Original Article

Association of *Kaphaja* and *Kapha-Pittaja Prakriti* and methylenetetrahydrofolate reductase C677T allele with type 2 diabetes

Archana Gupta, Akhtar Ali[1], Priyadarshini Tewari, Neeraj Kumar Agrawal[2], Rashmi Patel[1], Parameswarappa Shivappa Byadgi

Department of Vikriti Vigyan, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, [1]Center for Genetic Disorders, Faculty of Science, Banaras Hindu University, [2]Department of Endocrinology and Metabolism, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Abstract

Background and Objectives: Type 2 diabetes is a multifactorial disorder that results from the interaction between genetic predisposition and environmental factors. Different *Prakriti* (body constitution) individuals have different susceptibility for the diseases, and this *Prakriti* is determined by both genetic and environmental factor. This study was undertaken to determine the association status of Methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C with type 2 diabetes and *Prakriti*. **Materials and Methods:** After informed consent, 54 patients with type 2 diabetes and 56 individuals as normal controls were analyzed. Their constitution and pathological data were collected and MTHFR C677T and A1298C genotypes were determined. **Results:** *Kapha*/*Kapha-Pittaja Prakriti* were associated and found to be strong risk factors (Chi-square test = 39.67, $P < 0.00001$, odds ratio [OR] = 16.133, 95% confidence interval [CI] = 6.32–41.20) for type 2 diabetes. MTHFR C677T was associated (Chi-square test = 7.743, $P = 0.02$) with type 2 diabetes where the major CC genotype was found to be a risk for type 2 diabetes (OR = 3.78, 95% CI = 1.14–12.45). A1298C was not associated with type 2 diabetes (Chi-square test = 2.264, $P = 0.322$). None of the *Prakriti* was associated with C677T and A1298C variants. **Interpretation and Conclusion:** In the present study, an extremely strong association between *Prakriti* (*Kaphaja*/*Kapha-Pittaja*) and type 2 diabetes ($P < 0.00001$) was detected. The present study gives a strong clue for the association of *Prakriti* (body constitutional) and clinical phenotype.

Keywords: Methylenetetrahydrofolate reductase, *Prakriti*, type 2 diabetes

Introduction

According to Ayurveda, *Dosha* (bio humors) are one of the factors; others are *Dhatu* (bodu tissue) and *Mala* (metabolic waste) responsible for the state of health or diseases. Among these *Vata*, *Pitta* and *Kapha* are the *Sharirika Dosha* (body humors).[1] *Vata* governs movements, *Pitta* is concerned with functions of digestion, metabolism and energy production and *Kapha* governs physical structure, fluid balance and immune response of the body.[2]

The natural constitution of the body (*Prakriti*) is determined by these three *Dosha* at the time of fertilization. One or more of these three *Dosha* may dominate in an individual resulting into seven types of body constitutions (*Prakriti*) i.e. *Vata, Pitta, Kapha, Vata-Pitta, Vata-Kapha, Kapha-Pitta* and *Vata-Pitta-Kapha*. This classification of an individual is based on their physical, physiological and psychological characteristics. Different *Prakriti* individuals have susceptibility for particular disease. As per Sushruta Samhita, *Sharira Sthana* 4/74, *Prakriti* of an individual does not change except when the end of life is approaching.

Prakriti, the inherent characteristic of an individual refers to the genetically determined physical and mental makeup and is determined by (a) sperms and ovum; (b) forming condition of the uterus; (c) food and regimens of the mother during pregnancy; and (d) nature of predominant *Mahabhuta* of the fetus. The fetus gets afflicted with one or more of

Access this article online

Quick Response Code:

Website: www.ayujournal.org

DOI: 10.4103/ayu.AYU_230_18

Address for correspondence: Dr. Parameswarappa Shivappa Byadgi, Department of Vikriti Vigyan, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi - 221 005, Uttar Pradesh, India.
E-mail: psbyadgi@gmail.com

How to cite this article: Gupta A, Ali A, Tewari P, Agrawal NK, Patel R, Byadgi PS. Association of *Kaphaja* and *Kapha-Pittaja Prakriti* and methylenetetrahydrofolate reductase C677T allele with type 2 diabetes. Ayu 2018;39:146-50.

the two *Dosha,* which are dominantly associated with the above-mentioned factors. *Dosha* dominating the sperms and ovum during the time of conception determine *Prakriti* of the individual. Food and regimens of the mother, which aggravates *Dosha* at that time, also determine the physical constitution. The *Dosha* that ultimately emerge as dominant factors actually determine *Prakriti*.[3] Therefore, the knowledge of *Prakriti* may be important for prevention and better management of diseases.

