Adult nontwin sib concordance rates for type 2 diabetes, hypertension and metabolic syndrome among Asian Indians: The Indian Atherosclerosis Research Study

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Abstract: Diabetes (DM), hypertension (HTN), and metabolic syndrome (MS) are established cardiovascular risk factors with a complex etiology. The aim of the present study was to estimate the rates of concordance for the above coronary risk factors between siblings in Asian Indian families with premature coronary artery disease (CAD). Spouse concordance rates were used to evaluate the relative contribution of shared genes and lifestyle towards these traits. A total of 508 families comprising of 1250 sib-pairs and 463 corresponding spouse-pairs were analyzed. Concordance rates were manually determined. Plasma lipids were estimated by standard enzymatic assay. The concordance rates among sib-pairs for DM, HTN, and MS was 11% (N = 136), 14% (N = 174), and 23% (N = 287), while the corresponding concordance for spouse-pairs was 2.8% (N = 13), 6.3% (N = 29), and 28.1% (N = 130), respectively. Employing Chi-square test, sib-pairs showed significantly higher concordance for diabetes ($p \leq 0.0001$) and hypertension ($p < 0.0001$) while spouse-pairs had higher concordance for metabolic syndrome ($p = 0.033$) in our study. These findings suggest a probable dominant genetic component in the causation of DM and HTN and a predominantly nongenetic component for metabolic syndrome among Asian Indians.

Keywords: sib-pairs, spouse-pairs, type 2 diabetes, hypertension, metabolic syndrome, concordance, CAD, Asian Indians

Introduction

Type 2 diabetes (T2DM) is a heterogeneous disease that results from a complex interplay between genes and environment. Developing countries like India share a major portion of the global burden of diabetes (King et al 1998). Out of the present estimated 150 million diabetics worldwide, India has the largest number of 19.4 million diabetic individuals. This number is expected to increase to almost 57.2 million by the year 2025 (Pradeepa et al 2002) while the worldwide incidence is expected to rise to 300 million around the same time (King et al 1998). Hypertension is another well-recognized multifactor trait. Out of the estimated 600 million people with hypertension, 180 million from high income and 420 million from low/middle income countries are at risk of developing coronary artery disease (CAD), myocardial infarction, stroke, and heart failure (WHO 2000). Hypertension is directly responsible for 57% of all stroke deaths and 24% of all deaths due to coronary heart disease in India. At a conservative estimate, there are 34 million hypertensive subjects in urban and 31.5 million in rural populations that constitute about 25% of urban and 10% of rural populations respectively (Gupta 2004).

Both T2DM and HTN have a genetic etiology. This is evident from familial aggregation and a high concordance for both traits in identical twins (Rewers and

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Hamman 1995). Individuals with diabetes have a relatively greater proportion of family members with the disorder (10%–30%) than amongst nondiabetics (1%–6%). Studies have shown 45%–96% concordance for diabetes among monozygotic twins (MZ) and 3%–37% for dizygotic twins (DZ) (Sceuner and Rotter 1997). A similar trend has been reported for concordance of hypertension in MZ (36%) as compared with DZ (8%) twin pairs (Berg 1987). Parental blood pressure (BP) levels are said to independently predict BP levels in offspring. A similar correlation was observed in male and female siblings whereas correlation coefficients obtained for spouse was lower than the values obtained for first-degree relatives (Havlik et al 1979; Chiang et al 2003).

Metabolic syndrome (MS) refers to the clustering of features such as abdominal obesity, glucose intolerance/insulin resistance, high plasma triglyceride (TG), decreased high-density lipoprotein-cholesterol (HDL-C), and high systolic blood pressure in the same individual more often than by chance alone (Ferrannini 1983). Those with the syndrome are more likely to have family members with components of the syndrome (Hunt et al 2000). Twin studies have shown higher concordance rates for glucose intolerance, obesity and low-HDL-C among MZ than DZ twins (Poulsen 2001), while sib-pair studies on sibs with early onset coronary artery disease (EOCAD) in the GENECARD study have shown significant concordance for presence of T2DM (78%), dyslipoproteinemia (67%), obesity (63%), and hypertension (56%) (Hauser et al 2003). In the same study, however, substantial sib-pair concordance ($p < 0.01$) was also reported for environmental traits with no known inherited predisposition such as smoking history (74%), alcohol consumption (81%), and sedentary life-style (63%). A common latent factor might be responsible for the clustering of MS traits among adult male twins that is influenced by both genetic (59%) and environmental factors (41%) (Loos et al 2003).

