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ORIGINAL ARTICLE

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The effectiveness of correcting abnormal metabolic profiles

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Abstract

Inborn errors of metabolism cause disease because of accumulation of a metabolite before the blocked step or deficiency of an essential metabolite downstream of the block. Treatments can be directed at reducing the levels of a toxic metabolite or correcting a metabolite deficiency. Many disorders have been treated successfully first in a single patient because we can measure the metabolites and adjust treatment to get them as close as possible to the normal range. Examples are drawn from Komrower's description of treatment of homocystinuria and the author's trials of treatment in bile acid synthesis disorders (3β-hydroxy-Δ^5-C_{27}-steroid dehydrogenase deficiency and Δ^4-3-oxosteroid 5β-reductase deficiency), neurotransmitter amine disorders (aromatic L-amino acid decarboxylase [AADC] and tyrosine hydroxylase deficiencies), and vitamin B6 disorders (pyridox(am)ine phosphate oxidase deficiency and pyridoxine-dependent epilepsy [ALDH7A1 deficiency]). Sometimes follow-up shows there are milder and more severe forms of the disease and even variable clinical manifestations but by measuring the metabolites we can adjust the treatment to get the metabolites into the normal range. Biochemical measurements are not subject to placebo effects and will also show if the disorder is improving spontaneously. The hypothesis that can then be tested for clinical outcome is whether getting metabolite(s) into a target range leads to an improvement in an outcome parameter such as abnormal liver function tests, hypokinesia, epilepsy control etc. The metabolite-guided approach to treatment is an example of personalized medicine and is a better way of determining efficacy for disorders of variable severity than a randomized controlled clinical trial.

KEYWORDS

bile acid synthesis disorders, homocystinuria, N = 1 trials, neurotransmitter amines, personalised medicine, pyridoxal phosphate, randomised controlled trials, treatment, vitamin B6

It is a great honor to be asked to give this lecture dedicated to George Komrower especially at a conference with the overall theme of Old Roads and New Connections. I think looking back at some of Komrower's work reminds us that Old Roads can still take us to important destinations (effective treatments) but also of the importance of New Connections—sharing data in validating new treatments for inborn errors that are very variable in their effects.

George Komrower worked as a pediatrician in Manchester between 1948 and 1975. He made many contributions to our knowledge of inborn errors of metabolism. In particular he understood and could undertake biochemical measurements

and introduced, in Manchester, screening for amino acid disorders using paper chromatography which allowed for the detection of homocystinuria as well as phenylketonuria (PKU).

In the early 1960s, Carson had described children with developmental delay, skeletal abnormalities, and sometimes dislocated lenses who had increased levels of methionine and homocystine in plasma and increased homocystine excretion in the urine.[1]

Subsequent investigations showed that brain cystathionine was absent,[2] the activity in the liver of cystathionine synthase was markedly reduced[3] and that cystine was required to maintain positive nitrogen balance.[4] In the introduction to his 1966 paper on homocystinuria, Komrower referred to the pathway for metabolism of methionine via homocysteine and cystathionine to cysteine with the block at the level of cystathionine synthase (Figure 1).[5]

He argued that if the accumulation of methionine and/or homocysteine was involved in pathogenesis of disease, the logical way to treat the disease would be to reduce methionine intake and supplement with cysteine. On the other hand, if the disease was caused by a deficiency of cystathionine, treatment should be by supplementation with cystathionine. His choice was somewhat pragmatic. He pointed out that cystathionine is very expensive and is rapidly excreted in the urine, and that no known function has been found for this compound. So he opted to try and treat a newborn with homocystinuria with a low methionine diet. Komrower showed that, by adjusting the methionine intake, it was possible to maintain plasma methionine in the normal range and substantially reduce homocystine levels (albeit without correcting cystine levels). At 2 years the child had an IQ of 97 and no features of homocystinuria (in contrast to her affected siblings). So this was a successful trial of treatment in a single patient similar to that achieved for the first time in phenylketonuria by Horst Bickel et al in 1953.[6]

Subsequent assessments of dietary treatment of homocystinuria involved, as in Komrower's description, adjustment of diet based on free homocystine measurements. Yap et al in Ireland analyzed the outcome for example for IQ according to whether or not a median lifetime free plasma homocystine less than 11 micromols/L was achieved. The mean IQ was 106 for children with good biochemical control but 81 for those in whom this was not achieved.[7]

I think most of us are now convinced that we can achieve a good IQ outcome and avoidance of complications in homocystinuria by maintaining low levels of plasma total homocysteine with pyridoxine in responsive patients, and diet +/− betaine in non-responsive patients.

However, if we use the criteria of the proponents of "Evidence-based Medicine" we find that the evidence is not of the highest level. The "level of evidence" for efficacy of methionine restriction +/− pyridoxine +/− betaine is 2c, defined as "Outcomes Research" (focused on end results of therapy for chronic conditions, including functioning and quality of life). In contrast, treatment of Niemann-Pick disease type C with Miglustat scores an evidence level of 1b (individual randomized controlled trial [RCT]).[8] Do we believe that the evidence that Miglustat is effective in NPC is significantly better than the evidence that diet +/− pyridoxine +/− betaine is effective in homocystinuria? Or is it time to include other equally important measures of efficacy?

Many of our best treatments for metabolic disorders have evolved from treatment of a single patient as described by George Komrower for homocystinuria and by Horst Bickel for PKU. Surprisingly, I do not think we have a name for the process. So I am suggesting names for the two stage process.

Stage 1 is the "Metabolic Profile Correction trial" (MPC trial),

- "Metabolic profile correction trial" (MPC trial) for individual (or individuals with the same disorder, genetically, biochemically, and clinically) but possible variation in severity.

