ORIGINAL ARTICLE

Monoamine Oxidase A gene polymorphisms and self reported aggressive behaviour in a Pakistani ethnic group

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Abstract

Objective: To investigate the association of monoamine oxidase Agene polymorphisms with aggression.

Methods: The study was conducted in an ethnic community in Lahore, Pakistan, from August 2008 to December 2009 on the basis of data that was collected through a questionnaire between August 2004 and September 2005. It analysed 10 single nucleotide polymorphisms of monoamine oxidase A in unrelated males from the same ethnic background who were administered a Punjabi translation of the Buss and Perry aggression questionnaire. SPSS 13 was used for statistical analysis.

Results: Of the total 133 haplotypes studied, 52(39%) were Haplotype A, 58(43.6%) B, 8(6%) C, 3(2.3%) D, 9(6.8%) E and 3(2.3%) F. The six haplotypes were analysed for association with scores of the four subscales of the aggression questionnaire and multivariate analysis of variance showed no significant differences ($p>0.05$ each) in the error variances of the total scores and scores for three of the sub-scales across the haplotypes. The variance was significantly different only for the anger sub-scale ($p<0.05$).

Conclusion: The association of an extended haplotype with low levels of self-reported aggression in this study should assist in characterisation of functional variants responsible for non-aggressive behaviour in male subjects.

Keywords: Aggression, Behaviour, Behavioural Genetics, MAOA. (JPMA 65: 818; 2015)

Introduction

The World Health Organisation (WHO) has declared violence as a major public health problem worldwide.[1] Violence is a manifestation of aggression, a personality trait that comprises hostile, threatening and physically violent behaviour towards persons and objects. Aggression is generally considered to be a complex behavioural phenotype with a major genetic component. Studies in humans and animals have implicated monoamine oxidase (MAO) with aggression. MAO is a mitochondrial enzyme that catalyses the oxidative deamination of the neurotransmitters serotonin (5-HT), dopamine and noradrenalin, which are involved in the regulation of aggressive behaviour. In humans, two MAO isozymes, MAOA and MAOB, are encoded by genes located on the short arm of X chromosome.[2]

Several lines of evidence indicate that MAOA (EC 1.4.3.4) plays an important role in human aggression and risk-taking[3] especially in male individuals, and inhibitors of this enzyme are widely used to treat mental depression, anxiety disorders and high blood pressure. Low MAOA and high testosterone levels were observed in individuals scoring high on the Brown-Goodwin Lifetime Aggression Scale.[4]

Positive association has been reported between MAOA gene polymorphisms and various behavioural and psychiatric phenotypes in humans and mice.[5-7] Association of MAOA with aggression was first demonstrated in a Dutch family with Brunner Syndrome (OMIM#300615).[8] More recently brain imaging techniques have indicated that individuals with a 30 base pair variable number of tandem repeat (VNTR) polymorphism in the promoter region of MAOA[9] have problems controlling their emotions, and display anti-social behaviour in the absence of a stable family environment.[10,11] A high expression variant of MAOA was reported to be associated with more aggressive behaviour in women in a sad mood compared to women carrying a low expression variant.[12] Another study found an association between three polymorphisms (rs909525, rs6323 and rs2064070) of the MAOA gene and aggression-related traits in suicidal males, while the single nucleotide polymorphism(SNP) rs6323 was also found associated with anger in females.[13]

The current study was planned to investigate the association of MAOA variants with aggressive behaviour

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in an ethnic group from the Punjab province of Pakistan.

Subjects and Methods

The study was conducted in an ethnic community in Lahore, Pakistan, from August 2008 to December 2009. The group was selected as it was more likely to be either victim or perpetrator of heinous crimes as ascertained by historical medico-legal records maintained at the Department of Forensic Medicine, King Edward Medical College, Lahore.

Blood samples were collected from unrelated male subjects aged between 18-65 years. Subjects were interviewed and administered the Buss and Perry Aggression Questionnaire[14] between August 2004 and September 2005. The 29-item self-reporting objective questionnaire measured four aspects of aggression: physical aggression, verbal aggression, anger, and hostility. Respondents indicated how characteristic of them each item was using a 5-point scale, i.e. from 1 (extremely uncharacteristic of me) to 5 (extremely characteristic of me). Selected subjects were also assessed under the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), classification by a certified psychiatrist and excluded if they had a history of mania, bipolar disorders, schizophrenia, psychosis, alcohol or drug abuse problems within the preceding 12 months. Informed consent was obtained from each subject and the study had the approval of the relevant institutional bioethical committees and conformed to the tenets of the Declaration of Helsinki.