All three chronic conditions mentioned above are important and independent risk factors for coronary artery disease (CAD) and show familial clustering. The plausible environmental precipitants appear to be the inter-related triad of obesity, lack of physical exercise, and improper diet. The role of environmental determinants and their mutual interaction may be subject to individual genetic determinants and may not act in a similar manner in all populations (King 1998).

Household aggregation refers to the occurrence of a disease more frequently among residents sharing the same house in comparison to the general population. Studies on spouse-pairs help to understand the determinants related to modifiable behavioral characteristics that are likely to be influenced by shared lifestyle and socio-economic environment but not due to close genetic inheritance.

Given the high prevalence of T2DM, hypertension and metabolic syndrome among Asian Indians, the aim of the present study was to determine their concordance rates among adult nontwin siblings in families with history of CAD. Spouse-concordance rates were determined for the above traits to account for shared lifestyle effects.

Materials and Methods

Families in the Indian Atherosclerosis Research Study [IARS] were ascertained through a proband (males ≤ 60 years; females ≤ 65 years at onset of CAD) admitted to Narayana Hrudayalaya, a multi-specialty hospital, other clinics and hospitals in Bangalore, and to the Asian Heart Hospital in Mumbai for undergoing treatment for CAD and its complications. Only probands with a positive family history of coronary disease or stroke were enrolled into the study. There were five hundred and eight families comprising of 2313 individuals, 527 sibships, and 1250 sib-pairs, with a mean of 4.35 individuals/family. Four hundred and sixty three corresponding spouse-pairs were present in this cohort.

A detailed case record form containing information on demography, anthropometry, life style habits, medical history of diabetes, hypertension, stroke, medications, reports of diagnostic tests, and family tree up to a minimum of three generations were recorded for all subjects. General physical examination along with vital parameters was performed. Relevant information was obtained by personal interviews and through medical records available with the subjects and/or from the hospital records. Prevalence of diabetes and hypertension was ascertained based on self-report of physician's diagnosis and/or use of prescription medications. None of the probands or family members had concomitant or past illness such as cancer, cardiomyopathy, rheumatic heart disease, liver or renal disease, or concomitant infection. All participants gave their written informed consent to participate in the study that was approved by the local Ethics Committee.

Laboratory assays

Fasting blood glucose was tested on the Glucometer (Johnson and Johnson, Ltd, New Jersey, USA). Venous blood was collected in evacuated tubes after an overnight fast of

12 to 14 hours (Vacuette®, Greiner Bio-One GmbH, Vienna, Austria). Serum, EDTA, and citrate plasma samples were separated by centrifugation and aliquots were preserved at –80 °C until analysis. Serum TG was estimated using reagents, standards and controls from Randox Laboratories Ltd. (Antrim, UK). Estimation of HDL-C levels was carried out by the phosphotungstate method using precipitating agents and buffer from Bayer Diagnostics, control from Randox Labs, and standards from Dade-Behring Limited, UK .The assays were carried out on Cobas-Fara II Clinical Chemistry Auto analyzer (F. Hoffman La Roche Ltd., Basel, Switzerland).

Of a total of 1250 sib-pairs available for the study, sib-pairs in which both members had T2DM, HTN, or MS were considered concordant for that trait. Corresponding spouse-pairs were included in the study after exclusion of those spouses with family history of DM, HTN, or a consanguineous alliance.

Prevalence of MS amongst the sib- and spouse-pairs was assessed on the basis of 2001 NCEP-ATPIII (National Cholesterol Education Programme–Adult Treatment Panel III [ATPIII]) guidelines (NCEP-ATP111 2001), wherein any three of the following traits in the same individual met the criteria for the syndrome: (1) Abdominal obesity as defined by a waist circumference >102 cm in men; >88 cm in women; (2) Serum triglycerides ≥150 mg/dL; (3) HDL-C <40 mg/dl in men and ≤50 mg/dl in women; (4) Blood pressure of ≥130/85 mmHg; (5) Fasting blood glucose ≥110 mg/dL.

Statistical analysis

Mean and SD are presented for age and BMI. Sib-pair and spouse-pair concordance for T2DM, HTN, and MS were calculated by manual counting of subjects with the trait. The difference in concordance between sib-pairs and spouse-pairs was tested by Chi square test with Yates correction on the basis of pair-wise concordance rates for T2DM, HTN, and MS among all affected pairs. A 'p' value less than 0.05 were considered as statistically significant. SPSS V.10 (Microsoft, Redmond, USA) statistical software was used for the analysis.

