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Association of Type II 5¢ Monodeiodinase Thr92Ala Polymorphism and Cardiovascular disease risk in Type 2 Diabetes Mellitus

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Abstract

Background: The physiological ratio of T3:T4 is essential to trigger the biological action since it is efficiently regulated by extrathyroidal selenodeiodinases. Thr92Ala is a common variant in the DIO2 gene which may have implication in decreased phenotypic expression. This study intends to find the effect of the SNP on CVD risk in type 2 diabetes. **Materials and Methods:** We included 130 T2DM patients without signs of CVD as controls and 106 CVD patients with T2DM as cases. All were genotyped for Thr92Ala of DIO2 gene. Fasting blood glucose lipid and thyroid profile, HDL subfractionations, type II deiodinase, malondialdehyde, paraoxonase, and superoxide dismutase were measured **Results:** The mean D2 level in Ala/Ala individuals was significantly lower than in Thr/Thr or Thr/Ala genotypes (122±39 VS 161±32ng/ml). The thyroid profile was significantly altered among Ala/Ala genotypes when compared with Thr/Thr and Thr/Ala genotypes. There is a significant decrease in T3:T4 ratio, HDL3:HDL2 ratio and paraoxonase activity among Ala/Ala homozygote's when compared with the/Thr+Thr/Ala genotypes. HDL3:HDL2 ratio is positively correlated with paraoxonase activity among Thr/Thr+Thr/Ala genotypes ($r=0.36$, $p<0.05$). **Conclusion:** Ala/Ala genotype plays a key role in thyroid dysfunction, dyslipidemia and the development of CVD risk in those which type 2 diabetes.

Key-words: DIO2 Thr92Ala; Thyroid hormones; Dyslipidemia; Oxidative stress; Type 2 diabetes mellitus; CVD risk

Introduction

The association between diabetes mellitus and thyroid dysfunction in well known.[1] Thyroid dysfunction in known to contribute to dyslipidemia which is a recognized risk factor for cardiovascular disease.[2-7] T_3 regulates various enzymes and receptors in lipid metabolism like HMG Co-A reductase, and LDL receptors to name a few. Low T3 levels is known to affect the quality of HDL.[8 -16] The production of T_3 from T_4 mainly occurs in extrathyroidal tissues by 'extrathyroidal deiodination'. The selenium dependant deiodinases D1,D2, and D3 influences the thyroid hormone levels.[17-19] Several non fatal single nucleotide gene polymorphism's (SNP) have been found in DIO genes.[20-21] Thr92Ala SNP in a common variant in DIO2 gene and known to be linked to insulin resistance and decreased phenotypic expression as in Mexican-Americans and Pima Indians.[22-23] We propose that a decrease in circulating T_3 levels in due to altered extrathyroidal deiodination of T_4. This study is undertaken and with the objective of finding the effect of Thr92Ala SNP in DIO2 gene on its phenotypic expression, on thyroid functions on HDL metabolism and its role in the development of risk of CVD among those type 2 diabetes.

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Materials and Methods

The study was conducted on patients attending the diabetes and cardiac out-patient clinics of a government general hospital in Chennai, Tamilnadu. IEC and pregnant women were obtained. The study groups included 130 patients with type 2 diabetes and without CVD risk (controls) and 106 patients which type 2 diabetes and with CVD (cases). Patients who were non-smokers and non–alcoholics were included for the study. From the blood samples fasting glucose levels (FBG), lipid profile, Apolipoprotein A1 (ApoA1), glycosylated

haemoglobin (HbA_1C) and thyroid profile was estimated. Serum HDL_2, HDL_3 and Type II 5^1 monodeiodinase (D2) was estimated by ELISA. Basal Paroxonase activity and malondialdehyde were measured by spectrophotometer using standard methods.[24-25] Superoxide dismutase (SOD) was determined by Marklund and Marklund method.[26]

Whole blood sediment with buffy coat in K_2EDTA tubes were used for isolating DNA and for SNP analysis. For molecular analysis the DNA purification kit, PCR master kit and primers from HELINI biomolecules, Chennai were used. Primers were designed using database software available at http://cedar.genetics.soton.ac.uk.

