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ASSOCIATION OF POLYMORPHISMS OF SOME DOPAMINE METABOLIZING ENZYME GENES AND PLASMA DOPAMINE LEVELS AMONG HEALTHY MALES

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ABSTRACT

Genetic variations of dopamine metabolizing enzymes have been shown to affect the activity of these enzymes. However, data relating these genetic variations, activities of their protein products and plasma catecholamines are rather scarce.

The present study aimed at investigating the relationship between the polymorphisms of the catechol-O-methyl transferase (COMT) and dopamine β-hydroxylase (DBH) genes, the respective enzyme activities and plasma dopamine levels.

Ninety eight healthy individuals were genotyped for COMT Val108Met, DBH -1021CT and DBH 5' Ins/Del polymorphisms using RFLP and the plasma dopamine levels were measured using ELISA. The frequencies of the Val and Met alleles of the COMT Val108Met polymorphism were 0.614 and 0.386 respectively. For the C and T alleles of the DBH -1021CT polymorphism; the Ins and Del alleles of the DBH 5' Ins/Del polymorphism, the frequencies were 0.723 and 0.277; 0.654 and 0.347 respectively. Only COMT Val108Met alleles were in Hardy-Weinberg equilibrium and these alleles were also in linkage disequilibrium with the Ins and Del alleles of the DBH 5' Ins/Del polymorphism. Individuals homozygous of the Met108 allele of the COMT gene had a significantly ($p<0.05$) higher mean plasma dopamine level (78.08 ± 14.04ng/L) compared to the other COMT genotypes. Likewise, individuals with homozygous T genotype of the DBH -1021CT polymorphism had significantly ($p<0.05$) higher mean plasma dopamine (245.50 ± 13.50ng/L) compared to other genotypes. However, for the DBH 5' Ins/Del polymorphism, there was no significant difference ($p>0.05$) across all the genotype groups.
This study shows that certain genotypes of the dopaminergic genes can be predictive of the dopaminergic activities of individuals.

Keywords: *Catechol-O-methyl transferase, Dopamine β hydroxylase, plasma dopamine, gene polymorphisms*

INTRODUCTION

Dopamine is an important monoamine functioning both as a neurotransmitter and hormone. Within the central nervous system (CNS), dopamine has a variety of functions such as cognition, appetite, motor and modulation of other neurotransmitters (Nieoullon, 2002; Mehta and Riedel, 2006; Di Giovanni, 2010).

While outside CNS dopamine is involved in several important physiological functions, the disorders of which are implicated in diseases such as hypertension and diabetes which have been reported to be more prevalent among the black populations (Zhang *et al*., 2011; Spanakis, and Golden, 2013). Renal dopaminergic system provides a negative regulation of reactive oxygen species (ROS). Dopamine D1, D2 and D5 receptors can exert antioxidant effects via direct and indirect inhibition of prooxidant enzymes, specifically NADPH oxidase and through stimulation of antioxidant enzymes such as superoxide dismutase (SOD) and hemeoxygenase (Yang *et al*., 2014).

Similarly, endocrine and immune modulation effects of dopamine have also been reported (Torres-Rosas *et al*., 2014; Zhang *et al*., 2015). Dopamine has been found to perform an exocrine or paracrine function, modulate the immune system by acting on the immune cells in the spleen, bone marrow and circulatory system (Missale *et al*., 1998). In spite of inability of dopamine to cross the blood brain barrier, a link has been established between plasma dopamine and behavioral disorder such as internet addiction (Liu and Luo, 2015), cocaine and cannabis abuse (Faraj *et al*., 1994; Olasore *et al*., 2014). This suggests that the plasma dopamine levels may also reflect the central dopaminergic activity and therefore a potential index of the central dopaminergic activity. Physiological role of dopamine as a natriuretic and antioxidant agent has been documented (Rukavina Mikusic *et al*., 2014; Yang *et al*., 2015).

