruited from five extended families of ~10 generations. Hemodynamic phenotypes were computed from beat-to-beat BP, electrocardiography and impedance cardiography. Multi-point linkage was performed for resting, mental (word conflict test, WCT) and cold pressor (CPT) stress and their reactivity scores (Δ), using variance components decomposition-based methods implemented in SOLAR. *Results:* Genome-wide scans for BP phenotypes identified quantitative trait loci (QTLs) with significant evidence of linkage on chromosomes 1 and 12 for WCT-linked cardiac output (LOD = 3.1) and systolic BP (LOD = 3.5). Evidence for suggestive linkage for WCT was found on chromosomes 3, 17 and 1 for heart rate (LOD = 2.3), DBP (LOD = 2.4) and left ventricular ejection time (LVET), respectively. For ΔWCT, suggestive QTLs were detected for CO on chr11 (LOD = 2.5), LVET on chr3 (LOD = 2.0) and EDI on chr9 (LOD = 2.1). For CPT, suggestive QTLs for HR and LVET shared the same region on chr22 (LOD 2.3 and 2.8, respectively) and on chr9 (LOD = 2.3) for SBP, chr7 (LOD = 2.4) for SV and chr19 (LOD = 2.6) for CO. For ΔCPT, CO and TPR top signals were detected on chr15 and 10 (LOD; 2.40, 2.08) respectively. *Conclusion:* Mental stress revealed the largest number of significant and suggestive loci for normal BP reported to date. The study of BP and its intermediate phenotypes under mental and physical stress may help reveal the genes involved in the pathogenesis of essential hypertension.

■ **Keywords:** genome-wide, linkage, Arab pedigrees, haemodynamics, stress

In spite of the rapid advances in genomic research, the search for the genetic determinants of hypertension is proving to be one of the most challenging tasks. A large and a steadily increasing number of genome-wide linkage studies of essential hypertension (EH), normal blood pressure (BP) and related phenotypes have been published (Garcia et al., 2003; Samani, 2003). The low statistical power of these studies can be attributed, first, to the different methodologies and genetic and environmental heterogeneity of the populations (Doris, 2002; Garcia et al., 2003; Glazier et al., 2002; Hamet & Seda, 2007; Samani, 2003; Sing et al., 2003) and, second, to the use of BP cut-off points used for EH as a phenotype (Caulfield et al., 2003), ignoring the intermediate and the complex regulatory

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mechanisms' phenotypes of normal BP (Guyton, 1991) before being shaped by genetic and environmental factors into the final disease state (Sing et al., 2003). It was proposed that the study of intermediate BP phenotypes and their regularity mechanisms, particularly during stress, may be advantageous for gene-finding studies of complex diseases such as EH (Snieder et al., 2002). A few studies have shown that during acute stress, intermediate cardiovascular phenotypes in challenged individuals were more heritable than their unchallenged counterparts (de Geus et al., 2007; Wu et al., 2010). Failure to identify the specific genes influencing BP and related cardiovascular phenotypes have been attributed to the small effect sizes of many gene variants and the presence of gene by gene and gene by environment interactions (Choh et al., 2005; Kupper et al., 2006; Mitchell et al., 2008). Such interactions can only be discerned by studies designed to assess the genetics of the cardiovascular response to controlled short-term interventions that mimic long-term exposures known to affect cardiovascular health (Mitchell et al., 2008).

Although several linkage studies did not achieve enough statistical power to detect small effects, meta-analyses of these studies showed several genomic regions that may contain EH loci (Samani, 2003) that were later validated by other studies in humans and rodents (Chang et al., 2007). It is probable that these loci and their clusters, attributed to high or normal blood BP, were those of BP as well as of its intermediate phenotypes and their regulatory mechanisms. We therefore hypothesize that the study of normal BP and its intermediate phenotypes during short-term stressful laboratory conditions may reveal a matrix of quantitative trait loci (QTLs) representing the different components that make up BP.

In our Oman Family Study (OFS; Hassan et al., 2005), which began in 2002, we combined the advantages of a unique homogeneous Arab population with extensive phenotyping of more than 215 cardiovascular and related parameters during rest and during laboratory physical and mental stress. Detailed heritabilities of anthropometric, metabolic and hemodynamic parameters were reported elsewhere (Bayoumi et al., 2007; Hassan et al., 2009). Now we are reporting genome wide linkage results of BP and of its intermediate phenotypes during rest, physical stress and mental stress. To our knowledge, this is the first study which reports QTLs of BP and its intermediate phenotypes during rest and stress in large isolated pedigrees.

Methods

Study Area and Population

The Interior Province of Oman, where the study has been conducted, is 140 km south of the capital Muscat. It is a mountainous region dotted by several oases in river beds where traditional agriculture of mainly date palm and subsistence farming as well as livestock breeding have been practiced by successive Arab generations. These Omani Arabs have been relatively isolated, with little contact with the peoples of the coast who, in contrast, had close associations with the Indian subcontinent and East Africa for over 800 years. Fifty per cent of the small population of the Interior Province (75,000) is below the age of 20 years. It is distributed between the Willayat (district) of Nizwa (57,626) and several small villages averaging 1,000–5,000 individuals each. While the older generation is still working in traditional agriculture and animal breeding, the younger and educated men and women have taken government or private sector jobs in Nizwa or the capital Muscat. Most of these jobs are office jobs and a few are light manual jobs such as light vehicle drivers and labor supervisors. Due to the 35-year oil boom, a dramatic change in the lifestyle of the population has been witnessed (Hassan et al., 2005).

