The relationship between the C677T polymorphism of the MTHFR gene and serum levels of luteinizing hormone in males with erectile dysfunction

Omar Šerý [1,2], Taťána Šrámková [3], Jitka Klempová [1], František Šťastný [1], Jan Lochman [1], Naim Akhtar Khan [4]

1 Laboratory of Neurobiology and Molecular Psychiatry, Laboratory of Molecular Physiology, Department of Biochemistry, Faculty of Science, Masaryk University, Brno, Czech Republic
2 Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Brno, Czech Republic
3 Trauma Hospital of Brno, Department of Clinical Psychology and Psychiatry, Brno, Czech Republic
4 UPRES EA4183 "Lipides & Signalisation Cellulaire", Université de Bourgogne, Dijon, France;

Correspondence to: Assoc. Prof. RNDr. Omar Šerý, PhD.
Laboratory of Neurobiology and Molecular Psychiatry,
Department of Biochemistry, Faculty of Science, Masaryk University,
Kotlářská 2, 611 37 Brno, Czech Republic.
TEL: +420 549 497 312; FAX: +420 542 213 827; E-MAIL: omarsery@sci.muni.cz

Submitted: 2012-05-31 Accepted: 2012-07-17 Published online: 2012-10-02

Key words: **MTHFR; C677T polymorphism; follicle-stimulating hormone; luteinizing hormone; erectile dysfunction**

Neuroendocrinol Lett 2012; **33**(5):499–504 **PMID:** 23090267 NEL330512A02

Abstract

OBJECTIVES: The methylenetetrahydrofolate reductase (MTHFR) enzyme activity plays an important role in the metabolism of folate within methionine-homocysteine pathway and, consequently, in the development of vascular diseases. The C677T polymorphism (rs1801133) of the *MTHFR* gene affects the MTHFR activity, modifies the homocysteine plasma concentration and, among others, increases the risks for idiopathic male infertility, including erectile dysfunction (ED). As this sexual dysfunction is related to sex hormone levels, we investigated a possible relationship between the C677T polymorphism of the *MTHFR* gene and plasma concentrations of follicle-stimulating hormone (FSH) as well as luteinizing hormone (LH) in male patients with ED.

METHODS: We conducted our study on 90 healthy men with ED between the age of 32 and 61 (mean age was 51.1 ± 11.5) years. The subjects were genotyped and their FSH and LH plasma levels were analysed.

RESULTS: The analysis results of ED patients and their genotypes of the *MTHFR* gene did not provide evidence supporting any causal association of T allele in CT and TT genotypes with studied clinical parameters. However, we found that patients with the CC genotype had significantly higher plasma levels of LH than patients with the CT and/or TT genotypes.

CONCLUSIONS: Our observations suggest that the C677T polymorphism of *MTHFR* gene has no direct relationship to erectile dysfunction, but does exhibit a relationship between this rs1801133 polymorphism and plasma LH concentrations.

ORIGINAL ARTICLE

Abbreviations:

ANOVA	- analysis of variance
BMI	- body mass index
bp	- base pair
C	- Celsius
CSS	- computer statistic software
DNA	- deoxyribonucleic acid
ED	- erectile dysfunction
FSH	- follicle-stimulating hormone
IIEF	- International Index of Erectile Function
IRMA	- immunoradiometric assay
LH	- luteinizing hormone
MTHFR	- methylenetetrahydrofolate reductase
PCR	- polymerase chain reaction
RIA	- radioimmunoassay

INTRODUCTION

The methylenetetrahydrofolate reductase (MTHFR, EC 1.7.99.5) is a key enzyme in the homocysteine-methionine pathway that catalyses the initial conversion of folic acid into the methyl donor, 5-methyltetrahydrofolate (5-methylTHF). Three frequent polymorphisms (C677T, A1298C, and G1793A) of this MTHFR gene results in reduced activity of this enzyme. The 677T allele of the gene has been associated with a thermolabile form of MTHFR whose decreased activity is responsible for a mild hyperhomocysteinemia (Boushey *et al.* 1995). Recent studies have also shown that individuals with 677TT genotype exhibit a greater genetic risk of vascular disease (Boushey *et al.* 1995; Sazci *et al.* 2006), including erectile dysfunction (ED) and male infertility (Lombardo *et al.* 2004; Safarinejad *et al.* 2010; 2011).