Dopamine action is terminated after its release by certain dopamine metabolizing enzymes such as Dopamine β hydroxylase (DBH) and cathecol-O-methyltransferase (COMT). DBH is responsible for the conversion of dopamine to norepinephrine and its activity is reflected in the ratio of norepinephrine to dopamine (Garland *et al*., 2007). DBH deficiency is a recessive disorder characterized by elevated dopamine in the absence of norepinephrine. DBH gene is a 23kb, 12-exon gene mapped to chromosome 9q34 (Kopecková *et al*., 2006). Certain polymorphisms have been reported in the DBH gene that affect the activity of the enzyme. A -1021C/T polymorphism in the 5´ flanking region of the DBH gene has been shown to account for 35–52% of the variation in plasma DBH activity (Zabetian *et al*., 2001; Köhnke *et al*., 2002). Similarly, DBH 5'Ins/Del which is a 19bp insertion/deletion polymorphism is reportedly associated with plasma DBH activity (Cubells *et al*. 2000). COMT is an enzyme that converts dopamine to 3-methoxytyramine which is then subsequently converted to homovanilic acid. COMT gene is a 27kb gene with 6 exons located on chromosome 22q11(Lewis *et al*., 2003). A functional polymorphism of COMT gene, involving a substitution of

Valine (Val) for Methionine (Met) at codon 108, resulted in a 4-fold reduction in enzyme activity (Lachman *et al*., 1996).

Considering the many physiological importance of dopamine, investigating the effects of the genetic variations of dopamine metabolizing enzymes on plasma dopamine level might help to better understand the role of these enzymes in dopamine related physiological disorders. If a significant correlation is found between the genetic variations in dopamine metabolizing enzymes and plasma dopamine levels, then knowing the individual's genotype can be a good predictor of the extranervous peripheral dopaminergic function. Consequently, the possibility of these enzymes being a target for design of new drugs and exploiting of the existing ones for the management of the dopamine related physiological disorders. Since similar dopamine metabolizing enzymes are found on various cell types both in the periphery and in the CNS, the effects of these enzymes on the plasma dopamine level is expected to mirror their effects in the CNS. Genetic polymorphisms of these enzymes are therefore expected to correlate with the central dopaminergic function if they do on plasma dopamine levels. The present study was aimed at assessing the relationship between the DBH 5'Ins/Del, DBH -1021C/T and COMT Val108Met polymorphisms; the plasma DBH and COMT enzyme activities and plasma dopamine levels among healthy males in Lagos, Nigeria.

MATERIALS AND METHODS

Subjects

The work was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans. After obtaining the approval of the Human Research Ethics Committee of The Federal Neuropsychiatric Hospital, Yaba, Lagos, a total of 98 healthy volunteers between the ages of 21 and 45 were recruited for the study. Informed consent was obtained from all individual participants included in the study. Venous blood (10ml) was taken in the morning after an overnight fast for DNA analysis, plasma dopamine and enzyme assays.

Assays for COMT and DBH Enzyme Activities

The assay for COMT was carried out by the spectrophotometric method described by (Borchardt, 1974). This is based on the principle that COMT catalyzes the reaction of dihydroxyacetophenone with S-adenosyl-L-methionine to form O-methylated products which can be measured by their absorbance at 344nm. The DBH activity was assayed according to the spectrophotometric method described by (Nagatsu and Udenfriend, 1972). This is based on the conversion of tyramine (a

DBH artificial substrate) to octopamine by DBH which is then oxidized by sodium periodate to parahydroxybenzaldehyde which can be measured by its absorbance at 330nm. The enzyme activities were presented in µmol/mg protein/min.

Protein Determination

Plasma protein was estimated by the method of Lowry *et al*. (1951) using bovine serum albumin (BSA) as standard protein.