DNA was extracted from blood samples using a standard organic extraction protocol. Ten SNPs, including 2 non-synonymous SNPs that were located in an 85 kb region of the X chromosome encompassing the MAOA gene were genotyped (Table-1). Eight SNPs were typed using Amplified Fragment Length Polymorphism Polymerase Chain Reaction (AFLP-PCR) using the appropriate restriction enzyme (Table-1). The remaining 2 SNPs could not be typed using restriction enzymes and were, thus, genotyped by Allele Specific PCR (AS-PCR). The allele specific primers were designed online using a web-based allele-specific PCR (WASP) website.[15]

PCR was carried out in 15-25 µl reaction volumes containing 1µM primer, 200 µM deoxynucleotide (dNTPs), 2.0 mM magnesium chloride (MgCl2), 1 Unit DNA polymerase obtained from the Thermus Aquaticus Bacterium commonly referred to as TAQ polymerase and 20-40 ng template DNA. PCR cycling conditions consisted of a denaturation cycle of 4 minutes at 94°C followed by

Table-1: Single nucleotide polymorphisms(SNPs)analysed, their position on the human X chromosome (build GRCh37), primer sequences, SNP alleles Amplified Fragment Length Polymorphism Polymerase Chain Reaction (AFLP-PCR) or Allele Specific PCR (AS-PCR), assay methodology along with the restriction enzyme (if used) and expected product sizes. The ancestral allele is shown on the left in the alleles column and non-synonymous (NS) amino acid changes, if any, are shown in brackets in the database SNP (dbSNP ID) and Alleles columns, respectively.

No.	dbSNP ID	Position GRCh37	Primer sequence	Alleles	Assay	Enzyme	Product size (bp)
1	rs 12556694 (intron 1)	43518067	F - TCTTGCACAGTAGTTCACACCTC R - GGCATATAAAACTGCCACAGG	C→T	AFLP-PCR	BsiI	C = 200; 155 T = 355
2	rs6610842 (intron 2)	43550026	F - TTTGGCTGTGGGTTGCTAAT R - TGATCCTCTTTGTTGGGTGTC	G→C	AFLP-PCR	PvuII	G = 433 C = 215; 218
3	rs3027392 (intron 2)	43551400	F - AGAGCCAGCGAGCTAACAAG R - CCTGAGAGGATTTGAAGCTG	G→A	AFLP-PCR	TasI	G= 313 A = 225; 88
4	rs3027395 (intron 3)	43553528	F - CTTTCTCACTGAAGCCAAACA R - TGCTTTTTCACATCTGTCACC	G→C	AFLP-PCR	Eco88I	G = 203; 178 C = 381
5	rs1799835 (exon 8, NS)	43591085	F - CCACAAAGACTGCAGCTCAC R - TCATTATGTGTGGCCAAGGA	T→G (F→V)	AFLP-PCR	Eco147I	T = 199; 184 G = 383
6	rs3027399 (intron 9)	43592722	F - CAATTAGGAGGCCCAGTTCA R - TGTGGTAGAATAAATCGCCTTT	G→C	AFLP-PCR	SsiI	G = 120; 219 C = 120; 130; 89
7	rs3027401 (intron 9)	43593062	FN - AGGTTGTGCTGCTGGTTACTACT FD - AGGTTGTGCTGCTGGTTACTACC R - GCTCTTCCATTCATAGTTGG	C→T	AS-PCR	-	276
8	rs2205718 (intron 10)	43597465	FN - ATAACAGAACCTAAGTGATGGA FD - ATAACAGAACCTAAGTGATGGC R - AGTGGTTCCCAGATCTGACT	G→T	AS-PCR	-	234
9	rs5905418 (intron 12)	43602965	F - ACCTTCCCCCGAGAAAGAC R - GGTTGACGAATCACCCTTCA	A→G	AFLP-PCR	DdeI	A = 233; 147 G = 233; 94; 53
10	rs1803986 (exon 13, NS)	43603113	F - TGAATTTCTGTGCCTTCTGC R - AGATGAGCCCCATGAGTGAG	G→T (M→I)	AFLP-PCR	NlaIII	G = 174; 170 T = 344

Table-2: Ancestral allele frequency of the Single nucleotide polymorphisms (SNPs) in Pakistani (PAK); this study, HapMap Gujarati Indians in Houston (GIH), and 1000 Genomes Project African (AFR) and European (EUR) populations.