Primer (A allele):
FW: ATTGCCACTGTTGTCACCTCCTTCGGT
Rv: CTATGTTGGCGTTATTGTCCATGCGGTC
Primer (G allele):
FW: AATTCCAGTGTGGTGCATGTCTCCATTG
Rv: TTTTGGGCCATTCTTTACATTACCTGCCA
Thr92Ala(c. 274A>G; p. T92A; rs225014)

Genotype analysis was done using tetra primer amplification refractory mutation system PCR (TP-ARMS PCR).[27]

Statistical analysis

ANOVA was used to compare the mean difference between study groups. Karl Pearson correlation was used to determine the trend between various parameters within the groups. A linear regression analysis was performed between selected independent and dependant variables within the groups. A two tailed t test $p< 0.05$ was considered as statistically significant. c^2 test $p> 0.05$ was considered for allelic frequency in consistent with Hardy Weinberg Equilibrium (HWE). All the statistical parameters were analyzed by using PASW version 18.0 (SPSS, Chicago, IL) and STATISTICA 7.0 (StatSoft Inc., USA) software packages.

Results

Table 1 shows the frequency of the genopypes and the allele considered namely Thr/Thr,Thr/Ala and Ala/Ala, for the two groups studied. Table 2 shows the distribution of the studied parameters among the patients according to the genotype. Allelic frequency in the control group for Thr92Ala was consistent with HWE (p=0.46), whereas it is violating in the case group (p=0.006). The Thr/Thr wild homozygous and Thr/Ala heterozygous genotypes shows similarity in studied biochemical characteristics.

Hence a recessive inheritance model was considered to analyze various statistical parameters between and within the Thr/Thr+Thr/Ala and Ala/Ala genotypes. Ala/Ala genotype has a substantial alteration in base line biochemical parameters when compared with Thr/Thr+Thr/Ala genotypes DIO2 levels thyroid profile HDL_3:HDL_2 ratio and basal PON activity significantly differed between Thr/Thr+Thr/Ala and Ala/ala genotypes. Pearson correlation (r) was performed between selected variables within the Thr/Thr+Thr/Ala and Ala/Ala genotypes.

Serum TSH levels wes significantly negatively correlated with D2 levels in all the study groups Table 3. The proportion of T_3:T_4 was significantly positively correlated with D2 levels among Thr/Thr+Thr/Ala genotype ($r=0.55$, $p<0.05$), whereas this relation is significantly negative in Ala/Ala genotypes ($r= -0.35$, $p<0.05$). Moreover the proportion of T_3:T_4 is positively correlated with HDL_3:HDL_2 ratio among Thr/Thr+Thr/Ala genotype ($r=0.33$, $p<0.05$), whereas it was found to be negative in Ala/Ala genotypes ($r= -0.16$), but it was not statistically significant. The association between HDL_3:HDL_2 ratio and basal PON activity was significant positive among Thr/Thr+Thr/Ala genotypes ($r=0.36$, $p<0.05$), but this association was not significant among Ala genotypes ($r=0.20$). Plasma MDA levels were significantly positively correlated with FBG ($r=0.48$ in Thr/Thr+Thr/Ala; $r=0.30$ in Ala/Ala), but significantly negatively correlated with PON activity among all the subjects ($r= -0.25$ for Thr/Thr+Thr/Ala genotypes; $r= -0.23$ for Ala/Ala genotype). Moreover, basal PON levels were significantly positively correlated with SOD and ApoA1 levels in all the study groups table 3. Table 4, showing linear regression analysis between selected dependent and independent variables within the Thr/Thr+Thr/Ala and Ala/Ala genotype groups.

Among thyroid profile parameters, T_3:T_4 ratio has best regression line when D2 considered as an independent variable ($R^2 = 0.294$), compared to TSH ($R^2 = 0.127$) and fT_3:fT_4 ratio ($R^2 = 0.10$). We also performed linear regression analysis between T_3:T_4 ratio, fT_3:fT_4 and HDL_3:HDL_2. We found best fit of regression equation line between T_3:T_4 and HDL_3:HDL_2 ratios among Thr+Thr/Thr/Ala genotypes ($R^2 = 0.11$) but this were not happened in Ala mutant homozygotes. We also extended linear regression analysis for HDL_3:HDL_2 ratio, PON and MDA. Basal PON activity is positively regressed on HDL_3:HDL_2 ratio ($R^2 = 0.133$), and MDA levels ($R^2 = 0.082$) were negatively regressed on PON levels among Thr/Thr+Thr/Ala genotypes.