DNA Extraction, Amplification and Genotyping

DNA Extraction Method

High molecular weight genomic DNA was prepared from venous blood using modified salting out method described by (Nasiri *et al*., 2005). Some slight modifications were made by using SDS instead of laundry detergent as used by the above authors. The DNA yield was determined using Nanodrop 1000 (Thermo Scientific, USA). The yield was found to be between 45-55µg/mL of whole blood.

Genotyping

The PCR reactions were carried out in 25µl total reaction volume with 5µl 5x Taq Master Mix (Jena Bioscience, Germany) and approximately 15 - 20ng template DNA. All the PCR products were separated on 2.2% precast agarose gel (FlashGel DNA Cassette, Lonza, USA) using FlashGel DNA 50bp Marker.

The COMT Val158Met polymorphism was genotyped by slight modification of the method described by (Light *et al*., 2007). This method was a single-tube allele specific amplification protocol involving the use of two primer sets specific for each allele (i.e. Val and Met alleles). The Val (G) allele was amplified using the following pair of primers:

Forward: 5'- TCACCCAGCGGATGGTGGATTTCGCTGGGG-3',

Reverse: 5'- AACGTGGTGTGAACACCTGGTGGGGAG – 3'

This yielded a 143bp fragment. The A (Met) allele was amplified using the following pair of primers:

Forward: 5'- CGGGTCAGGCATGCACACCTTGTCCTTCCT-3',

Reverse: 5'- TGCTGTCACCAGGGGCGAGGCTCATCA – 3'

This which yielded a 116bp fragment. The 30 cycles PCR profile was as follows: Initial denaturation: 94^{o}C, for 3min; Denaturation: 94^{o}C for 30sec; Annealing: 62^{o}C for 1min; Extension: 68^{o}C for 1min; Final extension: 72^{o}C for 2min.

The DBH -1021C/T polymorphism was genotyped by the method described by (Hess *et al*., 2009). A 131bp fragment spanning the DBH -1021C/T polymorphic site of the DBH was amplified using the following pair of primers:

Forward: 5'-GGAGGGACAGCTTCTAGTCC-3',
Reverse: 5'-CACCTCTCCCTCCTGTCCTCTCGC-3'.
The PCR amplification was run for 30 cycles with the profile: Initial denaturation: 94^{o}C for 3min; Denaturation: 94^{o}C for 30sec; Annealing: 60^{o}C for 1min; Extension: 72^{o}C for 1min; Final extension: 72^{o}C for 5min. Fifteen µl of the PCR product was then digested with 2 units of *Hha* I restriction enzyme for 24 hours at 37^{o}C to yield a 109bp and a 22bp fragments (restriction site present) or a whole uncut 131bp fragment (restriction site present).

The DBH*5'-Ins/Del polymorphism was genotyped by the method described by (Cubells *et al*. 2000). A short region spanning the DBH*5'-Ins/Del polymorphic site was amplified using the following pair of primers: Forward: 5'-GCAAAAGTCAGGCACATGCACC-3'; Reverse: 5'-CAATAATTTGGCCTCAATCTTGG-3'. The 30 - cycle PCR profile was as follows: Initial denaturation: 95^{o}C, for 5min; Denaturation: 94^{o}C for 1min, Annealing: 68^{o}C, for 1min; Extension, 72^{o}C, for 1min; Final extension: 72^{o}C for 2min. The appearance of a 163bp fragment revealed the Ins allele while the Del allele showed 144bp fragment.

Population Genetics Analyses
All the population genetics analyses were carried out using PopGene 3.2 software. This software was used to obtain the genotype and allele frequencies and Hardy Weinberg equilibrium (HWE).

Statistical Analyses
All the statistical analyses were carried out using the SPSS version 15. One way ANOVA was used to compare the mean plasma concentration of dopamine and norepinephrine across various genotype groups. Linear regression was used to evaluate the contributions of the various predictors to the variance in the mean plasma dopamine levels (R^2). All p values less than or equal to 0.05 were taken as statistically significant.

