**Cloud Publications**
International Journal of Advanced Diabetes Research
2013, Volume 1, Issue 1, pp. 09-15, Article ID Med-76

Research Article | Open Access

# Association of MT2A Gene Polymorphism in the Patients Suffering from Type 2 Diabetes Mellitus and Dilated Cardiomyopathy

**Kinnari N. Mistry, Sanjay Lal, Anjali Thakkar and Avani Solanki**

Department of Medical Biotechnology, Ashok and Rita Patel Institute of Integrated Study & Research in Biotechnology and Allied Sciences (ARIBAS), New Vallabh Vidyanagar, Anand, Gujarat, India

Correspondence should be addressed to Kinnari N. Mistry, kinnarinmistry@yahoo.com

Publication Date: 10 July 2013

Article Link: http://medical.cloud-journals.com/index.php/IJADR/article/view/Med-76



**Abstract** Metallothioneins (MTs) are a group of intracellular metal-binding and cysteine-enriched proteins and are highly inducible in many tissues in response to various types of stress. The aim of the present study was to evaluate the role of MT2A gene polymorphism as a risk factor in the pathogenesis of cardiomyopathy. Molecular analysis was done in 15 patients with dilated cardiomyopathy, 20 diabetic patients without clinical evidence for cardiovascular disease. There was a significant difference between the control group and T2DM group [p value=0.048, odd ratio=3.857, 95% confidence interval (0.998-16.083)]. There was a significant difference between the control group and the dilated cardiomyopathy group [p value= 0.032, odds ratio= 4.500, 95% confidence interval (1.092-19.888)]. We found a significantly higher AA genotype frequency for MT2A in patients with CAD compared with NCVDDP patients and significantly higher AG+GG genotype frequency in diabetic patients. These finding suggest that MT2A Gene Polymorphism is associated in the patients suffering from Type 2 Diabetes Mellitus and Dilated Cardiomyopathy.
**Keywords** *Diabetes Mellitus, Dilated Cardiomyopathy, Metallothionein, Single Nucleotide Polymorphism*

## 1. Introduction

Diabetes mellitus (DM), long considered a disease of minor significance to world health, is now taking its place as one of the main threats to human health in the 21st century [9]. “A myocardial disorder in which the heart muscle is structurally and functionally abnormal, in structural abnormality heart muscle characterized by abnormal finding of chamber size and wall thickness and in functional contractile abnormal findings mainly systolic or diastolic dysfunction in the absence of coronary artery disease, hypertension, valvular disease and congenital heart disease sufficient to cause the observed myocardial abnormality” [2]. Mainly dilated cardiomyopathy is associated with the inflammation and other diabetes complications. During inflammatory conditions or oxidative stress, MTs transfer zinc to other or apoproteins and activate zinc dependent antioxidant systems or zinc dependent signaling

pathways [3, 4]. It has also been shown that MTs play significant role in the pathogenesis of arterial hypertension or diabetes mellitus [5, 8, 11, 12,].

MT isoforms are classified based on various factors like molecular weight, metal which bind, encoded genes, chromosomes, binding atoms, amino acids environment etc. Broadly it is classified as major and minor groups. The major groups are MT-1 and MT-2; these are the unique structure which is identical for the two major isoforms binds 7g atoms of divalent metals like zinc and cadmium. The MT-3 and MT-4 are minor isoforms which are normally found in specialized cells. The MT-3 protein was first isolated as a growth inhibiting factor (GIF) from brain neurons, and the MT-4 protein was found in stratified epithelium [1]. Genetic significant of metallothionein 2A gene related with prevalence ischemic cardiomyopathy patients affected by carotid artery stenosis and type 2 diabetes mellitus [10].

## 2. Materials and Methods

### 2.1. Genomic DNA Extraction

Peripheral blood leucocytes taken from controls and patients were used for genomic DNA extraction by salting out extraction procedure [13]. Genotypes were determined by a Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism Analysis (RFLP) Technique.