Pedigrees: Five large, extended and highly consanguineous families, each living in a separate village, were selected within a perimeter of 20 km around Nizwa. The number of subjects interviewed and found eligible for the study in the five pedigrees was 327, 160, 230, 279 and 281, totaling 1,277 which represented roughly 10% of the total number of individuals in these five pedigrees (Figure 1). Several connections between these five separate pedigrees were found enabling us to merge them into a single large pedigree. Subjects were 16–80 years old and all voluntarily took part in the study, appeared healthy and had no clinical complaints as noted in the questionnaire. First cousin marriages represent > 50% of all marriages (Hassan et al., 2005; Sulaiman et al., 2001). Polygamy is widely practiced, with up to four wives. The consequent rapid population growth produced these fairly young isolates of 7–12 generations each. A more detailed description of the stratification of the cohort and the OFS design have been explained in earlier reports (Bayoumi et al., 2007; Hassan et al., 2005). Prevalence of hypertension, defined as daytime ambulatory blood pressure SBP ≥ 135 mmHg and/or DBP ≥ 85 or the use of antihypertensive medication, was 22% with 2% of both genders on medication. Exclusion criteria were illiteracy (for the word conflict test [WCT]), pregnancy, malignancy, renal failure, heart failure and myocardial infarction/stroke within six months.

Data Collection

Data included in the final analysis was for 1,139 volunteers who had clean hemodynamic and biochemical phenotypes (Figure 1). A 20-minute questionnaire, anthropometric measurements, blood samples for DNA, biochemical and hormonal parameters were administered, collected and measured in the village. A written, informed consent explained to participants and signed or thumb-print rubber-stamped was obtained. The study was approved by ethics committees of Sultan Qaboos University and the Ministry of Health. Quality assurance was ascertained by

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duplicate measurements of e.g., anthropometry and BP of all subjects.

Experimental protocol: After an overnight fast, subjects reported to the field research centre at 07:00 hours. After explaining the procedure, electrodes were attached and subjects were made to rest supine on a comfortable bed in a quiet room for 10 minutes. Beat-to-beat recordings of hemodynamic and cardiac parameters were then continued as follows: 10 minutes of recordings at rest, 3 minutes of word conflict test (WCT), 3 minutes of recovery or until recording returned to baseline, and 2.5–3 minutes of cold pressor test (CPT). Tests were administered by the same male and female research assistants for the respective gender throughout the study.

Hemodynamic phenotypes: Hemodynamic measurements were compiled using direct and derived signals computed within the Task Force Monitor (TFM, CNSystems, Austria). Basic signals of the TFM were a 6-lead electrocardiogram (ECG), beat-to-beat BP and the impedance signal. Beat-to-beat BP was acquired by the vascular unloading technique using finger cuffs, and it was automatically counterchecked and corrected every minute by the oscillometric BP measurements recorded from the contra lateral upper arm (Gratze et al., 2005). Non-derived hemodynamic and cardiac parameters were continuous heart rate (HR), SBP and DBP.

Impedance cardiography: Derived hemodynamic parameters were computed from continuous BP and HR and the impedance signal (Skrabal, 2004). The impedance signal was acquired from a small constant sinusoidal alternating current passing through the thorax between an electrode placed around the neck and another placed at the lower end of the sternum. The voltage between the electrodes is proportional to the thorax impedance. Left ventricular ejection time (LVET), the time between points 'B' and 'X' (opening and closure of aortic valve, respectively) of the impedance signal, is considered in further calculations of hemodynamic parameters using the standard Kubicek's formula (Kubicek et al., 1974). Hemodynamic parameters calculated were stroke volume (SV), cardiac output (CO), total peripheral resistance (TPR), end-diastolic index (EDI) and index of cardiac contractility (IC).

Laboratory Stress Tests

The Word Conflict Test (WCT): The WCT (aka Stroop Test) involves sensory rejection of names of a spectrum of colours, but written in colours different from that of the colour itself (Fauvel et al., 1996; Stroop, 1935). The right cerebral hemisphere recognizes the colours and the left hemisphere names the word. The verbal narration of the conflict of words and colours forms the basis of the WCT. This creates cerebral confusion and invokes cardiovascular responses through central cerebral stimulation (Stroop,