SNP	PAK	GIH	EUR	AFR
rs12556694	C = 0.98	Not available	C= 1.00	C = 0.99
rs6610842	G = 1.00	Not available	G = 1.00	G = 1.00
rs3027392	G = 0.93	Not available	G = 0.960	G = 0.90
rs3027395	G= 1.00	Not available	G= 1.00	G= 0.99
rs1799835	T = 1.00	Not available	Not available	Not available
rs3027399	G= 0.98	Not available	G= 0.93	G= 1.00
rs3027401	C = 0.57	C = 0.375	C = 0.71	C = 0.88
rs2205718	G = 0.58	G = 0.368	G = 0.71	G = 0.88
rs5905418	A = 0.94	Not available	A = 0.89	A = 0.88
rs1803986	G = 1.00	Not available	Not available	Not available

30 cycles of 94°C for 45 seconds, 56°C for 45 seconds and 72°C for 45 seconds. Restriction digestion was carried out according to the manufacturer's instructions. PCR products were separated on 2-3% agarose and visualised under ultraviolet (UV) transillumination.

The ancestral allele for each SNP was determined using the Ensembl (release 75) Comparapipeline[16] that uses a comparison with non-human primate genomic reference sequences. The chimpanzee allele was taken as the ancestral allele in case data for orangutan and macaque was unavailable.

The frequencies of each SNP allele in the study population, a HapMap (GIH), and the 1000 Genomes Project[17] European (EUR) and African (AFR) samples were calculated (Table-2). GIH are Gujarati Indians in Houston, Texas, but their origin is from Gujarat, India, that is geographically close to the study population. The population allele frequencies for GIH were obtained from the Database of Single Nucleotide Polymorphisms (dbSNP Build 137).[18]

Median joining networks of the inferred haplotypes obtained from the SNP data were constructed using Network 4.6.1.2 and used to compare evolutionary relationships between the MAOA haplotypes.[19] SPSS 13was used for univariate (Kruskal-Wallis one-way analysis of variance [ANOVA]) and multivariate analysis of variance (MANOVA). Cohen's d statistic was estimated as described in literature.[20]

Published data on the collaborative Human Genome Diversity Project-Centre d'etude du polymorphismehumain (HGDP-CEPH) panelsamples from Pakistan[21] were used to estimate Linkage Disequilibrium (LD) (r^2 >0.70) in a ~121 kb region encompassing the MAOA gene. This block encompassed the VNTR locus as well. PLINK toolset[22] was used to import data and HaploView[23] to generate LD plots using haplotype frequencies based on allele frequency data from 9 SNPs that spanned this region in 130 Pakistani samples (Figure-1A) in the HGDP-CEPH panel.[21] Haploview input data was loaded as two files; one containing unphased diplotypes in linkage format and the other containing the marker information. A minimum minor allele frequency of 0.001 and Hardy Weinberg p value cut-off of 0.001 were used to calculate r^2.

In the absence of complete sequences from south Asian populations we used the low coverage re-sequencing data obtained from unrelated European CEU (CEPH Collection of Utah Residents with Ancestry from Northern and Western Europe) males (n = 45) to explore the haplotype structure in this region. CEU individuals belong to the CEPH collection of Utah residents with ancestry from northern and western Europe. Like the GIH, the CEU are also genetically closer to populations from Pakistan and an appropriate surrogate for population substructure and linkage disequilibrium in this genomic region. Haplotype networks were constructed for the ~110 kb region that was in high linkage disequilibrium.

Since p values are dependent upon the sample size, we used an effect-size measure (Cohen's d statistic) that is independent of sample size, to test for the strength of haplotype association with the mean scores of the aggression questionnaire.

