

### 34.1 Introduction

Leukotrienes comprise a group of biologically highly active lipid mediators. They are derived from 20-polyunsaturated fatty acids, predominantly arachidonic acid, and synthesised through the 5-lipoxygenase pathway [1, 2]. They include the cysteinyl leukotrienes (LTC<sub>4</sub>, LTD<sub>4</sub>, and LTE<sub>4</sub>), formerly known as “slow-reacting substance of anaphylaxis” and the dihydroxyeicosatetraenoate, LTB<sub>4</sub>. Synthesis of the primary cysteinyl leukotriene, LTC<sub>4</sub>, from conjugation of the unstable LTA<sub>4</sub> with glutathione is mediated by LTC<sub>4</sub>-synthase. Stepwise cleavage of glutamate and glycine from LTC<sub>4</sub> by  $\gamma$ -glutamyl transpeptidase and membrane-bound dipeptidase yield LTD<sub>4</sub> and LTE<sub>4</sub>, respectively. Biosynthesis is limited to very few human cells including mast cells, eosinophils, basophils and macrophages.

During the last decade leukotrienes have been mainly investigated because of their role as inflammatory mediators. Indeed, they play an important role in a variety of disease states, including asthma in childhood. What was not clearly recognised is that the human brain tissue also has the capacity to synthesise large amounts of leukotrienes. Their role in the CNS is poorly understood, but there is increasing evidence that they are messengers or modulators of CNS activity.

A few disorders have been identified causing secondary disturbances in LT elimination and degradation, e.g. defective hepatobiliary elimination of cysteinyl leukotrienes as seen in the Dubin-Johnson syndrome [3], impaired  $\omega$ -oxidation of LTB<sub>4</sub> in the Sjögren-Larsson syndrome [4] or altered  $\beta$ -oxidation in disorders of peroxisome biogenesis such as the Zellweger syndrome [5]. The metabolic changes seen in these disorders are characterised by altered urinary excretion patterns of leukotrienes. However, in these conditions LT synthesis itself is not affected.

Recently, total absence of cysteinyl leukotrienes in the CSF of patients with a severe neurodevelopmental syndrome led to the discovery of LTC<sub>4</sub>-synthase deficiency [6, 7]. In the meantime there is evidence of two further defects in the biosynthesis of leukotrienes representing a new group of neurometabolic disorders [8].

At present, LTC<sub>4</sub>-synthase deficiency has been identified in two independent patients. Onset of symptoms started already in the neonatal period or infancy. The clinical picture was mainly characterised by severe muscular hypotonia, psychomotor retardation, failure to thrive, microcephaly and death in infancy. All general and specific biochemical investigations were unremarkable. Pathological findings were noted for the complete absence of the primary cysteinyl leukotriene, LTC<sub>4</sub>, and its metabolites in biological fluids, especially in the CSF, as well as in stimulated blood cells. It is thought that the absence of LTC<sub>4</sub>, especially in the brain, is at least in part responsible for the observed neurological symptoms. Consanguinity in the parents of both patients suggests an autosomal recessive trait. The human gene for LTC<sub>4</sub>-synthase has recently been cloned. Molecular studies have not yet been performed. At present there is no effective treatment strategy.

The second step in cysteinyl leukotriene synthesis is mediated by  $\gamma$ -glutamyl transpeptidase. Up to now, five patients with this enzyme defect have been reported. Besides elevated glutathione in urine and plasma, these patients are characterised by the unique finding of LTC<sub>4</sub> excretion in urine and absence of LTD<sub>4</sub> in plasma. Three of the five patients had CNS symptoms.

