Int J Hum Genet, 7(4): 277-284 (2007)
PRINT: ISSN 0972-3757 ONLINE: 2456-6360
DOI: 10.31901/24566330.2007/07.04.01

Anthropological Perspective of the Single Nucleotide Polymorphisms in the NPY and DRD2 Genes among the Socio-Economically Stratified Populations of Andhra Pradesh, India

B. Mohan Reddy[1*], A.N. Srikar Reddy[1], T. Nagaraja[1], L.V. K. S. Bhaskar[2], H.P. Singh[1], V. M. Naidu[1], K. Thangaraj[2], A.Govardhan Reddy[2], A. Nirmala[1] and Lalji Singh[2]

1. Biological Anthropology Unit, Indian Statistical Institute, Hyderabad, India
2. Centre for Cellular and Molecular Biology, Hyderabad, India

KEYWORDS Neuropeptide Y; DRD2; allelic variation; socioeconomic hierarchy; traditional behaviors; association with alcoholism

ABSTRACT We report single nucleotide polymorphisms (SNP) at the two sites each of NPY and DRD2-*Taq1* loci among the 28 populations from southern parts of Andhra Pradesh, India. The average allele frequency at these sites in these populations categorized into upper, middle and lower ranking castes, Muslims and tribes were also carried out in order to test if there is any trend in the allele frequency of some of these SNPs that are implicated in alcoholism. This was felt important as some of these hierarchical low ranking groups and tribes traditionally are known for addictive behavior, especially to alcohol, as against the prohibitive norms among most of the higher ranking castes. The trend in average allele frequency although suggests some association with these traditional behavioral patterns, the case control studies are required before inferring the exact nature of this association. The bivariate plot between the NPY-C and *Taq1*A1 mean allele frequencies in the 28 populations and the lack of significant association between the genotypes of these two SNPs at the individual level rules out any possibility of co-adaptation between these two alleles, despite both being somewhat implicated in alcoholism.

INTRODUCTION

Neuropeptide Y (NPY) is a 36-amino acid peptide neurotransmitter encoded by NPY gene located on the long arm (q15.1) of human chromosome 7q15.1 (Minth et al. 1986) and it is divided into and consists of four exons (Baker et al. 1995). Neuropeptide Y (NPY) exists in both the central and peripheral nervous system and is thought to modulate many functions such as regulating energy balance of the body (Billington et al. 1994) and insulin release (Moltz et al. 1985), stimulating lipoprotein lipase activity in the adipose tissue (Billington et al. 1991), inhibiting norepinephrine release and potentiating norepinephrine response (Walker et al. 1991). It is suggested that a T1128C polymorphism in the signal peptide of the NPY gene results in leucine (Leu) 7 to proline (Pro) 7 substitution. This substitution is likely to alter the properties of the signal peptide due to the different physico-chemical properties of Leu and Pro amino acids and was found to be associated with high serum cholesterol and LDL cholesterol levels (Karvonen et al. 1998), enhanced carotid atherosclerosis in elderly patients with type 2 diabetes and control subjects (Niskanen et al. 2000a), retinopathy in type 2 diabetes (Niskanen et al. 2000b), birth weight and serum triglyceride concentration in pre-school aged children (Karvonen et al. 2000) and alcohol dependence (Kauhanen et al. 2000; Lappalainen et al. 2002). The findings related to alcohol dependence are, however, hetero-geneous (Ilvesoski et al. 2001; Lappalainen et al. 2002).

The D2 dopamine receptor (DRD2) is one of the five human dopamine receptors (D1, D2, D3, D4, D5/D1b) for which the gene has been isolated (Grandy et al. 1989). The DRD2*Taq*1A site, located approximately10 kb from the 3'end of *DRD2*, exerts no known effect on gene expression but has been associated with alcoholism and other addictive behaviors (Blum et al. 1990; Comings and Blum 2000). The "*Taq1* A" polymorphism is a C/T single nucleotide polymorphism (SNP)

Corrsponding Author: Dr. B. Mohan Reddy, Ph.D.
Professor, Biological Anthropology Unit, Indian Statistical Institute, Street No. 8, Habsiguda, Hyderabad 500 007, India
Telephone: 91-40-27153984 / 27171906
Fax: 91-40-27173602
E-mail: bmr@isical.ac.in