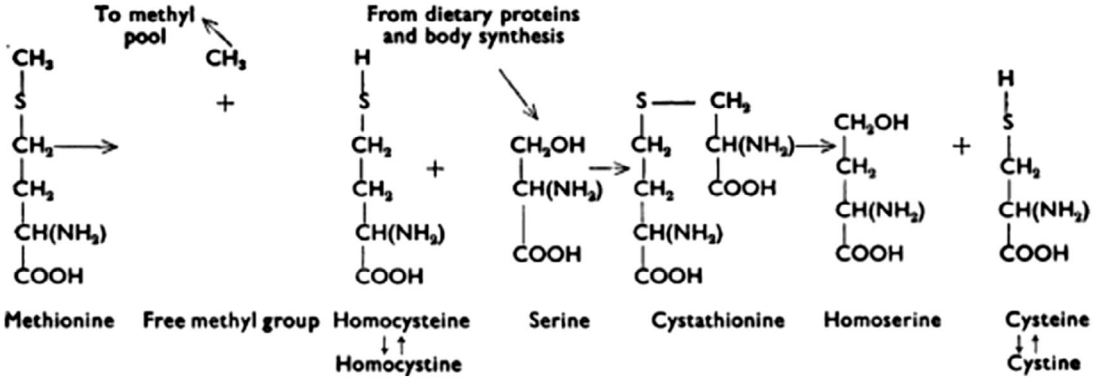

FIGURE 1 The pathway that is blocked in homocystinuria due to cystathionine β-synthase deficiency as depicted when Komrower undertook his trial of treatment. Taken from Ref. 1

- Monitor metabolites whose concentrations are probably pathogenic (toxicity or deficiency).
  - Measurements of metabolite concentrations are not subject to placebo effects.
- Allow adjustment of diet and/or drugs aiming for optimal correction of metabolic profile.
  - Spontaneous improvement can be detected.
- Test the hypothesis that correction of the profile is more than any observed with time; needs good natural history data.
- Repeat from n = 1 to n = all patients newly diagnosed.
  - NB need separate natural history data for patients diagnosed presymptomatically, for example, by neonatal screening.

This allows you to treat a patient according to the best current knowledge and determine whether it is possible to lower the levels of potentially toxic metabolites and/or correct metabolite deficiencies. As the outcome measures are laboratory measurements, there is no possibility of a placebo effect and if the disorder is one that corrects spontaneously or varies substantially, this can be detected by the metabolite measurements. So the two major reasons for undertaking a randomized controlled trial are eliminated. A meaningful randomized controlled trial requires either a homogeneous population or stratification by severity. The MPC trial allows for a wide variety of disease severity; the treatment is adjusted to the minimum necessary to keep metabolite levels in the target range, an example of personalized medicine. It is, however, important to recognize that this approach is based on the premise that you are monitoring a metabolite that is toxic at abnormally high or low levels. There have been a number of presumed "inherited metabolic diseases" which turned out to be non-diseases because biochemical changes were erroneously assumed to be pathogenetically relevant, for example, histidinemia, mistakenly even included in newborn screening programs in some centers from the 1960s to 1980s.

Stage 2 is the "Metabolic Correction Outcome trial" (MCO trial). The outcome measure can be a measure of disease progress, such as reduction in severity of a symptom, or improved weight gain, or improved disease markers such as liver size and liver function tests, or IQ. The hypothesis being tested is that the outcome is better if a defined correction of the metabolite profile is achieved. Non-compliance with treatment is not as serious as it is with an RCT; a non-compliant subject will be in the group that does not achieve the metabolite target(s).

During my career, I have had the opportunity on a few occasions to attempt what Komrower did with homocystinuria, and that is to try, in a single patient, to determine whether it might be possible to correct abnormal metabolite levels and thereby alleviate the symptoms and signs of the disease. I would like to share some thoughts on these. I want to emphasize (a) the importance of measurements of metabolites for guiding treatment; (b) the very marked variability in symptoms, signs and progress for most inborn errors; and (c) the importance of effect size in assessing evidence.

It should not be surprising that individuals with inborn errors show marked variability in levels of pathogenic metabolites and even more variability in clinical symptoms and signs. Different mutations can have very different effects on the amount of protein that is produced, its structure, thermostability, and ability to bind substrate(s) and cofacator(s), and it cellular location. This leads to different residual activities of an enzyme or transporter and differences in the degree to which activity can be restored by increasing substrate or cofactor concentration. When it comes to two individuals with the same mutations, there is still scope for variability. The activity of alternative pathways and the sensitivity of feedback control mechanisms may vary because of polymorphisms in genes other than that bearing the primary disease-causing mutation(s). Finally, there are many variable environmental factors that can contribute to pathogenesis such as diet, fasting, exercise, febrile illness etc.

1 | 3β-HYDROXY-Δ^5-C_{27}-STEROID DEHYDROGENASE DEFICIENCY

In 1987, we described a family in which two children had died of complications of liver disease and the third clearly had the same type of liver disease. We identified a group of unusual bile acids in the urine which contained the C5-C6 double bond that is present in cholesterol but not in normal bile acids.[9] We argued that these could only be made if there was a block in one of the steps required for the synthesis of normal bile acids (Figure 2). In the discussion of the paper I argued that it was likely that giving, as a medicine, the bile acids the child could not make (chenodeoxycholic acid and cholic acid) might completely cure all the problems. The decision to use chenodeoxycholic acid in the first patient was, like Komrower's decision for homocystinuria, rather pragmatic; chenodeoxycholic acid was available in pharmaceutical grade as it was still being used for gallstone dissolution. We worked out the dose that would be needed to match normal fecal losses of bile acids (3 mg/kg/d[10]), looked at the dose that had been used to suppress the synthesis of abnormal metabolites in another bile acid synthesis disorder, cerebrotendinous xanthomatosis (12.5 mg/kg/d[11]) and then looked at what doses were in the available tablets and capsules (125 mg). We opted to use two capsules (18 mg/kg/d) to build-up normal bile acid levels reducing after 2 months to one capsule per day (9 mg/kg/d).

