Further analysis of multicentre cystathionine beta synthase deficiency thrombosis data and metabolic pathways suggests potentially better treatment via improved cysteine supplementation, diet, antioxidant supplementation, follow-up and testing for thrombophilic mutations

David Vance *

ABSTRACT
Background
Homozygous or compound heterozygous Cystathionine-beta-synthase deficiency (CBS--) may result in thrombosis. Treatment has included various combinations of: low-methionine diets, cystine (cystine dimer)-enriched amino acid supplementation, vitamin B6, folic acid, vitamin B12 and betaine. Treatment compliance and outcomes even in the most-developed countries are mostly sub-optimal and variable, and the differing theoretical metabolic ramifications due to differing treatments have not been well addressed. The aim of this work was to further analyse the thrombosis events data of Yap et al (2001/2003), and to compare these with the rate of thrombosis in the general population, and to examine the theoretical significance of the metabolic pathways affected by CBS-- and its treatments, and so find any potential improvements in treatments, considering also less-developed areas.

Methods
Yap et al’s (2001/2003) data of the thrombosis outcomes of five major (CBS--)-treating centers: in Dublin, Sydney, Nijmegen, Manchester and London; were statistically compared with outcomes predicted by Mudd et al’s (1985) untreated natural history outcomes, and then Dublin versus the others; these rates were then compared with those of general populations; and treatments were examined regarding their theoretical metabolic ramifications.

Results
There were less thrombosis outcomes (P<.05) in the treated and followed CBS-- patient groups of each of the five centers, even when considered singly, than that expected in the absence of treatment by reference to the natural history data of Mudd et al (1985), but the reduction was less than half that claimed by Yap et al, and the remaining level of thrombosis is roughly 10 times that of the general population. The thromboses outcome (nil) of the Dublin group is better than that of the other four groups, but only at P ~ 0.16 with the other four groups combined, or P = 0.14 to 0.23 singly. Treatment regimens differ, including in the sub-optimality of metabolic outcomes, due not only to CBS-- but also its treatments.

Conclusions
It seems likely that substantial improvements in the treatment of CBS-- may be achieved through:
1) Cysteine supplementation (preferably on its own rather than in whole-diet formula mixtures), in accord with various uses of homocysteine-lowering nutrients other than VitB6, which have various effects on the metabolism of homocysteine to cysteine.
2) Better use of low-methionine, high-fruit and vegetable whole-food diets.
3) Supplementation with vitamin C and other antioxidants.
4) Better cultivation of patient compliance.
5) Testing for Factor 5 Leiden and prothrombin C20210A mutations.

Keywords: Cystathionine Beta Synthase, Homocystinuria, Methionine, Cysteine, Homocysteine, Diet, Vitamin C, FMD

INTRODUCTION
Homocystinuria resulting from severe homozygous or compound heterozygous Cystathionine-beta-synthase (CBS) (EC 4.2.1.22) deficiency (CBS--) (OMIM #236200) results in a range of pathologic outcomes in the absence of treatment, including variably: thrombosis (the focus of this work); premature atherosclerosis, increased Intima-Media Thickness and reduced Flow-Mediated Dilation of vasculature; various skeletal abnormalities including dolichostenomelia/ arachnodactyly (‘Marfanoid habitus’), osteoporosis, genu valgum and pectus excavatum; developmental delay/ mental retardation, coordination and pyramidal movement disorders; ocular ectopia lentis and myopia; and fatty liver changes.\(^1\)\(^-\)\(^3\)

Treatment is generally focused on reducing the homocysteine levels of the patient, with the focus on other altered metabolites having varied according to treatment locale or even individual practitioner, though less so lately, with more internally-homogenous treatments applied in locales (i.e. nations or major city-visited areas), which are accordingly better differentiable. Treatment has included low-methionine diets, cystine-enriched amino acid supplementation, vitamin B6 (responsibility to this, the major cofactor of CBS, is the criterion for dichotomising CBS--), folic acid, vitamin B12 and betaine.\(^4\)

CBS-- homocystinuria is coming more into the attention of countries with more less-developed areas, like India,\(^5\)\(^-\)\(^31\) which is expected to have roughly 1 case/ 100,000 people (10,000 cases nationally).\(^12\) Even very recent examinations of therapy,\(^33\) while now noting more high-tech possibilities, have not yet well dealt with the fundamentals of the variations in metabolic path disposition as function of vitamin B-6 responsiveness-based treatment, and the applicability of particular treatment components to various socio-economic situations such as in less-developed areas.

The aim of this work is to re-examine the work of Yap et al (2003)\(^4\) dealing with the thrombosis outcomes of five major CBS-- treating centers, in Dublin of Ireland, Sydney of Australia, Nijmegen of The Netherlands, and Manchester and London of the UK, and to further (i.e. statistically) compare them not only with Mudd et al’s (1985)\(^3\) untreated natural history outcomes, but with each other, and also with the thrombosis rate of the general population, and to examine the possible relationship of any differences in thrombosis outcomes of respective treatment regimens including with regard to the various metabolic ramifications of the various treatment regimes, and so to find any potential substantial improvements in the treatment of CBS--, considering also less-developed contexts.

METHODS
Outcome data collated by Yap et al (2003)\(^4\) of the thrombosis outcomes of five major CBS-- treating centers, in Dublin of Ireland, Sydney of Australia, Nijmegen of The Netherlands, and Manchester and London of the UK, were statistically compared (with an adjustment for proportions of VitB6-respondent/-nonrespondent CBS-- treatment years) with outcomes predicted by Mudd et al’s (1985)\(^3\) untreated natural history outcomes (using only that portion of Mudd et al’s time-to-thromboembolic event curve that is covered by the ageing period between the average age at commencement of treatment, and the average age at the Yap et al (2003)\(^4\) study present
time). The thrombosis outcome results of Dublin of Ireland are then compared to the thrombosis outcome results of the other four centres combined and then individually. The basic statistical method used is the method for comparison of proportions in independent samples provided by Snedecor and Cochran (1989), the essential equation being:

\[
 z = (p1\text{hat} - p2\text{hat}) / \sqrt{p\text{hat}(1-p\text{hat})(1/n1 + 1/n2)}
\]

where: \( p\text{hat} = (n \text{ of events1} + n \text{ of events2})/(n \text{ group1} + n \text{ group2}) \), \( q\text{hat} = 1 - p\text{hat} \), and \( p \) for statistical significance is obtained by referring \( z \) to the table for the normal distribution of \( z \). The possible relationship of any differences in thrombosis outcomes to respective treatment regimes is then discussed.

