Cerebrospinal fluid analysis in the diagnosis of treatable inherited disorders of neurotransmitter metabolism

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Dopamine and serotonin are involved in the control of a wide variety of neuronal functions. Within the CNS they regulate, among other things, psychomotor function (through involvement in the regulation of motor coordination), reward-driven learning, arousal, processing of sensory input, memory, appetite, emotional stability, sleep, mood, vomiting, sexual behavior and secretion of anterior pituitary and other hormones [1,2]. Peripherally, their involvement includes control of thermoregulation and modulation of pain mechanisms, regulation of vascular tone and blood flow [3], intestinal motility [4], hemostasis [5] and T-cell-mediated immune responses [6].

Synthesis & catabolism of dopamine & serotonin

Serotonin and dopamine are synthesized from the amino acids tryptophan and tyrosine, respectively. These are hydroxylated to form 5-hydroxytryptophan and L-dopa by tryptophan hydroxylase and tyrosine hydroxylase, respectively, and then a single enzyme – aromatic L-amino acid decarboxylase (AADC) – decarboxylates these to form the active neurotransmitters. In noradrenergic neurons dopamine can be hydroxylated further by dopamine β-hydroxylase to form norepinephrine. Following their action at the nerve synapse the neurotransmitters are metabolized rapidly. Dopamine is converted to homovanillic acid (HVA) and norepinephrine to 3-methoxy-4-hydroxyphenylglycol (MHPG) via the action of catechol-O-methyltransferase and monoamine oxidase; serotonin is converted to 5-hydroxyindoleacetic acid (5HIAA) via the action of monoamine oxidase. The concentration of these metabolites in cerebrospinal fluid (CSF) provides an overall picture of the functioning of the dopamine and serotonin systems (Figure 1).

CSF patterns in the disorders of dopamine & serotonin metabolism

Patterns in the deficiencies of tyrosine hydroxylase & aromatic L-amino acid decarboxylase.

Inherited defects of tyrosine hydroxylase and AADC have been described and characteristic metabolite patterns are observed in each disorder [7]. In tyrosine hydroxylase deficiency there are decreases in HVA and MHPG. In AADC deficiency both HVA and 5HIAA are decreased and there is accumulation of the two precursors L-dopa and 5-hydroxytryptophan. Subsequently, L-dopa is methylated to form 3-O-methyldopa (3-methoxytyrosine), which is found in very high concentrations (Figure 1) [8].

Patterns in the defects of tetrahydrobiopterin metabolism

Two cofactors are required for the synthesis of dopamine and serotonin: tetrahydrobiopterin (BH4) and pyridoxal 5’-phosphate. Tetrahydrobiopterin is the cofactor for tyrosine hydroxylase and tryptophan hydroxylase. It is synthesized in a three-step pathway from guanosine triphosphate (GTP) and, following its oxidation in the hydroxylase reactions, it is reduced back to the active cofactor via the action of dihydropteridinedi...
reductase and pterin-4α-carbinolamine dehydratase. Inherited disorders at the levels of GTP cyclohydrolase [9], 6-pyruvoyltetrahydropterin synthase [10], sepiapterin reductase [11], pterin-4α-carbinolamine dehydratase [12] and dihydropteridine reductase [13] have been described and all lead to a characteristic pattern of pterins (BH4 and neopterin) and neurotransmitter metabolites in the CSF (Figure 1).

GTP cyclohydrolase is the first enzyme required for BH4 synthesis; therefore, decreased activity therefore leads to low levels of the cofactor and its precursor molecule neopterin. Neopterin is the oxidized form of dihydroleptin, which is formed following dephosphorylation of dihydroleptin triphosphate, the product of the GTP cyclohydrolase reaction. 6-pyruvoyltetrahydropterin synthase converts dihydroleptin triphosphate
to 6-pyruvoyltetrahydropterin, and deficiency of this enzyme leads to the accumulation of neopterin and decreased concentrations of BH4. The last enzyme required for the synthesis of BH4 is sepiapterin reductase. Decreased activity of this enzyme results in a deficiency of BH4 and an accumulation of dihydrobiopterin and sepiapterin in the CSF [11]. Following the oxidation of BH4 in the hydroxylase reactions, it is reduced back to its active form by dihydropteridine reductase. Deficiency of this enzyme again results in an accumulation of dihydrobiopterin, but in general, the levels of BH4 are within, or only slightly lower than, reference ranges (Figure 1) [14].