We started the child on chenodeoxycholic acid at the age of 4 years.[12,13] Prior to treatment, the profile of bile acids in

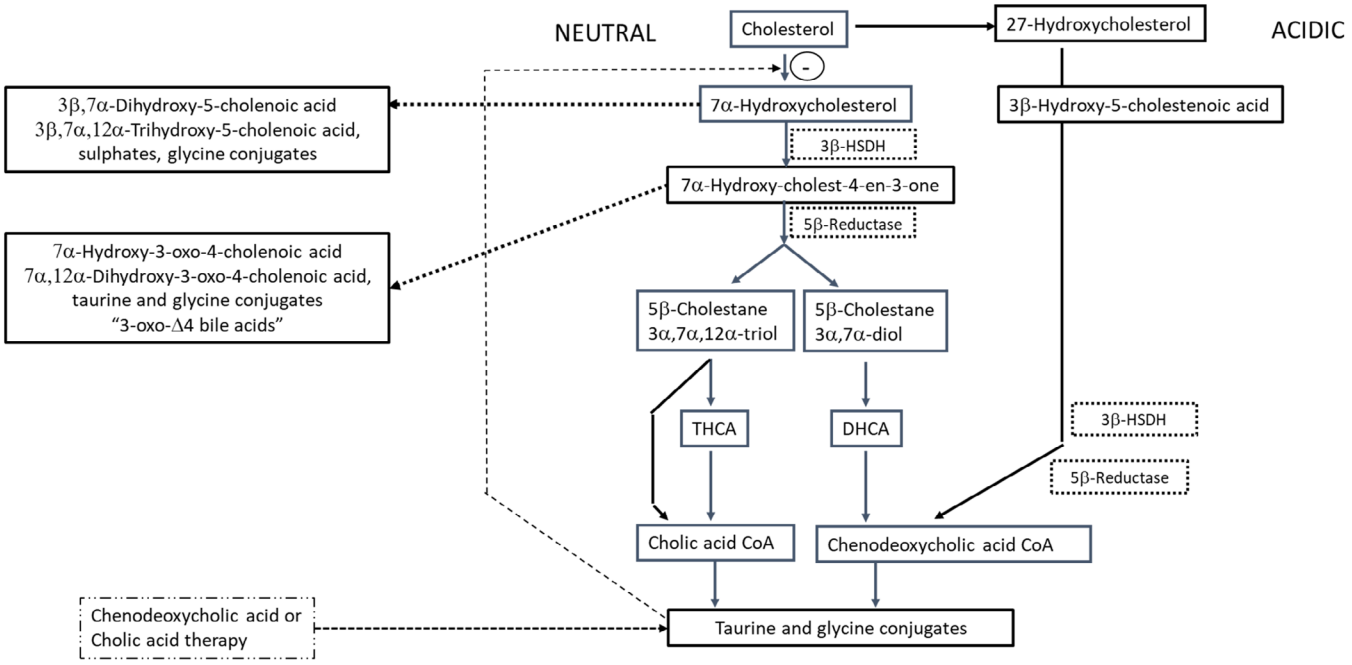

FIGURE 2 Simplified scheme of the bile acid synthetic pathways showing: (a) two main pathways, the neutral and the acidic; (b) feedback inhibition of cholesterol 7α-hydroxylase by the taurine and glycine conjugates of chenodeoxycholic acid and cholic acid (feedback inhibition of the acidic pathway is less well understood); (c) the effect of deficiencies of 3β-HSDH (3β-hydroxy-Δ5-C27-steroid dehydrogenase) and 5β-reductase (Δ4-3-oxosteroid 5β-reductase deficiency); block of the acidic pathway produces similar unsaturated bile acids to those produced from intermediates in the neutral pathway (not shown to simplify)

urine was similar to when we first investigated him at the age of 3 months, dominated by dihdroxy- and trihydroxy 5-cholenoic acids conjugated with sulphate and partially with glycine. Unsaturated bile acids also predominated in bile.

Following treatment there was a very major reduction in the concentrations of unsaturated bile acids in urine and they were barely detectable in plasma. In bile they had been completely replaced by the chenodeoxycholic acid, conjugated with glycine and taurine. The clinical response was equally dramatic. He had jaundice all his life and raised transaminases (indicating ongoing liver damage). Within weeks his liver function had completely normalized and it has remained normal for two decades. I would contend that the magnitude of the effect and the low likelihood of this occurring by chance are powerful evidence that this treatment was effective in this patient.

This response to treatment has since been repeated for the combination of chenodeoxycholic acid and cholic acid, and cholic acid alone.[14-16] We learned that 3β-HSDH deficiency shows significant variation in clinical presentation. There may be little evidence of liver disease, instead severe fat-soluble vitamin deficiency, for example, vitamin D deficiency producing rickets and hypocalcaemia or vitamin K deficiency producing a life threatening bleed. Even in the same family we can see one patient presenting with liver disease and another with vitamin K deficiency. Some individuals excrete the 3β-hydroxy-Δ^5 bile acids predominantly in non-sulphated form.[17] However, all our patients excreted unsaturated bile acids and in all patients this could be almost eliminated with bile acid therapy. This is associated with resolution of symptoms and signs of disease, maintained on long-term follow-up.