Results

Blood samples were collected from 159 unrelated male subjects, but due to limitation in amount or quality of the

Table-3: Means scores ± standard deviation (SD) for four factors of self-reported aggressive behaviour across the six Monoamine Oxidase A haplotypes.

Factors	MAOA Haplotypes						Total
	A (39.0%) n = 52	B (43.6%) n = 58	C (6.0%) n = 8	D (2.3%) n = 3	E (6.8%) n = 9	F (2.3%) n = 3	n = 133
Physical aggression	22.88 ± 6.38	22.93 ± 7.79	23.75 ±7.92	22.67 ± 8.50	21.33 ± 7.00	23.33 ± 4.04	22.86 ± 7.06
Verbal aggression	14.67 ± 5.00	14.98 ± 5.30	13.88 ± 4.45	13.33 ± 5.51	13.44 ± 5.41	15.00 ± 1.00	14.65 ± 5.03
Anger	14.00 ± 6.35	14.03 ± 6.79	17.12 ± 3.44	14.00 ± 6.24	12.44 ± 7.38	13.67 ± 8.39	14.09 ± 6.47
Hostility	18.10 ± 5.04	17.10 ± 6.47	17.38 ± 6.30	19.00 ± 5.20	15.33 ± 5.20	16.33 ± 7.37	17.41 ± 5.79

Table-4: A) Cohen's d Effect size statistic (below diagonal) with r values (above diagonal) for mean scores on the Buss and Perry Aggression Questionnaire across the six Monoamine Oxidase Ahaplotypes (A-F). B) Effect size measure for total and four factor scores for haplotype E in comparison with pooled samples from the remaining five haplotypes.

A)

Haplotypes	Monoamine Oxidase A Haplotype					
	A	B	C	D	E	F
A	0.00	0.01	0.06	0.02	0.22	0.10
B	0.03	0.00	0.07	0.00	0.17	0.03
C	0.13	0.15	0.00	0.09	0.15	0.02
D	0.04	0.00	0.17	0.00	0.16	0.02
E	0.34	0.29	0.46	0.32	0.00	0.13
F	0.07	0.03	0.19	0.04	0.27	0.00

B)

Factor	Total n = 133 Cohen's d (r values)
Physical Aggression	0.23 (0.12)
Verbal Aggression	0.25 (0.12)
Anger	0.26 (0.13)
Hostility	0.40 (0.20)
Total	0.33 (0.16)

DNA obtained, 26(16.35%) samples could not be genotyped for all SNPs and were dropped from subsequent analyses. Four (40%) of the 10 analysed variants, 2(20%) non-synonymous SNPs (rs1799835; rs1803986) and 2(20%) intronic (rs6610842; rs3027395) were fixed for the ancestral alleles. The fixation of the two non-synonymous amino acid changes in the MAOA gene in this population highlighted the functional constraints on the protein structure as these two positions are highly conserved in primates and across most human populations. The ancestral allele frequency was >0.93 in an additional 4(40%) intronic SNPs and in only 2(20%) (rs3027401; rs2205718) of the 10 SNPs there was an appreciably higher (>0.40) frequency of the derived allele. The derived allele frequencies for these two SNPs were higher compared to Africans (AFR) and Europeans (EUR), but lower in comparison with the HapMapGIH samples (Table-2).

The X chromosomal location of the MAOA gene allowed us to determine the haplotype in these male individuals without imputation. The combination of 10 SNPs identified 6 MAOA haplotypes designated A-F here: 52(39%) Haplotype A, 58(43.6%) B, 8(6%) C, 3(2.3%) D, 9(6.8%) E and 3(2.3%) F. Haplotype A was the ancestral haplotype. A median joining network of the 6 haplotypes (Figure-2) showed that two mutational steps separated the two major haplotypes A and B which together