Type 2 diabetes is a multifactorial disorder that results from the interaction between genetic predisposition and environmental factors. The risk for type 2 diabetes is increased when there is a positive family history of the disease. The genes that predispose to type 2 diabetes are poorly identified, but recent genome-wide association studies have identified several genes that contribute smaller risks to type 2 diabetes (relative risk, 1.1–1.5).[4] The extrinsic factors include advanced age, obesity, diet, sedentary lifestyle and increasing urbanization. The incidence of the disease is continuously increasing in developing countries like India. The World Health Organization (2003) has predicted that by 2030 the number of adults with diabetes will be almost doubled worldwide, from 171 million in 2000 to 366 million in 2030.[5] Methylenetetrahydrofolate reductase (MTHFR) is an enzyme involved in folate metabolism. It catalyzes the reduction of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a cosubstrate for the transformation of homocysteine to methionine through a transmethylation pathway. The gene encoding MTHFR is located on chromosome 1 at 1p36.6.[6] Reduction in the activity of MTHFR caused due to C677T change is known to be risks for various diseases. The T allele is also known to be associated with the elevated levels of plasma homocysteine which is also individually risks for many diseases.[7] MTHFR polymorphisms have been linked to type 2 diabetes and micro/macrovascular complications.[8] Numerous studies have demonstrated associations between MTHFR polymorphisms (C677T and A1298C) and risk of type 2 diabetes.[8,9]

MTHFR C677T and A1298C genetic variants have been found to be associated with type 2 diabetes both Indian as well as in other populations in the world.[10-13] The results are variable. These variants lead to an increase in homocysteine level and are a risk for various conditions including vascular diseases, ischemic stroke which in turn may increase the risk for type 2 diabetes.[14,15]

The present study also aims to determine the association of *Kapha Prakriti* with type 2 diabetes.[16,17] It also aims to determine whether the *Kaphaja Prakriti* have an association with MTHFR C677T and A1298C variants or not.

Aims and objectives of the study

The objectives of the study are (1) to determine whether MTHFRC677T and A1298C alleles are associated with *Madhumeha* (type 2 diabetes) or not, (2) to determine whether *Madhumeha* (type 2 diabetes) is associated with *Prakriti* or not, (3) to determine whether MTHFRC677T and A1298C alleles are associated with *Prakriti* or not.

Materials and Methods

The study was approved by the Ethical Committee of the Institute, faculty of Ayurveda, BHU, Varanasi, and informed consent was taken from each of the participants. A total of 54 type 2 diabetes patients (36 males and 18 females) and 56 normal controls (34 males and 22 females) (matched on geographic origin ethnicity, gender, age, environmental conditions and socioeconomic status) were registered for the study. The age of cases and controls were between 25 and 70 years and the median age of cases and controls was 54 and 44 years. The individuals were registered from the outpatient department and inpatient department and clinical laboratory of the hospital of faculty of Ayurveda, BHU, Varanasi. Detailed clinical examination and medical history were recorded. Additional investigations including plasma glucose level, lipid profile, hemoglobin A1c and urine investigations were also performed. *Prakriti* of each of the individuals was determined according to questionnaire-based scoring (Cronbach's alpha reliability of the selected questions after *Prakriti* assessment). Peripheral blood (3–5 ml) was collected for DNA isolation. MTHFR C677T, as well as A1298C genotypes of each of the participants, were determined by PCR followed by restriction digestion using the protocol as described in Kumari *et al*.[15] Statistical analysis was performed using computer programs quatpsy.org for calculation of Chi-square test and *P* value and hutchon.net for calculating odds ratio.

The selection of cases was done on the basis of fulfillment of diagnostic criteria of *Madhumeha* (type 2 diabetes).

Inclusion and exclusion criteria

Inclusion criteria

1. The age of patients between 25 and 75 years
2. Patients who fulfilled the criteria of diagnostic features of *Madhumeha* (type 2 diabetes)
3. Both male and female patients.

Exclusion criteria

1. Patients with type 1 diabetes
2. Patients with type 2 diabetes who were insulin dependent
3. Patients above the age of 75 years.

Results

Association analyses have been performed between different groups as follows:

Association analysis between *Prakriti*-type and type 2 diabetes

Association analysis was performed between various *Prakriti* - types and type 2 diabetes, where an extremely strong association between *Kaphaja*/*Kapha-Pittaja Prakriti* and type 2 diabetes was observed [Table 1].

