Dublin Institute of Technology
ARROW@DIT

Articles

School of Biological Sciences

2004-01-01

Increased Frequency of the MTHFR A1298C Mutation in an Irish Population

Cathal McCarthy
Dublin Institute of Technology

Fergus Ryan
Dublin Institute of Technology, fergus.x.ryan@dit.ie

Joseph Vaughan
Dublin Institute of Technology

Follow this and additional works at: http://arrow.dit.ie/scschbioart

Recommended Citation
McCarthy, Cathal; Ryan, Fergus; and Vaughan, Joseph, "Increased Frequency of the MTHFR A1298C Mutation in an Irish Population" (2004). *Articles*. Paper 57.
http://arrow.dit.ie/scschbioart/57

This Article is brought to you for free and open access by the School of Biological Sciences at ARROW@DIT. It has been accepted for inclusion in Articles by an authorized administrator of ARROW@DIT. For more information, please contact yvonne.desmond@dit.ie, arrow.admin@dit.ie.

This work is licensed under a Creative Commons Attribution-Noncommercial-Share Alike 3.0 License

Antenna & High Frequency Research Centre

Articles

Dublin Institute of Technology *Year* 2004

Increased Frequency of the MTHFR A1298C Mutation in an Irish Population

Cathal McCarthy* Fergus Ryan Dr.† Joseph vaughan‡

*Dublin Institute of Technology

†Dublin Institute of technology, fergus.x.ryan@dit.ie

‡Dublin Institute of Technology

This paper is posted at ARROW@DIT.

http://arrow.dit.ie/ahfrcart/1

— Use Licence —

Attribution-NonCommercial-ShareAlike 1.0

You are free:

- to copy, distribute, display, and perform the work
- to make derivative works

Under the following conditions:

- Attribution.
  You must give the original author credit.
- Non-Commercial.
  You may not use this work for commercial purposes.
- Share Alike.
  If you alter, transform, or build upon this work, you may distribute the resulting work only under a license identical to this one.

For any reuse or distribution, you must make clear to others the license terms of this work. Any of these conditions can be waived if you get permission from the author.

Your fair use and other rights are in no way affected by the above.

This work is licensed under the Creative Commons Attribution-NonCommercial-ShareAlike License. To view a copy of this license, visit:

- URL (human-readable summary):
  http://creativecommons.org/licenses/by-nc-sa/1.0/
- URL (legal code):
  http://creativecommons.org/worldwide/uk/translated-license

structural issues and can be resolved. The *p53* resequencing microarray is currently undergoing a redesign (Roche Molecular Diagnostics, personal communication). Given the cost-effectiveness of chip technology, it is reasonable to expect a next-generation Chip in which most of these limitations have been addressed. Such improvements could lead to a more useful and productive tool in cancer research and diagnostics.

References

1. Cooper M, Li S-Q, Bhardwhaj T, Rohan T, Kandel RA. Evaluation of oligonucleotide arrays for sequencing of the *p53* gene in DNA from formalin-fixed, paraffin-embedded breast cancer specimens. Clin Chem 2004;50:500–8.
2. Ahrendt SA, Halachmi S, Chow JT, Wu L, Halamachi N, Yang SC, et al. Rapid p53 sequence analysis in primary lung cancer using an oligonucleotide probe array. Proc Natl Acad Sci U S A 1999;96:7382–7.

Antonette C.P. Allen*
Francis A. Chiafari

BRT Laboratories, Inc.
400 West Franklin St.
Baltimore, MD 21201

*Author for correspondence. Fax 410-383-0938; e-mail allen@brtlabs.com.

Previously published online at DOI: 10.1373/clinchem.2004.038158

Drs. Kandel and Rohan respond:

To the Editor:

We would like to thank Drs. Allen and Chiafari for their comments. They reiterate many of the points we made in our report *(1)*. We are glad that they also had similar findings. However, they suggest that we differ in the detection of polymorphisms. In the Discussion section of our report *(1)*, we speculated that our cut-off score might be too high because we observed that a polymorphism in exon 6 had a score of 6. In their study, the GeneChip detected one of six exon 4 (codon 72) polymorphisms, whereas we were unable to detect any in 12 cases from which we obtained a PCR product. We would argue that this was not a significant difference because both sample sizes are small. In fact, it actually supports our conclusion that a combination of both microarray and sequencing is required to identify *p53* alterations, as they would have missed five polymorphisms. We also look forward to being able to use the next-generation *p53* microarray, as we stated in our report *(1)*, because this methodology definitely has a role to play in sequencing of the *p53* gene.

Reference

1. Cooper M, Li S-Q, Bhardwhaj T, Rohan T, Kandel RA. Evaluation of oligonucleotide arrays for sequencing of the *p53* gene in DNA from formalin-fixed, paraffin-embedded breast cancer specimens. Clin Chem 2004;50:500–8.

Rita Kandel[1*]
Thomas Rohan[2]

[1] *University of Toronto*
Toronto, Canada

[2] *Albert Einstein College of Medicine*
Bronx, NY

* Address correspondence to this author at: Pathology and Laboratory Medicine, Mt. Sinai Hospital, 600 University Ave., Toronto, ON M5G1X5 Canada. Fax 416-586-8628; e-mail rkandel@mtsinai.on.ca.

