

Homocysteine-Related Metabolites in Control and Down Syndrome Amniotic Fluid

P J Baggot, R M Baggot, D Sorapuru, T Kim, J D Shoemaker

Citation

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Abstract

Introduction: Cystathionine-beta-synthase is an enzyme coded by a gene on chromosome 21. This enzyme combines serine and homocysteine to form cystathionine, which is further metabolized to cysteine. Evidence for a gene-dosage effect was sought. The goal was to understand the biochemistry of Down syndrome in the fetuses, not to propose a method for prenatal diagnosis.

Materials and Methods: Archived amniotic fluid specimens from forty-one control fetuses and twenty-two fetuses with Down syndrome were examined by gas chromatography/mass spectrometry. Assayed metabolites included serine, homocysteine, homocystine, cysteine, cystine, and cystathionine. Mann-Whitney rank sum test was employed to analyze the data.

Results: Serine was decreased, but was not statistically significant. Cystathionine was significantly increased. Cysteine, cystine and total cysteine (cysteine + cystine) were increased in Down syndrome, with borderline significance. These results were consistent with a gene dosage effect. Unexpectedly, homocysteine was elevated in Down syndrome.

Discussion: The elevated homocysteine suggests deficiency in vitamin B6, which is also consistent with prior work. The other results are consistent with a gene dosage effect. These two different effects may coincide.

INTRODUCTION

Down syndrome is a multi-faceted disorder accompanied by numerous abnormalities, among which are structural, biochemical, and functional. Clinically it is associated with mental retardation, cardiac malformations, characteristic facies, and many other metabolic and anatomic derangements. An extra copy of chromosome 21 causes these characteristics. This results in one extra copy of each gene on chromosome 21. One proposed pathophysiologic mechanism in Down syndrome is that one extra copy of each gene causes a gene dosage effect.

We studied metabolites of an enzyme coded by a gene on chromosome 21: cystathionine-beta-synthase. For enzymes coded on chromosome 21, one might expect an approximately 50% increase in enzyme activity in Down syndrome, since three copies of the gene will be present instead of two. This increase is known as a gene dosage effect. Cystathionine-beta-synthase combines the substrates serine and homocysteine to form the product cystathionine. Gamma-cystathionase metabolizes cystathionine to cysteine (the downstream product).^[1] Under oxidizing conditions, the sulfhydryl groups of cysteine can form a disulfide bridge, transferring 2 protons, and resulting in cystine, the dimer of

cysteine. In a similar fashion, dimerization of homocysteine forms homocystine. Evaluation of cystine and homocystine were included so that the results are not affected by dimerization.

If a gene dosage effect were present, a 50% increase in cystathionine-beta-synthase activity could occur in trisomy 21. Our hypothesis was that serine and homocysteine would be reduced and that cystathionine and possibly cysteine would be increased in the amniotic fluid of fetuses with trisomy 21.

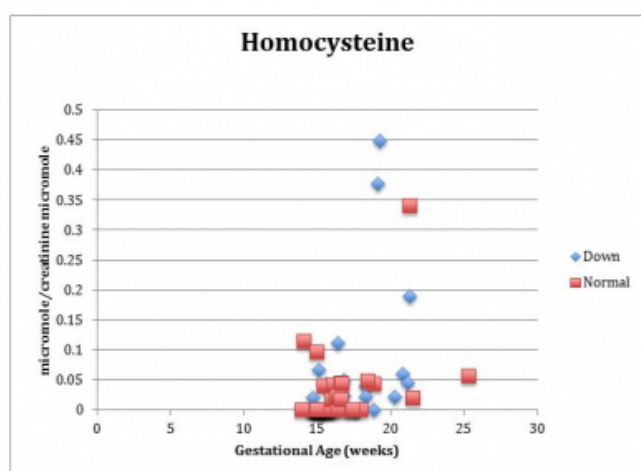
MATERIALS AND METHODS

Archived specimens of amniotic fluid from normal pregnancies and those with Down syndrome were shipped on dry ice from the Cytogenetic Laboratory of Virginia Commonwealth University to the Metabolic Screening Laboratory at Saint Louis University. The samples were collected prior to the mandatory supplementation of flour in the US with folate (2002). The control samples were collected from March to April 1996 and the Down syndrome samples were collected between May 1993 to October 1995. The median gestational age for the control group was 15.7 weeks. The median gestational age for the Down Syndrome

group was 16.7 weeks. Amniotic fluid samples were prepared and analyzed as previously described for urine.^[2] Forty-one control amniotic fluid specimens and twenty-two Down syndrome specimens were analyzed by gas chromatography/mass spectrometry, using a trimethylsilyl derivatizing agent. The relevant components were identified, and the amounts of each were quantified. Since archived specimens were used for the study, the Institutional Review Boards of both institutions waived further review.

Figure 1

Homocysteine



RESULTS

The data were not normally distributed and therefore violated the normality assumptions of the parametric T-test. This was analytically verified with the Lilliefors test. Lilliefors test of normality indicated that the data was not normally distributed, and therefore, non-parametric evaluation was performed. Mann-Whitney rank sum tests were used to analyze the data, with $p \leq 0.05$ as the criterion of statistical significance. Total cysteine is the sum of cysteine and its oxidized dimer cystine. Total homocysteine is the sum of homocysteine and its oxidized dimer homocystine.