The following is the background information from Mudd et al (1985)3 necessary for thorough comparative considerations:

Mudd et al (1985),3 in a seminal work, mailed a standardized questionnaire to each clinician known from a 1981 study18 of theirs to be caring for patients with CBS--. Physicians were asked to complete a questionnaire for each such individual about whom they had appropriate information. Further questions focused upon the factor(s) that led to ascertainment, whether the patient was responsive to VitB6, and upon the presence and age of appearance of major clinical manifestations. A detailed history of therapy was requested, as well as a reproductive history. Additional sources of information were identified by a review of the literature and by contacting centers around the world specializing in diagnosis and management of inborn errors of metabolism, as well as by soliciting physician cooperation by notices in appropriate journals. For some patients for whom recent information could not be obtained, published material only was used, if details were available to prove that they did not overlap. Data collection occurred during 1982 and early 1983 – duplication was searched for, and redundant information deleted.

For their 1985 survey, updated information was received concerning 532 homocystinuric patients with proven or presumed CBS--. To this group was added material from an additional 97 patients obtained primarily from published reports (they cite 37 references), bringing the total to 629 patients. All patients admitted to the study had been demonstrated to be excreting homocystine in conjunction with either enzyme assay or hypermethioninemia or dislocated optic lenses:

<table>
<thead>
<tr>
<th>Enzyme Assay</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ectopia Lents HyperMet</td>
<td>147 (68%)</td>
<td>274 (66%)</td>
</tr>
<tr>
<td>Ectopia Lents Only</td>
<td>21 (10%)</td>
<td>75 (18%)</td>
</tr>
<tr>
<td>Hypermethioninemia Only</td>
<td>39 (18%)</td>
<td>64 (16%)</td>
</tr>
<tr>
<td>Neither of the Above</td>
<td>9 (4%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Total</td>
<td>216 (100%)</td>
<td>413 (100%)</td>
</tr>
</tbody>
</table>

\( \text{Hypermet= Hypermethioninemia} \)

58 were discovered during screening of newborns, and an additional 88 were discovered by screening all siblings (the balance ascertained on clinical features). Of the 629 patients, 307 were females and 321 males. Of the 629 patients, 231 (37%) were classified as biochemically responsive to VitB6 when not folate depleted; 231 (37%) were classified as nonresponsive to VitB6; 67 (11%) were judged intermediate in response; and 100 (16%) had not been classified. For their subsequent analyses only the 231 VitB6-responsive and the 231 VitB6-nonresponsive were analysed. Of newborn screening ascertainment, 78% were VitB6-nonresponsive, whereas of untreated nonhypermethioninemic cases 90% were VitB6-responsive. The clinical features leading to investigation for homocystinuria (\( n = 472 \) not ascertained by newborn screening or proband sib screening, irrespective of VitB6-responsiveness) were:
Table 2 Clinical Features of Patients Involved in the Study

<table>
<thead>
<tr>
<th>Clinical Feature</th>
<th>Sole Cause (%)</th>
<th>Contrib Cause (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ectopia Lentis</td>
<td>21</td>
<td>65</td>
<td>86</td>
</tr>
<tr>
<td>Mental Retardation</td>
<td>4.0</td>
<td>52</td>
<td>56</td>
</tr>
<tr>
<td>Developmental Retardation</td>
<td>1.5</td>
<td>21</td>
<td>22.5</td>
</tr>
<tr>
<td>Early Thromboembolism</td>
<td>1.1</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>Marfanoid Characteristics</td>
<td>0.9</td>
<td>36</td>
<td>37</td>
</tr>
<tr>
<td>Bony Abnormality</td>
<td>0.2</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>Seizures</td>
<td>0.2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Behavioral/Psychiatric</td>
<td>0</td>
<td>2.8</td>
<td>3</td>
</tr>
<tr>
<td>Other</td>
<td>0.4</td>
<td>10.6</td>
<td>11</td>
</tr>
</tbody>
</table>

They presented the following time-to-event graph (projection lines added here) for first thromboembolic event in untreated patients, both combining and differentiating the VitB6-responders from the -nonresponders. All three curves have a substantially sigmoidal characteristic, such that under the age of very roughly 10 years, the slope of the curves are very roughly half of the maximum slope, that is reached at very roughly 20 years. By 28 years of age the slopes have decreased again. As they note, an idea of the differences between the VitB6-responders and -nonresponders is given by the chances of having had the initial clinically detected event by age 15 years being 12% and 27% respectively:

Fig 1 Modification (projections added) of Mudd et al’s (1985)³
Time-to-event graph for first thromboembolic event in untreated cystathionine beta synthase deficiency.
The following is the background information from Yap et al (2001)4 necessary for thorough comparative considerations. Selected data from that they collated, and selected results of theirs relevant to my study here, appear in Table 1 in the Results section below:

Now, Yap et al’s (2001)4 abstract is reproduced here with additions by the present author in parentheses “(... ... DJV)”: “Abstract.....We performed a multicenter (Dublin, Ireland; Sydney, Australia; Nijmegen, The Netherlands; Manchester, UK; London, UK DJV) observational study to assess the effectiveness of long-term Hcy-lowering treatment in reducing vascular risk in 158 patients. Vascular outcomes were analysed and effectiveness of treatment in reducing vascular risk was evaluated by comparison of actual to predicted number of vascular events, with the use of historical controls from a landmark study (Mudd et al 1985,3 DJV) of 629 untreated patients with CBS–. The 158 patients had a mean (range) age of 29.4 (4.5 to 70) years; 57 (36%) were more than 30 years old, and 10 (6%) were older than 50 years. There were 2,822 patient-years of treatment, with an average of 17.9 years per patient. Plasma Hcy levels were markedly reduced from pre-treatment levels but usually remained moderately elevated. There were 17 vascular events in 12 patients at a mean (range) age of 42.5 (18 to 67) years: pulmonary embolism (n = 3), myocardial infarction (n = 2), deep venous thrombosis (n = 5), cerebrovascular accident (n = 3), transient ischemic attack (n = 1), sagittal sinus thrombosis (n = 1), and abdominal aortic aneurysm (n = 2). Without treatment, 112 vascular events would have been expected, for a relative risk of .09 (95% CI .036 to 0.228; P<.0001).”

Yap provided additional comment on these findings in 2003.16

RESULTS
Now, firstly, it was not quite proper for Yap et al (2001)4 to aggregate thromboembolic events in with aneurysms, as they may well have differing metabolite etiologies, and also as the rates of (un)diagnosis may differ.