DOI: 10.1373/clinchem.2004.043117

Increased Frequency of the *MTHFR* A1298C Mutation in an Irish Population

To the Editor:

The enzyme methylenetetrahydrofolate reductase (MTHFR) catalyzes the reduction of methylene tetrahydrofolate to 5-methyltetrahydrofolate, the cosubstrate required for the remethylation of homocysteine to methionine. Mutations in the MTHFR enzyme are reported as causes of hyperhomocysteinemia *(1)*. Hyperhomocysteinemia is generally, although not universally, seen as an independent and graded risk factor for venous thrombosis and neural tube defects *(2)*. Several polymorphisms have been reported in the *MTHFR* gene, but two particular mutations generate the most interest, the recently described A1298C *(3)* and the most-characterized C677T *(4)*. The A1298C polymorphism in the *MTHFR* gene encodes for a glutamate to alanine substitution and leads to a decrease in enzyme activity. Combined heterozygosity for the C677T/A1298C polymorphisms in some studies *(5)* is associated with higher homocysteine concentrations and decreased plasma folate.

Amplification Refractory Mutation System (ARMS) PCR determination of the *MTHFR* C677T mutation has been described by Hessner et al. *(6)*. To determine the frequency of the A1298C mutation in the Irish population, we developed a reliable and rapid ARMS PCR method. We compared the results with those obtained with the standard method for detection, PCR followed by restriction fragment length polymorphism (RFLP) analysis *(3)*.

Our cohort consisted of 120 blood donors, none of whom had experienced any past or current thrombotic events or had a family history of thrombosis. Informed consent was obtained from all study participants. Total genomic DNA was isolated from blood leukocytes, and *MTHFR* A1298C was analyzed by PCR-RFLP *(3)*.

ARMS PCR was also used to determine the frequency of this mutation. A typical ARMS PCR set-up for the wild-type reaction consisted of 200 ng of genomic DNA, 2.5 mM $MgCl_2$, 0.4 mM each deoxynucleotide triphosphate (Invitrogen, BioSciences), 2.5 μL of 10× buffer [200 mM Tris-HCl (pH 8.4), 500 mM KCl; Invitrogen], 1.5 U of Platinum Taq polymerase (Invitrogen), and 50 mL/L dimethyl sulfoxide (Sigma-Aldrich). ARMS PCR primers used in the wild-type reaction were as follows: A1298C forward consensus primer (5′-CCTTTGGGGAGCTGAAGGACTACTAC-3′); A1298C wild-type reverse primer (5′-CAAAGACTTCAAAGACAGTC-3′); cystic fibrosis 22 (CF22) forward primer (5′-AAACGCTGAGCCTCACAAGA-3′), and CF22 reverse primer (5′-TGTCACCATGAAGCAGGCAT-3′; Sigma-Aldrich). The mutant reaction

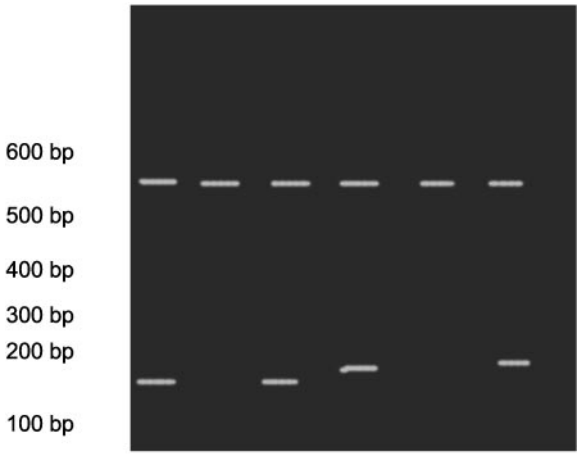

Fig. 1. ARMS PCR for *MTHFR* A1298C.

Reactions are shown in pairs. Products of the wild-type reaction are analyzed in the first lane of each pair, products of mutant reaction are analyzed in the second lane of each pair. Each pair contains the CF22 internal control, which generates a 578-bp product. *Lane 0*, 100-bp DNA ladder (Invitrogen, Bio-Sciences); *lanes 1* and *2*, presence of the 120-bp product in *lane 1* indicates wild type for the mutation; *lanes 3* and *4*, presence of 120-bp product in *lane 3* and 127-bp product in *lane 4* indicate heterozygous carrier of the mutation; *lanes 5* and *6*, presence of the 127-bp product in lane 6 indicates a homozygous carrier of the mutation.

had the same components as above, with the replacement of A1298C wild-type reverse primer with A1298C mutant reverse primer, which had the following sequence: 5′-GGTAAAGAACAAAGACTTCA-AAGACACTGTG-3′ (Sigma-Aldrich).