In general, autosomal recessively inherited defects of BH4 synthesis and regeneration lead to profound decreases of HVA and 5HIAA in the CSF (Figure 1). There is a mild form of 6-pyruvoyltetrahydropterin synthase deficiency, where the effects seem only to be manifest in the periphery. Neurotransmitter metabolite and pterin levels in the CSF are normal [15]. Pterin-4α-carbinolamine dehydratase deficiency leads to an accumulation of 7-biopterin, as opposed to the normal 6-biopterin, and does not lead to any neurological problems or changes in CSF metabolites [12].

Tetrahydrobiopterin is also a cofactor for phenylalanine hydroxylase, which converts phenylalanine to tyrosine in the liver. In general, the BH4 deficiencies can therefore be detected at the time of newborn screening due to the presence of hyperphenylalaninemia. Autosomal dominant GTP cyclohydrolase deficiency [16], certain compound heterozygotes for GTP cyclohydrolase deficiency [17,18] and autosomal dominant sepiapterin reductase deficiency [11] do not lead to hyperphenylalaninemia, and they are therefore missed on newborn screening. However, they all lead to characteristic pterin and neurotransmitter metabolite patterns in CSF (Figure 1).

Patterns in pyridox(am)ine 5′-phosphate oxidase deficiency
Pyridoxal 5′-phosphate (vitamin B₆) is required for the activity of aromatic L-amino acid decarboxylase. To date, no B₆-responsive cases of primary aromatic L-amino acid decarboxylase deficiency have been described, but it is still recommended that all patients receive a trial with this vitamin. Although B₆-responsive aromatic L-amino acid decarboxylase deficiency has not been found, there are reports of a secondary deficiency of this enzyme arising as a result of a lack of pyridoxal 5′-phosphate. In these cases the pyridoxal 5′-phosphate deficiency resulted from a defect in its synthesis at the level of pyridox(am)ine 5′-phosphate oxidase [19]. These patients present with early-onset seizures (probably in utero), have a burst suppression pattern on electroencephalogram and respond to therapy with pyridoxal 5′-phosphate, but do not respond to pyridoxine [19–21]. Pyridox(am)ine 5′-phosphate oxidase deficiency can be detected by investigating neurotransmitter metabolites and amino acids in CSF. The neurotransmitter metabolite pattern resembles that seen in aromatic L-amino acid decarboxylase deficiency. HVA and 5-HIAA levels tend to be decreased and there may be an elevation of 3-O-methyltyrosine; amino acids requiring B₆ for their catalobolism are also elevated. The most diagnostically significant is threonine, although elevations of glycine are also present [19–21].

When to consider serotonin & dopamine metabolism & pterin analysis in CSF
Our knowledge of the clinical phenotype spectrum in the disorders of neurotransmitter metabolism is ever expanding and we are constantly being surprised. Table 1 shows the major signs and symptoms in these disorders. It has been recommended that a neurotransmitter defect be considered in all patients with unexplained extrapyramidal signs or unexplained infantile ‘floppiness’ and, in selected cases, with progressive encephalopathy [22]. There are also some general features that should be considered. The possibility of a neurotransmitter disorder should be considered in any child having one or a mixture of the following: oculogyric crises, temperature instability in the absence of infection, proxis of the eyelids, severe irritability or some kind of movement disorder. Unfortunately, the absence of these does not rule out a neurotransmitter defect.

Seizures in the inherited defects affecting dopamine & serotonin metabolism
There is often a misconception that seizures are prominent in the disorders affecting dopamine and serotonin metabolism. This is only the case in the disorders affecting the cofactors BH4 and pyridoxal 5′-phosphate. Seizures are prevalent in the untreated, autosomal recessively inherited deficiencies of GTP cyclohydrolase, 6-pyruvoyltetrahydropterin synthase, sepiapterin reductase and dihydropteridine reductase, and they consist of grand mal, myoclonic or tonic–clonic attacks [11,23]. There are two examples of defects in BH4 metabolism where seizures are not reported as part of the clinical phenotype. These are dominantly inherited GTP cyclohydrolase deficiency
It is important to re-emphasize that the inherited defects of monoamine metabolism involving mutations in tyrosine hydroxylase and AADC are only rarely associated with seizures. However, there are still reasons to measure CSF neurotransmitter metabolites and pterins in infants and children who present with seizures, as abnormalities in the neurotransmitter metabolite profiles can be used to identify a folinic acid-responsive seizure disorder [24,25] and pyridox(am)ine 5′-phosphate oxidase-deficiency [19–21]. In addition, elevated levels of BH4 and neopterin can indicate the presence of an atypical form of the Aicardi–Goutières syndrome [26] and an increase in just neopterin can indicate that neurological symptoms are the result of an immune-, rather than metabolic-based etiology [27].