The epidemiology of ED has not been rigorously characterized, though it has been reported that, in the USA, there are approximately 18% of men suffering from male infertility (Selvin *et al.* 2007). In fact, the Massachusetts Male Aging Study has reported that prevalence of ED is approximately 52% in US males between 40 and 70 years (Levine 2000). As most of the men are reluctant to discuss ED, more than 70 % of ED cases remain undiagnosed (Müezzinoğu *et al.* 2007). Even though this sexual disorder evidently has a neurogenic/psychosocial and endocrine basis, its most common cause is thought to be related to vascular abnormalities of the penile blood supply, frequently associated with coronary heart disease, limited physical activity and androgen deficiency (Fung *et al.* 2004; Johannes *et al.* 2000; Kostis *et al.* 2005; Traish *et al.* 2007).

In the last several years various studies have also underlined a role of sex hormones in ED. From them it is obvious that androgens are important for the penis growth and for its erectile regulation (Müezzinoğu *et al.* 2007; Traish *et al.* 2007), but in fact no significant correlation was ascertained between serum testosterone levels and ED, while a relationship was found between serum testosterone and FSH as well as between serum FSH and LH in patients with ED (Müezzinoğu *et al.* 2007). In contrast to the male patients, women with MTHFR 677CC genotype showed higher serum estradiol levels than heterozygous or homozygous carriers of the 677T allele (Thaler *et al.* 2006) and recurrent pregnancy loss (Ozdemir *et al.* 2012; Wu *et al.* 2012). From this it is evident that genetic factors could be involved even in male infertility (Gava *et al.* 2011) and/or ED is an endocrine disorder comprising some genetic factors. Therefore, the aim of this study was to evaluate a role of C677T polymorphism of the MTHFR gene in relation to erectile dysfunction and to analyse changes in the FSH and LH hormone levels in ED patients with this polymorphism.

MATERIALS AND METHODS

Subjects

The sample included a total of 90 males of the Czech nationality, aged from 42 to 61 (51.1 ± 7.5) years, with a previously diagnosed erectile dysfunction. All males underwent a routine examination and measurements – blood pressure, BMI, waist circumference and routine laboratory tests. The ED diagnosis was based on sexual history (time of onset and possible cause of ED), somatosexual examination, laboratory tests and the IIEF-5 questionnaire (International Index of Erectile Function) with the total less than 21 points (out of 25) giving evidence of ED. All patients signed an informed consent. The study was approved by the Ethical Committee of the Trauma Hospital of Brno (Czech Republic).

Laboratory analyses

The C677T MTHFR genotype was analyzed by PCR using a forward primer 5`- CAT CCC TAT TGG CAG GTT A and a reverse primer 5`- AGG ATG TGT CAG CCT CAA AGA AA. The amplified DNA was digested by TaqI restriction enzyme at 65 °C for 5 hours. Electrophoresis on agarose gel stained with ethidium bromide was used for the visualization of genotypes. Two fragments at the length of 85 bp and 50 bp occurred in the presence of T allele, unrestricted fragment has the length of 135bp and it revealed to be C allele.

The levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in plasma were determined by kits (Cat. No. IM1381, IRMA, for LH, and Cat. No. IM3301, IRMA, for FSH). The radioimmunoassay (RIA) and immunoradiometric assay (IRMA), according to manufacturer's instruction manual, were followed on STRATEC SR 300 instrument by standardized kits (Beckman-Coulter intended for clinical diagnostics).

Statistics

For statistical analysis, the CSS Statistical software (StatSoft, USA) was used. After testing the hormonal levels for normal distribution, the t-test was used for comparison. One-way analysis of variance (ANOVA) was used for correlation of hormonal levels and genotypes.