RESULTS
The result for the population distribution of alleles and genotypes of the polymorphic sites under study are as shown in Table 1. The Val allele was more frequent (0.629) than the Met allele (0.371) while the heterozygous Val/Met genotype was the most frequent genotype (0.441). The observed heterozygosity was 0.441 while the expected heterozygosity was 0.469. The population did not deviate significantly from Hardy-Weinberg equilibrium (χ^2 = 0.344, df =1, p = 0.558). For

the DBH-1021C/T polymorphic site, the C allele was more prevalent compared to the T allele (0.723 and 0.277 respectively) while the heterozygous C/T genotype was observed to be the most frequent (0.511). The values for the observed and expected heterozygosity were 0.510 and 0.402 however the population was found to significantly deviate from Hardy-Weinberg equilibrium (χ^2 = 6.924, df =1, p = 0.009). Considering the DBH 19-bp Ins/Del gene polymorphism, the frequency of the Ins allele was higher than the Del allele (0.553 and 0.447 respectively). Homozygous Ins genotype was the highest frequency with the frequencies of 0.432. The observed heterozygosity was 0.242 while the expected heterozygosity was 0.497. The alleles were found to deviate from Hardy Weinberg equilibrium (χ^2 = 25.267, df =1, p = 0.000).

Table 1. Population genetics of COMT Val108Met, DBH -1021 C/T and DBH 5' Ins/Del polymorphic sites

	COMT Val108Met			**DBH -1021 C/T**			**DBH 5' Ins/Del**		
Alleles	**Val**	**Met**		**C**	**T**		**Ins**	**Del**	
	0.629	0.371		0.723	0.277		0.553	0.447	
Genotypes	Val/Val	Val/Met	Met/Met	C/C	C/T	T/T	Ins/Ins	Ins/Del	Del/Del
	0.409	0.441	0.151	0.468	0.511	0.021	0.432	0.242	0.326
Homozygosity	Obs.	Exp.		Obs.	Exp.		Obs.	Exp.	
	0.559	0.531		0.485	0.597		0.758	0.503	
Heterozygosity	Obs.	Exp.		Obs.	Exp.		Obs.	Exp.	
	0.441	0.469		0.510	0.402		0.242	0.497	
Hardy - Weinberg	χ^2	df	P	χ^2	df	p	χ^2	df	p
	0.344	1.000	0.558	6.924	1.000	0.009	25.267	1.000	0.000

Table 2 shows the distribution of plasma dopamine levels among the various genotype groups. For COMT Val108Met and DBH Ins/Del polymorphisms, there were significant differences in the plasma dopamine levels among the genotype groups as shown by the p values of 0.005 and 0.051 respectively. However, the difference was most significant across DBH -1021 C/T genotype groups in which the T/T genotype group had a much higher mean plasma dopamine level of 245.5ng/L compared to the other two genotype groups – C/T and C/C, which had 51.82 and 40.15ng/L respectively.

Table 2: Plasma dopamine levels across various genotype groups

	Plasma Dopamine (mean ± S.E.M) ng/L	**F**	**p**
COMT Val108Met			
Val/Val	38.35 ± 3.40	5.571	0.005
Val/Met	54.33 ± **6.91**		
Met/Met	**78.08 ± 4.22**		
DBH -1021 C/T			
C/C	40.15 ± **3.02**	68.02	<0.001
C/T	**51.82 ± 4.21**		
T/T	**245.50 ± 4.22**		
DBH 5' Ins/Del			
Ins/Ins	39.69 ± **3.72**	3.09	0.051
Ins/Del	61.55 ±**10.48**		
Del/Del	**57.89 ± 8.18**		

The comparison of the mean enzyme across the various genotype groups is presented in Table 3. Individuals in the Val/Val group had the highest mean COMT activity (1.91nmol/mg protein/min) while those homozygous of the Met variant had the lowest mean activity (0.99nmol/mg protein/min) with p value of less than 0.005. There was also a significant difference in the mean DBH activity values across the DBH -1021 C/T genotype groups ($p < 0.005$) with the T/T genotype group having the lowest mean value (0.20 nmol/mg protein/min) while individuals with the DBH C/C genotype had the highest mean DBH activity of 0.56nmol/mg protein/min. The mean DBH activity across the DBH 5' Ins/Del genotype groups also varied significantly. Individuals in the Ins/Ins group had the highest mean DBH activity (0.51 nmol/mg protein/min) while the Del/Del group had the lowest (0.31nmol/mg protein/min).