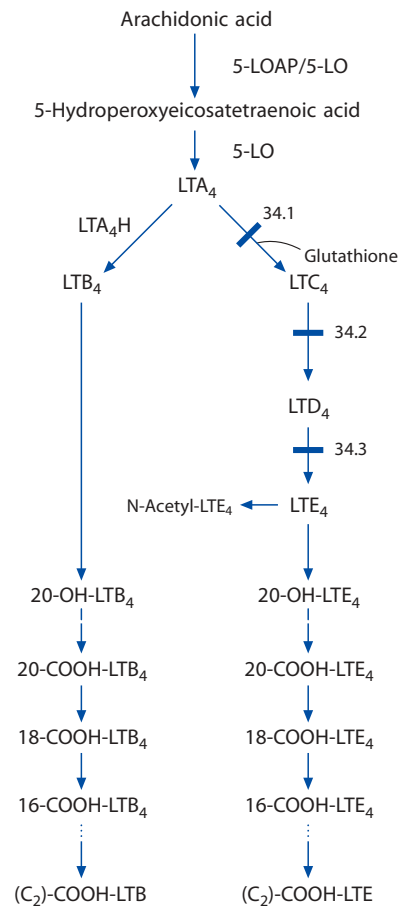
In the meantime a patient with cystinylglycinuria and, as it was only recently demonstrated, excretion of LTD<sub>4</sub> in urine has been identified [9]. Under physiological conditions, these metabolites are always not detectable. Even definite enzyme measurements could yet not be performed, this 15 year old male seems to be the first patient with membrane-bound dipeptidase (cysteinyl-glycinase) deficiency. This enzyme is involved in the third step of the synthesis of cysteinyl leukotrienes resulting in the synthesis of LTE<sub>4</sub>. Endogenous urinary LTE<sub>4</sub> represents the index metabolite for the generation of cysteinyl leukotrienes *in vivo*.

Defects in the pathway of the synthesis of LTB<sub>4</sub> has not been reported yet. Since LTB<sub>4</sub> is a highly potent chemotactic factor and activator of leucocytes, patients with LTB<sub>4</sub> synthesis deficiency are expected to present with recurrent infections and immunological problems in infancy.

34.2 Nomenclature

No.	Disorder – affected component	Tissue distribution	Chromosomal location	MIM
34.1	LTC <sub>4</sub> -synthase deficiency	Nucleated cells	5q35	246530
34.2	γ-Glutamyl transpeptidase deficiency	Nucleated cells, kidney, liver, small intestine, pancreas, testis, brain	22q11.1-q11.2	231950
34.3	Membrane-bound dipeptidase (cysteinyl-glycinase) deficiency	Kidney, liver, placenta, epithelium and endothelial cells (trachea)	16q24.3	179780

34.3 Metabolic Pathway



**Fig. 34.1.** Metabolic pathway of leukotriene biosynthesis and metabolism including known metabolic defects: 34.1, LTC<sub>4</sub>-synthase; 34.2, γ-glutamyl transpeptidase; 34.3, membrane-bound dipeptidase. LT, leukotriene; 5-LOAP, 5-lipoxygenase-activating protein; 5-LO, 5-lipoxygenase; LTA<sub>4</sub>H, leukotriene A<sub>4</sub> hydrolase

## 34.4 Signs and Symptoms

**Table 34.1.** LTC<sub>4</sub>-synthase deficiency

System	Symptoms/marker	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Special laboratory	LTC <sub>4</sub> (CSF, P)	↓	↓			
	LTD <sub>4</sub> (CSF, P)	↓	↓			
	LTE <sub>4</sub> (CSF, P, U)	↓	↓			
	LTB <sub>4</sub> (CSF, P)	n-↑	n-↑			
	Glutathione (RBC)	n	n			
	LTC <sub>4</sub> -synthesis in nucleated cells (WBC)	↓	↓			
Routine laboratory	Abnormal EEG	+	+			
	Abnormal EMG	+	+			
	Psychomotor retardation	+	+			
	Progressive deterioration	+	+			
	Hypotonia	±	+			
	Minimal spontaneous movements	+	+			
	Absent head control	+	+			
	Lack of facial expression	+	+			
	Reduced deep tendon reflexes	+	+			
	Microcephaly	±	+			
Other	Dysmorphic features	±	±			
	Symmetric extension in the legs	±	+			
	No visual contact	+	+			
	Failure to thrive	+	+			
	Death		<1 yr			

**Table 34.2.** γ-Glutamyl transpeptidase deficiency

System	Symptoms/marker	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Special laboratory	Glutathione (U)	↑	↑	↑	↑	↑
	Glutathione (RBC)	n	n	n	n	n
	γ-Glutamyl transpeptidase in nucleated cells (WBC)	↓	↓	↓	↓	↓
	LTC <sub>4</sub> (U, P)	↑	↑	↑	↑	↑
	LTD <sub>4</sub> (U, P)	↓	↓	↓	↓	↓
	LTE <sub>4</sub> (U, P)	↓	↓	↓	↓	↓
	LTB <sub>4</sub> (U, P)	n	n	n	n	n
	LTD <sub>4</sub> -synthesis in nucleated cells (WBC)	↓	↓	↓	↓	↓
CNS	Mental retardation			±	±	±
	Psychosis			±	±	±