ARMS PCR conditions were 95 °C for 5 min; 40 cycles of 95 °C for 30 s, 57 °C for 30 s, and 72 °C for 30 s; and 72 °C for 5 min. The wild-type reaction produces a 120-bp product, whereas the mutant reaction produces a 127-bp fragment (Fig. 1). The CF22 primers were used as internal control primers and produce a 578-bp fragment in each reaction. Statistical analysis was performed using the *Z*-test for two independent proportions. Statistical significance was set at $Z > 1.96$.

Of the 120 healthy Irish participants, 56 were heterozygous carriers, giving a genotype frequency of 46.7%, whereas 11 (14.2%) were homozygous for A1298C. Of the *MTHFR* C677T/A1298C genotype combinations, 28 participants (23.3%) were double heterozygotes. The prevalence of 1298CC homozygotes in this Irish study is significantly higher than that reported for most European populations, including a UK study ($Z = 1.97$) *(7)*, an Italian cohort ($Z = 2.51$) *(8)*, and an American study ($Z = 1.99$) *(9)*. Only two studies from Northern Scotland *(10, 11)* have reported a higher prevalence.

The results of the ARMS PCR were in complete concordance with the results obtained by standard PCR-RFLP. The ability of the ARMS PCR to distinguish between genotypes for the A1298C mutation is much higher than that of the standard method.

We thank Dr. A Davern, Dublin Blood Transfusion Centre, for the blood samples. We would like to also thank Drs. Valeria Capra (Laboratorio del Servizio di Neurochirugia, Instituto Scientifico G. Gaslin, Genova, Italy) and Nurit Rosenberg (Department of Hematology, Institute of Thrombosis and Hemostasis, The Chaim Sheba Medical Center, Tel-Hashomer, Israel) for the A1298C positive controls.

References

1. Lievers KJA, Kluijtmans LJA, Blom HJ. Genetics of hyperhomocysteinemia in cardiovascular disease. Ann Clin Biochem 2003;40:46–59.
2. Makris M. Hyperhomocysteinemia and thrombosis. Clin Lab Haematol 2000;22:133–43.
3. Van der Put NJM, Gabreels F, Stevens EMB, Smeitink JAM, Trijbels FJM, Eskes TKAB, et al. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural tube defects? Am J Hum Genet 1998;62:1044–51.
4. Frosst P, Blom HJ, Milos R. A candidate genetic risk factor for vascular disease, a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995;10:111–3.
5. Weisberg I, Tran P, Christensen B, Sibani S, Rozen R. A second genetic polymorphism in MTHFR associated with decreased enzyme activity. Mol Genet Metab 1998;64:169–72.
6. Hessner MJ, Luhm RA, Pearson SL, Endean DJ, Friedman KD, Montgomery RR. Prevalence of prothrombin G202010A, factor V G1691A (Leiden), and methylenetetrahydrofolate reductase (MTHFR) C677T in seven different populations determined by multiplex allele-specific PCR. Thromb Haemost 1999;81:733–8.
7. Dekou V, Whincup P, Papacosta O, Ebrahim S, Lennon L, Ueland PM, et al. The effect of the C677T and A1298C polymorphisms in the methylenetetrahydrofolate reductase gene on homocysteine levels in elderly men and women from the British regional heart study. Atherosclerosis 2001;154:659–66.
8. De Marco P, Grazia Calevo M, Moroni A, Arata L, Merello E, Finnell RH, et al. Study of MTHFR and MS polymorphisms as risk factors for NTD in the Italian population. J Hum Genet 2002; 47:319–24.
9. Hanson NQ, Aras O, Yang F, Tsai MY. C677T and A1298C polymorphisms of the methylenetetrahydrofolate reductase gene: incidence and effect of combined genotypes on plasma fasting and post-methionine load homocysteine in vascular disease. Clin Chem 2001;47: 661–6.
10. Sharp L, Little J, Schofield AC, Pavlidou E, Cotton SC, Miedzybrodzka Z, et al. Folate and breast cancer: the role of polymorphisms in methylenetetrahydrofolate reductase (MTHFR). Cancer Lett 2002;181:65–71.
11. Sharp L, Little J, Brockton N, Cotton SC, Haites NE, Cassidy J. Dietary intake of folate and related micronutrients, genetic polymorphisms in MTHFR and colorectal cancer: a population-based case-control study in Scotland [Abstract]. J Nutr 2002;132(Suppl 11):3542S.

Cathal Mc Carthy*
Fergus Ryan
Joseph Vaughan

Department of Biological Sciences
Dublin Institute of Technology
Dublin, Ireland

*Address correspondence to this author at: Department of Biological Sciences, Dublin Institute of Technology, Kevin St., Dublin 8, Ireland. Fax 353-1-402-2833; e-mail cathal_mccarthy@yahoo.com.

DOI: 10.1373/clinchem.2004.041517

Interference in Measurement of Potassium Caused by Bacterial Contamination of an Analyzer

To the Editor:

We encountered a problem with potassium measurement after periods of standby on a Beckman Synchron LX20 Pro. Initial potassium measurements were rejected because of excessive reference drift, and potassium results for quality-control (QC) samples were as much as 2.5 mmol/L above the previous mean. Replicate analyses of control material immediately after standby revealed a decreasing trend in potassium concentration, whereas simultaneously