Table 3: Differences in COMT and DBH activities across various genotype groups

	Mean DBH Activity ± SEM (nmol/mg protein/min)	Mean COMT Activity ± SEM (nmol/mg protein/min)	F	p
COMT Val108Met				
Val/Val		1.95 ± 0.07	25.75	<0.001
Val/Met		1.64 ± 0.07		
Met/Met		1.00 ± 0.04		
DBH -1021 C/T				
C/C	0.70 ± 0.02		28.648	<0.001
C/T	0.42 ± 0.04			
T/T	0.06 ± 0.02			
DBH 5' Ins/Del				
Ins/Ins	0.60 ± 0.04		1.822	0.168
Ins/Del	0.56 ± 0.06			
Del/Del	0.49 ± 0.05			

Figure 1 shows the relationship between plasma dopamine levels and the activity of catechol-O-methyl transferase (COMT). COMT activity was shown to contribute 19% of the variance in plasma dopamine level as shown by the R^2 value. In Figure 2, DBH activity was shown to contribute over 40% of the variance in plasma dopamine as shown by the R^2 value.

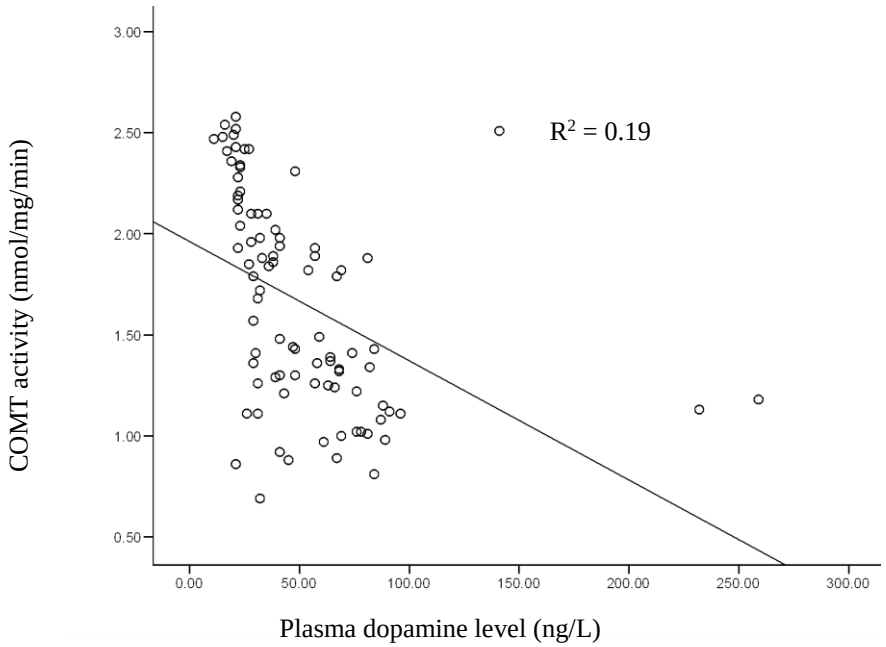

Figure 1: Relationship between COMT activity and plasma dopamine levels

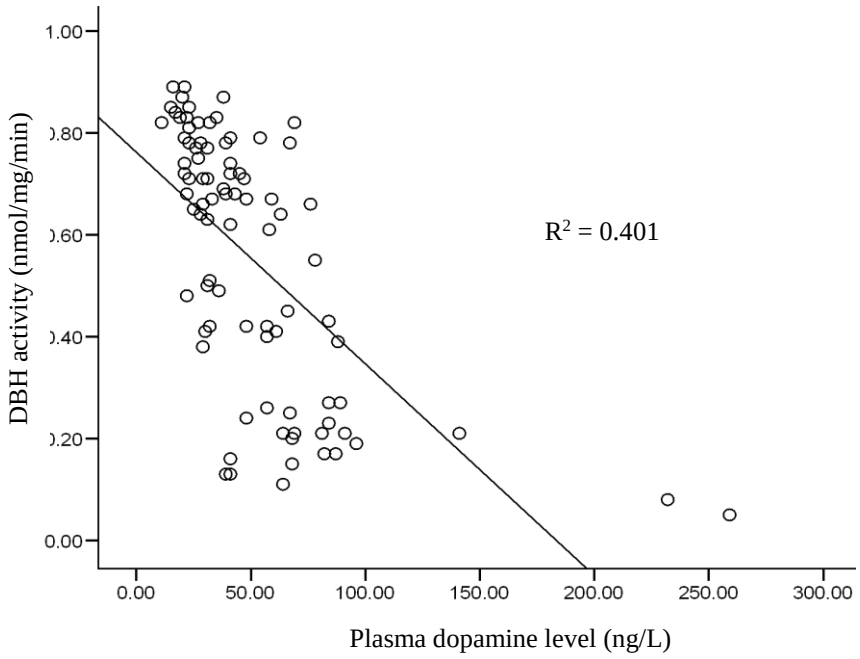

Figure 2: Relationship between DBH activity and plasma dopamine level

DISCUSSION

Although many genetic variations are silent ones, quite a number of them can have profound effects on the observed phenotypes and may explain why certain individuals are more prone to certain disorders than others. Polymorphisms of the genes coding for proteins involved in the signal transduction pathways can have adverse effects on normal physiological functions of neurotransmitters. Plasma dopamine level is a reflection of peripheral dopaminergic activity, extranervous dopamine biosynthesis and degradation as a result of inability of dopamine to cross the blood brain barrier (Rubí and Maechler, 2010). Although many studies have focused on the central nervous effects of dopamine imbalances, however the role of dopamine in the pathophysiology of some peripheral and non-nervous disorders cannot be ignored. In the present study, we found a considerable level of similarity in the frequencies of the alleles of dopamine metabolizing enzyme genes and those reported across various populations (Todt *et al*. 2009).