about 10 kb centromeric downstream to the stop codon of DRD2 on 11q23 (SNP rs1800497). The C allele results in a *Taq1* restriction site and the T allele does not. The T allele was earlier designated as the A1 allele, which we follow consistently in this article. The presence of the A1 allele is associated with a 40% reduction in the expression of D2 receptors in the striatum and perhaps in other cortical regions, without change in receptor affinity (Thompson et al. 1997, Ritchie and Noble 2003). This association is most likely a result of linkage disequilibrium between this polymorphism and a functional DRD2 polymorphism, since rs1800497 itself is a nonsynonymous coding polymorphism in the adjacent but functionally unrelated gene ANKK1 (Ankyrin repeat and kinase domain containing 1) ANKK1. The alternative hypothesis that ANKK1 regulates DRD2 levels has not been ruled out, however (Neville et al. 2004). The A1 allele of the *DRD2* gene has been associated not only with alcoholism (Noble, 2003) but also with other substance use disorders, including cocaine (Noble et al. 1993), nicotine (Spitz et al. 1998) and opiate (Lawford et al. 2000) dependence, polysubstance abuse (Smith et al. 1992) and obesity (Spitz et al. 1998) indicating that it is not a specific risk factor for alcoholism but rather a broad substance dependence genetic risk marker. In any case, the studies on its association with alcoholism yielded contradictory results so far (Bolos et al. 1990; Lawford et al. 1997; Ishiguro et al. 1998; Sander et al. 1999; Shaikh et al. 2001) as A2 allele also showed strong association in certain studies. However, this variant of the DRD2 gene has functional significance as subjects carrying the DRD2 A1 allele, compared with findings for those without this allele, have reduced brain D2 dopamine receptors (Jonsson et al. 1999).

A critical appraisal of the earlier studies on the nature and extent of the association of the above polymorphisms with alcoholisms, obesity and other complex disorders suggests that the results are contradictory and no definite inference can be deduced. Nevertheless, a wide ethnic variation is observed in the allele frequency. While the frequency of the Pro7 allele has been reported to be 0.08 in Finns, 0.03 in Dutchmen (Karvonen et al. 1998) and virtually absent in Japanese (Drube et al. 2001; Makino et al. 2001; Ding et al. 2002), the *Taq1*A allele frequencies vary more than fourfold among the European Samaritans population (Castiglione et al. 1995; Goldman et al. 1992). The frequency of the A1 allele was found to be as low as 0.09 in a Middle Eastern Caucasian population of Yemenite Jews to as high as 0.75 in Muskoke Amerindians. Arinami et al. (1993) found significant A1 frequency differences between their Japanese unscreened controls and unscreened white controls reported by others (Grandy et al. 1989; Bolos et al. 1990; and Gelernter et al. 1991). The frequency of A1 allele in the control samples varies from 6 to 21%, a 3.5 fold variation. When no significant association between A1 allele and alcoholism was found, the control A1 allele frequency ranged from 15 to 21%, whereas when significant association was observed the A1 allele frequency ranged from 6 to 11% (See Buckland 2001 for review).

To the best of our knowledge only one attempt has been made so far in India to study the association between *Taq1*A and alcoholism in the hospital sample at Bangalore (Sheik et al. 2001) and the results suggested lack of association. Further, the allele frequency was found to be somewhat similar to the East Asian population frequency. On the other hand, Viswanathan et al. (2003,2004) studied 5 populations of Nilgiri hills for the distribution of allele frequency of *Taq1*A, B and D and the three marker systems in their study are highly polymorphic in all the five tribal populations; *Taq1*A1 frequency ranged from 0.21 to 0.51. We report here the single nucleotide polymorphism at the 4 sites, two each from the NPY and DRD2 genes among the 28 populations from southern parts of India, which represent almost entire gamut of socioeconomic and occupational variation inherent in the Indian population structure. Since the studied populations are clearly stratified into upper, middle and lower castes and tribes with certain traditional cultural and behavioral patterns concerning drinking alcohol etc. known specific to each of them, we further endeavor to examine if the frequency of any of these alleles implicated in alcoholism, obesity etc. follow a trend consistent with the known traditional behavioral/lifestyle patterns that are somewhat related to alcoholism and other lifestyles in these hierarchical groups.

MATERIAL AND METHODS

Intravenous blood samples (about 5 ml each) were collected, with the informed written consent,

from 948 individuals belonging to 28 endogamous groups distributed in the contiguous areas of the 6 southernmost districts of Andhra Pradesh (Chittoor, Cuddapah, Ananthapur, Kurnool, Prakasam, and Nellore) (Fig. 1). All the subjects were apparently normal healthy volunteers and no diagnosis for any disorder was performed on them. All the samples were anonymized and have no individual specific identity. The names of the populations studied, the number of samples drawn from each population, and their socioeconomic backgrounds are furnished in Table 1. Even though this study area can be considered culturally, linguistically, and geographically homogeneous, it is inhabited by a wide array of caste and tribal populations, representing almost the entire spectrum of socioeconomic variation in the state.