Secondly, the method of calculation of the number of events expected in the treatment period had treatment not been applied is by no means made clear. The following is an example of what I posit is the most correct method without more complex computational requirements that would add little more precision to the outcome; it uses only that portion of Mudd et al’s (1985)3 curve that is covered by the ageing period between the average age at commencement of treatment, and the average age at the Yap et al study present time:

Taking the average age of 29.4 years, minus the total treatment time divided by n (2822/158 = 17.9 years) one derives that on average treatment commenced at age 29.4y – 17.9y = 11.5y. Extrapolating from the (“All types combined”, on the basis that over all five study centres combined there are very roughly similar numbers of VitB6-responsive and – nonresponsive CBS–) thromboembolic event time-curve of Mudd et al (1985),3 it can be seen that for an average individual of this group, the probability of having a thromboembolic event, in the absence of treatment, was: 0.52 (the probability of having a thromboembolic event, in the absence of treatment, by age 29.4y) minus 0.10 (the probability of having a thromboembolic event, in the absence of treatment, by age 11.5y) = 0.42.

And, 0.42*158 = 66.4 thromboembolic events expected during the treatment period had treatment not been applied, compared to the 112 given by Yap et al.

Now, take away the 2 abdominal aortic aneurysms, and the 5 repeat thromboses, to leave 10 vascular events instead of the 17 noted (It seems Yap et al did this).

The relative risk would in fact be closer to 0.2 than the .09 claimed. And furthermore, as Mudd (Skovby) et al made very explicit in 2010,17 the untreated natural history CBS– time-to-event (thrombosis) curve of Mudd et al (1985)3 was very likely substantially affected by ascertainment bias, such that the CBS– cases it was derived from were probably more predisposed to thrombosis than CBS– cases are on average, such that later estimates of
CBS— treatment efficacy with groups including CBS—ascertained including by newborn genetic or biochemical screening, made on the basis or comparing treatment outcomes with that curve, are likely to be substantially inflated above the actual treatment efficacy – accordingly the actual relative risk was probably closer to 0.3 than the 0.2 of my calculation just above (which 0.3 figure Mudd did not refute in personal communication in 2013), suggesting an exaggeration of efficacy estimate in relative risk of roughly threefold, increasing the implication that substantial improvements in CBS—treatment yet remain to be found and applied.

Relevant important detail here is that the treatments applied by these different groups of practitioners are quite different, in particular the Irish compared to the Dutch and the Australian (see also Yap et al 2000), and regarding the usually somewhat ignored cysteine-enriched amino acid supplementation. It seems that with later average age of treatment commencement it is probable that cysteine supplementation is less attempted, and still less achieved of compliance, than in the commendably thorough treatment/monitoring protocols of the Irish group. Analysis would better have compared on this aspect and sought biochemical theoretical basis for the differences amongst them, rather than proceeding as they have done.

I provide in the following table, data reproduced from tables in Yap et al (2001), as well as the results of my own corresponding, and further, analyses (all delineated by “according to Vance”), which includes the further and appropriate refinement of adjusting the number of first thromboses expected in the absence of treatment from the graph of Mudd et al (1985) for any compared group by a factor addressing the ratio of Total VitB6-responsive:-nonresponsive treatment years, derived in essence from proportionate movement away from the “all types combined” graph line of Mudd et al (1985) some commensurate distances whichever of the VitB6-responsive/-nonresponsive graph lines was appropriate, before projecting across to the y axis. The fact that the number of VitB6-responsive/-nonresponsive CBS— are closely proportional to the number of respective treatment years meets one condition necessary for the validity of that procedure. My tests of statistical significance are by the method for comparison of proportions in independent samples provided by Snedecor and Cochr (1989), done by hand. The Yap et al (2001) data was drawn by them from: Yap & Naughten (1998), Kluitmans et al (1999), Wilcken & Wilcken (1997), Yap et al (2000), and Walter et al (1998).

<table>
<thead>
<tr>
<th>Table 3 Yap et al (2001) data, and comparative/further analyses on it by Vance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n of CBS—homocystinuria patients</strong></td>
</tr>
<tr>
<td>n of CBS—patients followed</td>
</tr>
<tr>
<td>n of VitB6-responsive CBS— followed</td>
</tr>
<tr>
<td>Total VitB6-responsive treatment years</td>
</tr>
<tr>
<td>Average VitB6-responsive treatment years</td>
</tr>
<tr>
<td>n of VitB6-nonresponsive CBS— followed</td>
</tr>
<tr>
<td>Total VitB6-nonresponsive treatment years</td>
</tr>
<tr>
<td>Average VitB6-nonresponsive treatments ys</td>
</tr>
<tr>
<td>Age/years at start of treatment</td>
</tr>
<tr>
<td>Age/y at Yap et al study time (1998)</td>
</tr>
<tr>
<td>n of thrombosis events</td>
</tr>
<tr>
<td>Study Information</td>
</tr>
<tr>
<td>----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>p for n thrombosis events vs Mudd-predicted n of thromboses according to Yap et al</strong></td>
</tr>
<tr>
<td>Total treatment years ratio of VitB6-responsive: VitB6 nonresponsive according to Vance</td>
</tr>
<tr>
<td>Mudd-predicted % thrombosis according to Vance</td>
</tr>
<tr>
<td>Mudd-predicted n thromboses according to Vance</td>
</tr>
<tr>
<td><strong>p for n thrombosis events vs Mudd-predicted n of thromboses according to Vance</strong></td>
</tr>
<tr>
<td><strong>p for n thrombosis events Dublin vs the other four centers combined (by Vance)</strong></td>
</tr>
<tr>
<td><strong>p for n thrombosis events Dublin vs the other four centers singly (by Vance)</strong></td>
</tr>
<tr>
<td>VitB6-responsive Free homocysteine dimer (homocystine) in plasma (&quot;Free Hcy(ine)&quot;)</td>
</tr>
<tr>
<td>Total free homocysteine in plasma (Hcy-Hcy dimer Hcy plus Hcy-Cys dimer Hcy)</td>
</tr>
<tr>
<td>tHcy (total blood homocysteine) (value means a derived value)</td>
</tr>
<tr>
<td>VitB6-responsive Free homocysteine dimer (homocystine) in plasma (&quot;Free Hcy(ine)&quot;)</td>
</tr>
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</tr>
<tr>
<td>tHcy (total blood homocysteine) (value means a derived value)</td>
</tr>
<tr>
<td>VitB6-nonresponsive Free homocysteine dimer (homocystine) in plasma (&quot;Free Hcy(ine)&quot;)</td>
</tr>
<tr>
<td>Total free homocysteine in plasma (Hcy-Hcy dimer Hcy plus Hcy-Cys dimer Hcy)</td>
</tr>
<tr>
<td>tHcy (total blood homocysteine) (value means a derived value)</td>
</tr>
<tr>
<td>Dietary methionine restriction (mg/day)</td>
</tr>
<tr>
<td>VitB6 (mg/day)</td>
</tr>
<tr>
<td>Folate (mg/day)</td>
</tr>
<tr>
<td>VitB12</td>
</tr>
<tr>
<td>Betaine (g/day)</td>
</tr>
<tr>
<td><strong>Note: No further detail differentiating VitB6-</strong></td>
</tr>
</tbody>
</table>
responders from VitB6-nonresponders was supplied