### 2.2. Molecular Analysis of -209 A/G MT2A Polymorphism

The locus -209 in the promotor region of human MT2A gene corresponding to A/G transition was studied. This SNP (PubMed References rs1610216) could be found in dbSNPdatabase http://www.ncbi.nlm.nih.gov/SNP

### 2.3. PCR and RFLP Reaction Condition for -209A/G MT2A Polymorphism

The primer sequences for -209 MT2A polymorphism is sense 5’ GGC TCA GGT TCG AGT ACA GG and antisense 5’ AAG TCA CTT GCG GCT CCA [10].

PCR mixture at final volume 15 microliters was composed from genomic DNA samples (100ng), 10mMol dNTPs, 10x taq polymerase buffer, 250 nMol of each primer per lit, 3U of taq polymerase (Genie Bangalore). Amplification was performed by initial incubation at 95°C for 8 min and the following 35 cycles were at 95°C for 45 sec, 63.2°C for 45 sec, 72°C for 1.30 min and final extension at 72°C for 10 min. PCR products were digested with 3U *Sma I* (New England Biolabs, UK) at 25°C for 3 hours. After digestion of the PCR products of 247 bp fragments for AA homozygotes, 131 bp and 115 bp for GG homozygotes and all three fragments for heterozygotes were registered by separating on 3% agarose gel containing 0.5 µg/ml ethidium bromide and visualized with UV light.

### 2.4. Statistical Analysis

Statistical analysis involves determination of genotypic and allelic frequencies which were performed using the statistical package SPSS version 12. Allele frequencies were deduced from genotype frequencies. The differences in alleles and genotype between the groups were tested by Fischer’s exact test. The clinical variables were examined by one way ANOVA.

## 3. Results and Discussion

The human peripheral blood samples of 45 individuals were collected belonging to three categories in this Group-1 control(c), Group-2 diabetes mellitus (T2DM), Group-3 dilated cardiomyopathy. Table 1.1 indicates biochemical parameters of different study groups. Samples are isolated and then amplified by particular primers and visualized under 2% agarose gel by using ethidium bromide staining which is shown in Figure 1.2 and it contains different lanes l1 to l8 which contains different samples like l1 and l2 contain control individuals, l3, l4, l5 contains DCM samples then l7 and l8 lanes contain T2DM samples and 100bp marker run in to the l6 lane which is run for the analyzed the specific band size. The band size of the samples is 247bp which is mention in Figure 1.2. After the amplification process restriction digestion of the DNA samples done by specific enzyme at specific temperature and for specific time. After the digestion process the samples are run in to the 3% agarose gel and visualized under the UV transilluminator which is shown in Figure 1.3. Different lanes contain different type of the samples which contain different band size of the product .For the analyzing the band size DNA ladder is also run with the samples. Three bands are form after the digestions which contain 247bp 131bp and 115bp size which is shown in Figure 1.3. The biochemical values of all the patients belonging to different groups were analyzed by using one-way ANOVA method. P-values <0.005 were considered significant.

The p-values of HbA1c, CRP level, Serum cholesterol, serum triglyceride, HDL, VLDL and urea in comparison of control with other groups were found to be significant. Between control and other two groups the p-values of fasting sugar, postprandial sugar, creatinine, & LDL were insignificant. Similar was the case with the diabetes and dilated cardiomyopathy patients with insignificant p-values for fasting sugar, postprandial sugar, LDL & creatinine. While p-value for serum cholesterol level was found to be significant between control group and T2DM group also in dilated cardiomyopathy group.

Genotyping analysis for the association of control with T2DM, control with dilated cardiomyopathy is a comparison is shown in Table 1.2. The chi-square analysis showed a significant difference in the frequency distributions of the allelic gene for MT2Agene between the control group and dilated cardiomyopathy group ($p$<0.05, Table 1.3). There was a significant difference between the control group and the dilated cardiomyopathy group [$p$ value= 0.032, odds ratio= 4.500, 95% confidence interval (1.092-19.888)].

The distribution of MT2A in the T2DM and dilated cardiomyopathy are summarized in Table 1.2, indicating details of genotyping distribution of MT2A in the control and dilated cardiomyopathy are summarized in Table 1.2, indicating that the point mutation of the A and G allele is significant between control group and dilated cardiomyopathy group. Association between the control and T2DM is significant for MT2A gene (p<0.05). This is indicate that there was significant difference in the frequency distribution of the genotype for MT2A gene between control and T2DM (p>0.05).