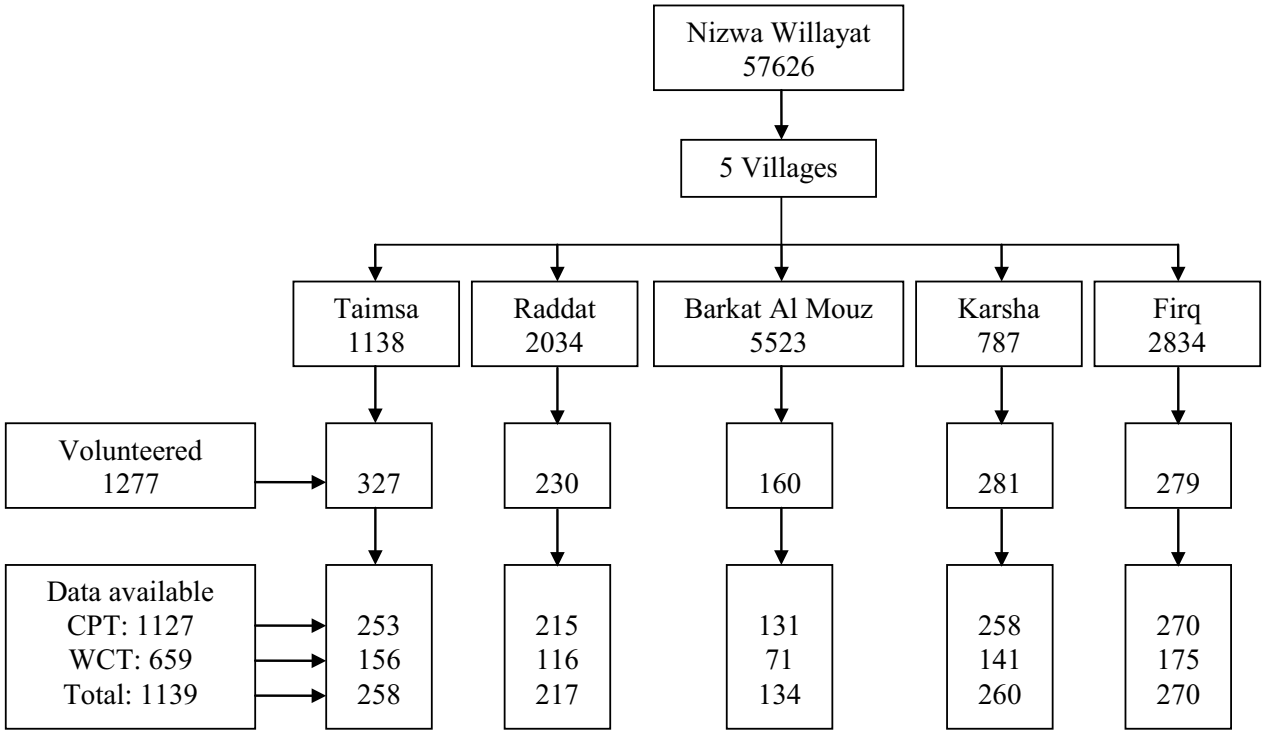

FIGURE 1
Flow chart of the population of Nizwa Willayat (district), the five study villages, volunteers and subjects with valid data.

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1935). The original English names of colours were translated into Arabic and the same incongruent colours were displayed on a monitor. The observer selected the words at a constant speed of one word/sec for a period of 3 minutes. The subject was asked to say the colour of the word and not read the word. Subjects were encouraged to respond faster and to concentrate fully throughout the test. Out of the 1,277 OFS subjects, only 659 (52%) participated in this test mainly due to illiteracy.

The Cold Pressor Test (CPT): The CPT is based on stimulation of pain receptors, which induces cardiovascular reactions (Wolff, 1951). The left foot was immersed in cold water with crushed ice (temperature 4° Celsius) up to the ankle joint for 3 minutes or until the test was aborted due to intolerable pain, but no less than 2 minutes (Kahn et al., 1993). The foot was wrapped in a towel after the test was over. Out of the 1,277 OFS subjects, full CPT data were available in 1,127 (88%).

Reactivity: Cardiovascular reactivity (Δ) was calculated as the difference between respective unweighted average resting values and the average of minutes 2 and 3 for both CPT and WCT.

Genotyping

DNA samples for 1,277 participants were genotyped for 343 microsatellite markers for a 10 cM genome-wide scan by the Mammalian Genotyping Services at Marshfield Clinic Research Foundation (http://research.marshfield-clinic.org/genetics). The mean sex-averaged distance between adjacent markers was 8.6 ± 6.5 cM with a predicted marker heterozygosity of 0.74 ± 0.11.

Statistical Analysis

Descriptive and comparative analyses were performed using the software package SOLAR v.4.0 (Almasy & Blangero, 1998). Likelihood ratio tests were used to calculate gender differences. A p value $< .05$ was considered to be statistically significant. The computer program Loki (Heath, 1997) was used to compute multipoint identity-by-descent (IBD) matrices, and the marker map positions were drawn by DeCode genetics (Kong et al., 2002). The phenotypes, cardiovascular resting values, stress values and reactivity to stress, were transformed using inverse normalization to meet the assumptions of normality and adjusted for age and sex for genetic analysis purposes. A variance components decomposition-based approach, implemented in the software package SOLAR v.4.0 (Almasy & Blangero, 1998), was used to perform a multipoint linkage analyses on related individuals. In short, it is an extension of the variance components approach in which variance due to a specific QTL is added to the basic model. It is based on estimating the effect of a specific QTL on the variation in phenotype, and can be modeled as a function of the IBD relationship at the marker locus between family members (Almasy & Blangero, 1998). Traditionally, a logarithm of the odds (LOD) score, which is computed directly from the likelihood ratio tests, is reported in linkage analyses (Almasy & Blangero, 1998). A LOD ≥ 2 was regarded as suggestive linkage and LOD ≥ 3 as significant linkage according to linkage analysis thresholds of Haines & Pericak-Vance (1998).

