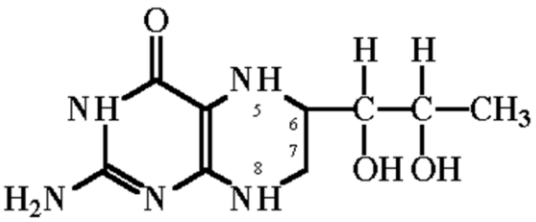
DEFECTS OF TETRAHYDROBIOPTERIN (BH4)

WHAT ARE THE DEFECTS OF BH4?

They are a group of diseases caused by mutations in the genes involved in the **synthetic or recycling pathways of BH4**. The mutated enzyme proteins cause a **BH4 deficiency** which in turn leads to defective activity of enzymes which need BH4 as the essential cofactor: phenylalanine hydroxylase, tyrosine hydroxylase and tryptophan hydroxylase and three isoforms of nitric oxide synthase. BH4 deficiency causes a defect in the **synthesis of neurotransmitters** (serotonin and L-Dopa) and may or may not be accompanied by hyperphenylalaninemia.

WHAT IS BH4?

Tetrahydrobiopterin (BH4)

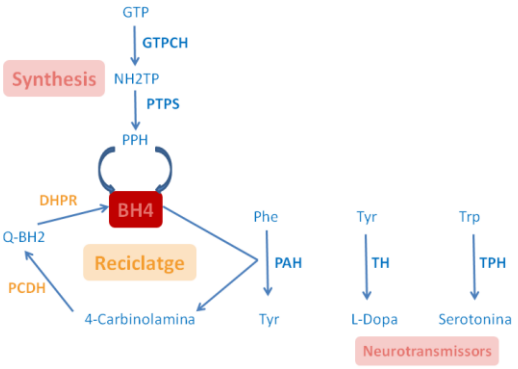


BH4 is a pterin which acts as **essential cofactor** in some enzymatic reactions of great metabolic importance, such as the **hydroxylation of aromatic amino acids** (phenylalanine, tyrosine and tryptophan), as well as in the three isoforms of **nitric oxide synthase**.

Phenylalanine hydroxylase (PAH) is the enzyme which converts phenylalanine to tyrosine and its genetic defect cause **phenylketonuria** (PKU).

Tyrosine hydroxylase (TH) is involved in the **synthesis of L-Dopa** from tyrosine and its genetic defect causes a deficiency of this neurotransmitter.

Synthesis and recycling of BH4



Tryptophan hydroxylase (TPH) is involved in the **synthesis of serotonin** from tryptophan and their genetic defect causes a deficiency of the neurotransmitter.

The **nitric oxide synthases** (NOS) are enzymes involved in the **synthesis of nitric oxide** from arginine and involved in cellular communication (neuronal isoform), endothelial function (endothelial isoform) and the immune defense system and cardiovascular inducible isoform), among other functions.

HOW DO BH4 METABOLIZE?

BH4 is synthesized from guanosine triphosphate (GTP) by the action of three enzymes, **GTP cyclohydrolase (GTPCH), 6-pyruvoil-tetrahydrobiopterin synthase (PTPS) and sepiapterin reductase (SR).**

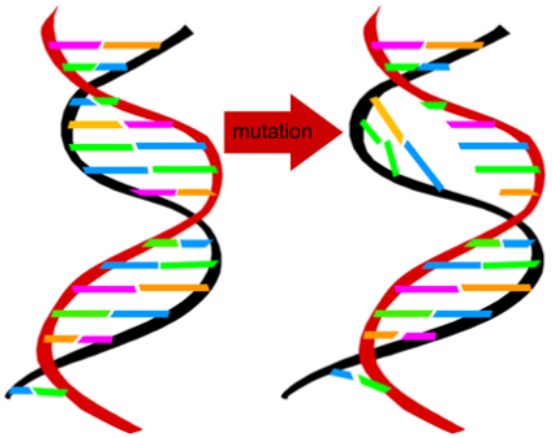
As BH4 synthesis do not cover the important BH4 functions the cofactor must recycle, by means of two enzymes: **pterin-4-carbinolamine dehydratase (PCD)** and **dihydropteridine reductase (DHPR).**

WHICH ARE THE DEFECTS OF BH4 METABOLISM?

BH4 defects are caused by the deficient activity of the enzymes involved in the **synthesis (GTPCH, PTPS and SR)** and **recycling (PCD and DHPR)** of BH4.