Having said this, I would like to emphasize the continuing importance of measuring metabolites. We recently used a new HPLC-MS/MS method[18] to measure bile acids in dried blood spots in a newly diagnosed patient with 3β-HSDH deficiency. Using cholic acid at the manufacturer's recommended dose of 15 mg/kg/day we observed a marked reduction in the blood spot concentrations of the unsaturated bile acids by 6 weeks. However, when we looked at the concentrations of taurine- and glycine-conjugated cholic acid on the first day of treatment, we noted that that they were very high (glycocholate 80 μM, taurocholate 25 μM). These levels are similar to those seen in children with progressive familial intrahepatic cholestasis due to mutations in the *BSEP* gene encoding the bile salt export pump in which high bile acid concentrations in the liver are thought to contribute to progression of the disease.[19] They are also similar to the levels seen in mice with failure of localization of BSEP to the canalicular membrane who develop progressive liver disease when fed cholic acid.[20] This can be explained by the

fact that at the start of treatment patients with 3β-HSDH deficiency have a bile salt pump that is inhibited by the accumulated 3β,7α-dihydroxy-5-cholenoic acid.[21] Looking back at the results of treatment of the first patient with chenodeoxycholic acid, it was apparent that there was a brief rise in bilirubin and transaminases before these normalized. So, monitoring metabolite levels could prevent over-treatment with cholic acid or chenodeoxycholic acid in the early stages.

2 | Δ^4-3-OXOSTEROID 5β-REDUCTASE DEFICIENCY

The second bile acid disorder for which I conducted a single patient trial of treatment was Δ^4-3-oxosteroid 5β-reductase deficiency.[22] The patient had been treated with ursodeoxycholic acid with no reduction of bilirubin and rising transaminases.

Ursodeoxycholic acid is good at fueling bile flow but cannot be expected to feed back on cholesterol 7α-hydroxylase and reduce the production of the 3-oxo-Δ^4 bile acids that, like the Δ^5 bile acids that accumulate in 3β-HSDH deficiency, can inhibit the bile salt export pump.[21]

So we changed to treatment with cholic acid and chenodeoxycholic acid. The 3-oxo-Δ^4 bile acids disappeared from plasma being replaced by conjugates of chenodeoxycholic acid and cholic acid.[22] After a spike in AST (similar to that I have described for 3β-HSDH deficiency), we saw a normalization of bilirubin and transaminases. At the time we undertook the trial we were not able to undertake molecular confirmation of the diagnosis but this became possible in 2003 and the patient we described was shown to be homozygous for a missense mutation in *AKR1D1* (p.P198L)[23]

You might think that we were dealing with a disorder that requires lifelong treatment but when we caught up with this girl again at the age of 13 we discovered that she had become poorly compliant and eventually the treatment was stopped altogether. Despite this she was thriving with normal liver function tests.[24] Analysis of plasma bile acids indicated that the 3-oxo-Δ^4 bile acids were no longer present in plasma; rather the major bile acids were allo or 5α(H) bile acids which can be produced from 3-oxo-Δ^4 acids if they are reduced by a 5α-reductase rather than a 5β-reductase.

Although allo bile acids are not as good detergents as the 5β(H)-bile acids, they are not thought to be inhibitors of the bile salt export pump like the 3-oxo-Δ^4 bile acids so induction of 5α-reductase could lead to an improvement in cholestasis. It is possible that this is more likely to occur in girls as 5α-reductase is higher in females, at least in the rat.[25]

I have encountered further examples of asymptomatic individuals with 5β-reductase deficiency. In a family from Birmingham which I have looked at with Prof Deirdre Kelly, in which 4 siblings and a cousin were shown to have homozygous mutations in *AKR1D1* (c.587delG), the cousin and one sibling died of progressive liver disease, another sibling presented with cholestatic liver disease and improved with cholic acid treatment but two further siblings tested in infancy had a urine bile acid profile which showing marked excretion of 3-oxo-Δ^4 bile acids but they have remained asymptomatic with normal liver function tests.

A second family from Norway was equally remarkable. Dr Barbro Stadheim undertook genome sequencing in a trio in which the propositus was a 37-year-old lady with learning difficulties but no signs of liver disease. She was found to be homozygous for a predicted pathogenic mutation in *AKR1D1* (c.580-1G>A); her parents were heterozygotes. The interesting history from this family was that a brother born in 1978 died of progressive liver disease in infancy for which no specific cause was found. I think it highly likely that he, like his sister, was homozygous for the splice site mutation in *AKR1D1* but whereas she has never had any detected liver disease, he died of liver failure.

So, what I have learned from this disorder is that the same mutations in *AKR1D1* in the same family can produce very different phenotypes from death from liver failure in infancy to no evidence of liver disease in the fourth decade. Measurement of bile acid profiles can demonstrate that treatment with chenodeoxycholic acid and cholic can lead to disappearance from plasma/urine of the 3-oxo-Δ^4 bile acids that are known to inhibit the bile salt export pump and when this happens liver function tests normalize. However, liver disease may not occur or it may resolve spontaneously. So measurements of toxic metabolites has been very useful but major questions remain about when and how *AKR1D1* mutations produce liver disease or do not.

The second group of disorders for which I was involved in $n = 1$ or $n = 2$ trials of treatment when the disorder was first recognized are the disorders of monoamine neurotransmitters:

3 | AROMATIC L-AMINOACID DECARBOXYLASE (AADC) DEFICIENCY

Aromatic amino acid decarboxylase is a pyridoxal-phosphate-dependent enzyme required for the conversion of L-Dopa to dopamine and 5-hydroxytryptophan to 5-hydroxytryptamine (Figure 3). We suspected this disorder when we found low CSF levels of the dopamine breakdown product, homovanillic acid (HVA), and the serotonin breakdown product, 5-hydroxyindoleacetic acid (5HIAA), but normal pterins, in twins with profound hypotonia, hypokinesia, and oculogyric crises.[26] We found all the compounds predicted from a loss of enzyme activity (eg, L-Dopa, 3-methoxytyrosine, and 5-hydroxytryptophan; Figure 3) and showed the activity of AADC in plasma was reduced.[27,28]