Heterozygosity is a genetic parameter that provides a very good index of the amount of genetic variation (Ibeagha-Awemu and Erhardt, 2006). Variation is very

necessary for organisms to adapt to the ever changing environment and very important for the health of a population (Khan *et al*. 2007). All the polymorphic sites analyzed in this study have relatively high level of heterozygosity and this high variability is an indication that the populations have adapted well to the environment. Analysis showed that the COMT Val108Met and DBH*5'-Ins/Del polymorphisms were found to be in linkage disequilibrium. By implication, there was a statistical association between these loci and they were therefore not likely transmitted independently (Lewis and Knight , 2012). Furthermore, only COMT Val/Met genotypes were in Hardy Weinberg equilibrium. By implication, these alleles distribution have been constant over time and are not subject to evolutionary forces (Yu *et al*., 2012). The remaining loci have genotype frequencies which deviated significantly from Hardy-Weinberg equilibrium.

A significant difference in the mean plasma dopamine level was observed across the COMT Val108Met genotype groups. Individuals with homozygous Val genotype had significantly lower mean plasma dopamine. This might be due to higher dopamine degradation in this group. The Val allele has been reported to be associated with higher enzyme activity and reduced dopamine level (Light *et al*., 2007), compared to the Met allele and a similar trend was replicated in this study. There was a significant difference in the mean COMT activity across the three genotype groups with the lowest activity found among the individuals with Met/Met genotype. This is in line with our finding that the highest plasma COMT activity was found in the group homozygous for the Val allele.