**Table 34.3.** Membrane-bound dipeptidase deficiency

	Symptoms/marker	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Special laboratory	Cystinylglycine (U, P)				↑	
	Glutathione (RBC)				n	
	Cysteine, bound (U)				↑	
	Half-cystine (U)				↑	
	LTD <sub>4</sub> (U)				↑	
	LTE <sub>4</sub> (U)				↓	
Routine laboratory	Abnormal EEG				+	
	Abnormal EMG				+	
	Mental retardation				+	
	Motor impairment				+	
	Peripheral neuropathy				+	
CNS						
Other	Foot deformity				+	
	Partial deafness				+	

### 34.5 Reference Values

Metabolite	Value	Unit
LTC <sub>4</sub> (CSF)	37.2–100.5	pmol/l
LTC <sub>4</sub> (P)	13.8–17.2	nmol/l
LTC <sub>4</sub> (U)	<5	nmol/mol creat
LTD <sub>4</sub> (CSF)	31.6–69.6	pmol/l
LTD <sub>4</sub> (P)	23.2–28.4	nmol/l
LTD <sub>4</sub> (U)	<5	nmol/mol creat
LTE <sub>4</sub> (CSF)	46.1–124.2	pmol/l
LTE <sub>4</sub> (P)	27.0–32.9	nmol/l
LTE <sub>4</sub> (U)	15.8–45.2	nmol/mol creat
LTB <sub>4</sub> (CSF)	55.5–183.3	pmol/l
LTB <sub>4</sub> (P)	27.3–35.4	nmol/l
LTB <sub>4</sub> (U)	<5	nmol/mol creat
20-Hydroxy-LTB <sub>4</sub> (U)	<5	nmol/mol creat
20-Carboxy-LTB <sub>4</sub> (U)	<5	nmol/mol creat
Cystinylglycine (U)	<1	mmol/mol creat
Cystinylglycine (P)	<16	μmol/l

Leukotrienes were measured either by specific immunoassay or GC-MS after purification with Sep-Pak C<sub>18</sub>-extraction and reverse-phase HPLC. There exist no significant age-specific differences of leukotriene concentrations in CSF, plasma and urine [10].

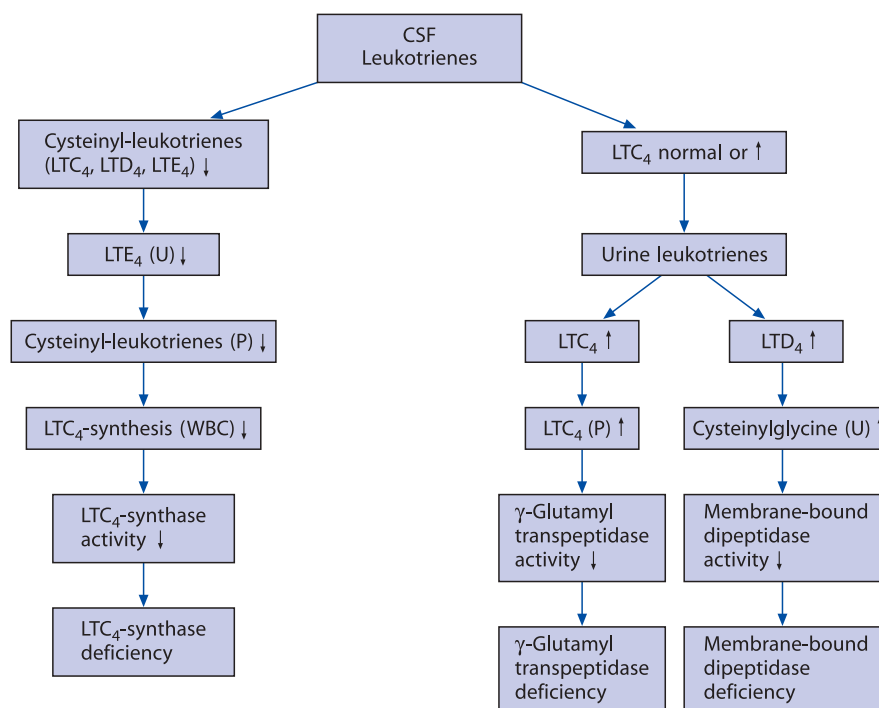
### 34.6 Pathological Values/Differential Diagnosis