RESULTS

Table 1 shows the genotypes of the C677T polymorphism of the MTHFR gene in 90 males. We observed that 47 of them had the CC genotype, whereas 32 had the CT and 11 had the TT genotype. We also determined the plasma levels of luteinizing hormone and follicle-stimulating hormone in the blood of these subjects (Table 1). Hence, we observed a significant positive relationship ($p<0.016$) between the plasmatic levels of LH and the C677T polymorphism (Figure 1). The CC genotype shows a statistically significant association with high level of LH. On the other hand, plasma levels of FSH are not significantly associated with C677T genotype (Figure 2). In the present study, we did not find any direct relationship between ED and the C677T polymorphism of the MTHFR gene. We also did not observe any relationship between the MTHFR gene polymorphism and the age of onset of ED, the cause of ED or the IIEF-5 questionnaire as per International Index of Erectile Function (results not shown). Besides, our results show the correlation between the plasma levels of FSH and LH ($p<0.00001$).

DISCUSSION

Erectile dysfunction (ED) is defined as the inability to achieve and/or maintain an erection required for sexual intercourse. Different severity of this dysfunction, based on the International Index of Erectile Function (IIEF-5) questionnaire, is one of the most common, usually long-lasting health issues, which affect men mainly after 35 years of age. Recent clinical studies have revealed an influence of human MTHFR gene polymorphisms on the idiopathic male infertility and ED (Lombardo *et al.* 2004; Safarinejad *et al.* 2010; 2011; Singh *et al.* 2010). Patients with early onset of vasculogenic ED, with significantly higher serum levels of homocysteine, also exhibited higher frequency of 677TT, 1298CC, and 1793GG genotypes (Safarinejad *et al.* 2010). Odds ratios suggested a possible risk for ED in individuals with the MTHFR 677TT genotype and the 677TT+1298AC combined genotype. The relationship between ED and C677T haplotype has been taken into consideration with respect to an impairment of the vascular wall by means of increased homocysteine concentration and from resulting intima-media thickening accompanied by a reduced blood flow in the erectile tissue of corpora cavernosa (Lombardo *et al.* 2004). However, the relationship between the polymorphism and ED might be modified by some ethnic differences as well as by differences at age and gender that change frequencies of the C677T MTHFR polymorphism in a population (Mayor-Olea *et al.* 2008; Russo *et al.* 2003).

The increased plasma levels of LH in our study is associated with CC genotype, which has been associated with decreased plasma levels of homocysteine (Castro *et al.* 2006). Hence, we assume that the C677T polymorphism might interfere with the vesicle formation and/or exocytosis of LH through the regulation of homocysteine metabolism. Two possible explanation of our observation could be stated. First, the methylation reactions at the intracellular level can be influenced, second, homocysteine itself acts as glutamate agonist/antagonist.

Methylation reactions at the intracellular level can influence LH levels

FSH and LH are non-covalently linked dimers of glycoprotein subunits: the glycoprotein-α subunit is common to both gonadotrophins, and the distinct β-subunits confer specificity to each dimer (Farnworth 1995). Gonadotrophs in the anterior pituitary synthesize and store the gonadotrophins like FSH and LH, which are secreted in accordance with the body's physiological requirements. Gonadotrophins have distinctly different

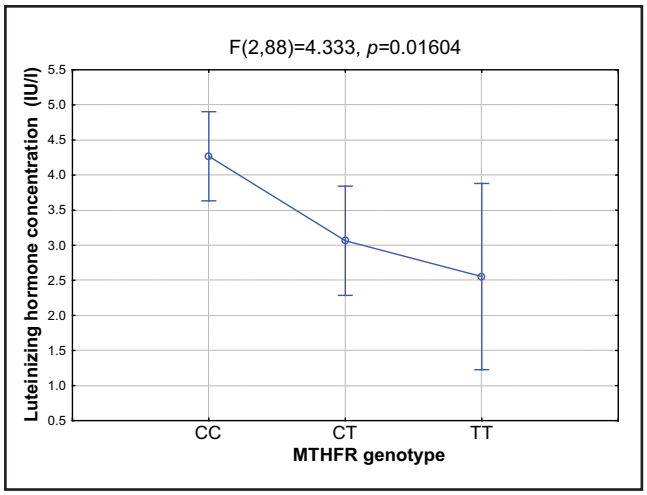

Fig. 1. The relationship between luteinizing hormone concentration in serum and MTHFR genotype.