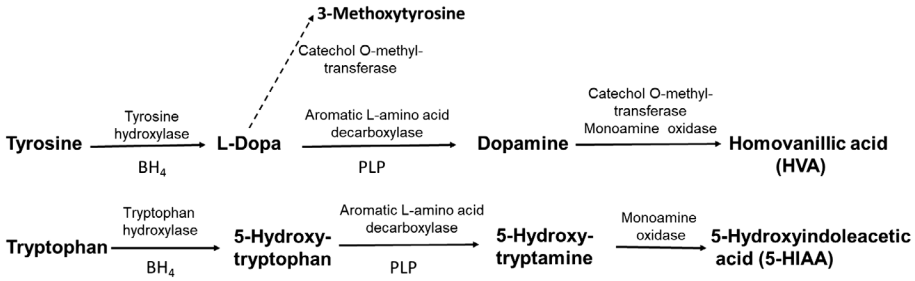

FIGURE 3 Simplified scheme of the pathways for synthesis and breakdown of the monoamine neurotransmitters, dopamine, and 5-hydroxytryptamine (serotonin). Abbreviations: BH_4, tetrahydrobiopterin; PLP, pyridoxal 5′-phosphate

We hoped that we might increase AADC activity, and hence aminergic neurotransmission, to a useful extent by supplying increased amounts of the pyridoxal phosphate (PLP) cofactor. Treatment with pyridoxine led to a reduction in CSF L-Dopa and 3-methoxytyrosine and an increase in HVA, all suggesting that enzyme activity had been increased. Unfortunately, there was no obvious clinical improvement. In a bid to activate post-synaptic dopaminergic receptors, we tried the dopamine agonist bromocriptine; this was successful in stopping the abnormal eye movements. When we tried to prevent catabolism of dopamine by using a monoamine oxidase inhibitor (tranylcypromine) this also stopped the abnormal eye movements. When we combined the three treatments, we observed a sustained improvement in tone and voluntary movements. The twins are alive in their 20s whereas an affected sibling died at 9 months. What happened when these treatments were tried on subsequently identified individuals with AADC deficiency?

The first thing that became clear with identification of further cases was that there was a large variation in severity and, sadly, only 15 out of 78 patients reviewed by Brun et al in 2010 clearly improved on a combined therapy with pyridoxine/PLP, a dopamine agonist, and a monoamine oxidase B inhibitor.[29] There are some mutations including one common in Taiwan that give rise to a severe form of AADC deficiency that does not respond to the triple therapy.[30]

Fortunately very important progress is being made on gene therapy for this disorder. Earlier in this meeting Prof Hwu presented some very exciting results of intraputaminal injections of adeno associated virus containing *AADC* cDNA.[31] They showed sustained de novo dopamine production on fluoro-dopa PET scans as well as improved motor function compared to baseline and after 2 years to a natural history cohort. Other trials by the same group have documented improvements in the CSF levels of HVA and 5HIAA.[32] Those that are firmly wedded to evidence-based medicine scores might be disappointed that the trial conducted by Chien et al[31] was an open label study and part of the evaluation used historic controls. In my view, where the only outcome measure possible is a clinical score, the most scientifically valid clinical trial is a double-blind randomized, crossover trial but where it is possible to measure a pathogenic metabolite, less rigid approaches with smaller numbers are equally valid.

4 | TYROSINE HYDROXYLASE DEFICIENCY

When we encountered an infant with infantile parkinsonism with low HVA but normal 5HIAA and pterins, we suspected that this must be tyrosine hydroxylase deficiency[33] (Figure 3). So here we needed to supply L-Dopa protected from peripheral metabolism by carbidopa. We measured the CSF HVA before treatment and on increasing doses of L-Dopa. At a dose of 10 mg/kg/d in five divided doses we could achieve a CSF HVA concentration in the middle of the normal range for age. The effect on the motor disorder was very dramatic with improvement in head control, facial expressions, tremor of limbs and tongue, ptosis and drooling.[34] This young lady remains well on slow release L-Dopa in her twenties and is studying for her Masters in Education. So how have subsequently diagnosed patients fared?

Once again, identification of subsequent cases revealed considerable heterogeneity with two main phenotypes:[35] Type A: An infantile onset progressive hypokinetic rigid syndrome with dystonia similar to our patient with 23 out of 25 individuals showing a good or moderate response to L-Dopa treatment within 2 weeks but in addition, a Type B: A complex encephalopathy with neonatal onset with a slow response to L-Dopa (which was good or moderate in only 6/11).

If someone had undertaken a randomized controlled trial of L-Dopa treatment in all patients with tyrosine hydroxylase deficiency they might have concluded that it was ineffective if they had a lot of Type B patients in their cohort.

To me the magnitude of the effect in my original patient leaves me in no doubt that the treatment was effective, and again, the measurement of CSF metabolites provided additional evidence.

I would like now to consider some examples of trials of treatment in a third group of disorders—those affecting vitamin B6.