Disorders of dopamine & serotonin biosynthesis are treatable conditions
Patients with dominantly inherited GTP cyclohydrolase (GTPCH) deficiency are usually cognitively intact and respond to low-dose

| Table 1. Signs and symptoms of patients with disorders of dopamine and serotonin metabolism. |
|-----------------------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Clinical signs & symptoms                     | GTPCH (recessive) | GTPCH (dominant) | 6PTPS (severe) | 6PTPS (mild) | SR | DHPR | PCD | TH | AADC | MAO | DβH |
| Mental retardation/developmental delay        | +              | +              | +              | +              | +  | +   | +   | +  | +   | +   | +/  |
| Limb hypertonia                              | +              | +/–            | +              | +              | +  | +   | +   | +  | +   | +   | +/  |
| Hypotonia                                    | +              | +/–            | +              | +              | +  | +   | +   | +  | +   | +   | +/  |
| Seizures                                     | +              | +              | +/–            | +              | +  | +   | +   | +  | +   | +   | +/  |
| Microcephaly                                 | +              | +/–            | +/–            | +              | +  | +   | +   | +  | +   | +   | +/  |
| Temperature instability                      | +              | +              | +/–            | +              | +  | +   | +   | +  | +   | +   | +/  |
| Hypersalivation                              | +              | +              | +              | +              | +  | +   | +   | +  | +   | +   | +/  |
| Excessive sweating                           | +              | +              | +              | +              | +  | +   | +   | +  | +   | +   | +/  |
| Chorea/athetosis                             | +              | +              | +/–            | +              | +  | +   | +   | +  | +   | +   | +/  |
| Oculogyric crises                            | +              | +              | +/–            | +              | +  | +   | +   | +  | +   | +   | +/  |
| Swallowing difficulties                      | +              | +              | +              | +              | +  | +   | +   | +  | +   | +   | +/  |
| Irritability                                 | +              | +              | +/–            | +              | +  | +   | +   | +  | +   | +   | +/  |
| Hypokinesia                                  | +              | +              | +/–            | +              | +  | +   | +   | +  | +   | +   | +/  |
| Tremor                                       | +/–            | +              | +/–            | +              | +  | +   | +   | +  | +   | +   | +/  |
| Gastroesophageal reflux                      | +              | +/–            | +              | +              | +  | +   | +   | +  | +   | +   | +/  |
| Ptosis                                        | +              | +              | +              | +              | +  | +   | +   | +  | +   | +   | +/  |
| Dystonia                                     | +              | +              | +/–            | +              | +  | +   | +   | +  | +   | +   | +/  |
| Orthostatic hypotension                      | +              |                                |                |                |    |      |      |      |      |      |      |
| Torticollis                                   | +/–            |                                |                |                |    |      |      |      |      |      |      |
| Spasticity                                   | +/–            |                                |                |                |    |      |      |      |      |      |      |
| Hypothermia                                  | +/–            |                                |                |                |    |      |      |      |      |      |      |
| Aggression                                    | +/–            |                                |                |                |    |      |      |      |      |      |      |
| Diurnal fluctuations of symptoms              | +/–            |                                |                |                |    |      |      |      |      |      |      |
| Hypersomnolence                               | +/–            |                                |                |                |    |      |      |      |      |      |      |

*Mild in the neonatal period.