Discussion

Serum D2 levels were significantly decreased in Ala/Ala genotypes compared to Thr/Thr+Thr/Ala genotypes (122±39 vs 161±32 ng/ml; $p<0.001$). Our results concurred with previous studies.[21] Thyroid profile among Ala/Ala genotypes was significantly deviated from Thr/Thr+Thr/Ala genotypes, although their thyroid profile is in the normal range Table 3. Interestingly, total T_3 and fT_3 levels were near the lower limit of the reference range (0.81±0.14ng/ml & 1.77±0.32 pg/ml, respectively (Fig 1), whereas TSH and total T_4 levels were close to an upper limit of reference range among Ala homozygote's (5.23±4.53µIU/ml & 6.82±2.76 µg/dl respectively). This phenomenon ultimately leads to decrease in T_3:T_4 ratio among Ala/Ala variants. Serum D2 levels significantly negatively correlated with total T_4 levels among Ala/Ala genotypes confirms the effect of T_4 on the levels of D2. Pituitary D2 activity is indispensable for the negative feedback regulation of hypophyseal TSH secretion by circulating T_4. Thus, a subtle change in T_3:T_4 ratio may have a profound effect on hypothalamus pituitary thyroid (HPT) axis and vice versa. Experiments on D2 knockout mice shown that, there is an impaired feedback regulation on HPT axis and elevated levels of T_4 and TSH but normal circulating T_3.[34] In our study, serum T_3:T_4 ratio and TSH were negatively regressed on D2 levels ($r = -0.41$ & $R^2 = 0.154$; $r = -0.39$ & $R^2 = 0.137$ respectively, Table-4) among Ala/Ala genotypes. In the study of Olga Gumieniak et.al, Ala homozygous found to be hypertensive and having higher normal levels of TSH.[31] Thus, Ala homozygous variant has a profound outcome on the phenotypic expression of D2 and thyroid function. Anti-atherogenic properties of HDL are solely dependent on its composition.

In the study of Camont L et.al was found that, the oxidation of LDL was reduced when serum samples were incubated with ApoA1, LCAT and PON in the presence or absence of HDL.[35] Therefore HDL associated proteins play a key role in its anti-atherogenic functions. The inter conversion and remodelling of HDL is dependent on the activity of lipoprotein lipase, hepatic lipase and CETP etc, which in turn regulated by T_3. Therefore the physiological concentration of circulating T_3 is very important in the metabolism of anti atherogenic lipoproteins. In our study, the ratio of T_3:T_4 and fT_3 levels were significantly positively correlated with the ratio of HDL_3:HDL_2 among Thr/Thr+Thr/Ala genotypes ($r=0.33$, $p<0.05$), whereas this was negative in Ala variants. Total HDLc levels did not significantly differ among the groups, but the HDL_3:HDL_2 ratio significantly decreased in Ala homozygote's (2.78±1.7 vs 3.36±0.73). The total HDLc is normally rises in subclinical hypothyroidism patients; which is due to rise in HDL_2 fraction.[36] In the present study, we found significant positive correlation between paraoxonase activity and HDL_3 levels among wild and heterozygote's (r=038) when compared with Ala mutant homozygote's (r=20). From the study results it is clear that, the physiological proportion of T_3:T_4 is important in the metabolism and remodelling of HDL. We also analyzed serum levels of ApoA1, which did not significantly differ between the groups, D2 catalyzed pituitary T_3 is important for regulation of HPT axis, whereas skeletal muscle T_3 is important for up regulation of insulin dependent GLUT4.[37] In the study of Mentuccia et.al found that, the novel Ala/Ala variant was associated with a 20% decreased glucose disposal rate and higher body mass index (BMI).[23] Therefore, it is thought that thyroid dysfunction may cause the low glucose disposal rate in diabetics with Ala/Ala genotype. From the study results, we propose that, there is a doubling of both hyperglycemia and dyslipidemia among Ala/Ala genotypes. Moreover, hyperglycemia induces the production of reactive oxygen species (ROS) through the auto oxidation of glucose. These ROS irreversibly damages several bio molecules which include lipoproteins and convert them to lipid peroxides.

The well known lipid peroxide end product is malondialdehyde (MDA), which is irreversibly bound to several bio molecules and damages them. In the present study all the subjects were found to be poor glycemic status (FBG-150±51 mg/dl) and which positively correlated with MDA levels (r=48 in Thr/Thr+Thr/Ala genotypes & r=30 in Ala/Ala genotypes). On the other hand, plasma levels of MDA were significantly negatively correlated with PON activity ($r= -0.25$ & $r= -0.23$). In the present study, PON activity is mostly associated with higher HDL_3:HDL_2 ratio among the Thr/Thr+Thr/Ala genotypes, this indicates that HDL_3 fraction of total HDL is playing a key role in anti-atherogenic function than HDL_2. In the present study, we also determined the serum activity of SOD in all subjects; we did not find any significant difference between the groups. However the SOD activity positively correlates with BPON activity and negatively correlating with both FBG and MDA levels in all the subjects. The similar results were shown in our previous study.[13] Therefore, we propose that, Ala/Ala genotype with lower D2 activity would decrease relative concentration of T_3 in situ and could create a state of intracellular hypothyroidism, decreasing the expression of genes involved in energy, lipid metabolism and exacerbate the diabetic complications leading to risk of lCVD.