5 | PYRIDOX(AM)INE PHOSPHATE OXIDASE (PNPO) DEFICIENCY

In 2003 we were faced with a newborn on the intensive care unit whose sister had died at a few weeks of age of uncontrolled epilepsy and was himself suffering from seizures that could not be controlled with conventional anticonvulsant drugs or pyridoxine.[36] The CSF chemistry indicated deficiency of aromatic amino acid decarboxylase in the brain with reduced concentrations of HVA and 5HIAA and raised 3-methoxytyrosine (3MT) but there were other indications of deranged amino acid metabolism such as raised glycine and threonine. The best unifying hypothesis for these biochemical changes and for the epilepsy was that cells in the brain were deficient in PLP. There was a case report from 2002 of an infant with epileptic encephalopathy who failed to respond to pyridoxine but responded to PLP.[37] We did not have pharmaceutical grade PLP available so we got some PLP tablets from the local healthfood store, ground one up dissolved it in water and administered it via a nasogastric tube (Figure 4). Within an hour seizure activity was dramatically reduced and within a few days, the abnormal biochemistry was corrected. On regular PLP this child is still alive at the age of 15y albeit with some handicap (wheelchair-bound and with limited speech); other early-treated PNPO-deficient patients are neurodevelopmentally normal.[38]

The work of Philippa Mills in the lab subsequently showed that the infant had mutations in the gene encoding PNPO, that prevented the enzyme from converting pyridoxine phosphate to PLP (Figure 4).[39] As with some of the disorders discussed above, when it became possible to diagnose PNPO deficiency by gene sequencing, it became clear that there was considerable variation in the phenotype.[40] In addition to PLP-responsive neonatal epileptic encephalopathy, PNPO deficiency could present with infantile spasms at 5 months, spasms that responded immediately to treatment with PLP. Furthermore it could also lead to a seizure disorder in early infancy that responded to pyridoxine; in some patients pyridoxine was better at controlling the seizures than PLP.

I would like to say something more about the two ends of the spectrum of PLP-responsive PNPO deficiency. First the severe end: One of the patients with neonatal epileptic encephalopathy responsive to PLP has done very well from a developmental point of view but he has been completely dependent on high doses of PLP given regularly every 6 hours. If a dose was late he would experience an aura or have a frank seizure. Unfortunately this boy developed liver cirrhosis[41] and then hepatocellular carcinoma requiring a liver transplant (Richard Webster, unpublished observation). We do not know if these hepatic problems are part of the disease when survival is prolonged from the neonatal period into adolescence, or a result of high doses of PLP or a result of impurities in PLP caused by photodegradation.

At the other end of the spectrum is a family involving a brother and sister who both have homozygous p.R116Q mutations in *PNPO*. The boy who presented with PLP-responsive infantile spasms (Case 7) has required a lower PLP dose to control his seizures than other patients with PNPO deficiency (10 mg/kg/day compared to the other patients who needed 2-10 × this dose). His sister born a couple of years later has remained asymptomatic without supplementation of PLP in pharmacological doses. The mild phenotype in these siblings is not explained by relatively preserved enzyme activity as the red cell enzyme activity was very low and even the heterozygous parents had slightly reduced activity.[42]

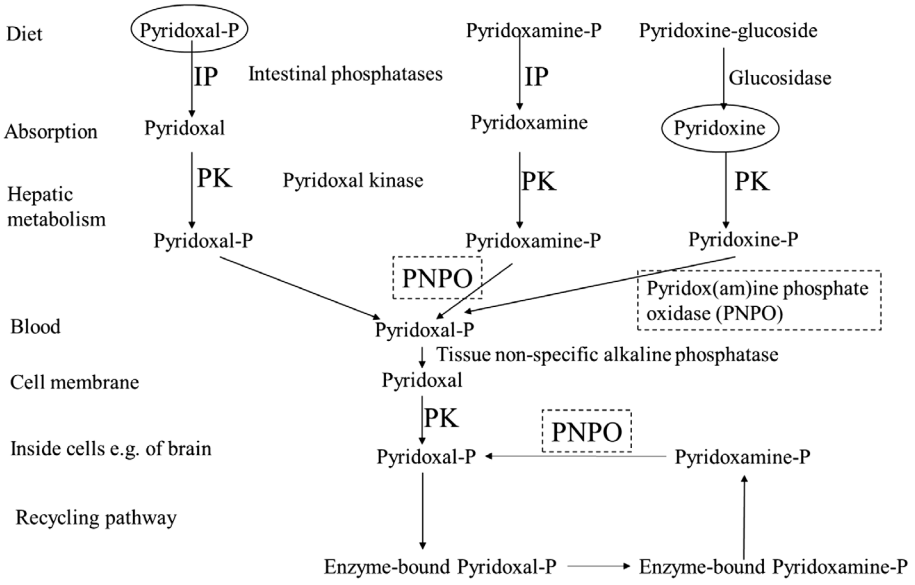

FIGURE 4 Simplified scheme of the pathways for conversion of B_6 vitamers in the diet (or given as treatment, circled) to the active cofactor, pyridoxal 5′-phosphate (PLP) and for recycling to salvage PLP. Pyridox(am)ine phosphate oxidase (PNPO) is required for conversion of pyridoxine (in the diet or given as a medicine) to PLP and for the salvage pathway but conversion of PLP given orally to PLP in body cells does not require PNPO

The phenotype of patients with the p.R116Q/p.R116Q genotype is not always mild; another patient in our cohort with p.R116Q/p.R116Q presented with seizures on day 1 and responded to pyridoxine (Case 8) but others have confirmed that onset of seizures may not be until 8 months to 3 years.[43] DiSalvo et al[43] showed that the mutation reduces the thermal stability of the enzyme, reduces its affinity for FMN and impairs the transfer of PLP to PLP enzymes.

Thus, what we see in PNPO deficiency is that it is possible by trials of treatment in each individual patient to determine which vitamer provides effective treatment and at what dose. If someone conducted a clinical trial of PLP and pyridoxine in patients with *PNPO* mutations it would produce some odd results: some patients asymptomatic on placebo, some doing well on pyridoxine some better on PLP. I believe it would have been impossible to gain the information we have about the disease if we had set out to do a one dose, one vitamer, randomized clinical trial.

