Homocysteine and related genetic polymorphisms in Down’s syndrome IQ


Objective: Down's syndrome (DS) is the most frequent genetic cause of Alzheimer-type dementia. Its metabolic phenotype involves an increased trans-sulphuration of homocysteine. The aim of the present study was to investigate the influence of homocystinaemia (t-Hcys), folate, vitamin B12, and related polymorphisms on intelligence quotient (IQ) in DS.

Methods: The IQ of 131 patients with trisomy 21 from a specialist centre in Sicily was determined and classified according to DSM-IV. The effects of age, folate, vitamin B12, t-Hcys, and genetic polymorphisms on IQ were evaluated separately and in combination using regression analyses.

Results: IQ was significantly lower in DS patients with t-Hcys > 7.5 μmol/l (median) and in those who were carriers of methylenetetrahydrofolate reductase (MTHFR) 677 T allele and of methylenetetrahydrofolate reductase 677 T and transcobalamin 776 G combined alleles (p = 0.0013, p = 0.0165, and p = 0.0074, respectively). The IQ correlated significantly with t-Hcys and folate in single and multiple regression analyses, independently of age. In addition, t-Hcys > 9.6 μmol/l (upper quartile) was found to be associated with low IQ (median of study group) with an odds ratio of 2.61 (p = 0.0203). The odds ratio was increased by threefold in carriers of MTHFR 677 T allele. The MTHFR 677 T allele/transcobalamin 776 G allele combination was associated with the risk of DS patients to have an IQ less that the median with an odds ratio of 2.68 (95% CI 1.26 to 5.70, p = 0.0104).

Conclusion: This study found evidence of an association between t-Hcys and MTHFR 677 T and transcobalamin 776 G alleles with IQ in patients with DS. The association may be related to a defective remethylation of homocysteine, affecting IQ.

Patients and Methods

Patients

The 131 outpatients were recruited after obtaining the family’s and patient’s informed consent in a specialist centre receiving patients only from Sicily. The centre’s ethical committee approved the study.

Karyotyping showed full trisomy 21 in 100% of patients. The clinical characteristics taken into account when analysing the data were body mass index (BMI; only for patients older than 10 years), coeliac disease, cardiopathy, thyroid status, and epilepsy. None of the patients smoked, had renal failure (creatinine < 15 mg/l in all cases), or took vitamin supplements or food fortified with vitamins. In all patients, the thyroid status was either normal or rendered euthyroid by replacement therapy. The IQ was tested with the Wechsler scales for adults and children, as described previously.

After testing, all subscale scores were transformed into age-scaled scores, the standard IQ calculated, and the mental retardation classified according to the DSM-IV, as specified by the American Society of Psychiatry (2000).

Abbreviations: AD, Alzheimer’s disease; ApoE, Apolipoprotein E; BMI, body mass index; DS, Down’s syndrome; IQ, intelligence quotient; MTHFR, methylenetetrahydrofolate reductase; MTR, methionine synthase; MTRR, methionine synthase reductase; t-Hcys, homocysteine; TCN, transcobalamin


Received 24 February 2004
Revised version received 3 September 2004
Accepted 17 September 2004

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PAPER
Table 1  Influence of homocysteine and genetic polymorphisms of methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), methionine synthase reductase (MTRR), transcobalamin (TC) and apolipoprotein E (ApoE) on intelligence quotient (IQ; median (25th–75th centile)) in patients with Down’s syndrome