Association analysis between methylenetetrahydrofolate reductase C677T and A1298C variants and type 2 diabetes

The present study revealed a significant association of MTHFR C677T single-nucleotide polymorphisms (SNP) with type 2 diabetes (Chi-square test = 7.743, $P = 0.02$) where its major genotype "CC" is associated (and not the minor allele) with type 2 diabetes (odds ratio [OR] =3.78, 95% Confidence interval = 1.14–12.45) in the population studied [Table 2]. Whereas, MTHFR A1298C was not associated with type 2 diabetes (Chi-square test = 2.264, $P = 0.322$) [Table 3].

Association analysis between methylenetetrahydrofolate reductase C677T and A1298C variants and *Prakriti*-type

No association was found between any of the *Prakriti* - types and MTHFR C677T as well as A1298C variants [Table 4].

Discussion

Methylenetetrahydrofolate reductase C677T and A1298C variants and type 2 diabetes

Being a multifactorial disease, type 2 diabetes is caused due to more than one genetic risk factors together with the effect of extrinsic factors. So far, there have been several genetic risk factors identified for the type 2 diabetes and still being unravelled.

Table 1: Frequencies of K/KP and non-K/KP in type 2 diabetes cases and controls

Prakriti	Cases (%) ($n=54$)	Controls (%) ($n=56$)
K/KP	44 (81.48)	12 (21.43)
Non-K/KP	10 (18.52)	44 (78.57)
χ^2	39.67	Reference
P (df=1)	<0.00001	Reference
K/KP versus non-K/KP or	16.133	Reference
95% CI	6.32-41.20	Reference

CI: Confidence interval, K/KP: *Kapha/Kapha-Pittaja*

Table 2: Allelic and genotypic frequencies of methylenetetrahydrofolate reductase C677T in type 2 diabetes cases and controls

Frequencies	T2D cases	Controls
Allele	108	112
C	103 (95.37)	99 (88.39)
T	5 (4.62)	13 (11.60)
χ^2	3.563	Reference
P (df=1)	0.059	Reference
Genotype	54	56
CC	50 (92.6)	43 (76.78)
CT	3 (5.55)	13 (23.21)
TT	1 (1.85)	0 (0.00)
χ^2	7.743	Reference
P (df=2)	0.02*	Reference
CC versus (CT + TT)		
OR	3.78	Reference
95% CI	1.14-12.45	Reference

OR: Odds ratio, CI: Confidence interval, T2D: Type 2 diabetes

The frequency of T allele of MTHFR *C677T* observed in type 2 diabetes cases was lower than that of normal controls. Although this difference was not significant ($P = 0.059$). Other studies on Indian populations have also documented the lower frequency of this allele.[14-17] The frequency of MTHFR gene mutation is quite variable in different geographic and ethnic groups. The genotype frequency of MTHFR C677CC described in the different populations fluctuates between 40% and 49% as in type 2 diabetes cases of Turkey (49%), Tunisia (45%), Brazil (46%) and China (44%).[8,16-18] In the present study, the CC frequency was 92.6%.

There are a very few studies on MTHFR and type 2 diabetes.[18,19] Variable results are found for the association of MTHFR *C677T* with type 2 diabetes. This variability could be due to population-specific genetic variations and effect of their environment. Most of the studies have shown T allele as a risk for type 2 diabetes in Caucasian populations[20] and found a significant association between MTHFR *C677T* polymorphism and type 2 diabetes in Moroccan population with a significant difference in the T allele frequency between the diabetic and control groups (26.06% vs. 33.20%, respectively). However, this group also did not find an association between *A1298C* polymorphism and type 2 diabetes. Lunegova *et al.* studied among ethical Kirghizes that the frequency of CT and TT genotypes was significantly higher in patients with insulin resistance than in controls ($P = 0.027$).[21] 677T allele was also associated with obesity, hypertriglyceridemia and low level of high-density lipoprotein cholesterol. A study from Taiwanian population has shown that T is not a risk to type 2 diabetes. No significance difference in the distribution of MTHFR genotypes between healthy and type 2 diabetes individuals is found in Taiwanese patients.[11] Besides, no significant associations between lipid/glucose metabolic indices with MTHFR genotypes among diabetic patients are observed. Whereas similar to results; a study from Indian population has shown that CC is a risk for type 2 diabetes.[19] This suggests that probably CC has different effects depending on other genetic modifiers and extrinsic factors.