DNA of the above samples was isolated using the protocol discussed by Sambrook et al. (1989). The Forward (5-CCG TCC GTT GAG CCT TCT GT-3) and reverse (5-CTA CCA GCC TGG GGA ACC TGA AT-3) primers were used to amplify 464 bp length covering the exon 2 of NPY. The primers 5014 (5' CCC TTC CTG AGT GTC ATC A 3') and 971 (5' CGG CTG GCC AAG TTG TCT A 3') were used to amplify a 310 bp fragment spanning the polymorphic sites of the DRD2 gene. PCR was carried out in a Gene Amp 9600 Thermal cycler (Perkin Elmer) in 10m1 volume and using 1.0 U of AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA). PCR amplicons were checked on 2% agarose gel.

PCR amplicons (70ng) were directly sequenced using the BigDye Terminator Cycle Sequencing Kit® (Applied Biosystems, Foster City, CA) using 5PM primer. Extended products were purified by alcohol precipitation followed by washing with 70% alcohol. Purified products were then dissolved in 10µ1 of 50% Hi- Di formamide and analysed in ABI3730 automated DNA Analyser (Perkin Elmer, USA).

Each genotyped site had two co-dominant alleles. Allele frequencies were determined by direct gene counting method. To test the significance of the degree of heterogeneity in the genotype frequencies among the hierarchical groups χ^2 test was performed.

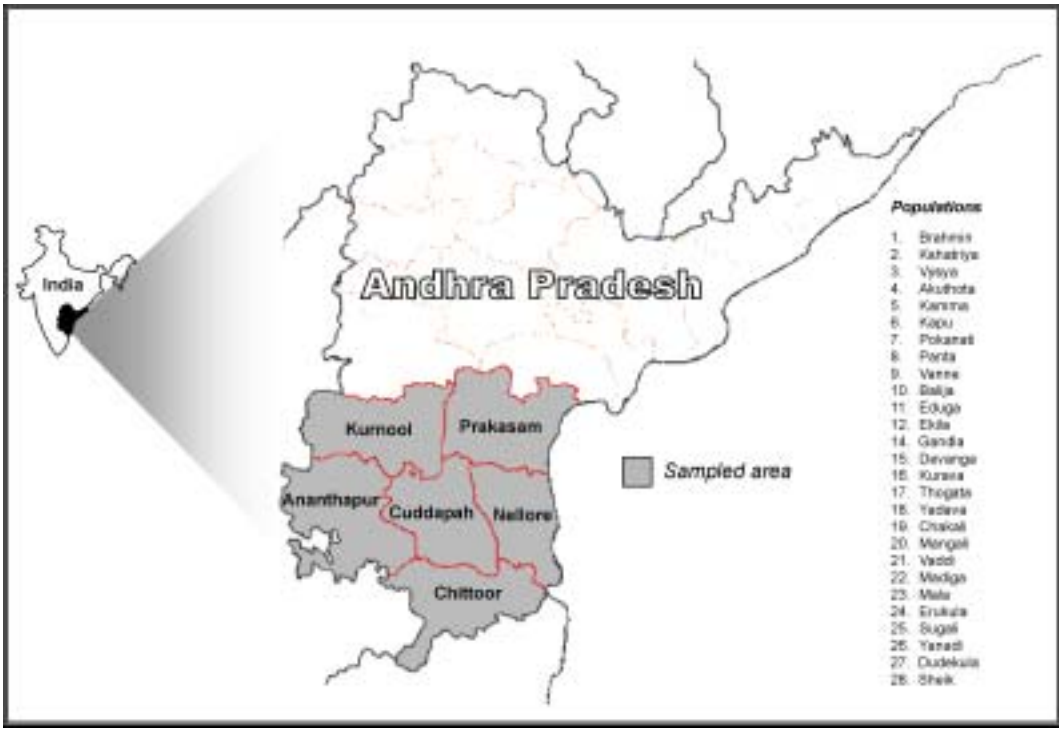

Fig.1. Map of Andhra Pradesh showing the sampled area and the names of populations studied.

RESULTS AND DISCUSSION

Sequencing of 464 bp fragment of NPY gene revealed two polymorphic sites. NPY T1128C is the polymorphism (rs16139) located in the signal peptide region of exon 2 changing the amino acid Leucine 7 to Proline 7, whereas G1258A is a synonymous SNP (rs9785023) located in the carboxyl terminal peptide (exon 2) without amino acid change at position 50 (Ser 50 ser). Analysis of 310 bp fragment of DRD2 gene also revealed two polymorphic sites, one is a *Taq1*A site, which is located 10 kb down stream of DRD2 stop codon (rs1800497) and another one is present exactly 100 bp down stream to this site (rs17115461).