<table>
<thead>
<tr>
<th>n of venepuncture incidents per year</th>
<th>28-10</th>
<th>1-4</th>
<th>1-2</th>
<th>1-4</th>
<th>2-4</th>
</tr>
</thead>
</table>

\[ a = \text{not adjusted for Total treat-years ratio of VitB6-responsive:-nonresp} \]
\[ b = \text{adjusted for Total treat-years ratio of VitB6-responsive:-nonresp} \]

Total free Hcy = free Hcy(ine) + plasma Cys-Hcy, tHcy (value = derived) = signifies that the underlined value has been derived by Vance from the other values not underlined, presented by Yap et al (2003) \(^{16}\) but implicitly pertaining to the Yap et al (2003) \(^{4}\) data.

The derivations use the following algorithms:

For Manchester and London a change of the Bonham et al (1997) \(^{70}\) algorithm of tHcy = 60uM + 4.5 * (Hcy(ine) <20uM) + 2*(Hcy(ine)>20uM), to that more consistent with the Given Dublin correlates, that is, tHcy = 50uM + 3.5 * (Hcy(ine) <20uM) + 2*(Hcy(ine)>20uM); For Sydney, a rough mean of the tHcy = 40uM + fHcy rule (Wiley et al (1989) \(^{71}\) with derivations involving the amount of protein per blood volume) and the relationship of the Nijmegen correlates.

So, there are statistically significantly (at the P<.05 level) less thromoses outcomes in the treated and followed CBS-- patient groups of each of the five groups, even when considered singly, than that expected in the absence of treatment by reference to the natural history data of Mudd et al (1985) \(^3\). There is a substantial suggestion that the thromboses outcome of the Dublin group is better than that of the other four groups, but this does not attain statistical significance at the P<.05 level (P ~ 0.16 with the other four groups combined, or P ranging from 0.14 to 0.23 with the other four groups each taken singly), though this lack of statistical significance is very much in the context of low numbers of thromboses amongst low numbers of patients. The possible relationship of any differences in thrombosis outcomes to respective treatment regimes is addressed in the Discussion section below, as is the fact that other than Dublin the CBS-- treatment centers all have thromboses outcomes roughly 10 times that of the general population of the same age.

### DISCUSSION

Firstly, it is essential to compare the rate of thrombosis events in the CBS-- groups studied here with the rate for the general population of a similar age. Recall from Table 1 above that the number of thrombosis events and other information crucial here for the CBS-- groups studied were as extracted for consideration here:

| Table 4 Number of Thrombosis Events and Other Information Crucial for the CBS groups |
|-----------------------------------------------|----------------|-------------|-------------|----------------|-------------|
| n thrombosis in n CBS-cases                  | Dublin  | Sydney     | Nijmegen   | Manchester | London      |
| n VitB6-responsive followed                  | 0 in 27  | 2 in 32    | 1 in 28    | 3 in 30    | 9 in 4/41    |
| n VitB6-nonresponds followed                 | 1       | 17         | 19         | 8           | 25          |
| n VitB6-nonresponds followed                 | 26      | 15         | 9          | 22          | 16          |

And also that the treatment time for the VitB6-respondents was not much different from that for the VitB6-nonresponders, so there is no substantially modifying data not having been brought down from Table 1 above, for the purpose of comparison here with the general population.

Now, it was derived from a graphing of thrombosis incidence by age from a 2010 retrospective, population-based cohort study in northern Sweden \(^{23}\), by taking the average yearly incidence of venous thromboembolism in the ages 0 -30 years and multiplying that by 30 years, that the cumulative incidence in that general population by age 30 years would be approximately 1 venous thromboembolic event per 200 people. Using an exactly analogous calculation on a 1998 retrospective, population-based cohort study in Minnesota, USA \(^{24}\), it was derived that the cumulative incidence in that general population by age 30 years would be approximately 1 venous thromboembolic event per 160 people. Further, again using an exactly analogous calculation, on a 2010 retrospective, national South Korean population-
based study, it was derived that the cumulative incidence in that general population by age 30 years would be approximately 1 venous thromboembolic event per 3,000 people. Now, even allowing that the South Korean population might be actually lower in thrombosis rate or have more undiagnosed thrombosis, one suggestion from comparison of these three rates is that there might not be high rates of undiagnosis of thrombosis in the western studies, even though some substantial undiagnosis is considered to be generally likely. Now before proceeding further we address the extent to which CBS- thromboses are a component of the general population thromboses. The actual rate of homozygosity or compound heterozygosity for CBS-- in the general population of the countries of the centers studied by Yap at al (2001) and further here are probably not more than 1 in 20,000, but might be only a fifth or less of that. Therefore it is not necessary to carry out subtraction from the rate of thrombosis in the general populations before comparison as done here. There is however, the question of whether the increased index of suspicion in the case of CBS-- might lead to less undiagnosis of thrombosis, such that the difference in rates of thrombosis between the CBS-- cases here and the general populations noted here might be less in reality than is apparent from mere comparison of the rate numbers here. There are also questions remaining as to whether some level of non-compliance has resulted in any exclusion of non-compliant cases from reported outcomes by the treating centers, and also here, that non-compliance might be more explicitly determinable in the case of VitB6-nonrespondent CBS-- treatment than in the case of VitB6-respondent CBS--. Furthermore, given the frequency of genetic mutations for thrombophilia (in particular Factor 5 Leiden and prothrombin G20210A) in the general population (notwithstanding that by survival selection these might well be less in the CBS-- population) are fairly common and the number of CBS-- cases studied here and still more so the number of thromboses are small, it is quite possible that differential rates of thrombophilic mutations has played a role in the differential thrombosis outcomes of the five study centers. Nevertheless, and particularly due to the fact that rates of thrombosis in the general population increase dramatically (over an order of magnitude) by later years, particularly with aging beyond the age of the CBS-- subjects studied here, as well as that most of the general population thromboses are preventable by e.g. diet and physical activity, and given that with the exception of Dublin (nil thromboses) the thrombosis rates in the CBS-- treatment groups here are roughly 10 times that of the general population of the same age (which will certainly be statistically significant at the P<.05 level due to the large numbers in the general population studies), it seems safe to assume that the rates of thromboses observed in the five (four) groups studied here are substantially enough in excess of (roughly 10 times) the real rate in the (non-CBS--) general population for these age groups to warrant attention regarding both studied age groups and what is likely in the future with ongoing aging.