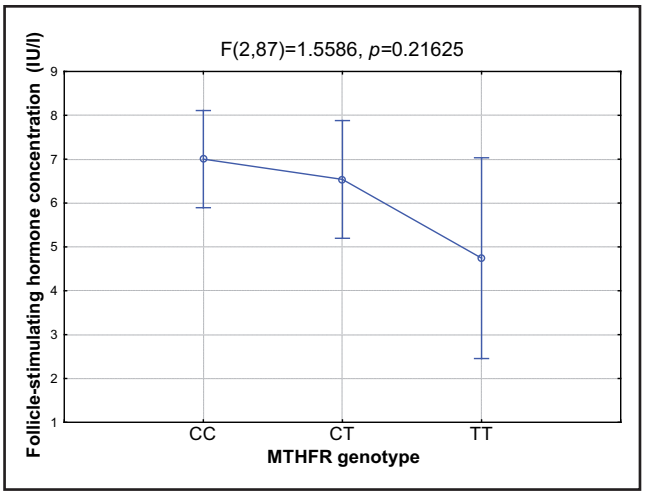

Fig. 2. The relationship between follicle-stimulating hormone concentration in serum and MTHFR genotype.

Tab. 1. LH serum concentration, FSH serum concentration and MTHFR genotype of each sample.

Sample No.	LH concentration (IU/l)	FSH concentration (IU/l)	MTHFR genotype
1	4.9	3.3	CC
2	2.43	3.6	TT
3	4.73	9.1	CT
4	3.14	8.3	CC
5	1.76	6.9	CT
6	4.25	8.2	CC
7	7.7	9.9	CC
8	3.82	13.9	CT
9	2.17	4.6	TT
10	3.83	4.6	CT
11	1.51	1.9	CC
12	7.15	9.71	CC
13	1.2	4	CC
14	3.55	2.5	TT
15	1.98	4.8	CC
16	0.15	1.01	CC
17	2.14	7.97	CT
18	5.25	8.37	CC
19	7.49	15	CT
20	2.10	9.8	TT
21	1.03	5	TT
22	3.84	7.2	CT
23	1.24	11.65	CT
24	1.77	4.15	CC
25	12.28	21.19	CC
26	2.42	0.39	CC
27	0.39	7.12	CC
28	0.9	1.9	TT
29	1.99	4.88	CC
30	0.89	3.57	CC
31	4.11	13.2	CT
32	5.7	9.8	CT
33	3.87	4.2	CT
34	4.6	9.4	CC
35	2.46	3	TT
36	4.2	8.2	CT
37	3.14	5.5	CT
38	3.45	9.1	CT
39	1.49	2.5	CC
40	2.75	5	CT
41	1.96	3.9	CC
42	4.89	8.9	CC
43	3.83	4.6	CT
44	0.7	3.9	CT
45	2.01	6.8	CC
46	3.08	3.48	TT
47	1.37	2.5	CT
48	7.88	8.9	CC
49	3.97	9	CC
50	3.39	5.9	CC
51	4.04	7.9	CT
52	3.14	7.5	CT
53	4.1	3.16	CC
54	4.62	6.9	CC
55	0.2	0.2	CT
56	6.52	6.9	CC
57	2.18	4.6	CT
58	3.36	9.1	CC
59	2.28	2.1	CT
60	4.95	8.2	CT
61	2.02	5.1	CT
62	6.68	9	CC
63	5.4	10.4	CC
64	4.22	8.9	CC
65	2.06	8.2	TT
66	3.52	6.1	CC
67	3.54	4.7	CC
68	4.35	4.3	CT
69	6.3	4.8	CC
70	5.7	6.14	CC
71	5.79	8.9	CC
72	4.51	5.4	CC
73	10.78	22.6	CC
74	3.5	12.9	CC
75	2.29	9.21	CT
76	3.71	6.1	CT
77	2.67	3.55	CC
78	6.9	5.4	TT
79	4.55	9.06	CC
80	2.57	3.5	CC
81	1.41	4.7	TT
82	1.1	5.1	CC
83	0.2	0.2	CT
84	2.32	3	CT
85	5.22	7.4	CC
86	1.98	3.71	CT
87	8	7.7	CC
88	2.43	5	CC
89	2.42	4.8	CT
90	3.21	5.8	CC