Population specific allele frequencies at these 4 sites are presented in Table1. From the functional perspective, NPY 1128C is expected to confer protection against alcoholism and DRD2 *Taq1*A1 is susceptible to alcoholism although the empirical data are often inconsistent. The NPY 1128C allele could not be traced in 9 of theout of 28 populations studied and the highest level of polymorphism was observed in the Kshatriya (11%), one of the high-ranking castes. There seems to be also a week trend of decrease in the mean frequency of NPY 1128C from upper to lower castes and tribes (Fig. 2). At the other polymorphic site of NPY (1258A) the A-allele varies from 0.4 in one of the Muslim groups to as high as 0.769 in the Jangam, one of the lower middle ranking castes. Overall, there appears to be a slight trend of decrease in the average frequency of allele A at this site from upper to lower ranking castes. On the other hand, at the *Taq1*A locus, while the A1 allele frequency varies from about 23% in Yanadi tribe to 50% in Chakali, a low ranked

Table 2: χ^2 test of homogeneity in the genotype frequencies of the 4 SNPs, two each of NPY and DRD2 Taq1A genes.

SNP	*df*	χ^2	*P value*
rs16139 (T1128C)	6	8.930	0.178
rs9785023 (G1258A)	12	8.357	0.757
rs1800497 DRD2 (*Taq1* A)	12	26.893	0.008
rs17115461	6	19.360	0.004

Table1: Population wise allele frequency at the 4 polymorphic sites, two each of NPY and DRD2-Taq1A genes screened among the 28 Indian populations.

Population (N)	*Socioeconomic Status (Caste or Tribe)*	*NPY*		*Taq1A*	
		rs16139 (1128C)	*rs9785023 (1258A)*	*rs1800497 [Taq1A1]*	*rs17115461 (C allele)*
Brahmin (21)	Upper	0.056	0.583	0.286	0.023
Kshatriya (25)	Upper	0.109	0.543	0.280	0.000
Vysya (19)	Upper	0.031	0.406	0.289	0.026
Akuthota (31)	Upper-middle	0.071	0.518	0.371	0.065
Kamma (48)	Upper-middle	0.023	0.500	0.333	0.031
Kapu (24)	Upper-middle	0.000	0.438	0.229	0.000
Pokanati (39)	Upper-middle	0.026	0.513	0.333	0.051
Panta (47)	Upper-middle	0.011	0.564	0.263	0.056
Vanne (32)	Upper-middle	0.047	0.500	0.317	0.117
Balija (34)	Lower-middle1	0.029	0.426	0.344	0.000
Ekila (35)	Lower-middle1	0.000	0.645	0.343	0.043
Kurava (50)	Lower-middle1	0.032	0.500	0.290	0.015
Thogata (16)	Lower-middle1	0.031	0.500	0.344	0.125
Yadava (31)	Lower-middle1	0.069	0.500	0.419	0.081
Ediga (32)	Lower-middle2	0.031	0.500	0.276	0.000
Gandla (21)	Lower-middle2	0.000	0.429	0.269	0.000
Jangam (14)	Lower-middle2	0.000	0.769	0.321	0.071
Devanga (62)	Lower-middle2	0.074	0.489	0.331	0.016
Chakali (29)	Lower1	0.000	0.457	0.500	0.086
Mangali (13)	Lower1	0.000	0.500	0.423	0.038
Vadde (40)	Lower1	0.025	0.475	0.338	0.005
Madiga (65)	Lower1	0.000	0.508	0.392	0.051
Mala (50)	Lower1	0.052	0.448	0.380	0.070
Erukala (80)	Proto-Australoid tribe	0.021	0.472	0.263	0.025
Sugali (39)	''Caucasoid'' tribe	0.045	0.470	0.308	0.038
Yanadi (106)	Proto-Australoid tribe	0.010	0.459	0.226	0.005
Dudekula (26)	Muslims	0.000	0.400	0.404	0.019
Sheik (21)	Muslims	0.000	0.447	0.381	0.214