Table 1 presents the data non-parametrically. Serine was reduced, but not significantly. Cystathionine was significantly higher in Down syndrome fluid ($p=0.047$). Cysteine, cystine, and total cysteine were also higher, and the differences were of borderline significance.

Table 1

Cystathionine-Related Metabolites (μM) in Control and Down Syndrome Amniotic Fluid (Mann-Whitney rank sum test)

Metabolite	Down Median	Control Median	P value*
<u>SUBSTRATES</u>			
Serine	12.50	16.50	0.286
Homocysteine	0.10	0.00	0.008
Homocystine	0.00	0.00	0.163
Total Homocysteine	0.18	0.00	0.045
<u>PRODUCTS</u>			
Cystathionine	0.10	0.05	0.047
<u>Downstream products</u>			
Cysteine	200.50	142.00	0.060
Cystine	0.15	0.05	0.061
Total Cysteine	200.75	142.00	0.056

Unexpectedly, homocysteine was significantly increased ($p=0.008$) in Down syndrome amniotic fluid. Total homocysteine was also significantly increased ($p=0.045$).

The excess in zeros could be of concern in the analysis of homocysteine. A chi square (2X2) was made to compare the zero and non zero values for both Down syndrome and controls. (Table 2) In this table the likelihood of non-zero values is greater in the Down syndrome group. Chi-square analysis revealed this comparison was also statistically significant ($p=0.014$). Thus, the finding of higher homocysteine levels in Down syndrome was sufficiently robust that it remained when the analysis was changed.

Table 2

Homocysteine 2x2 Chi-Squared Table. Zero and non-zero values in Down syndrome and control groups (P value=0.014).

Homocysteine
Down vs Normal

Observed	Zero	Nonzero	
Down	7	16	23
Normal	25	15	40
Grand total	32	31	63

Expected	Zero	Nonzero	
Down	11.68253968	11.31746032	23
Normal	20.31746032	19.68253968	40
Grand total	32	31	63

DISCUSSION

The most reliable finding was elevation of homocysteine (P=0.008). Homocysteine and total homocysteine were increased in Down syndrome rather than decreased. Elevation of homocysteine would not result from a gene dosage effect. These findings suggest that another homocysteine-related process controlled the level of homocysteine. Homocysteine may be elevated when there is deficiency of cobalamin (vitamin B-12), folate and/or pyridoxine (vitamin B-6).^[3,4] In previous papers, we found no evidence of folate deficiency or B12 deficiency.^[5,6] However, our previous work on pyridoxine-related metabolites suggested that vitamin B6 (pyridoxine) could be deficient.^[7] The current data is therefore consistent with the findings of B6 deficiency.

In Down syndrome, fetuses have characteristically short femurs around 40% of the time, but they are normal in length more often. Some fetuses with Down syndrome have major cardiac malformations (e.g. AV canal), but a majority do not. In clinical medicine one maintains a certain cognitive dissonance to accommodate the fact that individual cases both confirm and deny generalizations. Similarly, some fetuses with Down syndrome have elevated homocysteine but some do not. While this was an unexpected finding, the initial goal was to evaluate the potential gene dosage effect.

In a gene dosage effect, one could expect decreased serine and homocysteine and increased cystathionine and cysteine. Other findings are consistent with gene dosage effect but

with less statistical significance. Serine was decreased but not to a statistically significant degree. Cystathionine was increased to a statistically significant degree. Cysteine was elevated but with borderline statistical significance. The magnitude of these changes is generally consistent with a gene dosage effect.

In inborn errors of metabolism, where enzymes are deficient, most attention is focused on the accumulation of substrates. In a gene dosage effect, the relevant enzyme is in excess, and so more attention could be turned to the products- especially cystathionine. If product elevation is more important, then the fact that cystathionine was elevated would be support a gene dosage effect. Previously, other authors have found evidence of gene dosage effect for this enzyme in Down syndrome.^[8]

Homocysteine has recently been found to play a role in Alzheimer

CONCLUSION

The elevation of homocysteine may be consistent with impaired or deficient B6 metabolism. Other findings are consistent with gene dosage effect. It may be that a gene dosage effect and a pyridoxine (vitamin B6) deficiency could coincide. This work does not support a method of prenatal diagnosis.

ACKNOWLEDGEMENTS

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Author Information

Paddy Jim Baggot, M.D.

Guadalupe Medical Center
Los Angeles, California, USA
pjbaggot@hotmail.com

Rocel Medina Baggot, M.D.

Guadalupe Medical Center
Los Angeles, California, USA

Denease Sorapuru, B.S.

Guadalupe Medical Center
Los Angeles, California, USA

Theresa Kim, B.S.

Guadalupe Medical Center
Los Angeles, California, USA

James D. Shoemaker, M.D., Ph.D.

Metabolic Screening Laboratory, Saint Louis University School of Medicine
St. Louis, Missouri, USA