Results

Table 1 provides information on the relatedness of the study participants. Tables 2 and 3 show gender differences of subjects' age, body mass index (BMI) and hemodynamic values during rest, WCT, CPT and their reactivity. The younger age and smaller number of participants during WCT as compared to CPT was due to illiteracy, especially of older subjects.

In both rest and stress conditions, apart from HR (which was higher) and IC (which showed no difference), hemodynamic parameters were significantly lower in females than in males (Tables 2 and 3). The ΔWCT showed significantly smaller reactivities in females for HR, SV, and EDI and comparable values between males and females for other parameters (Table 2). In contrast, ΔCPT showed significantly higher reactivities in females than in males for HR, SBP, CO and LVET (Table 3).

Genome-Wide Scan of Blood Pressure Phenotypes

Table 4 presents the results of multipoint genome-wide linkage analysis of participants during rest, WCT and its reactivity (ΔWCT). All LOD scores of 1.0 or more are reported.

Rest

During the rest condition, we found evidence of suggestive linkage for SBP with the highest LOD score of 2.4 on chromosome 1q31 located between markers D1SA12F and D1S1660 (Table 4, Figure 2).

TABLE 1

Relative Pairs in the Five Pedigrees

Relationships	Size
Pedigree members	1851
Parent-offspring	2482
Siblings	1278
Grandparent-grandchild	3774
Avuncular	2815
Half-siblings	322
Grand avuncular	2928
Half avuncular	918
1st cousins	2610
1st cousins, 1 removed	932
Half 1st cousins, 1 removed	441
2nd cousins	4363
Other relationships	390
Total	25104

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TABLE 2

Characteristics (Mean [*SD*]) of the Participants and Gender Differences in Hemodynamic Parameters During Rest, WCT and its Reactivity (ΔWCT)

Phenotypes	Rest		WCT		Reactivity (ΔWCT)	
	Male *N* = 516	Female *N* = 625	Male *N* = 325	Male *N* = 334	Male *N* = 324	Male *N* = 331
Age	23.4 (8.0)	23.4 (5.4)				
BMI	23.6 (1.9)	23.4 (5.3)				
HR (beats/min)	68.0 (10.1)	75.0 (11.0)***	77.0 (13)	81.0 (12.1)***	13.6 (12.1)	9.0 (8.9)***
SBP (mmHg)	119.0 (14.4)	105.0 (11.3)***	128.0 (14.1)	112.0 (12.0)***	8.8 (10.5)	7.8 (9.3)
DBP (mmHg)	74.0 (12.1)	65.0 (9.0)***	82.2 (12.9)	72.2 (9.9)***	11.1 (12.8)	10.5 (13.2)
SV (ml)	89.0 (17.3)	76.2 (13.4)***	87.1 (17.1)	75.0 (1.33)***	-2.4 (11.0)	-0.7 (10.2)**
CO (L/min)	6.1 (1.5)	5.7 (1.2)	6.7 (1.7)	6.1 (1.3)***	10.7 (16.3)	7.8 (12.4)
TPR (dyne*s/cm5)	1196.3 (348.4)	1069.5 (236.5)***	1202.4 (352.4)	1111.3 (262.0)***	2.4 (16.0)	3.6 (14.3)
LVET (ms)	313.4 (16.3)	308.8 (16.9)**	301.5 (18.8)	299.6 (17.2)	-3.5 (3.9)	-3.1 (3.5)
EDI (ml/m2)	81.6 (16.6)	78.6 (12.9)**	78.8 (14.9)	78.0 (12.6)	-2.7 (9.7)	-0.4 (9.6)***
IC (1000/sec)	67.7 (22.9)	68.0 (19.6)	66.7 (21.7)	68.6 (20.0)	0.2 (15.9)	2.1 (15.7)*

Note: HR: Heart rate (bpm), SBP: Systolic BP, DBP: Diastolic BP, SV: Stroke volume (ml), CO: Cardiac output (L/min), TPR: Total peripheral resistance (dyne*s/cm^5), LVET: Left ventricular ejection time (ms), EDI: End diastolic index (ml/m^2), IC: Index of contractility (1000/sec), WCT: word conflict test ; *P < .05, **P < .001, ***P < .0001.