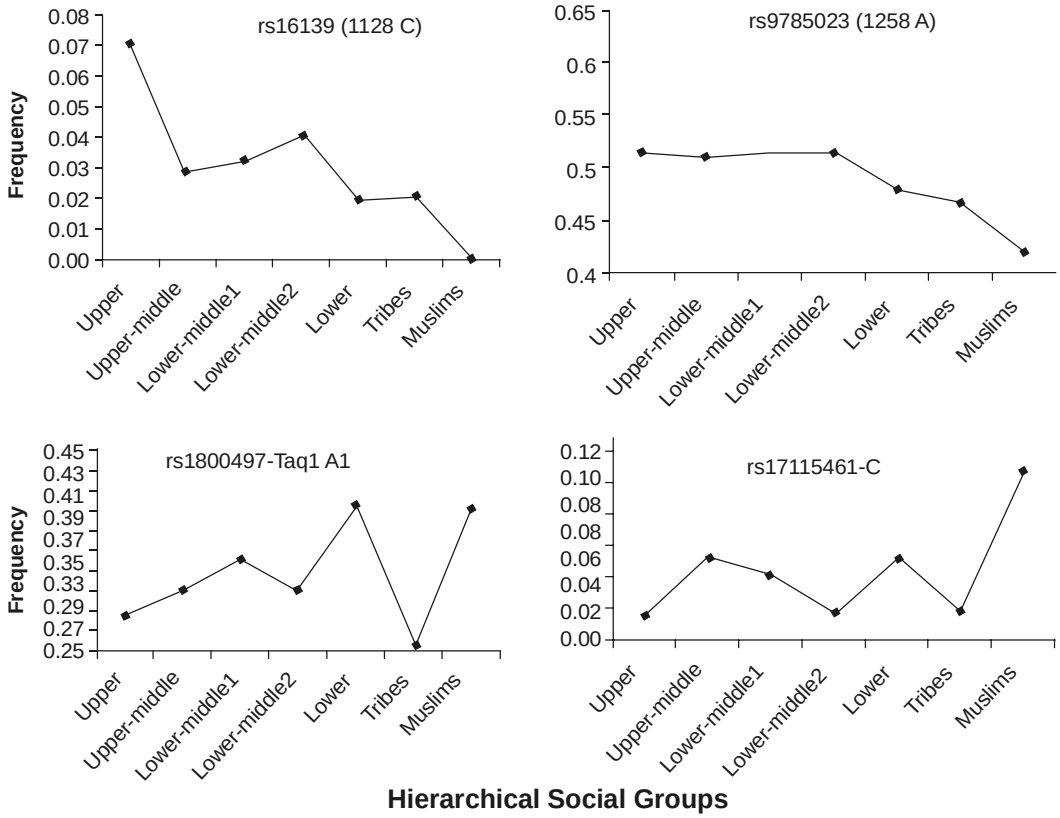

Fig. 2. Variations in the weighted mean allele frequencies in different socio economic categories of populations of Andhra Pradesh. While X-axis in the graphs represent socio economic category, Y-axis represents allele frequency.

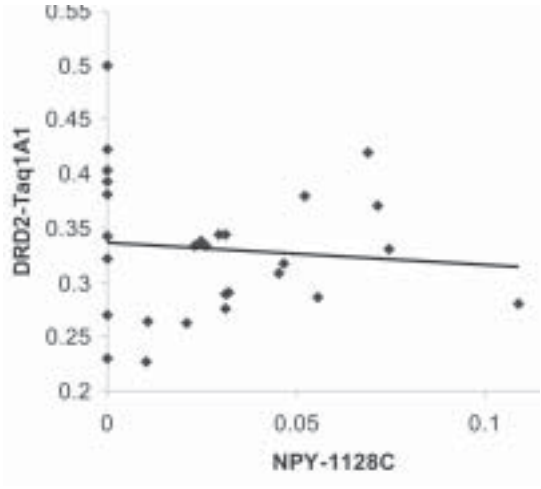

Fig. 3. Bivariate plot of allele frequency of NPY-1128C and DRD2-*Taq1*A1 in the 28 populations of India.

washermen caste at the rs17115461 site the allele C frequency varies from zero to 13% among the studied populations. A trend of increase in average frequency of the alleles from the upper to lower castes is evident at both the above sites. Further, the contingency χ^2 test for homogeneity in the distribution of genotypes among the hierarchical groups (Table 2) suggests significant heterogeneity for both the sites of DRD2 (χ^2 = 26.9 and 19.4; d.f. 12 and 6, $p < 0.01$) but not for NPY sites (χ^2 = 8.9 and 8.4; d.f. 6 and 12, $p > 0.15$). Since NPY 1128C as well as *Taq1*A1 alleles have been variously implicated in obesity, alcoholism and other substance abuse disorders, we tried to examine the nature of association of allele frequency at the population level and co-occurrence of these alleles at the individual level which may throw some light on the possibility of co-adaptation of these two alleles in the populations. The bivariate plot of allele

frequencies of NPY-C and *Taq1*A1 (T) at the population level (Fig. 3) suggests no association (r-value -0.09, p = 0.32).

Inherent in the traditional Indian population structure is the clearly defined roles and obligations for each of the hierarchical castes as they live in symbiotic relationship. Depending on the hierarchy of the castes their roles and value system varied distinctly. For example, while amongst most of the upper and upper middle castes drinking alcohol is considered a stigma and looked down upon, among the lower castes, scheduled castes and tribes, who traditionally worked mostly as laborers or rendered services as scavengers, barbers, leather workers, washermen, gold/iron smiths etc., assisting the upper castes in various activities, it is considered as quite a normal behavior; traditionally, most men and some of their women were used to drinking indigenously made liquor which is supposed to help these people to relax and have good nights sleep after the days hard physical labor inducing body aches etc. Further, typically, most men from these lower castes are known to spend greater part of their earnings on drinking alcohol, in additions to what the upper castes sponsor them for drinking during religious occasions like marriages, jatras, death ceremonies etc. Looking against these backgrounds there seems to be subtle trend in the mean allele frequency of both the NPY-C and *Taq1*A1 although one cannot be sure if there is genetic basis behind such average alcoholic behaviors of some of these service castes. Nevertheless, assuming that NPY-C gives protection to alcoholism and susceptibility to obesity, and the fact that relatively greater % of obese people are observed among the upper castes of Andhra Pradesh (Nirmala Reddy 1998a,, 1998b; Feitosa et al. 1999, 2000), the greater frequency of this allele among them may be expected and tempts one to surmise if there is not really genetic background to such cultural behaviors. Nevertheless, it appears imperative that suitable environment, by way of cultural norms that may dictate lifestyles, is necessary in order to manifest the genes responsible for developing addictive behaviors.

