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Brief Communication

A founder mutation causing a severe methylenetetrahydrofolate reductase (MTHFR) deficiency in Bukharian Jews

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ABSTRACT

Methylenetetrahydrofolate reductase (MTHFR) deficiency is a rare autosomal recessive disorder. A novel homozygous *MTHFR* c.474A>T (p.G158G) mutation was detected in two unrelated children of Jewish Bukharian origin. This mutation generates an abnormal splicing and early termination codon. A carrier frequency of 1:39 (5/196) was determined among unrelated healthy Bukharian Jews. Given the disease severity and allele frequency, a population screening for individuals of this ancestry is warranted in order to allow prenatal, or preimplantation diagnosis.

1. Introduction

Homocystinuria due to methylenetetrahydrofolate reductase (MTHFR) deficiency (OMIM ID: 236250) is a rare inborn error of folate metabolism resulting in elevated homocysteine levels in plasma and urine [1]. Methylenetetrahydrofolate reductase catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for homocysteine remethylation to methionine. The severe form of the disease is characterized by developmental delay, seizures and microcephaly [2], and may be associated with infantile epilepsy [3]. The disease is autosomally recessively inherited, caused by mutations in the *MTHFR* gene [4]. It has been suggested that early treatment with betaine may alleviate the effect of the disease to the brain in some cases [5].

In this study we demonstrate the existence of a common splicing founder mutation in the *MTHFR* gene, causing a severe MTHFR deficiency among individuals of Jewish Bukharic ancestry.

2. Material and methods

2.1. Patient recruitment

The study protocol and informed consent were approved by the local Institutional Ethical Committee. DNA and RNA samples were collected from affected individuals and their parents.

2.2. MTHFR gene sequencing

MTHFR gene (NM_005957.4) was screened for mutations using standard PCR and sequencing of both DNA strands of the coding region and the exon–intron boundaries followed the CMGS (Clinical Molecular Genetics Society) best practice guidelines (Centogene GmbH, Rostock, Germany). The mutation has been deposited in the NCBI dbSNP database (rs199476142).

2.3. RNA analysis

RT-PCR (reverse transcriptase PCR) was performed on cDNA generated from RNA extracted from peripheral blood leukocytes of the patients. Primers for the RT-PCR were located in exon 2 and 4 of the reference sequence to verify whether exon 3 and/or intron 3 are included in the mutated transcript of the patient and the carrier parents. The primers' sequences are available upon request.

2.4. Determination of the c.474A>T frequency among controls

We examined 196 unaffected and unrelated Bukharian Jews and 176 Ashkenazi Jews, by an allelic discrimination test (TaqMan, custom-made test, Applied Biosystems, Carlsbad, CA, USA). The carrier status was further confirmed in all these individuals by Sanger sequencing.

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3. Results

A 13-month-old boy was referred for genetic work-up with the diagnosis of MTHFR deficiency. He had microcephaly, seizures and severe developmental delay. MTHFR deficiency was suspected due to elevated homocysteine in the blood and urine, and was confirmed by testing the specific activity of methylenetetrahydrofolate reductase in extract of the patient's fibroblasts (0.05 nmol/mg protein/h). The parents were non-consanguineous Jews of Bukharian ancestry. They previously had a daughter with similar manifestations, who died at 7 months of age. The analyses revealed a previously unreported homozygous variant c.474A>T (p.G158G) that caused a synonymous change. The parents were found to be heterozygous carriers of this change (Figs. 1A–C).

The same homozygous mutation was detected in a 16-month-old girl, referred to our molecular genetics laboratory, with similar neurological and biochemical findings, suggestive of MTHFR deficiency. This girl was also an offspring of a non-consanguineous couple of Bukharian Jewish ancestry, unrelated to the family previously described. Her parents were found to be heterozygous carriers of the same mutation.

As the transversion is located at the 3′ extremity of exon 3, and does not alter an amino acid, aberrant splicing was considered to be a consequence of this alteration.