The two polymorphic sites of the DBH gene studied were both found to significantly affect the plasma dopamine level. Apart from the very significant differences in the plasma dopamine levels across the genotype groups of DBH*5'Ins/Del and DBH -1021C/T sites, these two sites were the only ones shown to account for the variance in the mean plasma dopamine using multiple linear regression. The homozygous Ins genotype of the DBH was associated with significantly lower plasma dopamine compared to the heterozygous and the homozygous Del genotypes. The Ins allele is reportedly associated with higher DBH enzyme activity than the Del allele (Cubells *et al*., 2000; Fernandez *et al*., 2006). By implication, the enzyme variant from this allele will convert more dopamine to norepinephrine per unit time compared to the variant from the Del allele. This was replicated in this work as the DBH enzyme activity was found to be significantly higher in the individuals homozygous for the DBH Ins allele compared to the other genotypes. The increase in the depletion of dopamine due to

its conversion to norepinephrine could explain at least in part the lower mean plasma dopamine associated with the homozygous Ins/Ins genotype.

The effect of the DBH-1021 C/T polymorphism was even more significant on plasma dopamine levels. We found that the less common T allele was associated with significantly higher plasma dopamine level. This allele has been reported to yield an enzyme variant with very low DBH enzyme activity. In fact, about 97% reduction in DBH activity has been reported in individuals with T/T genotype (Deinum *et al*., 2004). Conversely, the C allele has also been reported to confer high enzyme activity on the enzyme compared to the T allele (Zabetian *et al*. 2003). This was further confirmed by (Cubells and Zabetian, 2004) who also found an elevated dopamine and reduced norepinephrine in the presence of low DBH activity.

CONCLUSION

Genetic polymorphisms of the dopamine metabolizing enzymes and other genes involved in the dopaminergic signaling have been found to be good predictors of development and prognosis of mental disorders such as schizophrenia, depression, attention deficit hyperactivity disorder, substance abuse as well as certain motor disorders such as Parkinson's disease. However little attention has been given to the possible influence and the potential diagnostic and prognostic values of these genetic polymorphisms in non-nervous disorders. The present study has shown that there is a relationship between some genetic polymorphisms of COMT and DBH which are major dopamine metabolizing enzymes and plasma dopamine in healthy subjects. Nevertheless more data will be needed using specific populations of subjects with various dopamine related disorders to evaluate the relationship between these genetic variations and the pathophysiology of peripheral and extranervous dopamine system.

Competing interests

The authors declare they have no competing interests.