Without belabouring the point of the differences in outcomes between Yap et al’s (2001) and my analyses here, I turn to suggesting that the differences between the Irish outcomes (those included in the data here) and the other outcomes are worth noting, particularly in the light of small numbers overall plausibly being a factor in the statistical significances of the comparisons not quite reaching any (arbitrary, if generally reasonable) criteria set at P<.05.

Note that while on the one hand thromboses are a small part of the symptoms contributing towards ascertainment of the non-newborn-screening CBS-- cases that effectively do not constitute any of the Irish (approximately all newborn-screening ascertained) and therefore give an ascertainment bias towards the non-Irish groups having more thromboses, on the other hand the repeat thromboses of the English group have not been incorporated into the analysis, somewhat ameliorating such bias.

Furthermore, the VitB6-nonresponsive CBS-- cases (which constitute all of the Irish cases, but only very roughly half of the cases of the other four centers) have a worse expected outcome generally than the VitB6-responsive CBS-- cases (notwithstanding the accounting for this in numerical comparisons here).
Also, the Irish group of CBS-- experience much more venupuncture in the course of their metabolic monitoring, and venupuncture is known to promote thrombosis. I suggest that a fair adjustment for these factors would make the difference in thrombosis outcomes between the Irish and the combined other groups close enough to statistically significant to even more definitely warrant further discussion, as follows.

Consider the following diagram (Fig. 2) of the metabolic pathways central to considerations of the CBS-- situation:

![Fig 2 Metabolic pathways central to cystathionine beta synthase deficiency (adapted from Mudd et al (1985))](image)

**Sulfate**

Now firstly, inorganic sulfate is quite readily absorbed from the GIT \(^{28, 29}\), and this and renal secretion and reabsorption (this latter predominating over secretion, as the molecule is freely filterable \(^{30}\)) are quite the main mechanism of sulfate homeostasis. So although, for example, the family of recessively inherited osteochondroplasias, resulting from mutations in the Diastrophic Dysplasia Sulfate Transporter, are due to the undersulfation of proteoglycans in chondrocytes \(^{31, 32}\), it seems unlikely that derangement of sulfation is part of the mechanism of CBS-- pathology, unless in-situ generation of sulfate is a requirement, but this seems unlikely, as there are a number of sulfate-transporters seemingly supplying sulfate from ECF to the ICF of cells (effectively all types of cells?) that require sulfate for the production of 3-
phosphoadenosine 5-phosphosulfate (PAPS) for some use(s) or other. So, sulfate will not be considered any further here as an etiologic agent of CBS--pathology, notwithstanding its manifold and varied role in biology.

Cysteine
In accord with the enzyme defect in CBS it has been shown that requirement for cysteine is increased in CBS--.\(^{33-37}\)

Marfan syndrome (MFS, OMIM #154700) is an autosomal dominant disorder affecting multiple systems, including the cardiovascular, ocular and skeletal systems. The cardiovascular pathologies include aortic aneurysms and dissections, and mitral valve regurgitation/ prolapse. Ocular pathologies are ectopia lentis and myopia. Skeletal pathologies include dolichostenomelia, arachnodactyly, kyphoscoliosis, pectus deformities, pes planus, and highly arched palate with crowding of frontal teeth. It is noted that mutations in the gene for fibrillin-1, an elastic fiber, are responsible for most MFS cases.\(^{38}\)

Fibrillin contains 14% cysteine (an unusually high content among proteins), of which one third appeared to be in the free reactive sulfhydryl form.\(^{39}\) Disulfide bonds play a major role in the protein's structure.

CBS-- has in common with MFS many of the abovementioned pathological features (hence the term “Marfanoid habitus of CBS--”\(^{33, 36, 40-42}\) such that in times predating differentiation such CBS-- cases would be lumped in under the MFS diagnosis.

Investigations following on speculation that in CBS-- the altered plasma concentrations of homocysteine and/or cysteine may hinder the synthesis, deposition, or both, of fibrillin-1, reported that when arterial smooth muscle cells were cultured under conditions of cysteine deficiency fibrillin-1 deposition into the extracellular matrix was greatly diminished (and restored with addition of cysteine), but that excessive homocysteine in contrast had little if any effect on fibrillin-1 deposition. Type I collagen, the major matrix component synthesized by these smooth muscle cells, was not reduced by low cysteine concentrations nor by high homocysteine concentrations.\(^{43}\)

It may be seen that in VitB6-responsive CBS-- the use of VitB6, as a sole pharmacotherapy, such as to lower methionine to, and homocysteine near to, normal levels, must therefore also have increased cysteine to near normal levels, with regard to its coming from the CBS pathway represented at the bottom of Fig. 2.

Now, if it is necessary to lower methionine intake to lower methionine and (focally) homocysteine to near normal levels, the question must arise as to whether the products of the CBS pathway, in particular cysteine, become deranged, and to what extent – in this particular case, it would be expected that cysteine would be reduced – less homocysteine to drive the CBS pathway, resulting in less cysteine.

The use of methionine-restriction as a sole therapeutic measure would be very likely to result in cysteine deficiency of some level.

Now, if one adds folate, betaine, or VitB12 to the initially (however) successful VitB6-treatment of VitB6-responsive CBS--, then this would theoretically reduce the drive down the CBS pathway, resulting in less cysteine.

At this point it seems reasonable to suggest that a decrease in dietary methionine might benefit from an accompanying addition of cysteine to make good the resultant metabolic decrease of cysteine. And likewise, an increase in folate, betaine, or VitB12 might benefit from an accompanying addition of cysteine to make good the resultant metabolic decrease of cysteine. Using combinations of these would require increases in additional cysteine over that of the use of the single treatments of the same magnitude.

In VitB6-nonresponsive CBS-- methionine-restriction as a sole therapy would be very likely to result in an even more deficient level of cysteine than that pre-treatment, as even careful dietary composing must reduce cysteine also, as they are substantially positively correlated in foods, in both being protein

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constituents – in practice this compositing constitutes a move to a lower-protein diet, with plant protein sources predominating over animal protein sources.