To test the hypothesis that the c.474A>T mutation causes an aberrant splicing, we performed an expression study by RT-PCR. From the cDNA of the affected individuals we found an amplicon of about 1350 base pairs (bp), which was approximately 850 bp longer than the ~500 bp amplicon obtained in negative controls (Fig. 1F). The heterozygous parents had signs of both the wild-type allele and the mutant allele. Sanger sequencing of the large amplicon revealed the inclusion of the entire 838 bp sized intron 3 of the *MTHFR* gene (Figs. 1D and E). An early stop codon was generated in the novel aberrant transcript 22 codons downstream to the original exon–intron boundary, confirming the truncation nature of the mutation.

As these two affected individuals belong to the isolated ethnic group of the Jews coming from the province of Bukhara (Uzbekistan), we hypothesized that the c.474A>T mutation could be a founder allele. We therefore examined additional 196 unaffected and unrelated Bukharian Jews for the c.474A>T mutation and found it in five individuals, providing an estimated carrier frequency of 1:39. The mutation was not found among 176 Ashkenazi Jews. The prevalence of MTHFR deficiency due to this founder mutation in Bukharian Jews is expected to be ~1:6000.

4. Discussion

Our results show that a previously unreported mutation c.474A>T (p. G158G) is a common founder mutation among Jews of Bukharian ancestry, leading to aberrant splicing and resulting in generation of a premature termination codon. Currently, population-based screening programs are offered for a number of common genetic disorders world-wide, and are particularly prevalent in Israel [6]. Several criteria have been proposed to select genetic disorders for population screening. The agreed criteria include severity of disease, early onset, and high frequency of carriers, availability of genetic counseling and prenatal diagnosis, and an existence of a reliable test [7]. The MTHFR deficiency due to the c.474A>T mutation among couples of Bukharian Jewish origin clearly fulfills these criteria. Therefore, a population-based screening for the disease among this population is warranted, and will be added to the panel of ethnicity-specific mutations that are being offered in Israel. Furthermore, our results emphasize the importance of identifying rare founder mutations that lead to severe diseases among genetically-isolated populations. Such an approach