Indeed, if there is co-adaptation and/or interaction between these genes in effecting particular behavioral pattern of an individual this association should reflect in the genotypes of an individual at these two loci. However, the χ^2 value obtained does not reflect this ($\chi^2 = 1.1$, d.f. 4, $p > 0.5$), probably indicating that these two loci act independently and may not have joint role in developing alcoholism and/or obesity. On the contrary, it is quite interesting to note that when populations having zero % of NPY-C allele are excluded and plotted against the *Taq1*A1 as well as *Taq1*A2 alleles, respectively, it shows significant positive association ($r = 0.663$, $p<0.01$, d.f. 18) with the Taq1A1, while showing negative association ($r = -0.663$, $p< 0.01$, d.f., 18) with Taq1A2. This may provide subtle and indirect hint at possible joint action of these genes in developing the alcoholic phenotype and /or probably co-adaptation at the population level. On the other hand, if NPY1128C as well as *Taq1*A2 alleles are considered to have implication to alcoholism, as certain studies suggest, then these two genes may act as proxy to each other, which is perhaps reflected in the negative association at the population level. Nevertheless, without proper characterization of the individual alcoholic phenotypes in these populations and in the absence of family based and/or candidate gene approaches in conjunc-tion with population level screening of these markers these inferences may remain purely tentative. We plan to undertake such studies in the near future.

REFERENCES

Arinami T, Itokawa M, Komiyama T, Mitsushio H, Mori H, Mifune H, Hamaguchi H, Toru ML 1993. Association between severity of alcoholism and the A1 allele of the dopamine D2 receptor gene TaqI A RFLP in Japanese. *Biol Psychiatry*, **33**: 108-114.

Billington CJ, Briggs JE, Grace M, Levine AS 1991. Effects of intracerebroventricular injection of neuropeptide Y on energy metabolism. *Am J Physiol*, **260**: R321-327.

Billington CJ, Briggs JE, Harker S, Grace M, Levine AS 1994. Neuropeptide Y in hypothalamic paraventricular nucleus: A center coordinating energy metabolism. *Am J Physiol*, **266**: R1765-1770.

Blum K, Noble EP, Sheridan PJ, Montgomery A, Ritchie T, Jagadeeswaran P, Nogami H, Briggs AH, Cohn JB 1990. Allelic association of human dopamine D2 receptor gene in alcoholism. *JAMA*, **263**: 2055-2060.

Bolos AM, Dean M, Lucas-Derse S, Ramsburg M, Brown GL, Goldman D 1990. Population and pedigree studies reveal a lack of association between the dopamine D2 receptor gene and alcoholism. *JAMA*, **264**: 3156-3160.

Buckland PR 2001. Genetic association studies of alcoholism—problems with the candidate gene approach. *Alcohol*, **36**: 99-103.

Castiglione CM, Deinard AS, Speed WC, Sirugo G,

Rosenbaum HC, Zhang Y, Grandy DK, Grigorenko EL, Bonne-Tamir B, Pakstis AJ, Kidd JR, Kidd KK 1995. Evolution of haplotypes at the DRD2 locus**.** *Am J Hum Genet*, **57**: 1445-1456.

Comings DE, Blum K 2000. Reward deficiency syndrome: Genetic aspects of behavioral disorders. *Prog Brain Res*,**126**: 325-41.

Ding B, Bertilsson L, Wahlestedt C 2002. The single nucleotide polymorphism T1128C in the signal peptide of neuropeptide Y (NPY) was not identified in a Korean population**.** *J Clin Pharm Ther*, **27**: 211-212.

Drube J, Kawamura N, Nakamura A, Ando T, Komaki G, Inada T 2001. No leucine(7)-to-proline(7) polymorphism in the signal peptide of neuropeptide Y in Japanese population or Japanese with alcoholism. *Psychiatr Genet*, **11**: 53-55.

Feitosa M, Rice T, Nirmala A, Reddy PC, Rao DC 1999. Segregation analysis of regional fat distribution in families from Andhra Pradesh, India. *Int J Obes*, **23**: 874-880.