In a study performed by Qiangrong Liang and co-workers, they used MT-transgenic mice to test whether an antioxidant protein can reduce dilated cardiomyopathy in the OVE26 transgenic model of diabetes. OVE26 diabetic mice exhibited dilated cardiomyopathy characterized by significantly altered mRNA expression, clear morphological abnormalities, and reduced contractility under ischemic conditions. Diabetic hearts appeared to be under oxidative stress because they had significantly elevated oxidized glutathione. The MT transgene significantly reduced dilated cardiomyopathy in diabetic mice: OVE26MT hearts showed more normal levels of mRNA and glutathione. Typically, OVE26MT hearts were found to be morphologically normal, and elevated MT improved the impaired ischemic contractility seen in diabetic hearts. These results demonstrated that cardiomyocyte-specific expression of an antioxidant protein reduces damage to the diabetic complication [6].

A recent study was performed by Kozarova and co-worker analyzed association of MT2A polymorphism with coronary artery disease and diabetes mellitus in Bulgarian Cohort. Distribution of both genes is in Hardy Weinberg equilibrium for diabetic patients and patients with CAD (coronary artery disease) [7]. The investigations for the second MT2 A/G gene showed that patients with NCVDDP (Diabetic patients without cardiovascular disease) or CAD had significant increase in MT2A AG+GG genotype frequencies compared to control subjects with the same genotype. Diabetic and CAD patients did not show any significant difference in allele frequencies for both A and G alleles in MT2A gene when compared to controls. We also found a significantly higher AA genotype frequency for MT2A in patients with CAD compared with NCVDDP patients and significantly higher AG+GG genotype frequency in diabetic patients. The AA genotype in MT2A polymorphism was previous reported to be associated with ischemic dilated cardiomyopathy in diabetic patients with non-insulin dependent diabetes mellitus. Same authors also described no association between this polymorphism and hypertension in multiple regression analysis [10].

***Table 1.1:*** *Biochemical Parameter of Diabetes and Dilated Cardiomyopathy*

| **Biochemical Parameter** | **Groups** | | |
|---|---|---|---|
| | Group-1<br>Control(n= 10) | Group-2<br>T2DM(n= 20) | Group-3<br>DCM(n= 15) |
| Fasting sugar (mg/dL) | 96.10±3.08 | 121.9±6.38<br>NS[a] | 111.3±8.7<br>NS[a] |
| Postprandialsugar (mg/dL) | 121.9±6.38 | 182.35±9.7<br>NS[a] | 175.8±16.6<br>NS[a] |
| HbA1c (%) | 4.8±0.17 | 7.06±0.85<br>P<0.001[a] | 6.54±0.18<br>P<0.001[a] |
| Serum cholesterol(mg/dL) | 144.4±5.05 | 213.8±6.69<br>P<0.001[a] | 179.9±10.3<br>P=0.020[a\] |
| Serum triglyceride(mg/dL) | 136.7±4.20 | 210.2±11.8<br>P<0.001[a] | 128.1±5.35<br>NS[a] |
| CRP level (mg/dL) | 3.10±0.45 | 3.35±0.30<br>NS[a] | 13.33±1.3<br>P<0.001[a] |
| HDL cholesterol(mg/dL) | 24.80±1.58 | 37.7±3.59<br>P=0.019[a] | 42.60±1.76<br>p<0.005[a] |
| LDL cholesterol(mg/dL) | 117.6±2.96 | 125.7±3.52<br>NS[a] | 116±12.63<br>NS[a] |
| VLDL cholesterol(mg/dL) | 27.54±1.08 | 42.05±2.36<br>P<0.001[a] | 25.86±1.04<br>NS[a] |
| Creatinine (mg/dL) | 1.02±0.04 | 1.13±0.07<br>NS[a] | 1.18±0.03<br>NS[a] |
| Blood urea (mg/dL) | 21±1.26 | 31.3±1.19<br>P<0.001[a] | 27.4±2.05<br>P=0.037[a] |