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n (%)</th>
<th>Yes</th>
<th>No</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine &gt;7.5 μmol/l (median)</td>
<td>66 (50.3)</td>
<td>35 (25–45)</td>
<td>45 (35–55)</td>
<td>0.0013</td>
</tr>
<tr>
<td>Homocysteine &gt;9.6 μmol/l (75th centile)</td>
<td>34 (25.9)</td>
<td>35 (20–45)</td>
<td>40 (32–50)</td>
<td>0.0021</td>
</tr>
<tr>
<td>MTHFR 677 T allele</td>
<td>85 (65.4)</td>
<td>45 (30–45)</td>
<td>40 (32–50)</td>
<td>0.0165</td>
</tr>
<tr>
<td>MTHFR 1298 C allele</td>
<td>76 (58.0)</td>
<td>40 (32–50)</td>
<td>35 (25–50)</td>
<td>0.2762</td>
</tr>
<tr>
<td>MTR 2756 G allele</td>
<td>52 (40.0)</td>
<td>40 (32–50)</td>
<td>35 (30–50)</td>
<td>0.2849</td>
</tr>
<tr>
<td>TNC 776 G allele</td>
<td>93 (73.0)</td>
<td>40 (30–50)</td>
<td>35 (30–50)</td>
<td>0.1895</td>
</tr>
<tr>
<td>ApoE ε4 allele</td>
<td>15 (11.7)</td>
<td>45 (36–50)</td>
<td>40 (30–50)</td>
<td>0.1039</td>
</tr>
<tr>
<td>MTHFR 677 T allele/MTHFR 1298 C allele</td>
<td>39 (30.0)</td>
<td>40 (30–50)</td>
<td>40 (30–50)</td>
<td>0.4251</td>
</tr>
<tr>
<td>MTHFR 677 T allele/MTR 2756 G allele</td>
<td>32 (24.6)</td>
<td>40 (30–50)</td>
<td>39 (30–56)</td>
<td>0.0483</td>
</tr>
<tr>
<td>MTHFR 677 T allele/TNC 776 G allele</td>
<td>63 (48.5)</td>
<td>40 (32–45)</td>
<td>35 (32–54)</td>
<td>0.0074</td>
</tr>
<tr>
<td>MTHFR 677 T allele/ApoE ε4 allele</td>
<td>10 (7.8)</td>
<td>42 (35–45)</td>
<td>40 (30–50)</td>
<td>0.6222</td>
</tr>
</tbody>
</table>

*p values in bold are significant.

Table 2  Log rank Spearman’s correlation (r_s) between intelligence quotient (IQ) and either age, homocysteine, or folate in 131 patients with Down’s syndrome (DS) and in two subgroups divided by IQ median (IQ = 40)

<table>
<thead>
<tr>
<th>Group</th>
<th>No of cases</th>
<th>Age r_s</th>
<th>p value</th>
<th>Homocysteine r_s</th>
<th>p value</th>
<th>Folate r_s</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All DS</td>
<td>131</td>
<td>0.455</td>
<td>&lt;0.0001</td>
<td>-0.329</td>
<td>0.0002</td>
<td>0.110</td>
<td>0.2111</td>
</tr>
<tr>
<td>IQ&gt;40</td>
<td>69</td>
<td>-0.546</td>
<td>&lt;0.0001</td>
<td>-0.167</td>
<td>0.1689</td>
<td>0.088</td>
<td>0.4705</td>
</tr>
<tr>
<td>IQ&lt;40</td>
<td>62</td>
<td>-0.101</td>
<td>0.4285</td>
<td>-0.26</td>
<td>0.0355</td>
<td>0.328</td>
<td>0.0104</td>
</tr>
</tbody>
</table>

Laboratory assays
Blood was collected in the fasting state. Plasma t-Hcys was assayed by fluorescence polarisation immunoassay (FPIA), and vitamin B12 and folate by microparticle enzyme immunoassay (MEIA) using the Abbott IMx automated benchtop analyser system (Abbott Diagnostic, Rome, Italy). DNA was isolated from a lymphocyte enriched fraction of whole blood with NUCLEON BACC3 for extraction of genomic DNA (Amersham Pharmacia Biotech, Milan, Italy). The procedures for detecting the genomic DNA kit (Amersham Pharmacia Biotech, Milan, Italy). The procedures for detecting the methylation of MTHFR, as well as the 2756 A→G polymorphisms of MTR, and the 66 A→G polymorphisms were based on polymerase chain reaction (PCR) amplification, restriction cleavage and separation of the DNA fragments by 15% non-denaturant polyacrylamide gel electrophoresis (SDS-PAGE), as previously described. DNA samples corresponding to amplified DNA of the MTHFR, MTR, and MTRR genotypes were sequenced and subsequently used as controls in all series of genotype determination.

Statistical methods
Categorical variables are reported as counts and percentages, and continuous variables as median, 25th and 75th centiles. For categorical and continuous variables, a continuity corrected χ² test and the Mann–Whitney U test were used, respectively. Spearman’s rank correlation coefficient was used to estimate the correlation among IQ, t-Hcys, age, folate and vitamin B12. The significance and odds ratios of continuous and categorical variables regarding mental retardation were determined by stepwise multiple regression and logistic regression analyses, respectively. A p value higher than 0.10 was set to exclude variables in the stepwise analyses and a final p value lower than 0.05 was considered to indicate residual statistical significance. Data were collected and analysed using the Statview 5 software for Windows (SAS Institute, Berkley, CA, USA) and the SPSS 10.0 software for Windows (SPSS, Paris, France).