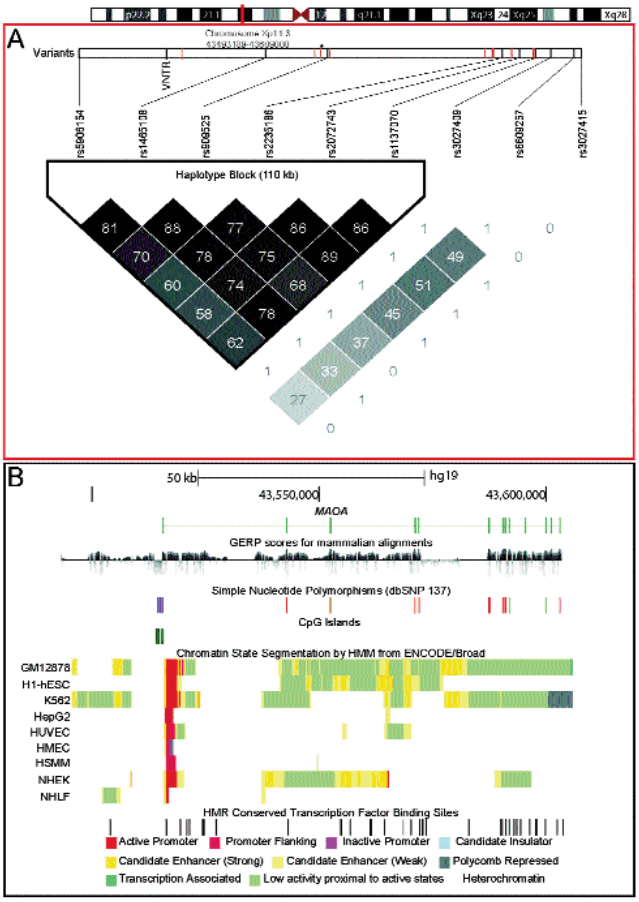

Figure-1: Linkage disequilibrium and regulatory elements in the genomic region surrounding Monoamine Oxidase A (MAOA). A) Genotyped variants (represented by vertical red lines) surrounding MAOA. The position of the rs3027392 SNP is indicated by an asterisk (*). The region in highLD (r2 ? 70%) in Human Genome Diversity Project-Centre d'etude du polymorphismehumain(HGDP-CEPH males from Pakistan is represented by the haplotype block. Linkage data is based upon 9 single nucleotide polymorphisms (SNPs) (vertical black lines and rs numbers given) in this region, that were genotyped in the HGDP-CEPH panel. The genomic location of the variable number of tandem repeat (VNTR) polymorphism is also shown. B). Regulatory features in the high LD region. The MAOA gene with its exonic (vertical bars) and intronic (horizontal lines) boundaries is shown in the ~110 kb high LD region. Evolutionary constraint in mammals is represented by the Genomic Evolutionary Rate Profiling (GERP) scores. Single nucleotide variants are from database (dbSNP) 137 (red - non-synonymous and essential splice site variants; green - synonymous; blue 5'UTR (Untranslated Region) variants). Encyclopedia of DNA Elements (ENCODE) annotation shows Chromatin State Segmentation in nine cell lines. The region is consistently associated with an active promoter site in all cell lines which is also supported by the presence of CpG islands upstream of the gene. There is strong evidence for candidate enhancer regions in GM12878, H1-hESC, K562 and NHEK cell lines. This is also supported by the presence of binding sites for transcription factors in the vicinity of the candidate regions.

comprised 110(83%) of the six variants. All the remaining haplotypes (C-F) were separated by one mutational step.

Haplotypes E was associated with the lowest total mean score of the questionnaire (Figure-2). The derived A allele