***Note:*** *In Table 1.1 values represent the mean ± SE. Statistical significance between different groups were evaluated by one way ANOVA method. P values < 0.05 were considered significant. The mean difference is significant at the 0.05 level.*
*Where;*
*a = comparison of control with group T2DM & Dilated Cardiomyopathy*

### 3.1. Amplification of MT2A Gene

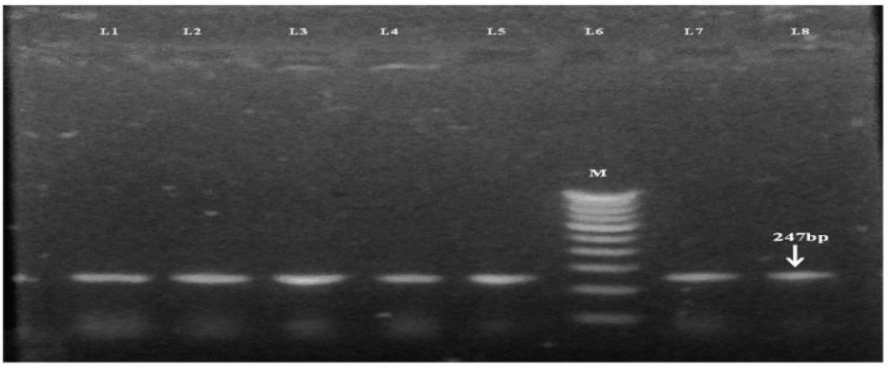


***Figure 1:*** *Amplification Product of MT2A Gene. Where L1 and L2 lanes represents control samples, L3, L4 and L5 lanes represents Dilated cardiomyopathy samples and L7 and L8 lanes represents Type 2 Diabetes samples, L6 lane represents 100bp marker*

### 3.2. Restriction Fragment Length Polymorphism (RFLP) Results

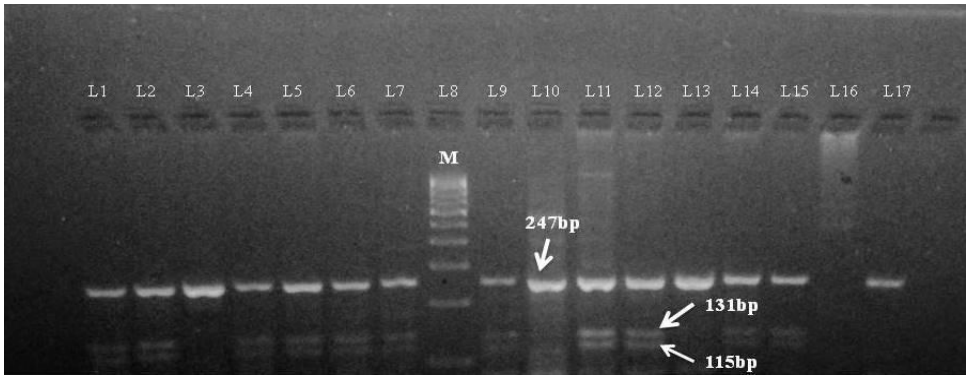


***Figure 2:*** *Restriction Digestion of MT2A Gene on 3% Agarose gel Here, L8 lane is contained 100 bp markers, lanes L1 – L18 Show the Digested Products. Lanes L1, L2, L4 are contain Control samples and lanes L5, L6, L7, L9, L11 are containsT2DM samples and lanes L12, L14, L15, L3, L13 are contains DCM samples*

***Table 1.2:*** *Genotype and Allelic Frequency of All Groups*

| Groups | Genotyping Results | | | Allelic Frequency | |
|---|---|---|---|---|---|
| | AA | AG | GG | A – allele | G – allele |
| Control | 16(80%) | 4(20%) | 0 | 36(90%) | 4(10%) |
| T2DM | 8(40%) | 12(60%) | 0 | 28(70%) | 12(30%) |
| Dilated Cardiomyopathy | 5(33.33%) | 10(66.66%) | 0 | 20(66.66%) | 10(33.33%) |