6PTPS: 6-pyruvoyltetrahydropterin synthase; AADC: Aromatic l-amino acid decarboxylase; DβH: Dopamine β-hydroxylase; DHPR: Dihydropteridine reductase; GTP: Guanosine triphosphate; GTPCH: GTP cyclohydrolase; MAO: Monoamine oxidase; PCD: Pterin-4α-carbinolamine dehydratase; SR: Sepiapterin reductase; TH: Tyrosine hydroxylase.
L-dopa/carbidopa [28]. In older patients, major depressive disorder and obsessive-compulsive disorder are seen more frequently than in the general population. These patients respond well to serotonergic agents and to L-dopa [29]. In the recessive disorders of BH4 biosynthesis the hyperphenylalaninemia present can be treated by giving low doses of BH4. This corrects the liver phenylalanine metabolism but does not prevent the inhibition of dopamine and serotonin synthesis in the CNS, as BH4 does not cross the blood–brain barrier unless given in large doses. The synthesis of dopamine and serotonin can be reinstated by giving L-dopa and 5-hydroxytryptophan. L-dopa is normally given in conjunction with carbidopa, which blocks the peripheral AADC, thus allowing more of the precursors to enter the CNS. The L-dopa and 5-hydroxytryptophan bypass the metabolic block and, in most cases, ameliorate the neurological symptoms. Occasionally, the use of 5-hydroxytryptophan has to be limited due to adverse gastrointestinal affects. In dihydropteridine reductase deficiency, BH4 (unless given in very large doses) is unable to correct the peripheral hyperphenylalaninemia as one molecule of BH4 is required for each molecule of phenylalanine converted to tyrosine. In this disorder a low phenylalanine diet is used to correct the hyperphenylalaninemia and, again, L-dopa and 5-hydroxytryptophan are used to bypass the metabolic block within the CNS. A secondary folate deficiency arises in dihydropteridine reductase deficiency, which is thought to occur as a result of competitive inhibition of 5-10-methylenetetrahydrofolate reductase by the elevated concentrations of quinonoid dihydrobipterin that accumulate in this disorder [30]. The folate deficiency requires the addition of folic acid to the therapeutic regimen [31]. Some patients with defects in BH4 metabolism have benefited from additional therapy with monoamine oxidase inhibitors, which slow down the catabolism of any neurotransmitter that may be formed [32].

The signs and symptoms of tyrosine hydroxylase deficiency (TH) deficiency can, in many cases, be ameliorated by administration of L-dopa/carbidopa, although there are early-onset forms of this disorder that do not respond to this therapy [33].

Unfortunately, most cases of AADC deficiency are not amenable to precursor therapy. All patients found so far have had some residual formation of dopamine and serotonin, as demonstrated by small amounts of 5HIAA and HVA in CSF. Therefore, management of this disorder is based on trying to prevent catabolism of the small amounts of neurotransmitters formed, by the use of monoamine oxidase inhibitors and by attempting to stimulate the postsynaptic receptors by administering receptor agonists [8,34,35]. In a single family with a k_m mutation affecting the L-dopa binding site, clinical benefit was obtained by the administration of L-dopa [36]. It is advisable to check the folate status within the CNS in patients with AADC deficiency, as there is a continual drain on carbon metabolism resulting from the methylation of L-dopa, using S-adenosylmethionine as the methyl group donor. Folic acid should be given if CSF 5-methyltetrahydrofolate levels are low [37]. The secondary deficiencies of AADC that result from pyridoxal 5′-phosphate deficiency respond rapidly when treated with this cofactor. They do not respond to pyridoxine [19].

No treatment has been found for monoamine oxidase deficiency; however, in dopamine β-hydroxylase deficiency, therapy with dihydroxyphenylserine is extremely effective [38].

Problems with treatment

Many of the signs and symptoms seen in patients with the inherited defects of neurotransmitter metabolism are similar to those that may be observed if too much medication has been employed. The administration of L-dopa in the inherited defects of neurotransmitter should be commenced carefully. It is likely that there is upregulation of the postsynaptic dopamine receptors due to the previous chronic deficiency of the neurotransmitter. Administration of excessive L-dopa can lead to firing of these receptors and, subsequently, severe dyskinetic episodes. In tyrosine hydroxylase deficiency and the defects of BH4 metabolism, a repeat lumbar puncture should be performed if symptoms persist in order to gauge whether or not the therapy has returned neurotransmitter metabolite levels to within the expected reference ranges.

CSF collection, processing & storage

The manner in which CSF is collected is critical if meaningful results are to be obtained. There is a rostrocaudal gradient for neurotransmitter metabolites and BH4 in lumbar spinal fluid. Therefore, the more spinal fluid that is drawn the higher the values for the metabolites go. Values double with approximately every 5 ml of CSF that is drawn. If the data obtained is not from the same fraction of CSF that was used to establish reference ranges, then either a false-negative or false-positive result may be obtained.
It is also essential to have appropriate age-related reference ranges to which results can be compared. Levels of metabolites are high in the newborn period, drop rapidly during the first year of life and then decrease slowly with age (Table 2). In our laboratory we send out spinal fluid collection tubes with the specific instructions included. We measure HVA, 5HIAA and 3OMD in the first 0.5 ml of CSF collected and BH4 and neopterin in the next 1 ml of CSF collected. Additional spinal fluid should always be collected and stored to allow for other testing, should it be required.