TABLE 3

Characteristics (Mean [*SD*]) of the Participants and Gender Differences in Hemodynamic Parameters During CPT and its Reactivity (ΔCPT)

Phenotypes	Rest		CPT		Reactivity (ΔCPT)	
	Male *N* = 516	Female *N* = 625	Male *N* = 502	Male *N* = 607	Male *N* = 502	Male *N* = 606
Age	32.3 (16.1)	34.3 (15.0)*				
BMI	24.8 (5.0)	25.2 (5.7)				
HR (beats/min)	68.0 (9.8)	73.0 (10.7)***	73.0 (10.9)	81.0 (12)***	7.6 (12.5)	10.4 (11.9)***
SBP (mmHg)	119.0 (15.4)	107.0 (12.9)***	132.0 (17.6)	122.0 (15.8)***	12.3 (12.6)	15.1 (11.9)*
DBP (mmHg)	76.0 (12.5)	67.0 (10.1)***	89.0 (14.6)	80.0 (12.1)***	17.3 (16.4)	21.3 (18.5)
SV (ml)	82.2 (19.9)	69.3 (15.6)***	79.6 (17.9)	66.5 (13.7)***	-3.2 (11.3)	-2.8 (11.7)
CO (L/min)	5.5 (1.5)	5.1 (1.4)	5.8 (1.5)	5.4 (1.3)***	3.4 (12.3)	6.8 (13.9)**
TPR (dyne*s/cm5)	1344.1 (450.0)	1269.2 (387.5)**	1492.8 (448.8)	1435.2 (414.6)*	15.0 (20.5)	14.7 (20.1)
LVET (ms)	314.4 (16.5)	319.7 (18)***	310.5 (16.8)	302.4 (17.6)***	-0.99 (4.1)	-2.4 (4.4)***
EDI (ml/m2)	74.6 (17.9)	70.8 (15.2)***	72.0 (16.4)	68.6 (13.8)***	-2.8 (9.9)	-2.2 (10.5)
IC (1000/sec)	58.9 (23.7)	56.9 (22.0)	55.7 (21.5)	54.7 (19.9)	-3.7 (15.5)	-1.3 (18.1)

Note: HR: Heart rate (bpm), SBP: Systolic BP, DBP: Diastolic BP, SV: Stroke volume (ml), CO: Cardiac output (L/min), TPR: Total peripheral resistance (dyne*s/cm5), LVET: Left ventricular ejection time (ms), EDI: End diastolic index (ml/m2), IC: Index of contractility (1000/sec), CPT: cold pressor test; *P < .05, **P < .001, ***P < .0001.

Word Conflict Test

For WCT, data were available for 659 individuals. Evidence of significant linkage was found for (a) for SBP, with a LOD score of 3.5 at position q15 to q23 on chromosome 12 between markers D12S1294 and D12S1052 (Table 4, Figure 2), and (b) for CO, with LOD score 3.1 at chromosome 1q near marker D1S1653 (Table 4, Figure 3). Furthermore, our analyses on WCT revealed three additional suggestive linkage peaks including one linkage region for HR, with a LOD score of 2.3 at chromosome 3p between markers D31766 and D3ST128 (Table 4), the other for DBP, with a LOD score of 2.4 at chromosome 17q between markers D12S1294 and D12S1052 (Table 4), and the last one for LVET with a LOD score of 2.2 at chromosome 1p between markers D1S79C10 and D1S3721 (Table 4, Figure 2). For WCT reactivity, we observed three suggestive linkage peaks. One for CO, with a LOD score of 2.5 on chromosome 11 between markers D11S1981 and D11S4E08, one for LVET with a LOD score of 2.0 on chromosome 3 between markers D3S2409-D3S1766-D3S3039, and one for EDI on chromosome 9 near marker D9S2169 (Table 4).

The significant locus linked to WCT CO (LOD = 3.1, one LOD support interval (OLSI) =149–164) on chromosome 1 overlaps the locus for SBP during rest, WCT HR (LOD = 1.7; Figure 2) and WCT-EDI (LOD = 1.5, OLSI = 149–167; Table 4). The suggestive locus for SBP during rest (LOD = 2.4, OLSI = 200–228), mentioned above, overlaps with a genetic region on chromosome 1 that showed some evidence of linkage to LVET during rest

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TABLE 4

Genome-Wide Scan for Rest, Word Conflict Test (WCT) and its Reactivity (ΔWCT)

Phenotype	Rest				WCT				ΔWCT			
	LOD	Chr (cM)	One-LOD interval	Marker(s)	LOD	Chr (cM)	One-LOD interval	Marker(s)	LOD	Chr (cM)	One-LOD interval	Marker(s)
HR (beats/min)	1.4	11 (12)	0–26	D11S2362 and D11S1999	**2.3**	3 (82)	72–90	D3S1766 and D3ST128	1.5	13 (87)	70–100	D13S317 and D13S793
SBP (mmHg)	**2.4**	1 (211)	200–228	D1S1660 and D1SA124F	**3.5**	12 (84)	71–91	D12S1294 and D12S1052	1.9	1 (122)	112–129	D1SA124C
DBP (mmHg)	1.7	20 (96)	82–101	D20S164	**2.4**	17 (83)	74–90	D17Sc5ZP	1.8	1 (102)	92–114	D1SA152F
SV (ml)	1.5	1 (122)	112–156	D1SA124C	1.5	7 (84)	74–95	D7S3046 and D7S2204	1.8	9 (14)	2–24	D9S2169
CO (L/min)	1.8	2 (220)	208–230	D2S434	3.1	1 (154)	149–164	D1S1653	2.5	11 (32)	20–44	D11S1981 and D11S4E08
TPR (dyne*s/cm^5)	1.1	1 (154)	143–163	D1S1653	—	—	—		—	—	—	—
LVET (ms)	1.4	1 (212)	196–239	D1S1660 and D1SA124F	**2.2**	1 (60)	48–68	D1S79C10 and D1S3721	**2.0**	3 (81)	73–90	D3S1766
EDI (ml/m2)	1.4	6 (134)	123–150	D6S1040	1.5	1 (154)	149–167	D1S1653	**2.1**	9 (14)	3–28	D9S2169
IC (1000/sec)	1.5	3 (59)	51–68	D3S2432	1.4	10 (169)	158–173	D10S1248 and D10S006Z	1.7	11 (73)	54–90	D11S2006 and D11S2371