Results

The clinical, demographic, and anthropometric data of the IARS families is presented in Table 1. A total of 508 families with 2313 individuals were included in the study after exclusion of 23 incomplete families. There were 1353 males (58.5%) and 960 females (41.5%) out of which 46.6% and 14.8% were affected with coronary artery disease (CAD) respectively. The mean age at onset of CAD was 50.3 ± 8.4 years for males and 53 ± 8.8 years for females. The proportion of subjects with T2DM or HTN was significantly higher in those affected with CAD (45% and 54.4%) compared with the unaffected family members (10.6 % and 17.3%) ($p < 0.0001$). The mean BMI was 25 ± 5 kg/m^2 for males and 25.4 ± 5.2 kg/m^2 for females.

Table 1 Details of IARS families

Descriptives	Number	Percent	Mean ± SD
Total number of families	508		
Total number of individuals	2313		
Average number per family	4.35		
Number of sibships	527		
Total number of males	1353	58.5	
Males with CAD	630	46.6	
Unaffected males	723	53.4	
Number of females	960	41.5	
Females with CAD	142	14.8	
Unaffected females	818	85.2	
Mean age at recruitment (in yrs) – affected males			55.5 ± 9.3
Mean age at recruitment (in yrs) – affected females			57.9 ± 9.3
Mean age at recruitment (in yrs) – unaffected males			37.2 ± 14
Mean age at recruitment (in yrs) – unaffected females			43.2 ± 13.7
Mean age at onset of CAD (in yrs) – males			50.3 ± 8.4
Mean age at onset of CAD (in yrs) – females			53 ± 8.8
CAD subjects with diabetes (N)	347	45	
Unaffected subjects with diabetes (N)	164	10.6	
CAD subjects with hypertension (N)	419	54.4	
Unaffected subjects with hypertension (N)	267	17.3	
BMI (kg/m^2) – males			25.5 ± 4.8
BMI (kg/m^2) – females			25.4 ± 5.2

The concordance rates for T2DM, HTN, and MS among the sib-pairs and spouse-pairs are provided in Table 2. Of the 1250-sib-pairs, 136 pairs (11%), 174 pairs (14%), and 287 pairs (23%) were concordant for T2DM, HTN, and MS respectively. Correspondingly, among the 463 spouse-pairs, 13 pairs (2.80%), 29 pairs (6.3%), and 130 pairs (28.1%) showed concordance for the three traits respectively. The concordance rates for T2DM (χ2- 26.71; OR = 4.226; 95% CI 2.367–7.544; $p < 0.0001$), and HTN (χ2- 18.23; OR = 2.420; 95% CI 1.608–3.642; $p < 0.0001$) were significantly higher among sib-pairs as compared with spouse-pairs. In contrast, concordance

Table 2 Concordance rates for diabetes (T2DM), hypertension (HTN), and metabolic syndrome (MS) among the sib-pairs and spouse-pairs

Descriptive	T2DM	HTN	MS
Sib-pairs (N)	1250	1250	1250
Sib-pairs both with the trait (N)	136	174	287
Sib concordance rate for the trait	11.00%	14.00%	23.00%
Corresponding spouse-pairs (N)	463	463	463
Spouse-pairs both with the trait (N)	13	29	130
Sib-spouse concordance for the trait	2.80%	6.26%	28.10%
Chi square values for difference in concordance rates between sib-pairs and spouse-pairs	26.71	18.23	4.531
'p' value	**<0.0001**	**<0.0001**	**0.0333**

Note: Metabolic syndrome diagnosed as per NCEP ATPIII Criteria (2001).

rate for the metabolic syndrome was significantly higher in the spouse-pairs as compared with sib-pairs (χ2- 4.531; OR = 0.7634; 95% CI 0.5994–0.9723; p = 0.033). Clustering of both diabetes and hypertension were observed in 29 sib-pairs (2.32%) and 9 spouse-pairs (1.9%) in our cohort.