Sample handling is also crucial. Blood contamination can lead to oxidation of metabolites if the red blood cells are allowed to hemolyse. Blood-contaminated samples should be centrifuged as soon as possible and the clear CSF transferred to new tubes that are labeled appropriately. 5-HIAA, HVA, 3OMD and neopterins are relatively stable and their values do not change if the sample has been kept at 4°C for up to 24 h. This is not the case for BH4; this cofactor is extremely labile and, if unprotected, rapidly oxidizes [39]. Addition of the antioxidants, dithioerythritol (DTE) and diethylene-triaminepentaacetic acid (DETAPAC) provides full protection, even at room temperature, for at least 24 h [39]. When stored at -70°C all the metabolites are stable for at least 5 years.

Caveats
Although all the enzyme deficiencies described above have characteristic patterns of pterins and neurotransmitter metabolites in CSF, these patterns are not specific for the disorders. Therefore, clinical signs and symptoms together with follow-up testing, should be utilized to ensure a correct diagnosis.

In the newborn period, a hypoxic/ischemic incident can lead to virtual elimination of HVA, 5HIAA and BH4 in CSF. This is often accompanied by an elevation of neopterin [40]. This pattern is also characteristic of 6-pyruvoyltetrahydropterin synthase deficiency. Neopterin is not always elevated and, in this situation, the metabolite pattern mimics that seen in GTP cyclohydrolase deficiency. Examination of plasma phenylalanine levels should allow discrimination between the pterin defects and the hypoxia/ischemia, as phenylalanine levels are not elevated in the latter disorder. However, GTP cyclohydrolase deficiency does not always lead to hyperphenylalaninemia [17,18]. Oral phenylalanine loading can be helpful in adding weight to the diagnosis [41]. Mutation analysis fails to detect abnormalities in many cases of suspected GTP cyclohydrolase deficiency and fibroblast enzyme assay is therefore the definitive test for this disorder [42].

A finding of an isolated decrease in HVA in the presence of normal levels of 5HIAA and pterins is characteristic for TH deficiency, but unfortunately it is not specific for this condition and there appears to be a multitude of different situations where CSF HVA levels are decreased [43]. Definitive diagnosis of TH deficiency can only be accomplished by the finding of a pathogenic mutation(s), as easily accessible tissue sources for tyrosine hydroxylase are not available for enzyme studies.

Aromatic L-amino acid decarboxylase deficiency results in a characteristic CSF pattern; low levels of HVA and 5HIAA and elevations of 3OMD, 5-hydroxytryptophan and L-dopa [8]. Tetrahydrobiopterin and neopterin concentrations are normal. This pattern may also be observed in pyridox(am)ine 5′-phosphate oxidase deficiency [19]. Differentiation between these two conditions can be made on clinical grounds and by measurement of AADC activity in the plasma. Early onset severe seizures are a prominent feature in pyridox(am)ine 5′-phosphate oxidase deficiency [19,20], whereas seizures are rarely found in AADC deficiency [34]. Saturating concentrations of pyridoxal 5′-phosphate are present in the AADC assay mixture, and in pyridox(am)ine 5′-phosphate oxidase deficiency AADC activity is normal, or even elevated, as the missing cofactor has been replaced [20]. In the primary deficiency of AADC, enzyme activity is low [8].

The normal reference ranges for neurotransmitter metabolites and pterins in the CSF vary widely across each age group and even subtle

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Table 2. Age-related reference ranges for homovanillic acid, 5-hydroxyindoleacetic acid, tetrahydrobiopterin and neopterin in cerebrospinal fluid.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>5HIAA</th>
<th>HVA</th>
<th>BH4</th>
<th>Neop</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–0.2</td>
<td>208–1159</td>
<td>337–1299</td>
<td>40–105</td>
<td>7–65</td>
</tr>
<tr>
<td>0.2–0.5</td>
<td>179–711</td>
<td>450–1132</td>
<td>23–98</td>
<td>7–65</td>
</tr>
<tr>
<td>0.5–2.0</td>
<td>129–520</td>
<td>294–1115</td>
<td>18–58</td>
<td>7–65</td>
</tr>
<tr>
<td>2–5</td>
<td>74–345</td>
<td>233–928</td>
<td>18–50</td>
<td>7–65</td>
</tr>
<tr>
<td>5–10</td>
<td>66–338</td>
<td>218–852</td>
<td>9–40</td>
<td>7–40</td>
</tr>
<tr>
<td>10–15</td>
<td>67–189</td>
<td>167–563</td>
<td>9–32</td>
<td>8–33</td>
</tr>
<tr>
<td>Adults</td>
<td>67–140</td>
<td>145–324</td>
<td>10–30</td>
<td>8–28</td>
</tr>
</tbody>
</table>