Note: HR: Heart rate (bpm); SBP: Systolic BP (mmHg); DBP: Diastolic BP (mmHg); SV: Stroke volume (ml); CO: Cardiac output (L/min); TPR: Total peripheral resistance (dyne*s/cm^5); LVET: Left ventricular ejection time (ms); EDI: End diastolic index (ml/m^2); IC: Index of contractility (1000/sec); LOD: Logarithm of the Odds for the top signal, Chr: Chromosome, cM: Cytogenetic location in centimorgan on DeCODE map, Markers: Nearest marker/flanking markers, CPT: Cold pressor test, WCT: World Conflict Test, ΔWCT: WCT reactivity, ΔCPT: CPT reactivity; Linkage analysis threshold: Suggestive ≥ 2 and Significant ≥ 3 (Haines & Pericak-Vance, 1998). LOD scores ≥ 2 are bolded.

(LOD = 1.4, OLSI = 196–239) and WCT (LOD = 1.3; Figure 2). The region significantly linked to WCT SBP (LOD = 3.5, OLSI = 71–91) on chromosome 12 also showed linkages to HR, DBP and LVET during WCT (Figure 3). LOD scores between 2 and 3 are considered evidence of suggestive linkage. LOD score less than 2.0 cannot be considered as suggestive. A locus on chromosome 8 showed suggestive linkage to both HR (LOD = 2.1) and LVET (LOD = 2.1) during WCT.

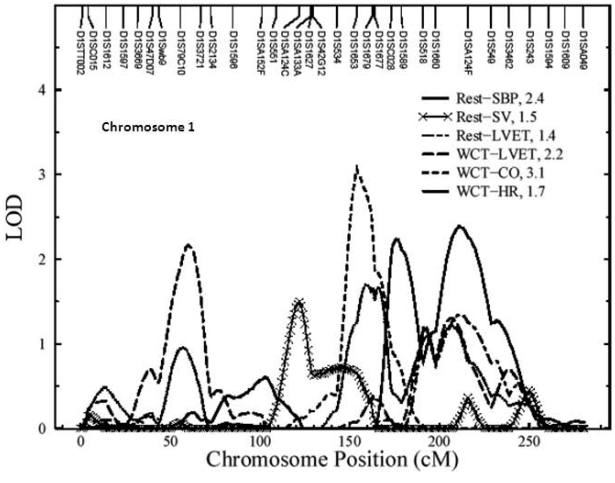

FIGURE 2

Chromosome 1 showing multipoint linkage plots for loci of resting (Rest) systolic BP (SBP), stroke volume (SV), left ventricular time (LVET) and for word conflict test (WCT) of LVET, cardiac output (CO), heart rate (HR) and their respective LOD scores as generated by SOLAR version 4.

Cold Pressor Test

Table 5 presents the results of the multipoint genome-wide linkage scan for CPT and its reactivity (ΔCPT). For CPT, data were available for 1,127 individuals. The genome-wide scan revealed five suggestive linkage peaks for HR (LOD=2.3; chr. 22; markers D22S1045 and D22S532), for SBP (LOD=2.3; chr. 9; marker D9S455A), for SV (LOD 2.4; chr 7; marker D7S3046), for CO (LOD 2.6; chr. 19; markers D19S589-D19S254), and LVET (LOD 2.8; chr. 22; markers D22S1045 and D22S532). For ΔCPT, we observed three suggestive linkage peaks; One for CO (LOD 2.4; chr. 15; markers D15S659), the other one for TPR (LOD 2.1; chr. 10, marker D10S1221) and the last one for IC (LOD 2.0; chr. 3, marker D3S1766 and D3ST128). Interestingly, the suggestive locus on chr.3 for WCT HR (LOD = 2.3, OLSI = 72–90) and for ΔWCTLVET (LOD = 2.0, OLSI = 73–90) showed also some evidence for linkage to CPT reactivity of SV (LOD = 1.6, OLSI = 70–105), EDI (LOD = 1.8, OLSI = 69–94) and IC (LOD = 2.0, OLSI = 69–94) (Tables 4 and 5). Suggestive loci for CPT HR (LOD = 2.3, OLSI = 48–69) and CPT LVET (LOD = 2.8, OLSI = 48–71) share the same chromosomal region of chromosome 22 (Table 5).