patterns of secretion (Nicol *et al.* 2004). The differential pattern of LH and FSH secretion predicts that the two hormones have different storage granules prior to their release. Exocytosis is mediated via the fusion of secretory vesicles to the plasma membranes, thus releasing the hormones to the extracellular environment (Savigny *et al.* 2007). Hence, it is possible that MTHRF may interfere with the exocytosis of LH. Our hypothesis is supported with the observations of Radeke *et al.* (1999) who have reported that *S*-adenosylhomocysteine inhibits secretion of proteins while *S*-adenosylmethionine increases the cellular secretory activity. Indeed, the C677T polymorphism of the MTHFR gene interferes with metabolism of these substances – the MTHFR inhibition leads to homocysteine accumulation and thus to a deficit of recyclable methionine required for *S*-adenosylmethionine production. The diminished MTHFR function would impair 5-MTHF production and thus also the recycling of methionine. Hence, TT genotype and particularly T-allele of the C677T polymorphism of the MTHFR gene will inhibit its enzymatic function and, consequently, would decrease the *S*-adenosylmethionine level, which would participate in decreased exocytosis of hormones. We observed a relationship between the CC genotype and increased plasma levels of LH. The CT genotype in this study is characterized by a lower level of LH.

The question arises: how does the decreased level of *S*-adenosylmethionine interfere with exocytosis? Gonadotropic cells use a number of proteins for the exocytosis of LH, including SNAP-25, munc-18 (Veeraragavan *et al.* 1988), secretogranin II and chromogranin A (Farnworth 1995). It is possible that methylation of proteins might participate in exocytosis same way as inhibitors of methylation decrease the exocytosis of insulin. Hence, a methyl acceptor protein and/or phospholipid play(s) a modulatory role in the coupling of cytosolic Ca^{2+} accumulation to exocytosis (Best *et al.* 1984). Moreover, the secretory proteins like chromogranins A, A1 and A2 in adrenal chromaffin granules were found to be methyl acceptor proteins (Veeraragavan *et al.* 1988).

Homocysteine as glutamate agonist/antagonist

Homocysteine acts as an agonist at the glutamate binding site of the NMDA receptor, but also as a partial antagonist of the glycine coagonist site (Lipton *et al.* 1997). The effect of homocysteine on NMDA receptor is dependent on physiological conditions, e.g. on glycine concentration. It was described that glutamate can stimulate LH secretion but glutamate did not alter FSH secretion (Mahesh & Brann 2005). Homocysteine is potentially neurotoxic by stimulation of glutamate binding site of NMDA receptor. The neurotoxicity of homocysteine was demonstrated at 10-100 micromolar concentrations (Lipton *et al.* 1997). The effect of homocysteine on LH secretion could be explained according to role of NMDA receptor in LH secretion when homocysteine would have similar activity as glutamate. Chronically elevated level of homocysteine could have cytotoxic effect via NMDA receptor activation on cell releasing LH. In our study observed higher concentration of LH in the relationship with CC genotype could be explained by chronically lower homocysteine concentration that could be related to non-cytotoxic effect on LH releasing cells. The situation is similar to our previous finding, when we found the association between protective role of BDNF polymorphism on color vision deficiency of alcohol dependent males based obviously on alcohol/glutamate interplay on neurotoxicity of retinal neurons (Šerý *et al.* 2011).