5HIAA: 5-Hydroxyindoleacetic acid; BH4: Tetrahydrobiopterin; HVA: Homovanillic acid; Neop: Neopterin.
changes below these ranges may indicate the presence of a defect. In the recessively inherited defects of BH₄ metabolism and in aromatic l-αmino acid decarboxylase deficiency there is not normally a problem, as the neurotransmitter metabolite and pterin patterns are clear and diagnostic. This is not always the case in dominantly inherited GTP cyclohydrolase deficiency and in tyrosine hydroxylase deficiency, where values may still be within, or only just below, the bottom end of the reference ranges. In this situation, if the clinical picture indicates the possibility of the defect, a repeat lumbar puncture should be performed and the analysis repeated.

Conclusion
The first description of an inherited defect in dopamine and serotonin synthesis was published in the early 1970s [44]. Since that time, over 500 cases of recessively inherited defects affecting BH₄ have been described [101]. There are likely an even greater number of autosomal dominantly inherited cases of GTP cyclohydrolase deficiency. The first case of AADC deficiency was described in 1990 [45], and that of TH in 1996 [46]. Since then, at least 20 further cases of each of these disorders have been detected. The disorders of dopamine and serotonin synthesis are all treatable, to some degree, therefore, it is unfortunate that there are likely to be many cases that remain undiagnosed. Darryl De Vivo coined the term morbid fear of lumbar puncture (MFLP). It is vital that in patients with appropriate neurological symptoms, where hyperphenylalaninemia is not present, that the physician first considers a neurotransmitter disorder on clinical grounds and then follows up the suspicion by CSF to initiate the investigation of neurotransmitter metabolism.

Future perspective
In this review, the topic has been limited mostly to the utility of CSF measurement for the detection of the disorders of BH₄ metabolism and the deficiencies of TH and AADC. Deficiencies of dopamine β-hydroxylase and monoamine oxidase have also been described and both have characteristic changes of neurotransmitter metabolites in CSF (Figure 1) [47,48]. There remains a multitude of disorders to be detected. The process of neurotransmission first requires synthesis of the neurotransmitter in the presynaptic nerve terminal and most of the defects in synthesis have been described. The major exception is an inherited disorder affecting tryptophan hydroxylase. In this situation one would expect to observe an isolated decrease in 5HIAA. Initially, a single gene localized to 11p15.3-p14 [49] was thought to encode tryptophan hydroxylase [50]. Using targeted ablation of this gene in mice, another isoform of tryptophan hydroxylase was discovered that was encoded by an additional gene located on chromosome 12 [51]. The original form (TPH1) was shown subsequently to be present predominantly in the pineal gland, thymus, spleen and gut, whereas the second (TPH2) was located mostly in the brain stem. It is possible in humans that the two isoforms lead to the formation of the brain serotonin pool and hence the CSF 5HIAA pool. In this situation, a decrease in 5HIAA caused by a mutation in TPH2 may be masked by the presence of the 5HIAA formed through the activity of TPH1. Certainly, decreases in CSF 5HIAA are observed frequently and it may be that the TPH2 and TPH1 genes have to be screened routinely in these patients if we are to locate inherited defects affecting tryptophan hydroxylase activity.