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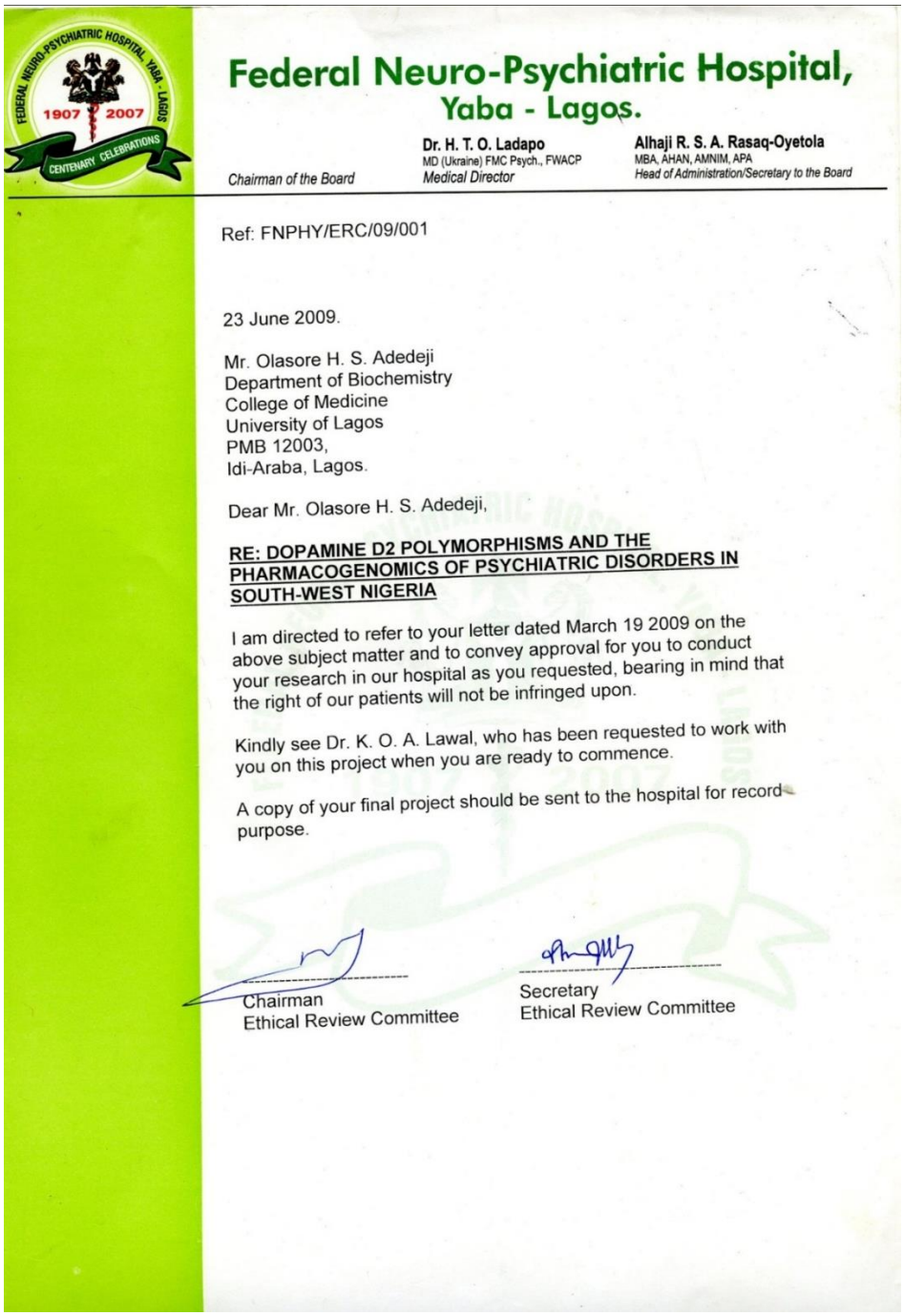

Federal Neuro-Psychiatric Hospital, Yaba - Lagos.

FEDERAL NEURO-PSYCHIATRIC HOSPITAL YABA - LAGOS
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Dr. H. T. O. Ladapo
MD (Ukraine) FMC Psych., FWACP
Chairman of the Board — *Medical Director*

Alhaji R. S. A. Rasaq-Oyetola
MBA, AHAN, AMNIM, APA
Head of Administration/Secretary to the Board

Ref: FNPHY/ERC/09/001

23 June 2009.

Mr. Olasore H. S. Adedeji
Department of Biochemistry
College of Medicine
University of Lagos
PMB 12003,
Idi-Araba, Lagos.

Dear Mr. Olasore H. S. Adedeji,

RE: DOPAMINE D2 POLYMORPHISMS AND THE PHARMACOGENOMICS OF PSYCHIATRIC DISORDERS IN SOUTH-WEST NIGERIA

I am directed to refer to your letter dated March 19 2009 on the above subject matter and to convey approval for you to conduct your research in our hospital as you requested, bearing in mind that the right of our patients will not be infringed upon.

Kindly see Dr. K. O. A. Lawal, who has been requested to work with you on this project when you are ready to commence.

A copy of your final project should be sent to the hospital for record purpose.

Chairman
Ethical Review Committee

Secretary
Ethical Review Committee