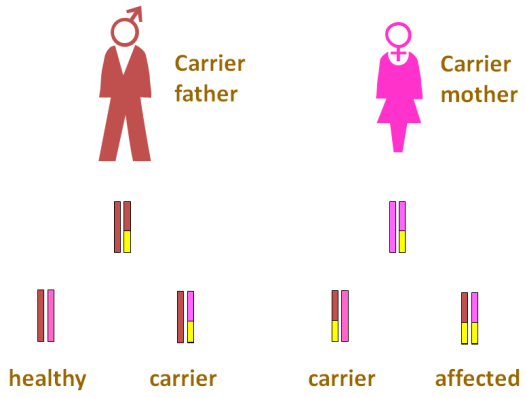
WHY DO THE DEFECTS OF BH4 METABOLISM OCCUR?

Each of the metabolism reactions that will lead to compounds that form our body is genetically determined (encoded). We inherit from our parents the right or altered information that determines each of these metabolic processes.



BH4 defects occur due to **mutations** (stable and heritable changes) in the (***GTPCH, PTPS, SR, PCD and DHPR***) genes encoding these enzyme proteins.

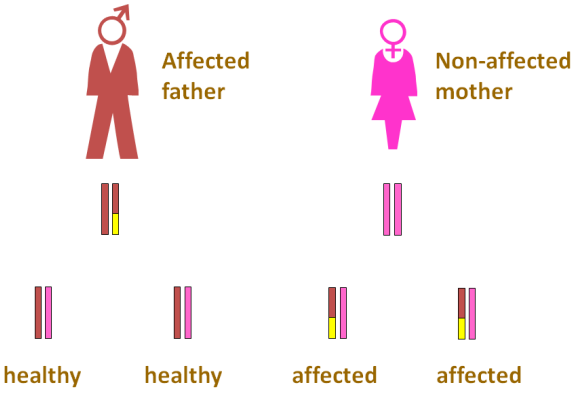
Autosomic recessive inheritance



These defects are genetic disorders of **autosomal recessive inheritance**, ie, parents carry mutations in these genes but not suffer the effects of the enzyme deficiency. If both parents pass a mutated gene to the baby, he /she will suffer a BH4 genetic defect.

However, GTPCH may also have a **dominant inheritance** (Segawa disease), which is the most common.

Autosomic dominant inheritance



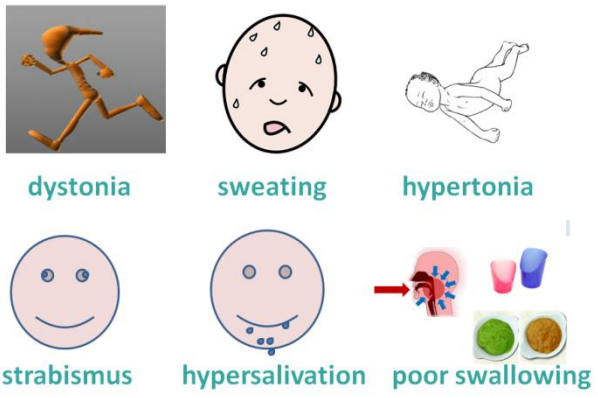
CLINICAL FEATURES OF BH4 DEFECTS

Defects in BH4 synthesis and recycling were first discovered in patients with hyperphenylalaninemia who suffered neurological impairment, despite optimal treatment and metabolic control with a phenylalanine-restricted diet. Therefore, they were initially called **malignant hyperphenylalaninemias** (no response to conventional treatment).

The onset of symptoms occurs in the first months of life, although some of them, such as poor suction and little movement, are already apparent from the first weeks of life. Symptoms are mainly due to the **neurotransmitters defect, poor phenylalanine hydroxylation, and deficient tetrahydrofolate synthesis** (only in DHPR deficiency).

In general, there are mild and severe forms in most defects.

Clinical symptoms of BH4 defects



However, common symptoms of these disorders are the neonatal hypotonia, alternating strabismus, dystonia, poor temperature control, seizures, changes in posture and tone, hypersalivation and difficulty swallowing.

The clinical features that may appear in BH4 defects presenting with hyperphenylalaninemia are summarized in the Table.

Clinical symptoms of BH4 defects

	AR GTPCH defect	PTPS defect	PCD defect	DHPR defect
Hypotonia/ hypertonia	Yes	Yes	Yes	Yes
Poor temperature control	Yes	Yes	No	Yes
Seizures	Yes	Yes	No	Yes
Microcefaly	Yes	No	No	Yes
Hypersalivation	Yes	Yes	No	Yes
Mental retardation	Yes	No	No	Yes
Choreoathetosis	No	Yes	No	Yes

As for **GTPCH deficiency** with **autosomal dominant inheritance**, also called **Dopa-responsive dystonia or Segawa disease**, presents with dystonia and Parkinsonism [association of tremor, rigidity and bradykinesia (slowness of movement)].