Following synthesis, the neurotransmitters are stored in presynaptic storage vesicles. Transport into these vesicles is an active process and requires the activity of the neuronal isoform of the vesicular monoamine transporter (VMAT)2. This transporter is critical for the process of neurotransmission, as it regulates location, mechanism and quantity of neurotransmitter release during synaptic transmission [52]. Knockout mice lacking VMAT2 have a virtual absence of dopamine and serotonin in the brain [53]. Only polymorphisms have currently been described in the human VMAT2 gene and none have been associated with clinical symptoms [54]. Again, it is fairly common to find decreased levels of both HVA and 5HIAA in situations where there is no obvious explanation for the low levels. Sequencing of the VMAT2 gene might produce some interesting findings. Following release into the synaptic cleft the neurotransmitters bind to specific receptors on the pre- and postsynaptic membranes. There are a multitude of these receptors for both dopamine and serotonin and polymorphisms in the genes controlling their synthesis have been associated with numerous neuropsychiatric conditions. Currently, it is unclear exactly what CSF metabolite picture might be seen if there are individual receptor mutations, but one might speculate on upregulation of neurotransmitter synthesis.
Catabolism of monoamines requires the activity of monoamine oxidase and catechol-O-methyltransferase. Monoamine oxidase-A knockout mice have elevated brain levels of serotonin and norepinephrine. These animals show an aggressive phenotype with reduced anxiety-like behavior [55]. This phenotype is similar to that described in a family with X-linked, nondysmorphic, mild mental retardation who harbored a point mutation in exon 8 of the monoamine oxidase-A structural gene [56,57]. Several polymorphisms have been described in the human COMT gene that appear to have functional consequences. A Val158Met substitution alters enzyme activity, with homozygotes demonstrating a three- to fourfold reduction in activity [58]. An Ala72Ser substitution also reduces COMT enzyme activity and has been associated with an increased risk for schizophrenia [59]. In CSF, one would assume that levels of 5HIAA would be low in monoamine oxidase deficiency and that HVA levels would be reduced in catechol-O-methyltransferase deficiency. This has yet to be documented.

Dopamine and serotonin are removed from the synaptic cleft by the action of two specific transporters, which take the neurotransmitters back into the presynaptic nerve terminals. Knockout mice lacking the dopamine transporter, DAT [60], and serotonin transporter, SERT [61], have been generated. In both situations, neurotransmitter synthesis is significantly downregulated with intracellular levels of dopamine and serotonin being only 5–20% of normal. Although dopamine and serotonin levels, within the synaptic cleft, are elevated, it is likely that the overall downregulation of synthesis would lead to low levels of CSF HVA in a case of DAT-deficiency and low levels of 5HIAA in a case of SERT-deficiency.

Many other processes are required to ensure the correct regulation of neurotransmission. It remains to be determined if CSF analysis of metabolites might be helpful in recognizing more of these, or if blanket molecular sequencing of all candidate genes might be a more specific and fruitful way to go in the future.
CSF analysis of inherited disorders of neurotransmitter metabolism – SPECIAL REPORT

### Executive summary

#### CSF collection, processing & storage
- Collection of CSF in a carefully controlled manner is vital.
- There are rostrocaudal gradients for metabolites, with levels increasing rapidly as more CSF is drawn.
- Hemolysis of red blood cells leads to oxidation of metabolites; therefore, blood cells should be removed by centrifugation prior to freezing and the clear CSF frozen in new tubes.
- Tetrahydrobiopterin is extremely labile, the CSF sample used to measure this metabolite should contain antioxidants.
- The inherited defects discovered so far that affect dopamine and serotonin neurotransmission have been limited to those involving synthesis and catabolism of these neurotransmitters. However, the process of neurotransmission requires synthesis of the neurotransmitter in the presynaptic nerve terminal, storage in presynaptic secretory vesicles, regulated release into the synaptic cleft, the presence of specific receptors on the pre- and postsynaptic membranes and a means of termination of the action of the released neurotransmitter. There are certainly inherited defects affecting all stages of the neurotransmission process.

#### Caveats
- Although CSF metabolite patterns are characteristic for each of the described disorders of dopamine and serotonin metabolism they are not specific for these disorders. Therefore clinical signs and symptoms together with follow-up testing should be utilized to ensure a correct diagnosis.

#### Future perspective
- The inherited defects discovered so far that affect dopamine and serotonin neurotransmission have been limited to those involving synthesis and catabolism of these neurotransmitters. However, the process of neurotransmission requires synthesis of the neurotransmitter in the presynaptic nerve terminal, storage in presynaptic secretory vesicles, regulated release into the synaptic cleft, the presence of specific receptors on the pre- and postsynaptic membranes and a means of termination of the action of the released neurotransmitter. There are certainly inherited defects affecting all stages of the neurotransmission process. In some, predictable changes in neurotransmitter metabolites might be expected and they may be detectable by CSF metabolite analysis. In others, gene sequencing or other molecular techniques are likely to be required if a diagnosis is to be made.

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