ONLINE RESOURCES

MAO-A promoter polymorphism and idiopathic pulmonary arterial hypertension

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Introduction

Monoamine oxidases (MAO) are mitochondrial enzymes that convert biogenic amines to their corresponding aldehydes. Two isoforms, MAO-A and MAO-B, exist which differ in their substrate affinities, inhibitor sensitivities and are coded for different genes residing on the X chromosome (Xp11.23). Serotonin is metabolized first in the liver and later in lungs by the enzyme MAO-A, via oxidative deamination. A variable number of tandem repeat (VNTR) polymorphism of 30-bp-repeat sequence present as 3, 3.5, 4, or 5 copies in the promoter region of the monoamine oxidase-A (*MAOA*) gene and are associated with its transcriptional activity (Sabol *et al*. 1998). The four-repeat *pVNTR* yields higher expression levels of a reporter gene than three repeats, *in vitro*. The *MAOA*-VNTR polymorphism has been mainly associated with neurological disorders such as panic disorder, anorexia nervosa etc. (Urwin *et al*. 2003; Guindalini *et al*. 2005).

Increased plasma 5-hydroxytryptamine (5-HT) levels are found in patients with idiopathic pulmonary arterial hypertension (IPAH) (Herve *et al*. 1995). The longer promoter variant (L) of 5HTT gene transporter, having higher transcriptional activity has been implicated in the pathogenesis of IPAH (Eddahibi *et al*. 2001). *In vitro* studies have shown that the ROS generated by the breakdown of 5-HT by MAO-A leads to proliferation of vascular smooth muscle cells (SMC) and cardiac hypertrophy (Bianchi *et al*. 2005; Coatrieux *et al*. 2007). DotBlot array of lung specimens from IPAH patients showed an upregulated *MAO-A* gene activity (Edgar *et al*. 2006). After its uptake and cellular internalization by the pulmonary artery smooth muscle cells, 5-HT is metabolized mainly by MAO-A. Therefore, it has been suggested that MAO-A may play a role in IPAH which could prove to be more useful in elucidation of the role of 5-HT in IPAH (Catalano 2001). Therefore, the main objective of the present study was to examine the distribution of *MAO-A* promoter VNTR alleles and its association with IPAH.

Materials and methods

This study was approved by the Institutional Ethics Committee of CARE Hospitals, Hyderabad, India, and an informed consent was obtained from all participants. IPAH is a rare condition and a total of 77 confirmed IPAH cases (females, 41; males, 36; mean ± age 28.6 ± 11.4 years) were obtained from the cardiology unit of CARE Hospital and 100 healthy volunteers (mean age 29.57 ± 7.75 years) without any history of heart and systemic disorders served as controls. Diagnosis was based on WHO criteria (Galie *et al*. 2004). From all participants 5 mL of venous blood was obtained and the genomic DNA was isolated from peripheral blood as described by Lahari and Nurunberger (1991). Polymerase chain reaction (PCR) amplification of the *MAO-A* pVNTR sequence was carried out using primers: forward: 5′-ACAGCCTGACCGTGGAGAAG-3′ and reverse: 5′-GAACGGACGCTCCATTCGGA-3′ (Bioneer Biosciences, Daejeon, Korea). Amplification was carried out in a final volume of 25 μL containing 50 ng genomic DNA, 200 μM dNTP, 10 pmol primers, 0.1% Triton X and 0.5 U *Taq* DNA polymerase (Biotools, Madrid, Spain) in the manufacturer's buffer with 1.5 mM $MgCl_2$. Cycling conditions: initial denaturation for 10 min, 35 cycles of 1 min at 95°C, 1 min at 59.8°C, and 1 min at 72°C , followed by a final extension of 7 min was carried out in Eppendorf Master Cycler Thermal Gradient (Hamburg, Germany). The PCR products were separated by electrophoresis on 10% polyacrylamide gels (BioProducts, Wilmington, USA) and visualized by silver staining (*3* allele 309 bp and *4* allele 339 bp). Statistical

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analysis was carried out using GraphPad Prism 5.0 software (San Diego, USA).