Discussion

The Oman Family Study aimed to perform a genome-wide scan in a homogeneous Arab population to identify genomic region linked to BP and its intermediate phenotypes during mental and physical laboratory stress tests. The study was conducted in isolated, highly consanguineous and multigenerational Arab pedigrees of 1,277

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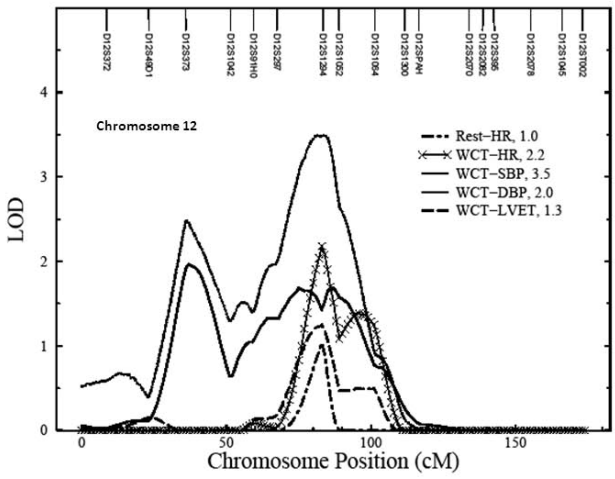

FIGURE 3

Chromosome 12 showing multipoint linkage plots for loci in of resting (Rest) and WCT HR, and WCT SBP, DBP and LVET and their respective LOD scores as generated by SOLAR version 4.

individuals with a mean age of 33.5 years, 60% of whom were below 20 years of age. The multipoint genome-wide linkage revealed two significant and several suggestive peaks with some sharing and overlap of the same chromosomal regions during rest, WCT and CPT. The WCT revealed two significant linkage peaks: one for SBP on position q15 to q23 on chromosome 12 and one for CO on chromosome 1q. Three additional suggestive linkage peaks were found for HR on chromosome 3p, for DBP on chromosome 17q and for LVET on chromosome 1p. The WCT reactivity revealed three suggestive linkage peaks. One for CO on chromosome 11, one for LVET on chromosome 3 and one for EDI on chromosome 9. The CPT revealed five suggestive linkage peaks: for HR on chromosome 22, for SBP on chromosome 9, for SV on chromosome 7, for CO on chromosome 19, and for LVET on chromosome 22. ΔCPT revealed three suggestive peaks: one for CO on chromosome 15, one for TPR on chromosome 10 and one for IC on chromosome 3. During rest a suggestive linkage peak was revealed for SBP in the chromosome 1q31 to 1q42region.

Several significant or suggestive loci mostly for OFS CPT and WCT hemodynamic phenotypes match with other linkage studies of EH, SBP or DBP or in meta-analyses of combinations of these phenotypes (e.g., EH+SBP). In OFS, a locus for resting SBP and WCT CO on chromosome1 replicated with DBP, with a significant LOD score of 3.2 in the GenNet study (Chang et al., 2007), and with EH+DBP and EH+SBP but with lower LOD scores in a meta-analysis (Koivukoski et al., 2004). In the same meta-analysis, OFS WCT HR and ΔWCT LVET overlaps with a locus on chromosome 3 for EH+DBP and EH+SBP and EH with significant LOD scores ranging from 3.3–4.69 (Koivukoski et al., 2004). In another meta-analysis, OFS WCT HR and ΔWCT LVET and ΔCPT IC overlaps with EH+DBP and EH+SBP on the same chromosome and locus of the later study (Wu et al., 2006). On chromosome 7, OFS CPT SV overlaps with a locus for DBP and also on chromosome 10, OFS ΔCPT TPR overlaps with a locus for DBP in Nigerian families (Cooper et al., 2002). On chromosomes 11, 12, 15; OFSΔWCT CO, WCT SBP and ΔCPT CO match with loci for EH in the NHLBI Family Heart Study (Hunt et al., 2002). On chromosome 17 OFS ΔWCT DBP matches with loci for SBP and EH respectively in two

TABLE 5

Genome-Wide Scan Results for Cold Pressor Test (CPT) and its Reactivity (ΔCPT)

Phenotype	CPT				ΔCPT			
	LOD	Chr (cM)	One-LOD interval	Marker(s)	LOD	Chr (cM)	One-LOD interval	Marker(s)
HR (beats/min)	**2.3**	22 (55)	48–69	D22S1045 and D22S532	1.6	10 (117)	96–122	D10S677
SBP (mmHg)	**2.3**	9 (0)	0–8	D9S455A	1.9	4 (155)	145–165	D4S1629
DBP (mmHg)	1.9	4 (121)	98–128	D4S2623 and D4SA006	1.4	15 (13)	0–30	D15S822
SV (ml)	**2.4**	7 (83)	75–90	D7S3046	1.6	3 (86)	70–105	D3ST128
CO (L/min)	**2.6**	19 (105)	89–113	D19S589-D19S254	**2.4**	15 (46)	38–59	D15S659
TPR (dyne*s/cm^5)	1.6	17 (91)	80–98	D17S45	**2.1**	10 (76)	70–85	D10S1221
LVET (ms)	**2.8**	22 (53)	48–71	D22S1045 and D22S532	1.2	16 (26)	16–37	D16S1E04 and D16S748
EDI (ml/m^2)	1.2	22 (51)	27–69	D22S1045	1.8	3 (84)	69–94	D3S1766 and D3ST128
IC (1000/sec)	1.2	18 (20)	0–28	D18ST060	**2.0**	3 (84)	69–94	D3S1766 and D3ST128

