C677t polymorphism of MTHFR and G80A polymorphism of RFC genes and their relation with homocysteine levels in obese tunisian children

Sonia Gara[1], Hanène Ochi[1], Abalo Chango[2], Latifa Najjar[2], Moncef Feki[3], Fattouma B'chir[4], Neziha Kaabachi[3], Saayda Ben Becher S[5], Samir Boukthir[6], Monia Abdennebi[1]

1: Unité de Recherche 04/UR/08-03. Faculté de Médecine. Tunis. Tunisie. - 2: Institut Polytechnique Lasalle, Beauvais, France.
3: Service de Biochimie. Hôpital La Rabta. Tunis. - 4: Laboratoire de radioimmunologie. Institut Pasteur, Tunis.
5- Service PUC. Hôpital d'Enfants. Tunis. - 6: Unité de Recherche 06SP16. Université Tunis El Manar

S. Gara, H. Ochi, A. Chango, L. Najjar, M. Feki, F. B'chir, N. Kaabachi, S. Ben Becher S, S. Boukthir, M. Abdennebi

Les polymorphismes c677t du gène de la MTHFR et G80A du gène RFC et leur relation avec la concentration en homocysteine chez les enfants tunisiens obèses.

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RÉSUMÉ

Buts: Déterminer les fréquences des polymorphismes C677T du gène MTHFR et G80A du gène RFC chez les enfants Tunisiens obèses et non obèses, et étudier leurs relations avec les taux sériques de l'homocystéine, des folates et de la vitamine B12.
Méthodes: Etude ayant concerné 31 enfants obèses et 22 enfants non obèses. Le taux sérique de l'homocystéine a été déterminé par méthode immunofluorescente, et ceux des folates et de la vitamine B12 par méthode radioimmunologique. Les mutations C677T et G80A ont été mises en évidence par pyroséquençage.
Résultats: Nous n'avons pas trouvé de différence significative entre les taux d'homocystéine chez les enfants obèses comparés aux non obèses, (10.34 ± 4.86 μmoll/l vs 11.00 ± 4.26 μmoll/l). Nous ne trouvons par ailleurs pas de différence dans les fréquences des allèles T du gène MTHFR et A du gène RFC, (29.03 % vs 30.95 % ; 64.52 % vs 59.52 %). Les taux moyens d'homocystéine, des folates et de la vitamine B12 ne sont pas corrélés aux génotypes du gène MTHFR et du gène RFC.
Conclusion: Nous avons montré qu'il n'existe pas de corrélations entre les taux d'homocystéine, des folates, de la vitamine B12 et les fréquences alléliques des polymorphismes C677T du gène MTHFR et G80A du gène RFC chez les enfants Tunisiens obèses et non obèses. Il semble que ces deux polymorphismes n'ont pas un impact sur le statut en homocystéine, folates et vitamine B12.

Mots-clés

Obésité, Homocystéine, polymorphisme MTHFR, polymorphisme RFC

SUMMARY

Aims: To investigate the frequencies of C677T polymorphism in MTHFR gene and G80A polymorphism in RFC gene in obese and no obese Tunisian children and to assess their relation with homocysteine (tHcy), folate and vitamin B12 levels.
Methods: We have studied 31 obese compared to 22 no obese children. tHcy was assessed by fluorescence-immunoassay ; folate and vitamin B12 by radioimmunoassay. C677T and G80A mutations were detected using pyrosequencing.
Results: There were no differences in tHcy levels between obese and no obese, (10,34 ± 4,86μmoll/l vs11,00 ± 4,26μmoll/l). We found no difference for the allelic frequencies of the C677T polymorphism (29.03 % vs 30.95 %) and of the G80A polymorphism (64.52 % vs 59.52 %). Mean levels of tHcy, folic acid and vitamin B12 were not significantly different according to MTHFR and RFC genotypes.
Conclusion: We demonstrated no difference in tHcy, folates, vitamin B12 levels and allelic frequencies of C677T and G80A polymorphisms in MTHFR and RFC genes between obese and no obese Tunisian children. These two polymorphisms don't seem to have any impact on homocysteine, folate and vitamin B12 status in the two populations.

Key-words

Obeity, Homocysteine, MTHFR polymorphism, RFC polymorphism

Recent epidemiologic studies have confirmed the well-known positive relationship between body mass index above 27 kg/m2 and cardiovascular morbidity (1). Although atherosclerotic heart disease does not become manifest until adulthood, several studies have clearly shown that the atherosclerotic process begins early in life, long before clinical disease is evident (2, 3, 4). Traditional risk factors explaining increased cardiovascular mortality in obesity include: hypertension, dyslipidemia with high triglycerides and low high density lipoprotein-cholesterol, insulin resistance and hyperinsulinemia which affect blood vessels and contribute to hypertension and coronary artery disease (5). There is ample evidence linking increased concentrations of plasma homocysteine to premature coronary and carotid atherosclerosis. Moreover, hyperhomocysteinemia seems to be an important factor of the obesity-induced early arterial atherosclerosis during childhood (6).