Prasher B and others observed that individuals from the three (*Vata, Pitta* and *Kapha*) most contrasting constitutional types exhibited striking differences with respect to biochemical and hematological parameters and at genome-wide expression levels.[22] They also reported that biochemical profiles such as liver function tests and lipid profiles and hematological parameters like hemoglobin levels exhibited differences between *Prakriti* types. Thus, they concluded that Ayurveda-based method of phenotypic classification of extreme constitutional types may be utilized to uncover genes that may contribute to physical, physiological and psychological differences in normal individuals and their susceptibility to the diseases.

Table 3: Allelic and genotypic frequencies of methylenetetrahydrofolate reductase A1298C in cases and controls

Frequencies	Cases	Controls
Allele	106	102
A	41 (38.68)	49 (48.04)
C	65 (61.32)	53 (51.96)
χ^2	1.855	-
P (df=1)	0.173	-
Genotype	53	51
AA	9 (16.98)	15 (29.41)
AC	23 (43.39)	19 (37.25)
CC	21 (32.00)	17 (33.33)
χ^2	2.264	-
P (df=2)	0.322	-

Table 4: Allelic and genotypic frequencies of methylenetetrahydrofolate reductase C677T and A1298C in cases and controls of two *Prakriti* groups

Frequencies	Case		Control	
	K/KP	Non-K/KP	K/KP	Non-K/KP
C677T				
CC	41	9	10	33
CT	2	1	2	11
TT	1	0	0	0
χ^2	0.673	-	0.367	-
P (df=2)	0.714	-	0.544	-
A1298C				
AA	8	0	4	11
AC	19	5	4	15
CC	13	6	3	14
χ^2	3.333	-	0.388	-
P (df=2)	0.189	-	0.824	-

MTHFR A1298C shows variable results in different studies. In the present study, it was not associated with type 2 diabetes.

Prakriti and type 2 diabetes

In the present study, an extremely strong association between *Prakriti* (*Kapha*/*Kapha-Pittaja*) and type 2 diabetes ($P < 0.00001$) was detected. This is the first study at the molecular level that confirms the *Kaphaja Prakriti* individuals are highly prone for diabetes. There is one more report confirming the association of *Prakriti* with clinical phenotypes (cardiovascular risk factors, inflammatory markers, and insulin resistance).[23] Mahalle *et al.* and the present study gives a strong clue for the association of *Prakriti* (body constitutional type) and clinical phenotype.[24] This will strongly help in the prevention and management of type 2 diabetes. This could be because these variants may not contribute to the constitution of *Prakriti* type, at least in this population. It is further required to test the association of *Prakriti* and genetic make-up at the whole genome level.

Conclusions

In present study, the major 'C' allele showed an association with type 2 diabetes in the present Indian population studied. However, no significant association was found between another SNP MTHFR A1298C and type 2 diabetes in this study. MTHFR variants did not show association with the *Prakriti* in the present study.

Limitations and further scope

The analysis on larger sample size from other Indian cohorts on type 2 diabetes is required to confirm the association findings. The inclusion of samples from other cohorts of Indian populations should also be incorporated in further studies on larger scale.

The present study will provide a way to further analyze the association status of MTHFR SNPs with type 2 diabetes as well as *Prakriti*.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Hankey A. A test of the systems analysis underlying the scientific theory of Ayurveda's Tridosha. J Altern Complement Med 2005;11:385-90.
2. Dwarakanath C. The Fundamental Principles of Ayurveda. Part III. Reprint edition. Varanasi, India: Krishnadas Academy; 2009. p. 102.
3. Sharma RK, Bhagvan D, editor. Charaka Samhita of Agnivesha. Vimana Sthana. Ch. 8, Ver. 95. Reprint Edition. Varanasi: Chowkhamba Prakahsan; 2007. p. 262.
4. Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J. Diabetes Mellitus. Harrison's Principles of Internal Medicine. James H. McGraw John A. Hill., Pennsylvania Plaza, New York City; 2012. p. 2968-3002.
5. Boon NA, Colledge NR, Walker BR. Diabetes mellitus. In: Davidson's Principles & Practice of Medicine. Edinburgh, UK: Churchill Livingstone Elsevier; 2006. p. 805-47.
6. Prescott NJ, Winter RM, Malcolm S. Maternal MTHFR genotype contributes to the risk of non-syndromic cleft lip and palate. J Med Genet 2002;39:368-9.
7. Mann NJ, Li D, Sinclair AJ, Dudman NP, Guo XW, Elsworth GR, *et al.* The effect of diet on plasma homocysteine concentrations in healthy male subjects. Eur J Clin Nutr 1999;53:895-9.
8. Settin A, El-Baz R, Ismaeel A, Tolba W, Allah WA. Association of ACE and MTHFR genetic polymorphisms with type 2 diabetes mellitus: Susceptibility and complications. J Renin Angiotensin Aldosterone Syst 2015;16:838-43.
9. Zhang D, Zhou Y, Han L, Ji H, Li J. The effect of MTHFR C677T polymorphism on type 2 diabetes mellitus with vascular complications in Chinese Han population: A meta-analysis. Endocr J 2014;61:717-26.
10. El-Baz R, Settin A, Ismaeel A, Khaleel AA, Abbas T, Tolba W, *et al.* MTHFR C677T, A1298C and ACE I/D polymorphisms as risk factors for diabetic nephropathy among type 2 diabetic patients. J Renin Angiotensin Aldosterone Syst 2012;13:472-7.
11. Chang YH, Fu WM, Wu YH, Yeh CJ, Huang CN, Shiau MY, *et al.* Prevalence of methylenetetrahydrofolate reductase C677T and A1298C polymorphisms in Taiwanese patients with type 2 diabetic mellitus. Clin Biochem 2011;44:1370-4.
12. Sun JZ, Xu Y, Lu H, Zhu Y. Polymorphism of the methylenetetrahydrofolate reductase gene association with homocysteine and ischemic stroke in type 2 diabetes. Neurol India 2009;57:589-93.