Feitosa M, Rice T, NirmalaA, Rao DC 2000. Major gene effect of Body Mass Index: The role of energy intake and energy expenditure. *Hum Biol*, **72**: 781-799.

Gelernter J, O'Malley S, Kranzler HR, Krystal J, Merikangas K, Kennedy JL, Kidd KK 1991. No association between an allele at the D2 dopamine receptor gene (DRD2) and alcoholism. *JAMA*, **266:** 1801-1807.

Goldman D, Dean M, Brown GL, Bolos AM, Tokola R, Virkkunen M, Linnoila M 1992. D2 dopamine receptor genotype and cerebrospinal fluid homovanillic acid, 5-hydroxyindoleacetic acid and 3-methoxy-4-hydroxyphenylglycol in alcoholics in Finland and the United States. *Acta Psychiatr Scand*, **86**: 351-357.

Grandy DK, Litt M, Allen L, Bunzow JR, Marchionni M, Makam H, Reed L, Magenis RE, Civelli O 1989. The human dopamine D2 receptor gene is located on chromosome 11 at q22-q23 and identifies a TaqI RFLP. *Am J Hum Genet*, **45**: 778-85.

Ilveskoski E, Kajander OA, Lehtimaki T, Kunnas T, Karhunen PJ, Heinala P, Virkkunen M, Alho H 2001. Association of neuropeptide y polymorphism with the occurrence of type 1 and type 2 alcoholism. *Alcohol Clin Exp Res*, **25**: 1420-1422.

Ishiguro H, Arinami T, Saito T, Akazawa S, Enomoto M, Mitushio H, Fujishiro H, Tada K, Akimoto Y, Mifune H, Shioduka S, Hamaguchi H, Toru M, Shibuya H 1998. Association study between the -141C Ins/Del and TaqI A polymorphisms of the dopamine D2 receptor gene and alcoholism. *Alcohol Clin Exp Res*, **22**: 845-848.

Jonsson EG, Nothen MM, Grunhage F, Farde L, Nakashima Y, Propping P, Sedvall GC 1999. Polymorphisms in the dopamine D2 receptor gene and their relationships to striatal dopamine receptor density of healthy volunteers. *Mol Psychiatry*, **4**: 290-296.

Karvonen MK, Koulu M, Pesonen U, Uusitupa MI, Tammi A, Viikari J, Simell O, Ronnemaa T 2000. Leucine 7 to proline 7 polymorphism in the preproneuropeptide Y is associated with birth weight and serum triglyceride concentration in preschool aged children. *J Clin Endocrinol Metab*, **85**: 1455-1460.

Karvonen MK, Pesonen U, Koulu M, Niskanen L, Laakso M, Rissanen A, Dekker JM, Hart LM, Valve R, Uusitupa MI 1998. Association of a leucine(7)-to-proline(7) polymorphism in the signal peptide of neuropeptide Y with high serum cholesterol and LDL cholesterol levels.*Nat Med*, **4**: 1434-1437.

Kauhanen J, Karvonen MK, Pesonen U, Koulu M, Tuomainen TP, Uusitupa MI, Salonen JT 2000. Neuropeptide Y polymorphism and alcohol consumption in middle-aged men. *Am J Med Genet*, **93**: 117-121.

Lappalainen J, Kranzler HR, Malison R, Price LH, Van Dyck C, Rosenheck RA, Cramer J, Southwick S, Charney D, Krystal J, Gelernter J 2002. A functional neuropeptide Y Leu7Pro polymorphism associated with alcohol dependence in a large population sample from the United States. *Arch Gen Psychiatry*, **59**: 825-831.

Lawford BR, Young RM, Noble EP, Sargent J, Rowell J, Shadforth S, Zhang X, Ritchie T 2000. The D(2) dopamine receptor A(1) allele and opioid dependence: Association with heroin use and response to methadone treatment. *Am J Med Genet*, **96**: 592-598.

Lawford BR, Young RM, Rowell JA, Gibson JN, Feeney GF, Ritchie TL, Syndulko K, Noble EP 1997. Association of the D2 dopamine receptor A1 allele with alcoholism: Medical severity of alcoholism and type of controls. *Biol Psychiatry*, **41**: 386-393.

Makino K, Kataoka Y, Hirakawa Y, Ikeda A, Yamauchi A, Oishi AR 2001. A leucine(7)-to-proline(7) polymorphism in the signal peptide of neuropeptide Y was not identified in the Japanese population. *J Clin Pharm Ther*, **26**: 201-203.

Minth CD, Andrews PC, Dixon JE 1986. Characterization, sequence, and expression of the cloned human neuropeptide Y gene. *J Biol Chem*, **261**: 11974-11979.