Total homocysteine levels (tHcy) are controlled both by mutations in its regulatory enzymes and by folate and vitamin B status (vitamin B12, vitamin B6). The 5, 10 methylenetetrahydrofolate reductase (MTHFR) is an enzyme that regulates 5-methyltetrahydrofolate, which is required for the methylation of homocysteine to methionine and S-adenosylmethionine, the common methyl donor for the maintenance of DNA methylation. The C to T substitution at nucleotide 677 (677C-T mutation), which leads to an exchange of an alanine for a valine in the gene encoding MTHFR, results in a thermolabile variant that produces a partially defective enzyme. In particular, raised tHcy concentrations have been related to homozygous 677T, especially in the presence of low folic acid concentrations. However, the plasma tHcy concentrations may increase as a result of nutritional deficiencies in essential cofactors
or enzymes substrates, including vitamin B12, folic acid and vitamin B6. The reduced folate carrier gene (RFC) seems to be a candidate gene for 5-methyltetrahydofolate internalization within cells (7). The human RFC gene encodes an integral membrane protein with a molecular weight ranging from 80 to 120 Kda. A common polymorphism at position 80 in exon 2 of RFC has been identified. This polymorphism changes a guanine to an adenine in the gene and its association with 677 C-T mutation has been found to increase tHcy levels in healthy adults (7).

The aim of the present study was to assess tHcy, folates, vitamin B12 levels and frequencies of MTHFR (C677T mutation) and RFC (G80A mutation) polymorphisms in obese children compared to non obese and to compare the plasma levels of tHcy, folic acid and vitamin B12 according to the genotypes in the two populations.

PATIENTS AND METHODS

We investigated 31 obese children, 21 boys and 10 girls aged 56-172 months, with BMI > 97th percentile for age and sex (8) who attended our department for obesity. At the time of the study, none of them was on vitamin or food supplementation, or on weigh reducing diet. The obese patients were compared to a control group of 22 normal weight children. Venous blood samples were taken after an overnight fast. Total homocysteine was analyzed in an Abbott® system according to fluorescence polarization immunoassay technology. Folic acid and vitamin B12 were assessed by radioimmunoassay.

DNA was extracted from white blood cells using the phenol-chloroform method. Pyrosequencing PSQ 96MA instrument (Pyrosequencing AB, Uppsala, Sweden) was used for allele frequency determination. DNA samples were amplified with PCR using primers HsMTH2.0-s: 5'-biotinAGGTTACCCCAAAGGCCACC-3' ; HsMTH2.0-as 5'-GCAAGTGATGCCCATGTCG-3' for MTHFR gene and HsRFC7.0-s5'-TGCAGACCATCTTCCAAGG-3'; HsRFC7.0-as 5'-biotinCCATGAAGCCGTAGAA GC-3' for RFC gene (9). The PCR products were purified using streptavidin-Sepharose HP beads (Amersham Biosciences, Orsay, France) and a Pyrosequencing Sample Preparation Kit. Purified samples were run on to a PSQ 96MA instrument containing a cartridge filled with dATPaS, dTTP, dCTP, dGTP, substrate, and enzyme as supplied in a PSQ Reagent Kit. Analysis of sequences was automatically performed by the Allele Quantification software. The intensity of light signal is directly proportional to the number of nucleotides incorporated (10). All measurements are presented as mean ± SD. Student's t-test was used for statistical analysis. P less than 0.05 represent statistical significance.

RESULTS

No significant difference was found for tHcy, folates and vitamin B12 levels between obese and non obese children (table 1). The two groups, obese and non obese, show no difference for the allelic frequencies of the C677T polymorphism of MTHFR (C: 70.97 % vs 69.05 %, p = 0.83; T: 29.03 % vs 30.95%, p = 0.83) nor of the G80A polymorphism of RFC gene (G: 35.48 % vs 40.48 %, p = 0.60; A: 64.52 % vs 59.52 %, p=0.60). Tables 2 and 3 show the genotypic frequencies of the MTHFR and RFC polymorphisms.

Mean levels of tHcy, folates and vitamin B12 were not significantly different according to MTHFR (Table 4) and RFC genotypes (Table 5).