***Table 1.3:*** *Logistic Regression Analysis of MT2A for Dilated Cardiomyopathy and T2DM*

| Groups | P- Value | CHI.Square Value | Odd Ratio | 95%C.I.Value |
|---|---|---|---|---|
| Control vs. Dilated Cardiomyopathy | 0.032 | 5.833 | 4.500 | 1.092-19.888 |
| Control vs.T2DM | 0.048 | 5.000 | 3.857 | 0.998-16.083 |

***Note:*** *p-value <0.05 odd ratios> 1 and 95% confidence interval > 1 is significant*

## 4. Conclusion

In our study, we found that the single nucleotide polymorphism in MT2A is significant, which determines its role in protecting from dilated cardiomyopathy. Additional studies, including large, population- based case control studies, will provide a better understanding of the protective role of Metallothionein 2A plays in dilated cardiomyopathy risk population.

## Acknowledgement

Authors are grateful to Charutar Vidya Mandal (CVM) Vallabh Vidyanagar, Gujarat, India for providing platform for this research work. We are also thankful to Director, Dr. Pradip Patel of Ashok and Rita Patel Institute of Integrated Study & Research in Biotechnology and Allied Sciences (ARIBAS) for providing the facilities for our research work. We owe reverence to Dr. Asha Ahuja (M.D. Cardiologist) and Dr. Prabhu Ahuja (M.S. General Surgeon), Shivneri hospital Bombay, for their valuable suggestions and help during our research work.

## References

[1] Cherian M. George et al. *Metallothioneins in Human Tumors and Potential Roles in Carcinogenesis*. Mutation Research. 2003. 533; 201-209.

[2] Elliott P., et al. *Classification of the Cardiomyopathies: a Position Statement from the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases*. European Heart Journal. 2008. 29; 270–76.

[3] Inoue K., et al. *Metallothionein as an Anti-Inflammatory Mediator.* Mediators of Inflammation. 2009. 1-7.

[4] Maret W. *A Role for Metallothionein in the Pathogenesis of Diabetes and its Cardiovascular Complications.* Molecular Genetics and Metabolism. 2008. 94; 1-3.

[5] Maret W., et al. *Cellular Zinc and Redox Buffering Capacity of Metallothionein/Thionein in Health and Disease.* Molecular Medicine. 2007. 13; 371-375.

[6] Qiangrong Liang, et al. *Over Expression of Metallothionein Reduces Diabetic Cardiomyopathy.* Diabetes. 2002. 51; 174-181.

[7] R. Kozarova et al. *Association of +1245 A/G MT1A and -209 A/G MT2A Polymorphysms with Coronary Artery Disease and Diabetes Mellitus in Bulgarian Cohort.* Medical Biotechnology. 2011. 26; 1310-2818.

[8] Sowers J.R., et al. *Diabetes, Hypertension, and Cardiovascular Disease*. Hypertension. 1995. 26; 869-879.

[9] Zimmet P. *Globalization, Coca-Colonization and the Chronic Disease Epidemic: can the Doomsday Scenario be averted?* Journal of Internal Medicine. 2000. 247; 301-310.

[10] Giacconi R., et al. *Novel −209A/G MT2A Polymorphism in Old Patients with Type 2 Diabetes and Atherosclerosis: Relationship with Inflammation (IL-6) and Zinc*. Biogerontology. 2005. 6; 407–13.

[11] Apostolova M., et al. *New Competitive Enzyme-Linked Immunosorbent Assay for Determination of Metallothionein in Tissue and Sera.* Talanta, 1998. 46; 325-333.

[12] Apostolova M., et al. *Metallothioneins in Spontaneously Hypertensive Rat Liver*. Japanese Journal of Medical Science & Biology. 1992. 45; 185-198.

[13] Miller S.A., et al. *A Simple Salting out Procedure for Extracting DNA from Human Nucleated Cells*. Nucleic Acids Research. 1988. 16; 1215.

[14] Thirumoorthy N., et al. *A Review of Metallothionein Isoforms and their Role in Pathophysiology*. World Journal of Surgical Oncology. 2011. 9; 54.