The addition of folate, VitB12, and betaine to the methionine-restriction and cysteine-supplementation treatment of VitB6-nonresponsive CBS-- would not be expected to result in less cysteine as would be the case in VitB6-responsive CBS--.

However, in the event of these latter treatments displacing, through either CBS-- non-compliance or physician non-emphasis, either the methionine-restriction of diet, or the cysteine supplementation, without some alteration in the other, then cysteine levels would be reduced accordingly.

Note that the Irish treatment regime is instituted from birth, and is reinforced thereafter in the most thoroughgoing manner of all the CBS-- treatment centres considered here (for which they are to be commended), and that it is probable that they accordingly have better compliance with cysteine supplementation than the other centers, particularly if it is over-connected in the minds of the patients/carers with use of the low-methionine (lower-protein) diet. Such over-connection is not valid, particularly in the case of VitB6-nonresponsive CBS-- in particular the case of non-compliance with any low-methionine diet that has been structured to include higher cysteine: methionine ratio, but also in any case (cysteine-methionine correlation in foodstuffs being sufficiently high as noted above), and it is erroneous to view non-compliance with the low-methionine diet as at all a good reason for non-compliance with cysteine supplementation. In the case of VitB6-responsive CBS-- that has been prescribed a low-methionine diet that has not been structured to include higher cysteine: methionine ratio, non-compliance with cysteine supplementation per se is less likely to be problematic than it would for VitB6-nonresponsive CBS--, but is still undesirable.

Hydrogen sulfide can be formed from cysteine via CBS, which is highly expressed in the hippocampus and cerebellum, and brain homogenates produce hydrogen sulfide, and physiological concentrations of hydrogen sulfide facilitate the induction of long-term potentiation in the hippocampus. More relevant to my considerations here, endogenous hydrogen sulfide regulates smooth muscle (e.g. vascular) tone in synergy with nitric oxide.

OTAMC pathway, hydrogen sulfide, methanethiol, 3-methylthiopropionate, formate, and formaldehyde

Discussion of other metabolites involved in CBS-- is limited here in scope to that of the general concept, but one that is worth bearing in mind as such nonetheless.

In VitB6-responsive CBS--, if only VitB6 has been used to successfully lower both methionine and homocysteine to near normal levels, then it may be reasonably said that the resultant levels of all metabolites central to CBS-- have been perhaps close to effectively normalised – and this situation (all metabolites normalised) is probably/possibly the most desirable, practically.

Now, if it is necessary to lower methionine intake to lower methionine and (focally) homocysteine to near normal levels, the question must arise as to whether (other) products of the CBS and oxidative transamination methionine catabolic (OTAMC) pathways become deranged, and to what extent.

Now, if one adds folate, betaine, or VitB12 to the initially (however) successful VitB6-treatment of VitB6-responsive CBS--, then this will reduce the drive down the CBS pathway – but, via raised (above threshold >350 uM) methionine, will increase the drive down the OTAMC pathway, producing raised and potentially harmful levels of hydrogen sulfide, methanethiol, 3-methylthiopropionate, formate, and formaldehyde.

In VitB6-nonresponsive CBS--, methionine-restriction as a sole therapy would be very likely to result in an even more deficient level of metabolites downstream of cysteine in the CBS than that pre-treatment, unless (as methionine and cysteine are associated in foodstuffs) careful dietary compositing had not reduced cysteine also, as noted above in the Cysteine section, but in a change back down towards normal
of OTAMC pathway metabolites, particularly if assuming that normally flux through the OTAMC pathway is minimal

Addition of sufficient cysteine to methionine-restriction-treatment of VitB6-nonresponsive CBS-- would perhaps seem likely to result in no change in OTAMC pathway metabolites.

The addition of folate, VitB12, and betaine to the methionine-restriction and cysteine-supplementation treatment of VitB6-nonresponsive CBS-- might increase OTAMC pathway metabolites via increased methionine. In the event of these latter treatments displacing, through either CBS-- non-compliance or physician non-emphasis, either the methionine-restriction of diet or the cysteine supplementation, then OTAMC pathway metabolites would be expected to further increase, or, metabolites downstream of cysteine in the CBS to decrease, respectively.

So, there are metabolites other than the ones commonly focused on in CBS-- that are also deranged and may contribute to some aspect of CBS- pathology.

Vitamin C
It is reported that after treatment of 5 CBS-- (mean age 26 y, all treated with vitamin B6 and folate, four treated with betaine, two treated with a "methionine-free protein mix", one treated with VitB12, such as to result in tHcy mean 100 uM vs 5 controls 9 uM), acutely with 2 g of vitamin C once, or chronically with 1 g of vitamin C/day for 2 weeks or 6 months, endothelium-dependent Flow-Mediated Dilatation (FMD) was restored from a baseline of mean 20 um (vs controls mean 116 um) to 160 um, 170 um, and 170 um, respectively. This was highly statistically significant (P<.001 for all time-points). Note that there was no blinding in this experiment, and that CBS-- behaviour in other regards, i.e. compliance to other treatment modalities i.e. including diet may have changed as a result.

FMD has been found to be strongly negatively associated with the following prothrombotic states:

Systemic hypertension; FMD 4.8% vs controls 8.6%, P<.001
Stable coronary artery disease; FMD 2.4% vs controls 8.5%, P<.001
Primary antiphospholipid syndrome
However, it is not clear whether differentiation of the FMD-reduction etiology has a bearing on tendency to thrombosis. Nevertheless, there is a fairly strong suggestion that reduced FMD might be a contributor to the thromboses found in untreated CBS--, and also to a lesser extent amongst CBS-- under some treatment regimes, although of course compliance is always a factor.