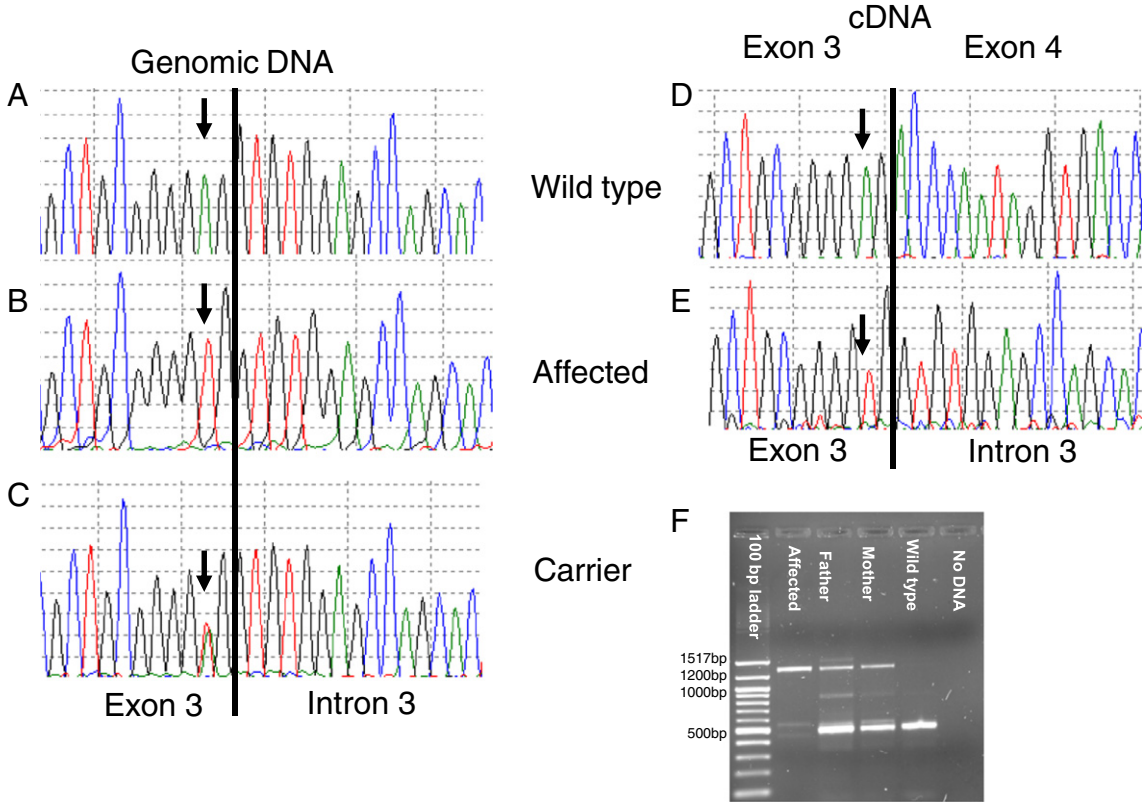

Fig. 1. Molecular analysis of the c.474A>T (G158G) mutation. On genomic DNA, compared with controls (A), affected individuals have a homozygous A>T transversion (B), while carrier individuals show the same change in the heterozygous state (C). The exon–intron junction is defined by the black vertical line. At the cDNA level, compared with unaffected controls (D), the original intron 3 is included in the cDNA of affected individuals immediately after exon 3 (E). RT-PCR of a cDNA fragment including exons 3 and 4 of the reference sequence revealed an expected ~500 bp band in controls and an enlarged ~1350 bp band in affected individuals. Both bands were detected in the heterozygous parents (F).

will assist in preventing and alleviating the burden of severe rare genetic disorders, in addition to the prevention of common severe recessive diseases, such as spinal muscular atrophy (SMA) [8].

References

[1] S.H. Mudd, H.L. Levy, J.P. Kraus, Disorders of transsulfuration, in: C.R. Scriver, A.L. Beaudet, W.S. Sly, D. Valle (Eds.), The Metabolic and Molecular Bases of Inherited Disease, McGraw-Hill, Inc, New York, 2001, pp. 2007–2056.
[2] F. Skovby, M. Gaustadnes, S.H. Mudd, A revisit to the natural history of homocystinuria due to cystathionine beta-synthase deficiency, Mol. Genet. Metab. 99 (2010) 1–3.
[3] A.N. Prasad, C.A. Rupar, C. Prasad, Methylenetetrahydrofolate reductase (MTHFR) deficiency and infantile epilepsy, Brain Dev. 33 (2011) 758–769.
[4] P. Goyette, J.S. Sumner, R. Milos, Human methylenetetrahydrofolate reductase: isolation of cDNA, mapping and mutation identification, Nature. Genet. (1994) 195–200.
[5] K.A. Strauss, D.H. Morton, E.G. Puffenberger, C. Hendrickson, D.L. Robinson, C. Wagner, S.P. Stabler, R.H. Allen, G. Chwatko, H. Jakubowski, M.D. Niculescu, S.H. Mudd, Prevention of brain disease from severe 5,10-methylenetetrahydrofolate reductase deficiency, Mol. Genet. Metab. (2007) 165–175.
[6] G. Rosner, S. Rosner, A. Orr-Urtreger, Genetic testing in Israel: an overview, Annu. Rev. Genomics. Hum. Genet. 10 (2009) 175–192.
[7] A.S.H.G., Ad Hoc Committee on Cystic Fibrosis Carrier Screening, Am. J. Hum. Genet. 51 (1992) 1443–1444.
[8] S. Ben-Shachar, A. Orr-Urtreger, E. Bardugo, R. Shomrat, Y. Yaron, Large-scale population screening for spinal muscular atrophy: clinical implications, Genet. Med. 13 (2011) 110–114.

Web-based resources

http://www.ncbi.nlm.nih.gov/omim
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http://www.ensembl.org
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