Results

Only two alleles, *3*-repeat and *4*-repeat, were found in our study population. Rare alleles *3a* and *5* were not observed either in patients or in controls. No deviation from Hardy–Weinberg equilibrium (HWE) was observed either in patients or control groups. The 3-repeat allele was found to be predominant in both controls (60.6%) and patients (62.7%). Allelic frequency of the *4*-repeat allele was higher in male IPAH patients than in controls (38.8% versus 32%, $P = 0.64$) whereas the *3*-repeat allele frequency in women was slightly higher in IPAH (63.4%) in comparison to controls (57%) ($P = 0.44$). The odds ratio of *4*-repeat allele was 1.352 (CI: 0.5521–3.312) in males and 0.7648 (CI: 0.4201–1.392) in female patients (table 1).

Discussion

MAO-A plays an important role in the onset and amplification of oxidative stress, as it is a major intracellular source of ROS. The H_2O_2 generated during tyramine oxidation by MAO-A has been shown to activate a stress-induced mitogenic signal via the metalloproteases (MMP2)/sphingolipid pathway, resulting in excessive remodelling and alteration of the vascular wall (Coatrieux *et al*. 2007). Oxidative stress can also induce the activation of transcription factors, pro-inflammatory genes, chemokine production, stimulate and recruit inflammatory cells, that are critical processes involved in vascular inflammation and injury (Touyz *et al*. 2002).

In our study, no significant association was observed among any of the particular alleles/genotypes with that of the disease, indicating that *MAO-A* promoter VNTR tandem repeat may not play a direct role in IPAH. Increased ROS production by MAO-A, could however, initiate/enhance the oxidative stress in IPAH, contributing to the remodelling of pulmonary vasculature. Studies on animal models have shown that oxidative stress plays a role in the development of hypoxia-induced pulmonary hypertension and pulmonary vascular remodelling (Hoshikawa *et al*. 2001).

MAO-A gene expression is known to be influenced by additional factors such as increased Ca^{2+} ions and/or epigenetic modifications. Ca^{2+} is a modulator of *MAO-A* regulation and increase in free intracellular Ca^{2+} above normal levels increases MAO-A activity in living hippocampal HT-22 cells (Cao *et al*. 2007). Elevated levels of intracellular Ca^{2+} are present in pulmonary artery SMCs (PASMCs) of IPAH patients, as a consequence of endothelial dysfunction and dysregulated voltage-gated K^+ channels. Epigenetic modifications of *MAO-A* gene have also been implicated in diseases such as lung cancers (Rybaczyk *et al*. 2008).

Hence, the copy number variation of the promoter may not be directly involved in upregulation of expression of *MAO-A* gene observed in IPAH but may contribute to the oxidative stress. However, replicated studies in larger/different populations are required, keeping in view the ethnic variation that exists in distribution of *MAO-A* repeats.

Table 1. Allelic and genotype frequencies of *MAO-A* promoter polymorphism in controls and IPAH.

		Alleles (%)			
	n	*3*-repeat allele	*4*-repeat allele	*P**	OR (95% CI)
Total group					
Controls	100	91 (60.6)	59 (39.34)		
IPAH	77	74 (62.7)	44 (37.2)	0.801	1.091 (0.6636–1.792)
By sex					
Males					
Controls	50	34 (68)	16 (32.0)		
IPAH	36	22 (61.1)	14 (38.8)	0.6479	1.352 (0.5521–3.312)
Females					
Controls	50	57 (57.0)	43 (43.0)		
IPAH	41	52 (63.4)	30 (36.6)	0.4477	0.7648 (0.4201–1.392)

	Genotypic frequencies (females only)			
	3R/3R	3R/4R	4R/4R	Total
Controls	19 (36%)	19 (38%)	12 (24%)	50
IPAH	19 (46.34%)	14 (34.1%)	8 (19.1%)	41

P, fishers exact probability; OR, odds ratio for *4* repeat versus *3* repeat allele, CI, 95% confidence intervals.

Further, a comparison of tissue expression profile of *MAO-A* for different VNTR alleles between controls and patient group would be more useful to rule out association.

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