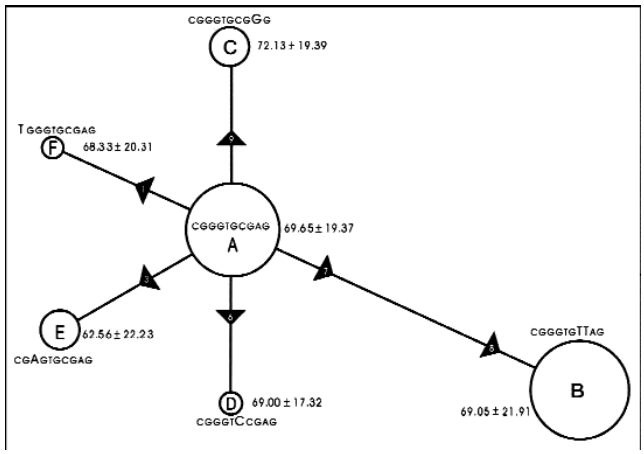

Figure-2: Median joining network of six Monoamine Oxidase A (MAOA) haplotypes observed in the sampled population. The haplotypes (A-F) and the given sequences were defined by 10 single nucleotide polymorphisms (SNPs) (numbered 1 - 10 in Figure1 and Table 1). Circles are proportional to the haplotype frequency in the population and mutational changes are shown along each branch. Mean scores for self-reported aggression (± Standard Deviation) are given besides each circle along with the haplotype sequence and the base change that characterises each haplotype is shown in large font size.

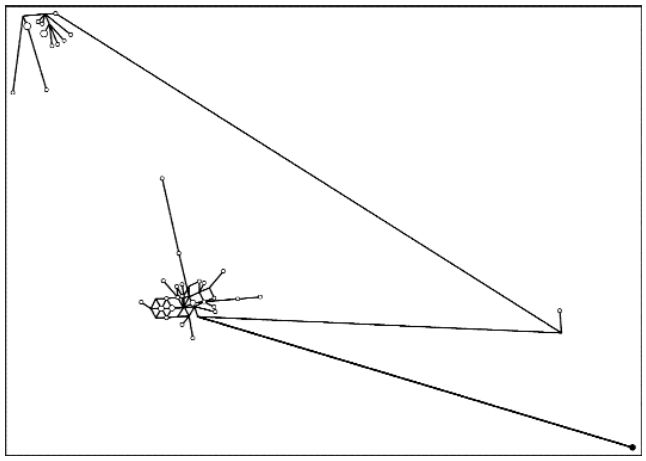

Figure-3: LHaplotype networks in the high LD region in CEU males. Median joining networks were constructed using the Network software for a ~110 kb region that is in high (r2 ?0.70) LD in CEU males. The network is based on 45 CEU males sequenced by the 1000 Genomes Project. The network shows a single haplotype (black circle) that contains the derived A allele for rs3027392. This haplotype is separated from the major haplotype cluster by a long branch characterised by 41 variants (bold line) and is present at a frequency of 4.4% in CEU males.

for SNP rs3027392 that characterises this haplotype is located in the second intron of the MAOA gene. Low mean scores were observed not only for the total scores, but also for the scores across three of the four subscales on the aggression questionnaire (Table-3) for this haplotype but the difference was not significant (p=0.95).

The six haplotypes were analysed for association with scores of the four subscales of the aggression questionnaire and MANOVA showed no significant differences (p>0.05 each) in the error variances of the total scores and scores for three of the sub-scales across the haplotypes. The variance was significantly different only for the anger sub-scale (p<0.05).

MANOVA analysis indicated that there was only a 37% probability of finding a significant effect given our limited sample size.

Haplotype E individuals showed a medium effect size association (d 0.27-0.46) with lower mean scores. However, only 2-5% of the variance in the mean scores could be accounted for by membership in haplotype E (Table-4A). Comparison of scores for haplotype E with the combined samples from the remaining 5 haplotypes (A-D and F)revealed a medium effect size for low score on the hostility subscale (Cohen's d statistic 0.40) and small effect sizes (Cohen's d statistic 0.23-0.26) for the remaining three subscales (Table-4B).

Discussion

Aggression is a complex personality trait that is likely to be associated with the action of several genes involved in brain function.[3] This is the first study investigating an association between scores on the self-reporting Buss and Perry Aggression Questionnaire and the candidate gene, MAOA, in male subjects from Pakistan. We analysed 10 SNPs on the MAOA gene in males belonging to a homogeneous ethnic group from Pakistan in order to identify any associated MAOA polymorphisms or extended haplotypes. The ethnic group was chosen because of its propensity for violence i.e. by being either perpetrators, or victims, of violent behaviour as ascertained by historical medico-legal records maintained by the provincial government. The Punjabi translation of the questionnaire that was administered to the study population was validated with Cronbach's coefficient alpha of 0.85.