I would like to talk about the commonest form of pyridoxine-dependent epilepsy. I certainly did not initiate trials of treatment for it; this dates back to 1954.[44]

6 | PYRIDOXINE-DEPENDENT EPILEPSY (PRESUMED ALDH7A1 DEFICIENCY)

In 1954 Hunt et al noted that an infant with neonatal onset intractable seizures stopped having fits when on a multivitamin preparation containing pyridoxine.[44] More than 50 years later, I was involved in the discovery in 2006 that the commonest form of PDE was ALDH7A1 (antiquitin) deficiency, a defect in lysine degradation that led to accumulation of α-aminoadipic semialdehyde and Δ^1-piperideine 6-carboxylate [P6C].[45] The P6C could form a complex with PLP thereby inactivating it (Figure 5). This led to new treatments monitored by measurements of these metabolites.[46] What I would like to consider today is how the dose of pyridoxine used to treat the disorder increased over half a century when no relevant biochemical measurements were possible.

So in 1954, Hunt was faced with the second child in a family to have severe seizure disorders starting within hours of birth. Hunt observed that the seizures stopped when the infant was started on a vitamin supplement that contained a generous amount of pyridoxine and worked out that the infant's seizures could be controlled with 2 mg of pyridoxine a day for the whole of the 21 months of follow-up. Several other early reports describe effective seizure control with similarly low doses ranging between 0.5 and 10 mg per day. However other cases were described that needed higher doses of pyridoxine to fully control seizures and so the dose that was recommended to see if an infant was pyridoxine-responsive rose to 50 to 100 mg per day.

In 2007 Peter Baxter described a 10-year-old with school failure who showed a dramatic increase in IQ when his dose of pyridoxine was increased from 50 to 150 mg/d.[47] Baxter went on to look at the effect of increasing the dose of pyridoxine on five other children; the effect of the increased dose was in four cases much less dramatic. He concluded that there was a benefit in most children from increasing the dose from 5 to 15 mg/kg/d but no definite benefit above that dose. Is there any risk to giving more than 15mgkg/d? I think there is.

In one of the families first shown to have ALDH7A1 (antiquitin) deficiency[45] there were three affected boys (R1,

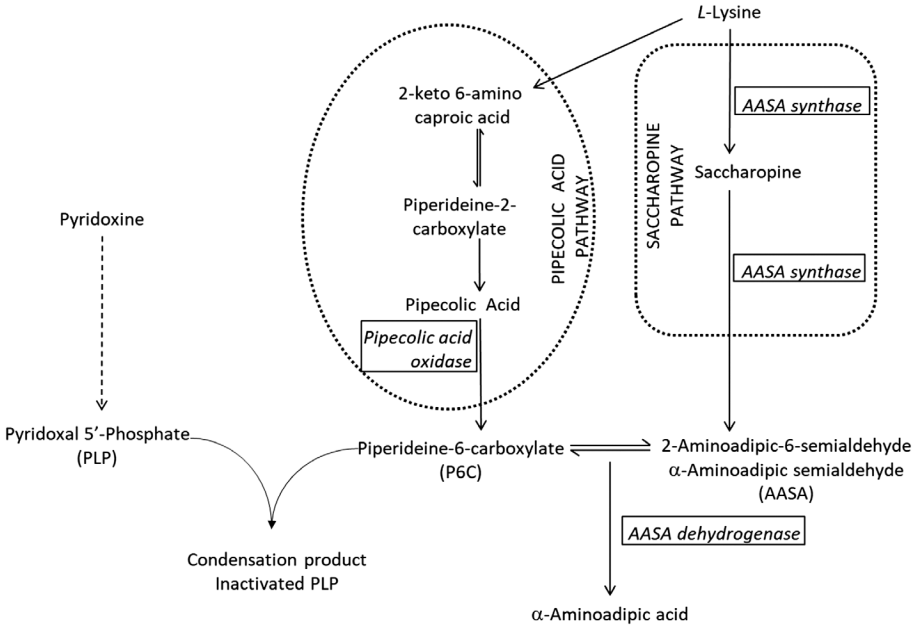

FIGURE 5 The commonest cause of pyridoxine-dependent epilepsy is a defect in the catabolism of lysine—a deficiency of α-amino-adipic semialdehyde encoded by *ALDH7A1*. Accumulating Δ^1-piperideine-6-carboxylate reacts with PLP causing PLP deficiency in the brain. Treatment with pyridoxine corrects the PLP deficiency in the brain and very effectively controls seizures. Additional treatments include dietary lysine restriction and arginine which competes with lysine for uptake across the blood brain barrier

TABLE 1 Plasma B6 vitamer profiles in four school age children with ALDH7A1 deficiency

Diagnosis and clinical information	Age	Medication** (dose documented where known)	Peripheral neuropathy?	PLP	PL	PA	PN	PNP	PMP	PM	PLP:PL	PL:PA
Reference range n = 24	4.3-16 y			46-321	4.6-18.1	16.4-139	nd-0.62	nd	nd-9.3	nd	5.2-18.6	0.1-0.7
PDE	10 y 5 mo	PN (100 mg BD)	+	**23.9**	**6351.4**	**5903.7**	**60.4**	nd	**14.9**	**107.6**	**0.0038**	**1.1**
PDE	12 y 2 mo	PN (100 mg BD)	+	**11.4**	**6475.8**	**5068.8**	**7.2**	nd	**23.57**	**143.8**	**0.0018**	**1.3**
PDE	8 y 7 mo	PN (100 mg BD)	–	**587.9**	**198.4**	**238.8**	0.35	nd	nd	nd	**3.0**	**0.8**
PDE	8 y 7 mo	PN (100 mg BD)	–	**603.3**	**202.6**	**320.9**	0.37	nd	nd	nd	**3.0**	0.6

Note. Modified from Footitt et al.[53]

Abbreviations: PA, pyridoxic acid; PDE, pyridoxine-dependent epilepsy; PL, pyridoxal; PLP, pyridoxal 5′-phosphate; PM, pyridoxamine; PMP, pyridoxamine 5′-phosphate; PN, pyridoxine; PNP, pyridoxine 5′-phosphate.