Moltz JH, McDonald JK 1985. Neuropeptide Y: Direct and indirect action on insulin secretion in the rat. *Peptides*, **6**: 1155-1159.

Neville MJ, Johnstone EC, Walton RT 2004. Identification and characterization of ANKK1: A novel kinase gene closely linked to DRD2 on chromosome band 11q23.1. *Hum Mutat*, **23**: 540-55.

Nirmala Reddy B 1998. Blood Pressure and Adiposity: A comparative evaluation among the Socio-economically diverse groups of Andhra Pradesh, India. *Am J Hum Biol*, **10**: 5-21.

Nirmala Reddy B 1998. Body mass index and its association with socioeconomic and behavioral variables: A study among socio economically heterogeneous populations of Andhra Pradesh, India**.** *Hum Biol*, **70**: 901-917.

Niskanen L, Karvonen MK, Valve R, Koulu M, Pesonen U, Mercuri M, Rauramaa R, Toyry J, Laakso M, Uusitupa MI 2000. Leucine 7 to proline 7 polymorphism in the neuropeptide Y gene is associated with enhanced carotid atherosclerosis in elderly patients with type 2 diabetes and control subjects. *J Clin Endocrinol Metab*, **85**: 2266-2269.

Niskanen L, Voutilainen-Kaunisto R, Terasvirta M,

Karvonen MK, Valve R, Pesonen U, Laakso M, Uusitupa MI, Koulu M 2000. Leucine 7 to proline 7 polymorphism in the neuropeptide y gene is associated with retinopathy in type 2 diabetes. *Exp Clin Endocrinol Diabetes*, **108**: 235-236.

Noble EP, Blum K, Khalsa ME, Ritchie T, Montgomery A, Wood RC, Fitch RJ, Ozkaragoz T, Sheridan PJ, Anglin MD,Paredes A, Treiman L, Sparkes RS 1993. Allelic association of the D2 dopamine receptor gene with cocaine dependence. *Drug Alcohol Depend*, **33**: 271-285.

Noble EP 2003. D2 dopamine receptor gene in psychiatric and neurologic disorders and its phenotypes. *Am J Med Genet B Neuropsychiatr Genet*, 116: 103-125.

Ritchie T, Noble EP 2003. Association of seven polymorphisms of the D2 dopamine receptor gene with brain receptor-binding characteristics. *Neurochem Res*, 28: 73-82.

Sambrook J, Fritsch EF, Maniatis T 1989. Molecular Cloning: A Laboratory Manual., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sander T, Ladehoff M, Samochowiec J, Finckh U, Rommelspacher H, Schmidt LG 1999. Lack of an allelic association between polymorphisms of the dopamine D2 receptor gene and alcohol dependence in the German population. *Alcohol Clin Exp Res*, **23**: 578-581.

Shaikh KJ, Naveen D, Sherrin T, Murthy A, Thennarasu K, Anand A, Benegal V, Jain S 2001. Polymorphisms at the DRD2 locus in early-onset alcohol dependence in the Indian population. *Addict Biol*, **6**: 331-335.

Smith SS, O'Hara BF, Persico AM, Gorelick DA, Newlin DB, Vlahov D, Solomon L, Pickens R, Uhl GR 1992. Genetic vulnerability to drug abuse: The D2 dopamine receptor Taq I B1 restriction fragment length polymorphism appears more frequently in polysubstance abusers. *Arch Gen Psychiatry*, **49**: 723-727.

Spitz MR, Shi H, Yang F, Hudmon KS, Jiang H, Chamberlain RM, Amos CI, Wan Y, Cinciripini P, Hong WK, Wu X 1998. Case-control study of the D2 dopamine receptor gene and smoking status in lung cancer patients. *J Natl Cancer Inst*, **90**: 358-363.

Thompson J, Thomas N, Singleton A, Piggott M, Lloyd S, Perry EK, Morris CM, Perry RH, Ferrier IN, Court JA 1997. D2 dopamine receptor gene (DRD2) Taq1 A polymorphism: reduced dopamine D2 receptor binding in the human striatum associated with the A1 allele. *Pharmacogenetics*, **7**: 479-84.

Vishwanathan H, Deepa Edwin, Cordaux R, Stoneking M, Usha Rani M.V, Majumder PP 2004. Genetic structure and affinities among tribal populations of southern India: A study of 24 autosomal DNA markers. *Ann Hum Genet*, **68**: 128-138.

Vishwanathan H, Deepa Edwin, Usha Rani MV, Majumder PP 2003. A survey of haplotype frequencies and linkage disequilibrium at the DRD2 locus in the Nilgiri hill tribes, South India. *Curr Sci*, **84**: 566-568.

Walker P, Grouzmann E, Burnier M, Waeber B 1991. The role of neuropeptide Y in cardiovascular regulation**.** *Trends Pharmacol Sci*, **12**: 111-115.