RESULTS
The IQ of the 131 patients with DS ranged from 70 to 10, with median, 25th and 75th centiles of 40, 30, and 60, respectively. The plasma homecysteine levels were close to those observed in a control population (median, 25th and 75th centiles 7.5, 5.7, and 9.6 μmol/l, respectively). The plasma levels exceeded the 15 μmol/l limit of moderate hyperhomocysteinemia in nine patients, including five with a severe IQ 35–25 and two with a profound IQ<25 degree of mental retardation, according to the four degrees of the DSM-IV classification. We also found that patients who had t-Hcys levels >7.5 μmol/l had an IQ significantly lower than that of those who had a concentration below this median value (p = 0.0013, table 1). Reciprocally, t-Hcys was higher in DS patients with an IQ <40 (median of the study group), compared with those with less mental retardation (median and interquartiles: 8.19, 6.20, 10.50 and 6.80, 5.37, 8.70 μmol/l, respectively; p = 0.004).

Since t-Hcys was also related to age (table 2), we performed the same analysis in two age matched subgroups with IQ<40 (n = 29, mean (SD) age 19.7 (9.3) years) and >40 (n = 40, mean age 20.3 (8.8) years). The t-Hcys plasma level was still higher in the age matched group with IQ<40 (median and interquartiles: 8.4, 7.1, 9.3 and 6.9, 5.4, 8.8 μmol/l, respectively; p = 0.005). The IQ correlated significantly with t-Hcys and folate in log rank Spearman’s univariate analysis (table 2). This association was seen mainly in the patients who had the lowest IQ (<median) (see table 2). Conversely, a significant correlation was found between IQ and age, mostly in the patients with the less severe degrees of mental retardation. Indeed, this correlation was not significant when only patients with IQ<40 were considered (table 2). In a stepwise multiple regression analysis that also included vitamin B12, transcobalamin,
thyroid stimulating hormone (TSH), BMI, and age, the IQ remained associated only with t-Hcys (initial and residual p values 0.0112 and 0.0037, respectively) and folate (initial and residual p values 0.0967 and 0.0359, respectively). In a stepwise logistic regression analysis, we found an association between t-Hcys >9.6 μmol/l and IQ <40 (median) with an odds ratio of 2.61 (95% confidence interval (CI) 1.16 to 5.88; p = 0.0203), and IQ <30 (10th centile) with an odds ratio of 3.21 (95% CI 1.40 to 7.37, p = 0.0057).

There was no difference with regard to t-Hcys, folate, and vitamin B12 between patients with coeliac disease and the other patients (p = 0.363, 0.720, 0.351, respectively). We investigated the independent determinants of t-Hcys by multiple regression analysis—age and folate were but vitamin B12, transcobalamin, TSH, and BMI were not significant determinants (p = 0.0034 and p = 0.0232, respectively).

The distributions of MTHFR, MTR, MTRR, and TCN genotypes were in Hardy–Weinberg equilibrium. We evaluated the influence of these polymorphisms on the IQ, both alone and in combination with each other. The patient with DS bearing the MTHFR 677 T allele and the MTHFR 677 T/TCN 776 G allele combination had an IQ significantly lower than those carrying the corresponding wild genotypes (table 1). This effect was not age related, as the MTHFR 677 T allele and the MTHFR 677 T/TCN 776 G allele combination had no influence on the IQ of carriers (p = 0.6299 and p = 0.7426, respectively). We also found a significant association of the MTHFR 677 T allele and TCN 776 G allele combination with the risk of DS patients to have a low IQ <median with an odds ratio of 2.68 (95% CI 1.26 to 5.70, p = 0.0104). There was no direct association of MTHFR 677 T allele with the risk of low IQ. However, this genotype increased the risk of low IQ associated with t-Hcys by about threefold with an odds ratio of 7.78 (95% CI 11.20 to 50.43, p = 0.0015).

**DISCUSSION**

Several clinical and experimental studies have hypothesised that patients with DS have disturbed one-carbon metabolism.6–10 25–27 t-Hcys, vitamin B12, and folate are metabolic and nutritional factors directly related to this metabolism. However, it remained unknown whether these factors and the associated genetic polymorphisms aggravate the age related mental retardation in DS.