Table 1. Genotype and allelic frequency for Thr92Ala SNP of DIO2 gene among the study groups

Groups	Genotype n (%)			Allele (%)	HWE P
	Thr/Thr	Thr/Ala	Ala/Ala		
Controls (n=130)	22 (16.9%)	58 (44.6%)	50 (38.5%)	Thr – 39% Ala – 61%	0.46*
Cases (n=106)	11 (10.4%)	65 (58.5%)	30 (28.3%)	Thr – 41% Ala – 59%	0.006

*indicates the allelic frequency consistent with HWE

Table 2. Distributions of biochemical values according to the genotype

Genotype	Thr/Thr		Thr/Ala		Ala/Ala			
Group	Control	Case	Control	Case	Control	Case	F	P
Parameter								
Age (Y)	58±11	49±9	60±9	55±9	63±9	56±9	-	NS
FBG (mg%)	137±40	150±29	152±61	164±44	138±58	160±43	-	NS
HbA_{1C} (%)	7.5±1	7.1±1.1	7.5±1.0	7.6±1.2	7.6±1.1	7.5±1.2	-	NS
TC (mg%)	152±19	128±35	158±94	141±37	161±40	153±51	-	NS
LDLc (mg%)	83±30	85±14	87±33	87±19	93±26	88±18	-	NS
HDLc (mg%/)	46±4	43±4	46±5	43±5	47±5	41±5	-	NS
HDL_2 (nM/ml)	15.5±1.3	17.9±2.7	15.2±2.4	15.9±3.6	20.3±6.6	30.2±53.2	2.98	0.013*
HDL_3 (nM/ml)	51.7±4.4	55.6±3.1	51.5±6.1	50.9±4	45.6±5.4	42.8±8.1	19.25	< 0.001
ApoA1 (mg%)	114±22	114±22	109±39	122±37	107±27	105±35	115±35	106±30
PON (nM/min/ml)	118±17	126±27	118±18	118±19	104±21	104±19	118±19	104±20
MDA (µM/l)	13±5	11±6	11±6	17±13	11±4	12±7	13±9	11.6±5.1
TSH (µIU/ml)	2.89±1.92	2.29±1.02	2.48±1.48	2.18±0.99	4.78±3.1	6.0±6.21	2.45±1.42	5.23±4.53
Total T_3 (ng/ml)	1.09±0.12	0.83±0.14	1.07±0.13	0.86±0.16	0.81±0.15	0.81±0.12	0.96±0.18	0.81±0.14
TT_3:TT_4	0.21±0.05	0.18±0.06	0.22±0.05	0.19±0.07	0.13±0.05	0.16±0.07	0.20±0.06	0.14±0.06
TotalT_4 (µg%)	5.26±0.91	5.01±1.47	5.19±1.17	5.08±1.47	7.17±2.06	6.24±3.62	5.18±1.27	6.82±2.76
D2 (ng/ml)	169±36	142±27	179±32	150±21	131±30	107±47	161±32	122±39
SOD (IU/ml)	12±3.7	11.5±3.2	11.6±4.8	9.0±2.9	11.1±4.	8.7±2.4	10.5±3.9	10.2±3.8

* P< 0.05, ** P<0.005, NS – not significant

Table 3. Pearson correlation (r) between selected parameters among the genotypes

Correlation (r)	Thr/Thr+Thr/Ala (n=156)	Ala/Ala (n=80)
D2 vs TSH	-0.37*	-0.38*
D2 vs T_3:T_4	0.55*	-0.35*
TSH vs T_3:T_4	-0.11	0.015
T_3:T_4 vs HDL_3:HDL_2	0.33*	-0.16
HDL_3:HDL_2 vs PON	0.36*	0.20
FBG vs MDA	0.48*	0.30*
PON vs MDA	-0.25*	- 0.23*
PON vs SOD	0.21*	0.29*
PON vs ApoA1	0.26*	0.27*