Discussion

Traditionally, MZ and DZ twin studies have provided a basis for understanding the proportional contribution of nature versus nurture for any given trait with a multifactor etiology, although they have their inherent limitations (Kyvik 1995; Guo 2001). In principle, given the 100% gene sharing between MZ twins, a concordance rate below unity for a given trait would imply an environmental component in the etiology of the disease. On the other hand, if the etiology was wholly nongenetic, the concordance rates for MZ and DZ twins should not differ. Twin studies have shown that both T2DM and HTN have a genetic component that is subject to environmental influences related to lifestyle and socio-economic conditions.

In practice, it is much simpler to study a cohort of adult nontwin siblings rather than twins since they are analogous to DZ twins; in both instances the pairs share 50% of the genetic material. However, concordance for a trait in non-twin sibs could be due to shared genes or common lifestyle. Spouse-pairs have been used as controls in our study for they allow the investigation of determinants of disease related to modifiable behavioral characteristics. Couples are more likely to share the same lifestyle and socio-economic environment but not close genetic inheritance in a nonconsanguineous mating (Bloch et al 2003). Therefore, to some extent, the concordance rates for a trait in nontwin sib-pairs and their corresponding nonconsanguineous spouse-pairs should be comparable if the trait had no genetic etiology. The statistically significant difference in concordance rates between sib and spouse-pairs for both T2DM and hypertension in our study support a partial genetic etiology of these traits. An interesting observation made by Simpson and Peek (2005) on the concordance of chronic conditions such as T2DM, HTN, and arthritis in elderly Mexican-American couples over 65 years of age underlines the reciprocal influence that marital partners have on each other which may be due to shared living arrangements and shared health risks (Simpson and Peek 2005).

The significant concordance observed for T2DM and HTN among the sib-pairs selected from the IARS cohort primarily assessed for premature CAD underline the importance of these traits as risk factors for CAD. A significantly higher prevalence of T2DM and HTN in those with CAD as compared with the unaffected family members in our study also lends weight to this observation.

The American Heart Association has termed male gender as a nonmodifiable risk factor for CAD. On an average, symptoms of coronary disease appear a decade earlier among males when compared to females (Meyer 2006). Lower incidence of CAD among pre-menopausal women as compared with men and post-menopausal women has been attributed to the cardio-protective properties of estrogen against endothelial dysfunction (Kannel and Wilson 1995; Thomas and Braus 1998), which includes protection against insults by oxidatively modified low-density lipoprotein and promotion of nitric oxide synthesis. Women at every age are said to have less CAD than men, even when various risk factors are accounted for but for the presence of diabetes, which carries equal mortality for both sexes. In our study, in spite of similar BMI and mean age across gender, a significantly higher incidence of T2DM and hypertension was observed among females (54%, 70%) as compared with males (43%, 51%) but with a relatively lower

incidence of CAD. Higher incidence of CAD among males is now considered as a universal phenomenon irrespective of the ethnicity of the population under study. This trend was also observed among the IARS cohort.

Metabolic syndrome refers to occurrence of high TG, low HDL-C, obesity, glucose intolerance and HTN in the same individual. It is worthwhile to note that the first three traits are effected and controlled to some extent by common lifestyle and socio-economic conditions, which should be evident by clustering of these traits in a spouse-pair cohort. This was reflected in our findings wherein spouse-pairs showed higher concordance for MS as compared with sib-pairs. In spite of an awareness of the need for modified definitive criteria for MS in different populations (Snehalatha 2003; Misra et al 2005) our objective was to primarily observe the distribution of MS qualitatively across the sib-pair and spouse-pair cohort. Nevertheless, there is a need to re-assess the criteria to arrive at a standard protocol for defining MS for the Asian Indian people.

In conclusion, employing nontwin sib concordance rates, the present study demonstrates a highly probable genetic component in the causation of T2DM and hypertension in this cohort of Asian Indian population known for high susceptibility to these traits. However, the nature and mechanism by which genes function in causing these multifactor traits are largely unknown. Recently, the 9p21 region has been implicated in T2DM using genome wide association approach on Caucasian population (Saxena et al 2007; Scott et al 2007). The same region has also been linked to premature CAD in three large independent studies (Helagadottir et al 2007; Mc Pherson et al 2007; Samani et al 2007) suggesting the intriguing possibility of common metabolic pathways and shared etiology between two complex disorders. Given the high concordance for T2DM and HTN among siblings in our study, it would be worthwhile to perform similar genome wide scan employing single nucleotide polymorphisms (SNP) as well as examine the identity by descent (idb) pattern of shared alleles among these sibling pairs with a strong family history of CAD to enable the identification of novel genetic elements involved in the etiology of these traits.

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