The relatively low blood levels of t-Hcys in our patients can possibly be explained by an overexpression of the chromosome 21 cystathionine-β-synthase enzyme, as has been observed by others.28 Despite these relatively low levels, we found a significant association between IQ and t-Hcys. Several hypotheses may explain this association. The relatively high t-Hcys levels in the subgroup of patients who had the lowest IQ could be an indirect consequence of mental retardation, since the latter may lead to reduced autonomy and subsequent deficient dietary intake. In fact, in our study, the influence of diet seemed to be limited to folate. We compared two age matched groups consisting of outpatients from an socioeconomic background. The BMI had no influence on the IQ average, and a significant correlation of IQ with folate was observed in only half of the patients with DS—those who had the highest level of mental retardation (see table 2). In addition, multiple regression analysis showed that neither vitamin B12 nor BMI were independent determinants of t-Hcys, and that folate was a weak determinant. Age is another factor that may be involved in the association between IQ and t-Hcys; it is known to be a determinant of both t-Hcys and cognitive function.29 However, our study suggests that age alone cannot explain the t-Hcys/IQ association in DS. Indeed, the influence of age on IQ was observed in only half the patients, those who had the lowest level of mental retardation. In addition, IQ was associated with t-Hcys independently of age in the multiple regression analysis.

We also investigated the role of genetic determinants of t-Hcys in the association between t-Hcys and IQ level. Carriers of the MTHFR 677 T allele had a lower IQ than those carrying the corresponding wild genotype (see table 1). This can be explained at least in part by its effect on the association of t-Hcys level with IQ since it increased the odds ratio of this association by threefold. The influence on IQ became slightly more significant when we considered the combination of MTHFR 677 T and TCN 776 G allele. TCN 776 G→G polymorphism has been reported to be a weak determinant of homocysteine in some but not all studies in healthy populations.23 24 It may influence the cellular availability of vitamin B12 because it is associated with a decreased concentration of transcobalamin, the carrier protein which delivers vitamin B12 to cells, and with an increased blood level of methylmalonic acid.25 26 The influence of MTHFR 677 T allele either alone or in combination with TCN 776 G allele on IQ may correspond, therefore, to decreased remethylation of homocysteine due to reduced activity of MTHFR, eventually accentuated by reduced availability of vitamin B12, cofactor of MTR. None of the other genetic polymorphisms of the one-carbon metabolism influenced the level of mental retardation.

The trisomy 21 phenotypic abnormalities of one-carbon metabolism could theoretically be caused by the overexpression of genes on chromosome 21.21–23 From this point of view, the increase in the activity of the trans-sulphuration pathway of t-Hcys, which results from the overexpression of cystathionine β-synthase on chromosome 21,24 25 26 27 28 may promote a “folate trap” by decreasing the cellular concentration of homocysteine and therefore its subsequent methylene tetrahydrofolate and vitamin B12 dependent remethylation and the cellular synthesis of tetrahydrofolate.29 30 Indeed, supplementation with either folic acid or methyl-B12 is very effective in increasing the defective cellular level of methionine and S-adenosylmethionine in lymphoblastoid cells of trisomy 21.31 In this metabolic context, a decreased activity of MTHFR, resulting from a 677 TT genotype, may act as an aggravating factor of the “folate trap” by decreasing the remethylation of homocysteine and the synthesis of tetrahydrofolate. The reduced activity of mutated MTHFR also decreases the level of S-adenosylmethionine,32 a substrate needed for pathways possibly involved in DS pathogenesis such as DNA and protein methylation and synthesis of choline.33 34 This polymorphism has been also described as a key genetic determinant of a folate imbalance between DNA synthesis on one hand and remethylation of homocysteine and DNA methylation on the other hand, which is effective only in the absence of folate repletion.35 36 The hypothesis of a link between DNA methylation and cognitive dysfunction should be further investigated in the light of the present association of t-Hcys with IQ because t-Hcys correlates significantly with genomic DNA methylation.37

Recently, a follow up study showed that t-Hcys was an independent risk factor for the occurrence of dementia of sporadic AD.38 t-Hcys interacts with several pathomechanisms of sporadic AD. It impairs DNA repair in hippocampal neurons, promotes apoptosis, hypersensitivity to excitotoxicity and oxidative stress and it potentiates the neurone toxicity of β-amyloid peptide, the proteolytic product of amyloid precursor protein.39 40 In addition to the overexpression of cystathionine β-synthase, another phenotypic abnormality related to chromosome 21 genes is the over-expression of amyloid precursor protein, which confers a predominant role to the pathway of amyloid precursor protein in the neurodegeneration in DS dementia.41 42 It has
been showed that homocysteine and folate deficiency increases the risk of neurodegenerative disorders.\(^2\) We also reported recently that MTR had an influence on the progression of AD, which may be enhanced by carriage of allele \(e4\) of \(ApoE\).\(^3\) Therefore, our previous results and the present data argue for investigating the potential interactions between homocysteine and \(\beta\)-amyloid fragment metabolism in the pathogenesis of DS dementia.

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Competing interests: none declared

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**REFERENCES**