To sum up, we can state that C677T polymorphism of the MTHFR gene could influence, through its impact on the LH levels, the pathogenesis of different diseases like cardiovascular abnormalities as it has been recently shown that high LH concentrations predict ischemic heart disease events in older men (Hyde *et al.* 2011). However, the role of MHTFR and LH levels in these diseases remains to be explored in future.

ACKNOWLEDGMENTS

This work has been supported by Internal grant agency of the Ministry of Health of the Czech Republic (IGA MZCR) No. NT/11152–6 and by by the Grant Agency of the Czech Republic (309/09/P361).

REFERENCES

1 Best L, Lebrun P, Saceda M, Garcia-Morales P, Hubinont C, Juvent M *et al.* (1984) Impairment of insulin release by methylation inhibitors. Biochem Pharmacol **33**: 2033–2039.
2 Boushey CJ, Beresford SA, Omenn GS, Motulsky AG (1995). A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA **274**: 1049–1057.
3 Castro R, Rivera I, Blom HJ, Jakobs C, Tavares de Almeida I (2006). Homocysteine metabolism, hyperhomocysteinaemia and vascular disease: an overview. J Inherit Metab Dis **29**: 3–20.
4 Farnworth PG (1995). Gonadotrophin secretion revisited. How many ways can FSH leave a gonadotroph? J Endocrinol **145**: 387–395.
5 Fung MM, Bettencourt R, Barrett-Connor E (2004). Heart disease risk factors predict erectile dysfunction 25 years later: the Rancho Bernardo Study. J Am Coll Cardiol **43**: 1405–1411.
6 Gava MM, Chagas Ede O, Bianco B, Christofolini DM, Pompeo AC, Glina S *et al.* (2011). Methylenetetrahydrofolate reductase polymorphisms are related to male infertility in Brazilian men. Genet Test Mol Biomarkers **15**: 153–157.
7 Hyde Z, Norman PE, Flicker L, Hankey GJ, McCaul KA, Almeida OP *et al.* (2011). Elevated LH predicts ischaemic heart disease events in older men: the Health in Men Study. Eur J Endocrinol **164**: 569–577.
8 Johannes CB, Araujo AB, Feldman HA, Derby CA, Kleinman KP, McKinlay JB (2000). Incidence of erectile dysfunction in men 40 to 69 years old: longitudinal results from the Massachusetts male aging study. J Urol **163**: 460–463.
9 Kostis JB, Jackson G, Rosen R, Barrett-Connor E, Billups K, Burnett AL *et al.* (2005). Sexual dysfunction and cardiac risk (the Second Princeton Consensus Conference). Am J Cardiol **96**: 313–321.
10 Levine LA (2000). Erectile dysfunction: a review of a common problem in rapid evolution. Prim Care Update Ob Gyns **7**: 124–129.