Now, 1 g of vitamin C would be supplied by (roughly): (Fruits) 0.42 kg of Hawaiian guava, less than 1 kg or 1 L of orange juice, 1.4 kg of kiwifruit, 1.7 kg of pawpaw, 2 kg of oranges/ other citrus, 2 kg of lychee fruit, 2.3 kg of custard apple, 2.9 kg of rockmelon, 3.6 kg of mango, 5 kg of pineapple, 5.6 kg of honeydew melon or prickly pear, 6.7 kg of bananas, 7 kg of persimmon, 17 kg of apples or grapes, and not at all by any even vaguely feasible quantity of pears; (“Vegetables”) 0.6 kg of raw red capsicums, 0.9 kg of raw broccoli or Brussels sprouts, 1.2 kg of boiled broccoli or Brussels sprouts, 1 kg of raw mustard cabbage or watercress, 1.4 kg of raw red cabbage or raw cauliflower or raw kohlrabi, 2.2 kg of raw common cabbage, 2.8 kg of boiled common cabbage, 3.4 kg of boiled broad beans, 5 kg of boiled potato or sweet potato, 5.3 kg of boiled fresh kidney beans, 5.6 kg of raw common tomato, 6.7 kg of Lebanese cucumber, 8 kg of raw bean sprouts, 8.3 kg of boiled pumpkin, 12 kg of common cucumber, and not at all by any even vaguely feasible quantity of lettuce and even less so of any legume dried then boiled
(second digits are of course of varying, if any, accuracy). Furthermore, amongst these and other foods are substantial amounts of other antioxidant nutrients such as vitamin E and carotenoids, which are very likely to reduce the absolute amount of vitamin C required for most of its biological functions, and certainly its antioxidative functions. It can thus be seen that dietary alterations on their own could very likely bring about near enough to the vitamin C related increase in FMD noted above. The question then follows as to whether CBS-- treatment regimes that achieve compliance with low-methionine diets, which in
practice have an increased amount of fruit and vegetables, are achieving an improved thrombosis outcome via improved FMD via increased intake of vitamin C and other antioxidant nutrients.

Note that the Irish treatment regime is instituted from birth, and is reinforced thereafter in the most thoroughgoing manner of all the CBS-- treatment centres considered here (for which they are to be commended), and that it is probable that they accordingly have better compliance than those other centers.

Acid-Base Balance
Acidification of the human body is more closely tied to higher food sulfur amino acid levels than to food amino acid levels per se. Based on this and other aspects of this review one would expect the low-methionine diet as commonly employed in CBS-- to lead to a reduction in acidity. However, the sparse literature (i.e. suggests that acidosis is associated with reduced, not increased, coagulation. What bearing any alkalizing effect of the low-methionine diet as commonly employed in CBS-- might have on thrombosis seems unclear.

Cystathionine
On autopsies on two CBS-- dying of postoperative pulmonary thromboembolism aged 9 years, and 14 controls, it was reported: “....the strikingly low concentrations of free cystathionine in all parts of the brains examined in homocystinurics (0.0-0.4 mg/100gWetW) as compared to normal brains from patients dying from entirely unrelated conditions...”, but also “If (n = 11) the adult control tissues alone are considered, the various areas of brain show striking differences in the concentration of free cystathionine from one area to another: Occipital lobe (12-90) > Frontal lobe (11,13) > Pons and Medulla (8,5) > lateral lobe of Cerebellum (3,5, 2)” in conjunction with “The other sulphur containing free amino acids showed no difference either between controls and homocystinurics or between various areas.”

The meaning of this is basically that cystathionine is potentially not merely an intermediate in the transsulfuration pathway, with no biochemical function of its own, in all tissues. How this might relate to CBS-- pathology is not well defined, even regarding the possibility of involvement in CBS-- clumsiness and gait disturbance, where homocysteic acid as a neural excitotoxin must also be considered a possibility.

Homocysteine
It is beyond the scope of this work to consider the likely involvement of the many postulated mechanisms of homocysteine pathogenesis, with the thrombosis outcome that is the focus of this paper, and this has not been established elsewhere, so I only list the following potential mechanisms noted by recent reviewers: The high pKa (10.0) of the homocysteine sulfhydryl moiety enabling it to form stable bonds with protein cysteine residues, impairing the function of proteins such as albumin, fibronectin, transthyretin, annexin II, and factor V, angiototoxicity, neurotoxicity, and inhibition of collagen cross-linking, impaired vascular endothelium-dependent Flow-Mediated Dilation, inhibition of endothelial nitric oxide synthase by its endogenous inhibitor asymmetric dimethylarginine, and oxidative inactivation of nitric oxide mediated by upregulation of prooxidant enzymes and downregulation of antioxidant enzymes, attenuation of GABA-A/B receptors and increasing redox stress, which activates a disintegrin and metalloproteinase that suppresses tissue inhibitors of metalloproteinase. Also note the report concluding that reduction of intramolecular calcium binding epidermal growth factor like (cEFG) domain (i.e. of the cysteine-rich fibrillin-1 elastic fiber protein) disulfide bonds by homocysteine and the resulting disruption of structure may contribute to the change in connective tissue function seen in homocystinuria, but that other cEFG-containing proteins may also be involved in CBS-- pathology. It seems plausible that both homocysteine excess and cysteine deficiency could play joint roles in at least this latter aspect of CBS-- pathology.

Unfortunately, comparison of the homocysteine levels of the five centers examined in this work provides little information - the algorithms used to derive those of the tHcy values that had to be derived, though reasonable, are fairly rough, and the tHcy values have little consistent association with the
thromboses outcomes – while the UK centers London and Manchester had both higher tHcy levels and more thromboses than the other three centers, Nijmegen and Sydney had lower homocysteine levels but more thromboses than Dublin. And here of course the comparison should incorporate VitB6-responsivity, but that differentiation of data is not available from Yap et al (2001) 4. Also it is not guaranteed that the different monitoring schedules (in conjunction with other aspects) of the different centers give rise to equal representativeness of the assayed homocysteine values of the average homocysteine values, nor that such averages are of more relevance than i.e. maxima. Any attempt to relate the tHcy values to the treatment regimens should incorporate VitB6-responsivity, but that differentiation of data is not available from Yap et al (2001) 4, and given the relative closeness of the five regimens, even guarded speculation has little to offer here. And finally, the tHcy values are not greatly different from each other.

Platelets
Regarding platelet stickiness, 5 CBS-- aged 4-15 y had 46%, 58%, 60%, 48%, 48%, average 54% of platelets remaining at 20 minutes, while the 5 similarly aged controls had 50%, 85%, 77%, 85%, 75%, average 74% of platelets remaining at 20 minutes 64. However an adjustment for platelet count (CBS-- average 296,000/ul, control average 216,000/ul) might have decreased the observed difference to little or nothing. During the course of nitrogen balance studies, in one CBS-- case, SM, “The platelets appeared to be equally abnormal in the first, third and fourth periods and no decreased stickiness had occurred when the last measurement was made 2 days before SM’s discharge although the plasma methionine levels had been near normal values for 5 days. The only change in platelet behaviour was in the second period (supplementary cysteine replaced with methionine) when temporarily they were more sticky than usual and clumped together to such an extent that it was not possible to count them.” They report to the contrary that in trials of a low-methionine, low-protein diet in another CBS--, RM, “Platelet stickiness was measured 3 times in RM, once before starting the diet, and on the 8th and 22nd day after starting it. On all three occasions the platelets were grossly abnormal in behaviour and the improvements induced by the diet in plasma amino acid concentration (reported: plasma methionine from 12 mg/dl to 1.2 mg/dl, free homocysteine from 130 uM to 100 uM) were not obviously associated with any decrease in platelet stickiness ... ... The dietary trial here was long enough to allow regeneration of all platelets, taking the life of a platelet to be 8 days, so that the failure to improve was not due to the persistence of permanently injured platelets in the circulation.” 35. However, note that the change in homocysteine levels was not great, and not equal to that able to be obtained by full or even substantial compliance with the specified regimens of any of the five centers studied here.