	34.1 LTC <sub>4</sub> -synthase deficiency	34.2 $\gamma$ -Glutamyl transpeptidase deficiency	34.3 Membrane- bound dipeptidase (cystinylglycinase) deficiency
CSF (pmol/l)			
LTC <sub>4</sub>	<1		
LTD <sub>4</sub>	<1		
LTE <sub>4</sub>	<1		
LTB <sub>4</sub>	n-↑		
Plasma (nmol/l)			
LTC <sub>4</sub>	<3	↑	
LTD <sub>4</sub>	<3	<3	
LTE <sub>4</sub>	<3	<3	
LTB <sub>4</sub>	↑	n	
Cystinylglycine	n	n	30
Urine (nmol/mol creat)			
LTC <sub>4</sub>	<5	↑	<5
LTD <sub>4</sub>	<5	<5	↑
LTE <sub>4</sub>	<5	<5	<5
LTB <sub>4</sub>	<5	<5	<5
Cystinylglycine (mmol/mol creat)	n	n	49–72
WBC			
LTC <sub>4</sub> -synthesis	↓	↑↑	
LTD <sub>4</sub> -synthesis	↓	↓	
			(1 patient)

### 34.7 Loading Tests

Not applicable.

### 34.8 Diagnostic Flow Chart



**Fig. 34.2.** Diagnostic flow chart for defects in the synthesis of cysteinyl leukotrienes. *LT*, Leukotriene

### 34.9 Specimen Collection

Test	Preconditions	Material	Handling	Pitfalls
Leukotrienes (CSF)	Before medication	CSF	0.5 ml, immediately kept in liquid nitrogen, store at $-70^{\circ}\text{C}$	Leukotrienes are very susceptible to oxidative degradation; collection and storage in polypropylene tubes, if possible under argon; long storage may result in lower contents
Leukotrienes (U)	Before medication	Urine	10 ml, freeze immediately (preferably $-70^{\circ}\text{C}$ )	As above; leucocytes or bacterial contamination may cause artificial higher contents
Leukotrienes (P)	Before medication	Plasma from heparinised blood	1 ml, centrifuge, remove and freeze immediately (preferably $-70^{\circ}\text{C}$ )	As above; leukotrienes are easily artificially generated and released from leucocytes during blood sampling
Leukotrienes (WBC)	Before medication	Monocytes Neutrophils	Stimulation with calcium ionophore A 23 187 (10 $\mu\text{mol/l}$ for 15 min at $37^{\circ}\text{C}$ )	As above
Cystinylglycine	Before medication	Urine/plasma	1 ml, frozen ( $-20^{\circ}\text{C}$ )	

### 34.10 Prenatal Diagnosis

Prenatal diagnosis has not been recorded.

### 34.11 DNA Analysis

DNA analysis has not been performed yet. It might be in principle possible from genomic DNA (e.g. extracted from blood cells or cultured skin fibroblasts) in 31.1 (LTC<sub>4</sub>-synthase deficiency) and 34.3 (Membrane-bound dipeptidase deficiency). In 34.2 ( $\gamma$ -Glutamyl transpeptidase deficiency) DNA analysis might be difficult since the human genome has at least seven different loci for  $\gamma$ -glutamyl-transpeptidase. Five of them are located on chromosome 22.

### 34.12 Initial Treatment (Management while Awaiting Results)

There is no special initial treatment.

### 34.13 Summary/Comments

In the synthesis of the leukotrienes hereditary primary defects have been detected in three of the enzymatic steps: LTC<sub>4</sub>-synthase,  $\gamma$ -glutamyl transpeptidase and membrane-bound dipeptidase. Deficiency of these enzymes results in abnormal levels and profiles of cysteinyl leukotrienes in CSF, urine and/or plasma. In LTC<sub>4</sub> synthase deficiency patients seem to be most severely affected including muscular hypotonia, psychomotor retardation, microcephaly and failure to thrive. A more profound understanding of the role of leukotrienes in the brain and their pathophysiological role is the prerequisite for the suggestion of possible therapeutic approaches. Although at present only a few patients have been identified with defects in the synthesis of leukotrienes it is possible that such disorders are underdiagnosed since leukotrienes are most often not yet included in the routine metabolic work up. Analysis of leukotrienes, preferably in the CSF, is recommended in all patients with neurological symptoms who have no apparently obvious metabolic cause.



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