11 Lipton SA, Kim WK, Choi YB, Kumar S, D'Emilia DM, Rayudu PV *et al.* (1997). Neurotoxicity associated with dual actions of homocysteine at the N-methyl-D-aspartate receptor. Proc Natl Acad Sci U S A **94**: 5923–8.
12 Lombardo F, Sgrò P, Gandini L, Dondero F, Jannini EA, Lenzi A (2004). Might erectile dysfunction be due to the thermolabile variant of methylenetetrahydrofolate reductase? J Endocrinol Invest **27**: 883–885.
13 Mahesh VB, Brann DW (2005). Regulatory role of excitatory amino acids in reproduction. Endocrine **28**: 271–80.
14 Mayor-Olea A, Callejón G, Palomares AR, Jiménez AJ, Gaitán MJ, Rodríguez A *et al.* (2008). Human genetic selection on the MTHFR 677C>T polymorphism. BMC Med Genet **9**: 104.
15 Müezzinoğu T, Gümüş B, Temeltaş G, Ari Z, Büyüksu C (2007). A relationship of sex hormone levels and erectile dysfunction: which tests should be done routinely? Yonsei Med J **48**: 1015–1019.
16 Nicol L, McNeilly JR, Stridsberg M, McNeilly AS (2004). Differential secretion of gonadotrophins: investigation of the role of secretogranin II and chromogranin A in the release of LH and FSH in LbetaT2 cells. J Mol Endocrinol **32**: 467–480.
17 Ozdemir O, Yenicesu GI, Silan F, Köksal B, Atik S, Ozen F *et al.* (2012). Recurrent Pregnancy Loss and Its Relation to Combined Parental Thrombophilic Gene Mutations. Genet Test Mol Biomarkers **16**: 279–86.
18 Radeke HS, Digits CA, Casaubon RL, Snapper ML (1999). Interactions of (-)-ilimaquinone with methylation enzymes: implications for vesicular-mediated secretion. Chem Biol **6**: 639–647.
19 Russo GT, Friso S, Jacques PF, Rogers G, Cucinotta D, Wilson PW *et al.* (2003). Age and gender affect the relation between methylenetetrahydrofolate reductase C677T genotype and fasting plasma homocysteine concentrations in the Framingham Offspring Study Cohort. J Nutr **133**: 3416–3421.
20 Safarinejad MR, Safarinejad S, Shafiei N (2010). Role of methylenetetrahydrofolate reductase gene polymorphisms (C677T, A1298C, and G1793A) in the development of early onset vasculogenic erectile dysfunction. Arch Med Res **41**: 410–422.
21 Safarinejad MR, Shafiei N, Safarinejad S (2011). Relationship between genetic polymorphisms of methylenetetrahydrofolate reductase (C677T, A1298C, and G1793A) as risk factors for idiopathic male infertility. Reprod Sci **18**: 304–315.
22 Savigny P, Evans J, Mcgrath KM (2007). Cell membrane structures during exocytosis. Endocrinology **148**: 3863–3874.
23 Sazci A, Ergul E, Tuncer N, Akpinar G, Kara I (2006). Methylenetetrahydrofolate reductase gene polymorphisms are associated with ischemic and hemorrhagic stroke: Dual effect of MTHFR polymorphisms C677T and A1298C. Brain Res Bull **71**: 45–50.
24 Selvin E, Burnett AL, Platz EA (2007). Prevalence and risk factors for erectile dysfunction in the US. Am J Med **120**: 151–157.
25 Serý O, Šťastný F, Zvolský P, Hlinomazová Z, Balcar VJ (2011). Association between Val66Met polymorphism of Brain-Derived Neurotrophic Factor (BDNF) gene and a deficiency of colour vision in alcohol-dependent male patients. Neurosci Lett **499**: 154–7.
26 Singh K, Singh SK, Raman R (2010). MTHFR A1298C polymorphism and idiopathic male infertility. J Postgrad Med **56**: 267–269.
27 Thaler CJ, Budiman H, Ruebsamen H, Nagel D, Lohse P (2006). Effects of the common 677C>T mutation of the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene on ovarian responsiveness to recombinant follicle-stimulating hormone. Am J Reprod Immunol **55**: 251–258.
28 Traish AM, Goldstein I, Kim NN (2007). Testosterone and erectile function: from basic research to a new clinical paradigm for managing men with androgen insufficiency and erectile dysfunction. Eur Urol **52**: 54–70.
29 Veeraragavan K, Coulombe R, Gagnon C (1988). Stoichiometric carboxyl methylation of chromogranins from bovine adrenal medullary cells. Biochem Biophys Res Commun **152**: 732–738.
30 Wu X, Zhao L, Zhu H, He D, Tang W, Luo Y (2012). Association Between the MTHFR C677T Polymorphism and Recurrent Pregnancy Loss: A Meta-Analysis. Genet Test Mol Biomarkers (in press).