Table 1 : Mean levels of tHcy, folic acid and vitamin B12 among obese and non obese children

	Obese	No Obese	p
tHcy (μmol/l)	10.34 ± 4.86	11.00 ± 4.26	0.61
Folates (nmol/l)	13.08 ± 13.51	12.28 ± 13.82	0.84
Vitamin B12 (pmol/l)	499 ± 383	666 ± 402	0.15

Table 2 : Genotypic frequencies of C677 polymorphism in MTHFR gene in obese and no obese children

Genotypes	Frequency (%)		p
	Obese	No obese	
CC	48.4	42.86	0.69
CT	45.2	52.38	0.60
TT	6.5	4.76	0.72

Table 3 : Genotypic frequencies of G80A polymorphism in RFC gene in obese and non obese children

Genotypes	Frequency (%)		p
	Obese	No obese	
GG	12.9	14.29	0.78
GA	45.2	52.38	0.60
AA	41.9	33.33	0.53

Table 4 : Mean levels of tHcy, folates and vitamin B12 according to MTHFR genotypes

	CC	CT	TT
tHcy (µmol/l)			
Obese	10.84 ± 4.87	9.16 ± 4.79	14.49 ± 4.62
Non obese	10.9± 5.07	11.33± 4.13	10.05
p	0.97	0.25	-
Folates (nmol/l)			
Obese	12.91 ± 13.85	12.85 ± 14.19	3.36 ± 4.57
Non obese	18.77 ± 18.54	8.13 ± 6.39	5.3
p	0.42	0.32	-
Vitamin B12 (pmol/l)			
Obese	537 ± 421	534 ± 374	207 ± 122
Non obese	459 ± 176	723 ± 430	1162
p	0.46	0.23	-

Table 5 : Mean levels of tHcy, folates and vitamin B12 according to RFC genotypes

	GG	GA	AA
tHcy (µmol/l)			
Obese	14.58 ± 6.23	10.59 ± 5.70	9.42 ± 3.55
Non obese	14.67 ± 3.05	11.92 ± 4.43	8.23 ± 3.20
p	0.98	0.53	0.46
Folates (nmol/l)			
Obese	15.80 ± 20.11	15.10 ± 11.49	10.31 ± 14.50
Non obese	29.33 ± 28.95	9.12 ± 6.62	12.7 ± 13.42
p	0.52	0.14	0.74
Vitamin B12 (pmol/l)			
Obese	643 ± 606	407 ± 317	553 ± 399
Non obese	511 ± 193	585 ± 405	758 ± 364
p	0.79	0.26	0.31

DISCUSSION

We demonstrated no significant difference in tHcy, folates and vitamin B12 levels between obese and non obese Tunisian children. The relationship between obesity and homocysteine is not clear. Some reports show no difference in tHcy levels between obese and non obese children (11, 12, 13) whereas others reported a significant difference between the two groups (14, 15). Homocysteine levels are the result of various nutrient and genetic factors effects. The most common cause of mildy elevated homocysteine concentrations is a deficiency in some nutrients that regulate homocysteine metabolism. We demonstrated neither folic acid, nor vitamin B12 deficiency in our patients. In studies conducted in adults and children, folic acid levels correlates inversely with homocysteine and contribute independently and significantly to the variance in homocysteine (16).

It was reported that folic acid itself is inversely related to fat mass and BMI probably because of reduced intake of vegetables in favour of fat (17, 18). Homocysteine levels are also controlled by mutations in its regulatory enzymes. Raised homocysteine concentrations have been related particularly to homozygous C677T in MTHFR gene especially in the presence of low folic acid concentration.

C677T mutation frequencies differ among ethnic populations. T allele frequency ranges from 0.06 to 0.59 (19).

In our series T allele frequency was 0.31 in non obese children which was similar to that reported in some healthy Mediterranean populations (20). However, we found no association between C677T genotype and homocysteine levels as reported by some (21, 22).

The frequency of G80A polymorphism in RFC gene has not been well investigated in different ethnic groups. Chango et al have reported frequencies of 0.52 for G allele and 0.47 for A allele in unrelated French healthy population. It's yet unclear which allele will prove to be more common in different populations.

As shown by Chango et al., G80A genotype did not have notable influence on homocysteine levels but a significant increase was found in doubly homozygous 80GG/677TT subjects. These observations could be useful for further studies on the genetic determinants of homocysteine levels.

Variance in plasma homocysteine levels might also be attributed to insulin, since previous report showed that insulin is a main correlate of homocysteine in obese children and adolescents (16). It was suggested that insulin might regulate homocysteine levels by a possible regulation on genes of transsulfuration enzymes cystathionine ß synthase and cystathionine Á lyase (23).

Thus, it was suggested that fat-mass-associated hyperinsulinism may contribute to impairment of homocysteine metabolism in childhood obesity.

CONCLUSION

This study demonstrates that tHcy, folates, vitamin B12 levels and frequencies of polymorphisms C677T in MTHFR gene and G80A in RFC gene show no difference between obese and non obese Tunisian children.

These two polymorphisms don't seem to have any impact on homocysteine levels in the two populations.

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