13. Al-Rubeaan K, Siddiqui K, Saeb AT, Nazir N, Al-Naqeb D, Al-Qasim S, *et al.* ACE I/D and MTHFR C677T polymorphisms are significantly associated with type 2 diabetes in Arab ethnicity: A meta-analysis. Gene 2013;520:166-77.
14. Sukla KK, Raman R. Association of MTHFR and RFC1 gene polymorphism with hyperhomocysteinemia and its modulation by Vitamin B12 and folic acid in an Indian population. Eur J Clin Nutr 2012;66:111-8.
15. Kumari P, Ali A, Sukla KK, Singh SK, Raman R. Lower incidence of nonsyndromic cleft lip with or without cleft palate in females: Is homocysteine a factor? J Biosci 2013;38:21-6.
16. Acharya YT, editor. Charaka Samhita by Agnivesha, Sutra Sthana. Ch. 10, Ver. 11. 5th edition. Varanasi: Chaukhamba Prakahsan; 2007. p. 205.
17. Kumar S, Byadgi PS. Critical appraisal of Madhumeha (diabetes mellitus). Int J Res Ayur Pharm 2011;2:687-93.
18. Ali A, Singh SK, Raman R. MTHFR 677TT alone and IRF6 820GG together with MTHFR 677CT, but not MTHFR A1298C, are risks for nonsyndromic cleft lip with or without cleft palate in an Indian population. Genet Test Mol Biomarkers 2009;13:355-60.
19. Abbas S, Raza ST, Ahmed F, Ahmad A, Rizvi S, Mahdi F, *et al.* Association of genetic polymorphism of PPARγ-2, ACE, MTHFR, FABP-2 and FTO genes in risk prediction of type 2 diabetes mellitus. J Biomed Sci 2013;20:80.
20. Benrahma H, Abidi O, Melouk L, Ajjemami M, Rouba H, Chadli A, *et al.* Association of the C677T polymorphism in the human methylenetetrahydrofolate reductase (MTHFR) gene with the genetic predisposition for type 2 diabetes mellitus in a moroccan population. Genet Test Mol Biomarkers 2012;16:383-7
21. Khodaeian M, Enayati S, Tabatabaei-Malazy O, Amoli MM. Association between genetic variants and diabetes mellitus in Iranian populations: A systematic review of observational studies. J Diabetes Res 2015;2015:585917.
22. Lunegova OS, Kerimkulova AS, Turdakmatov NB, Sovkhozova NA, Nabiev MP, Isakova ZhT, *et al.* Association of C677T gene polymorphism of methylenetetrahydrofolate reductase with insulin resistance among Kirghizes. Kardiologiia 2011;51:58-62.
23. Prasher B, Negi S, Aggarwal S, Mandal AK, Sethi TP, Deshmukh SR, *et al.* Whole genome expression and biochemical correlates of extreme constitutional types defined in Ayurveda. J Transl Med 2008;6:48.
24. Mahalle NP, Kulkarni MV, Pendse NM, Naik SS. Association of constitutional type of Ayurveda with cardiovascular risk factors, inflammatory markers and insulin resistance. J Ayurveda Integr Med 2012;3:150-7.