Sepiapterin reductase deficiency may present with microcephaly, mental and motor retardation, hypersalivation, dystonia, hypotonia of the trunk and limbs hypertonia, seizures, tremor, extrapyramidal signs, oculogyric crises and hypersomnolence.

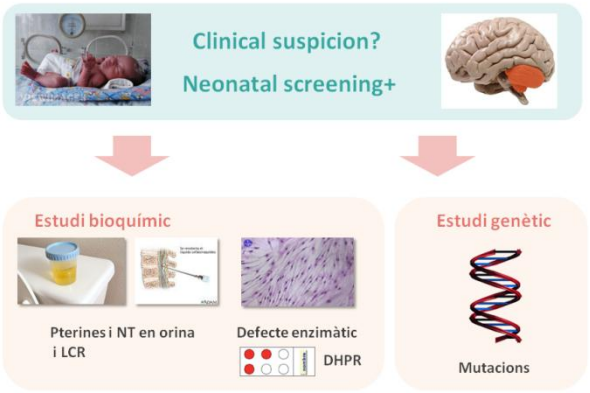
DIAGNOSIS OF BH4 DEFECTS

BH4 defects that occur with hyperphenylalaninemia (autosomal recessive GTPCH, PTPS, PCD and DHPR) use to be detected in the neonatal screening for phenylketonuria, although detection may fail in some cases with mild hyperphenylalaninemia.

When there is hyperphenylalaninemia in the newborn screening, we must proceed to the **differential diagnosis** of its etiology, ie to discover whether elevated phenylalanine originates due to phenylalanine hydroxylase deficiency (PAH) or to a BH4 defect.

Therefore, we proceed to a **20mg / kg of BH4 overload** under standardized conditions, performing an analysis of **amino acids** in plasma and urine, **pterines** in urine, **DHPR activity** in erythrocytes (dried blood on filter paper, in general), and blood samples are collected pre- and post-overload in pre-established time intervals (4 and 8 hours). This study allows the **differential diagnosis of BH4 defects** and, in addition, permits to rule out phenylketonuria.

Diagnosis of BH4 defects



The other **defects without hyperphenylalaninemia** (dominant GTPCH and SR) should be suspected on clinical grounds. For **dominant GTPCH deficiency** or Segawa disease a phenylalanine (100 mg / kg) loading test is useful by comparing the blood phenylalanine elevation of potential patients with the values of a healthy control group.

Pterins and neurotransmitter analysis in cerebrospinal fluid, enzymatic study and particularly the **genetic study** of the genes involved in the suspected defect confirm the diagnosis, and allow genetic counseling and prenatal diagnosis, if required.

TREATMENT OF BH4 DEFECTS

Treatment of BH4 defects is based on preventing hyperphenylalaninemia, deficiency of neurotransmitters (serotonin and L-Dopa), and tetrahydrofolate deficiency (only in DHPR deficient activity).

1. **Hyperphenylalaninemia** may be prevented by means of a **phenylalanine-restricted diet**, ie restriction of proteins of high and medium biological value containing phenylalanine (PCD and DHPR deficiencies) and by **oral administration of BH4** (GTPCH and PTPS deficiencies, whose mild forms respond to this treatment).
2. **Neurotransmitter defects** may be prevented by the administration of **L-Dopa and 5-hydroxytryptophan** (serotonin and dopamine precursors, respectively). L-Dopa is administered with **carbidopa**, an inhibitor of L-Dopa decarboxylase which prevents its degradation. To maintain the concentration of L-Dopa in the central nervous system, monoamine oxidase (MAO) can be used, such as **selegiline**. More

recently, **entacapone** is also used for the same purpose.

3. **Tetrahydrofolate deficiency** occuring in DHPR deficiency is prevented by the administration of **folinic acid**.

Treatment of BH4 defects

Treatment	GTPCH AR defect	PTPS defect	PCD defect	DHPR defect	GTPCH AD defect	SR defect
Neurotransmitters L-Dopa & 5-OH-Trp	Yes	Only severe forms	No	Yes	Yes	Yes
BH4	Yes	Yes	No	No	No	Yes
Phenylalanine restricted diet	No	No	Yes	Yes	No	No
Folinic acid	No	No	No	Yes	No	No

Defects in BH4 synthesis and recycling are serious neurological diseases if not treated properly. However, early diagnosis and treatment improve the prognosis and quality of life of patients.

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