It was reported of a trial of a calcium cystinate-supplemented low-protein branched-chain ketoaciduria diet which generally achieved approximately undetectable free homocysteine levels (other than those attending infrequent indiscretions), that is to say tHcy levels approximately <40 uM, in a CBS-- case treated from infancy to age 2.3 years with good outcome, that “platelet stickiness of 72%” was “considered well within the normal range of this technique.” 34.

The following groups were reported on: Two VitB6-nonresponsive CBS--, diagnosed at age 20 years and 16 years, one of whom had tHcy>200 uM and the other probably likewise, had a platelet count of mean 297,000/ul and platelet survival of mean 5.0 days; Two VitB6-responsive treated CBS--, diagnosed at age 8 years and 16 years, with tHcy of approximately <100 uM and 50 uM and a platelet count of mean 197,000/ul and platelet survival of mean 8.6 days; Thirty five normal controls with a platelet count of mean 250,000/ul and a platelet survival of mean 9.5 days.

They carried out further platelet function tests and homocysteine infusion experiments using baboons, to further report that platelet destruction was not due to a direct toxic effect of homocysteine on platelets, since dipyridamole therapy blocked platelet consumption, nor was it due to enhanced platelet reactivity. They therefore concluded that the underlying process of CBS-- thrombosis probably
involves formation of platelet thrombus on altered, nonendothelialized endarterial surfaces. 65

Another work reported that in three VitB6-responsive CBS-- cases, collagen-, ADP-, and adrenaline-induced platelet aggregation was decreased (not a misprint here) before treatment started, and returned to normal when homocystine (the free dimer) disappeared from plasma. They concluded that the platelet alterations in untreated patients result from their refractory stage after a release reaction has already taken place, as maybe caused by homocysteine-caused endothelial damage 66.

Platelets were harvested from six VitB6-nonresponsive CBS-- (treated with VitB6 and folate), six VitB6-responsive CBS-- (treated with VitB6), and 11 normal volunteers, and labelled with 111Indium and reinfused. There were no substantial differences in platelet survival time (a maximum difference between any two groups of approximately 5% of the survival times found) according to all of three different mathematical models 67.

A 20 year-old female CBS-- case diagnosed eventually (and later found to be VitB6-responsive) after thrombosis sequela following one month after first parturition, whose platelets while on warfarin treatment (no CBS-- treatment) following on serial thrombectomies, had a very high count at 674,000/ul (normal <450,000/ul), and had a stirred, EDTA-added, whole-blood platelet disappearance of 20% in 6 minutes (normal <5%). However molondialdehyde-generation in clotted whole blood was normal, as was separated platelet rich plasma behaviour with regard to arachidonic acid-induced malondialdehyde production, and response to challenge with ADP, adrenalin and collagen 68.

In conclusion, it seems reasonable to assume that platelets are more likely to generate thrombi when/after contacting damaged endovascular epithelium, and that reduced FMD causes pressure phenomena that probably increase thrombogenesis (as well as vascular endothelial damage). These phenomena are likely to apply not only to normal platelets, but also to deranged platelets. The other obvious inconsistencies in, and incompleteness of, the information discussed here on platelet behaviour in CBS-- I am unable to resolve in general, and in particular with regard to the differences in thromboses outcomes of Dublin versus the other four groups examined here.

**Thrombophilic Mutations**

It is obvious enough that it is generally desirable for CBS-- patients to be tested for thrombophilic mutations, at least the common Factor 5 Leiden and prothrombin G20210A mutations, and, notwithstanding the limitations of small numbers, the question as to whether such mutations are additive or multiplicative in their effects should be examined 69.

**CONCLUSION**

Rates of Thrombosis in relatively young treated CBS-- groups in most-developed countries are probably roughly 10 times that of the general population, clinically significant even then and likely to become much more so with aging. The efficacy of current CBS-- treatment even in most-developed countries is not high enough to warrant ignoring potential improvements. There seems enough evidence to warrant consideration by those treating CBS-- that cysteine supplementation may be of more importance than generally acknowledged, and that its dose should perhaps be varied more systematically as a function of the use of homocysteine-lowering treatment modalities other than VitB6, that reduce metabolic flux down the CBS transsulfuration pathway in VitB6-responsive CBS--, when it becomes even more important.

Furthermore, in less-developed contexts there is the desirability of keeping cysteine supplementation separate in a capsule that is more readily associated with a treatment for a particular disease, and therefore less likely to be consumed by others than the CBS-- person, rather than attempting to provide whole-diet bulk formula that can very likely be mistaken for supposedly generally-beneficial formula food or be consumed in any case by non-CBS-- persons out of desperation. Likewise, that low-methionine, high-fruit and vegetable diets may be of more importance than generally acknowledged, and that where compliance with this is compromised (and
perhaps in any case, to some extent), supplementation with at least vitamin C and probably also other antioxidants should be implemented, though hopefully not in a manner so as to further displace the diet. There is also scope for finding locally-grown or available proteinaceous foods that are low enough in methionine to be able to be usefully included in the diet in suitable proportions. Also, the apparently meticulous thoroughness of the Dublin group with regard to the reinforcement/cultivation of compliance with e.g. cysteine supplementation and low-methionine high-fruit and vegetable diet is probably worthy of study and emulation.

Furthermore, all CBS-- patients should be tested for thrombophilic mutations, at least the common Factor 5 Leiden and prothrombin G20210A mutations, and thought should also be given to rarer mutations. It also well behoves to consider that these CBS--cohorts studied here are not yet into older age, when pathologic outcomes in general, and very much so thrombosis 22-25, are greatly increased in the general population.

Further study of these matters, at least via ongoing monitoring of treatments/outcomes and updating of data, and improved detail of data collected, is obviously desirable. Clarification of platelet behaviour and mechanisms would potentially be helpful.

It is redundant to note that further inquiry into the role of homocysteine in disease is desirable, but certainly some more attention to related other metabolites seems warranted.

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