* p<0.05

Table 4. Linear regression analysis between selected independent and dependent variables among the genotypes

		R^2		Adjusted R^2		SEE		Beta		F		P	
Constant	**D.V**	**A/A + A/G**	**G/G**	**A/A + A/G**	**G/G**	**A/A + A/G**	**G/G**	**A/A + A/G**	**G/G**	**A/A + A/G**	**G/G**	**A/A + A/G**	**G/G**
	TSH	0.133	0.148	0.127	0.137	1.32	4.2	-0.37*	-0.39*	23.7	13.6	<0.0001	<0.0001
D2	**T_3:T_4**	0.299	0.165	0.294	0.154	0.05	0.055	0.547*	-0.41*	65.7	15.41	<0.0001	<0.001
	fT_3:fT_4	0.106	0.003	0.10	-0.009	0.75	0.464	0.326*	-0.06	18.34	0.24	<0.0001	0.62
T_3:T_4		0.12	0.017	0.11	0.004	0.69	1.69	0.35*	-0.13	21.12	1.36	<0.0001	0.24
fT_3:fT_4	**HDL_3: HDL_2**	0.004	0.0005	-0.002	-0.012	0.733	1.69	-0.07	-0.02	0.7	0.039	0.4	0.84
HDL_3: HDL_2	**PON**	0.138	0.045	0.133	0.033	18.15	19.68	0.372*	0.213	24.78	3.7	<0.0001	0.058
PON	**MDA**	0.088	0.04	0.082	0.028	8.6	5.006	-0.30*	-0.20	14.85	3.27	<0.001	0.074

D.V- dependent variable; R^2 – coefficient of determination; SEE – Standard Error of Estimate; F – Fisher's ratio of ANOVA
*p<0.05

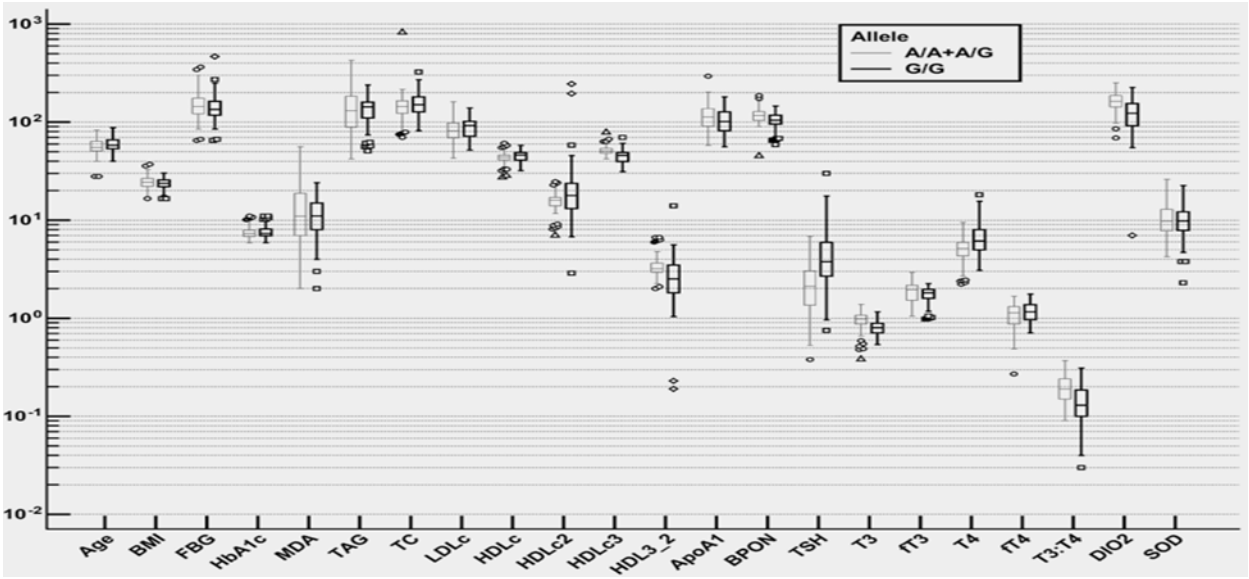

Fig 1. Box and whisker plot of the parameters among Thr/Thr+Thr/Ala and Ala/Ala genotypes

Conclusion

Ala/Ala mutant homozygosity for D2 Thr92Ala polymorphism is associated with decreased phenotypic expression of D2 activity and altered thyroid profile which resembles "intrinsic thyroid disease".

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