R2, and R3). All had developed a sensory neuropathy as school age children on doses of pyridoxine between 12 and 26 mg/kg/d.[48] The literature suggests clinical signs of neuropathy in adults without an inborn error of metabolism are associated with a pyridoxine intake in excess of 1000 mg/day (15 mg/kg/d)[49] although there are some reports of mild symptoms occurring at lower doses.[50] Peripheral neuropathy has been described in patients with homocystinuria receiving pyridoxine at a dose over 900 mg/d[51] leading to the recommendation that the dose should not exceed 10 mg/kg/d (500 mg total).[52] However many doctors are using doses of 20 to 30 mg/kg/d and not reporting neuropathy. Can measurements help to determine whether there is a subgroup of antiquitin deficient patients who are at risk? I think they possibly can.

Table 1 shows the plasma B6 vitamer profiles in four children with ALDH7A1 deficiency all receiving 100 mg of pyridoxine twice daily.[53] The two children in family 1 (upper two rows) had had their dose reduced to 200 mg/d because they had developed ENMG evidence of a sensory neuropathy. The two children in family 2 had had no signs of neuropathy. In the children who developed neuropathy, PLP levels in plasma were still below the normal range on treatment and there was marked elevation of pyridoxine, pyridoxamine, and pyridoxamine phosphate, suggesting that these children did not have the capacity to convert pyridoxine and pyridoxamine to PLP, presumably because of relatively low activity of PNPO. The most severe neuropathy that I know of in patients with a B6 disorder was in a girl whose seizures were pyridoxine-responsive and who was treated with pyridoxine for many years but developed a severe sensory-motor neuropathy. She was subsequently shown to have PNPO deficiency (Patient 10,[40]).

In the two children who did not develop neuropathy, plasma PLP levels were well above the normal range and there was no accumulation of pyridoxine, pyridoxamine or pyridoxine. So could the build-up of non-PLP vitamers contribute to neuropathy? I think perhaps it could.

We have always been puzzled by the paradox that B6-deficiency, for example that induced by drugs, can cause neuropathy but high doses of B6 in the form of pyridoxine can also cause neuropathy A possible explanation can be found in the paper of Vrolijk et al.[54] The authors exposed neuroblastoma and colon cancer cell lines to different B6 vitamers and measured cell viability and expression of apoptosis. Of all the B6 vitamers only pyridoxine induced cell death. They then looked at two PLP-dependent enzymes in vitro and showed that pre-incubation of enzyme with pyridoxine reduces the activity of a bacterial tyrosine decarboxylase and inclusion of pyridoxine in the assay reduced the activity of alanine aminotransferase. Although this study can be criticized on the basis of the measurement of activity of

purified enzymes rather than their activity in cells, and using a bacterial PLP-dependent enzyme, it does fit nicely with our clinical data suggesting that it is the patients in whom pyridoxine accumulates that get the neuropathy.

7 | CONCLUSIONS

I hope that I have convinced you that, for me, the approach pioneered by George Komrower and Horst Bickel is alive and well. That approach is to:

- Measure blood/CSF/urine levels of metabolites that are probably contributing to the disease process through toxicity or deficiency.
- Adjust treatment to the minimum necessary to keep metabolite concentrations in the target range and control acute symptoms (eg, epilepsy, movement disorder).
  - Laboratory measurements are not susceptible to placebo effects and will detect spontaneous improvement (one can reduce or stop treatment).
  - This approach is an example of "personalized medicine"—finding what is best for the individual patient.
- The process can be repeated with every subsequent patient with the same disease and metabolite profile.
- With sufficient numbers it will be possible to demonstrate that this improves long term outcome measures (eg, IQ, progression to liver failure, survival).

This is an approach that can cope with the extreme variability of disease that can be seen in many inborn errors of metabolism.

In comparison, in a randomized controlled trial without metabolite measurements:

- Large numbers and a homogeneous patient group are required.
  - This is rarely achievable with inborn errors of metabolism.
- Patients in the treatment arm often all receive the same treatment at the same dose.
  - This is not appropriate for many inborn errors.
- Patients in the placebo arm may be denied treatment that is probably effective.
- The result provides information on the efficacy of the intervention on the group as a whole.
  - This may not be applicable to the individual patient about whom you are trying to make decisions.
    So this is not the gold standard for a highly variable disorder.

This needs to be understood by bodies making decisions on funding treatments for rare disease.

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I have only been involved in successful trials of new treatments because of the skilled laboratory scientists who could make the vital measurements and confirm diagnoses. So a particular thanks to Kevin and Philippa Mills who have both worked alongside me in the ICH for many years now, to Simon Heales and his team of chemical pathologists both in Great Ormond Street and the National Hospital labs.

Of course, no consultant acts alone and I am deeply indebted to my fellow consultants and our highly gifted nurses and dietitians in looking after patients with rare disorders.

Where we have been able to introduce a new treatment, we obviously did it after a full explanation to parents (and the child if old enough). During most of my career, parents have listened to the scientific rationale behind the proposed treatment and agreed to go ahead but I do thank them sincerely for putting their trust in me.

CONFLICT OF INTEREST

I have received funding for research and consultation fees from Actelion (now owned by Johnson & Johnson) who market Miglustat used for the treatment of Niemann-Pick C disease which is mentioned in this article.

ETHICS STATEMENT

Details of ethical permissions are given in references where applicable. This article describes the use of medicines off license for newly defined, and often life-threatening, disorders. Doctors in the UK have always had the right to prescribe off-license or even unlicensed products (https://psnc.org.uk/dispensing-supply/receiving-a-prescription/who-can-prescribe-what/).

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