Using a candidate gene approach, unrelated males were typed for MAOA variants and 6 extended MAOA haplotypes were observed two of which comprised 83% of the sample, which may reflect the high degree of consanguinity in this population. One haplotype in particular (haplotype E in Figure-2) was associated with lower mean total and sub-scale scores for physical aggression, anger and hostility on the questionnaire. In comparison with the remaining five haplotypes, this haplotype (E) was associated with medium effect size for low scores on the hostility (Cohen's d statistic 0.404) and small effect for low scores on the remaining three

subscales (Cohen's d statistics 0.233, 0.249 and 0.256 respectively, for physical aggression, verbal aggression and anger) of the self-reporting questionnaire. The Aallele for SNP rs3027392 that characterised this haplotype was located in the second intron of the MAOA gene but its functional significance is unclear. It lies in the vicinity of the MAOA VNTR polymorphism in a ~110 kb region that is in strong linkage disequilibrium in the HGDP-CEPH South Asian population panel (Figure-1A). The linkage interval includes two (rs909525 and rs6323) of the three SNPs associated with aggression-related traits in suicidal males.[13] The MAOA VNTR has been associated with behavioural phenotypes and although this variant could not be genotyped in this study the pattern of H3K4Me3 histone modifications surrounding it are suggestive of it being associated with the MAOA promoter region (Figure-1B).The promoter signal is found consistently across all nine diverse human cell lines and there are deoxyribonuclease (DNase I) hypersensitivity sites in its vicinity in several neuronal cell lines examined by Encyclopedia of DNA Elements (ENCODE)[24] and a postmortem human brain tissue.[25] In addition, there are H3K4Me1 and H3K27Ac histone modifications in several cell lines in the vicinity of rs3027392 which characterises the haplotype associated with lower self-reporting aggression scores. H3K4Me1 and H3K27Ac histone modifications are hallmarks of enhancer or regulatory activity (Figure-1B). Although expression quantitative trait loci (eQTL) evidence is absent, but there are numerous binding sites besides rs3027392 for several DNA binding proteins and transcription factors such as E1A binding protein p300 (EP300) and RE1-silencing transcription factor (formerly called Neuron Restrictive Silencer Factor [NRSF]), a transcriptional repressor that represses neuronal genes in non-neuronal tissues.[26] EP300 functions as a histone acetyl transferase and transcription co-activator that regulates chromatin remodelling and mutations in this gene are reported to cause Rubinstein-Taybi syndrome, a disorder with many behavioural phenotypes.[27,28]

In the absence of whole genome sequences from South Asia, we examined the haplotype structure in the high ($r^2 \geq 0.70$) LD region in CEU males that were sequenced by the 1000 Genomes Project.[17] The derived allele for rs3027392 was part of a single haplotype separated by a long branch from the major haplotype cluster (Figure-3). This haplotype was present at a frequency of 7% in the CEU population (4.4% in males) and is expected to be associated with the functional variant associated with low scores on the aggression questionnaire.

It is unlikely that a single gene would stand out in an association study of human behavioural traits[28] but the association of a MAOA haplotype with lower self-reporting scores on the Buss and Perry Aggression Questionnaire in this population is an intriguing observation and requires replication in other population groups. It suggests that some humans may have been evolutionarily conditioned to curb their aggressive tendencies, thus enabling them to form social groupings. Such studies will help to unravel the role of candidate genes, epigenetics, gene-environment and gene-gene interactions in the development of aggressive human behaviour.[29] Genome-wide association studies or whole genome sequencing of several thousand selected individuals with carefully defined behavioural phenotypes may offer several additional candidates for replication studies and functional analyses.

Our study was under-powered due to limitations in sample size and due to the fact that some samples had to be dropped because they could not be genotyped due to limited amount or poor quality of the DNA available.

Conclusion

The study identified an extended haplotype associated with low levels of aggression and that might contain functionally relevant variants. Although the present study was unable to find a strong association of self-reported aggressive behaviour with MAOA variants in the Pakistani male population, yet it offers a starting point for more extensive work in this field.

Acknowledgement

We are grateful to all the DNA donors, and to Luca Pagani, S. Rehman and O. M. Saeed for technical assistance and S. Amin for help with the illustrations. Two of the authors were supported by a grant from the Shifa College of Medicine, for which they are grateful, while one of the authors is currently supported by the Wellcome Trust.

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