Note: HR: Heart rate (bpm); SBP: Systolic BP (mmHg); DBP: Diastolic BP (mmHg); SV: Stroke volume (ml); CO: Cardiac output (L/min); TPR: Total peripheral resistance (dyne*s/cm^5); LVET: Left ventricular ejection time (ms); EDI: End diastolic index (ml/m^2); IC: Index of contractility (1000/sec); LOD: Logarithm of the Odds for the top signal, Chr: Chromosome, cM: Cytogenetic location in centimorgan on DeCODE map, Marker(s): Nearest marker/flanking markers, CPT: Cold pressor test, WCT: World Conflict Test, ΔWCT: WCT reactivity, ΔCPT: CPT reactivity; Linkage analysis threshold: Suggestive ≥ 2 and Significant ≥ 3 (Haines &Pericak-Vance, 1998). LOD scores ≥ 2 are bolded.

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separate studies (de Lange et al., 2004; Kristjansson et al., 2002). On chromosome 19, OFS CPT SV overlaps with a locus for SBP in Nigerian families (Cooper et al., 2002). On chromosome 22 OFS CPT HR and LVET match with a locus with a significant LOD score for EH in diabetic families (Avery et al., 2004).

Of interest are the two chromosomal regions that were associated with CO on chr 1 and SBP on chr 12 that harbour interesting candidate genes (Fisher et al., 1986). The one-LOD confidence interval for the QTL for CO spans a region of 15 cM and contains two positional candidate genes. The gene Lamin (*LMNA*) encodes lamin A and lamin C, which are components of the inner membrane that regulates nuclear shape and size. Mutations in this gene have been associated with several neuromuscular disorders, including diseases of cardiac muscle (Benedetti et al., 2007). Associations of mutations in the LMNA gene with dilated cardiomyopathy and defects in cardiac conduction have been reported by several studies (Charniot et al., 2003; Fatkin et al., 1999; Meune et al., 2006; Sebillon et al., 2003; van der Kooi et al., 2002). In addition, mutations in the nitric oxide synthase 1 (Neuronal) adaptor protein (*NOS1AP*) gene have been associated with the QT interval, a measure of the duration of cardiac depolarization and repolarization (Arking et al., 2006; Eijgelsheim et al., 2009; Post et al., 2007), indicating the importance of the chromosomal region 1q22-q23.1 in cardiac function. The QTL for SBP is located on chromosome 12q13.1-q21.3 and includes the arginine vasopressin receptor 1A (*AVPR1A*) gene, which encodes the receptor of the antidiuretic hormone vasopressin (Thibonnier et al., 1994). Vasopressin is involved in the regulation of blood volume and BP. A study conducted by (Koshimizu et al., 2006) reported lower BP in Avpr1a-null mice than wildtype mice; however, data is limited in humans.

The strengths of the OFS includes the accessibility and authenticity of the genealogical records; the close family ties of all five pedigrees guaranteed more homogeneous environmental exposures with similar socioeconomic status, similar health-related habits such as diet, habitual physical activity, and the strict religious abstinence from alcohol and smoking (Hassan et al., 2005; Sulaiman et al., 2001). In addition, we applied stringent criteria for extensive phenotyping of cardiovascular traits contributing to the regulation of BP by dissecting BP into its primary and intermediate phenotypes in a supposedly normal population. There are some limitations to the study as well, of which one is the much smaller sample size available for the WCT data ($n = 659$) compared to the resting and CPT data ($n = 1127$). This was caused by exclusions due to illiteracy, especially of older subjects. It is important to note that, compared to OFS loci, all other studies and meta-analyses without exception used SBP, DBP in normal and EH cohorts or EH as phenotypes. Our results propose that those studies unknowingly detected loci for some of the OFS intermediate BP phenotypes; most of the significant and suggestive loci in this study were exposed during mental and physical stress. Studies in the cardiovascular reactivity literature have applied several modalities of mental stress stimuli. These include mental arithmetic, serial subtractions, mirror image tracing, video games, and so on. Stroop WCT involves sensory rejection to study the phenomenon of inhibition or interference (Stroop, 1935). This test has been reported to provoke steady increase in HR, BP (Fauvel et al., 1996), and plasma adrenaline and noradrenaline (Hoshikawa & Yamamoto, 1997). The test is reproducible, produces better sympathetic activation compared with the mental arithmetic test (Freyschuss et al., 1990) and is a good psycho-physiological indicator of stress reactivity (Hamer et al., 2006). The CPT induces reflex hemodynamic and autonomic responses through stimulation of cold nociceptors (Menkes et al., 1989; Yamamoto et al., 1992). Since the 1940s both tests have been extensively used and described in the scientific literature to understand cardiovascular reactivity.

Conclusion

Significant and suggestive QTLs for BP and for its intermediate phenotypes were obtained during laboratory stress tests. Laboratory mental stress, which revealed the only significant BP loci, may represent a replay of the environmental conditions that contribute to the development of EH and may help identify the genes of this complex condition. In addition, the different loci detected during rest, mental, physical stress and during reactivity in the whole genome further confirm the complexity of BP control as well as the oligogenicepistatic nature of the genetic components of BP determination.

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