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CDG-syndrome type 1 with normal phosphomannomutase activity and unusually mild clinical manifestations

We report on a boy showing a CDG-syndrome type 1, normal phosphomannomutase activity and the following clinical course: the boy of consanguinous parents, now aged 5.5 years, had an uneventful pregnancy, delivery and newborn period. No abnormal subcutaneous fat distributions were noted. At 1 month of age peculiar ocular movements appeared. A delay of psychomotor development became obvious with repeated periods of apathia; the tendon reflexes, however, were exaggerated. Cerebellar ataxia developed and bouts of infections became troublesome. Nevertheless the boy began to walk at 21 months of age. *Diagnostic investigations* during the first 2 years were unremarkable, especially for transaminases, TSH and proteinuria. CCT scan showed slight cortical atrophy, ophtalmologic examination confirmed spontaneous nystagmus and strabismus. At age of 3.5 years CDG-syndrome type 1 was diagnosed by IEF with specific immunofixation in serum, but phosphomannomutase activity in cultured fibroblasts and lymphocytes was normal. Detailed investigations revealed the following typical findings: subnormal factor XI (40%), elevated insulin, inconstantly elevated TSH, slight intermittant proteinuria. But MRT was normal, the cerebellum being at the lower limit of normality. His mental and motor developments are about half of his age. With 5.5 years of age apathic episodes become rare, susceptibility for infections and ataxia are reduced (typical feature of the desease). Growth was never impaired. *In summary,* our clinical and laboratory data of the boy point to an atypical CDG-syndrome type 1 probably indicating a new subtype.

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CDG-syndrome type 1 with normal PMM activity: is α2,6N- sialyltransferase affected?

Carbohydrate-deficient glycoconjugate (CDG) syndromes are a group of genetic multisystemic diseases. Four family members with moderate psychomotor retardation showed a CDGS type 1 serum sialotransferrin pattern but normal phosphomannomutase activity in fibroblasts. One of us (JJ) suspected a sialylation deficiency on the basis of unusually low blood clotting factors.

The α2,6N-sialyltransferase (ST6Gal I) activity was slightly decreased compared to age-matched controls and in fibroblasts the activity was also decreased. For comparison α2,3N-sialyltransferase and β1,4-galactosyltransferase were normal. Fibroblasts were analysed by immunofluorescence microscopy using antibodies to ST6Gab I. The expected Golgi staining was observed proving the enzyme to be expressed and to be correctly localised to the Golgi apparatus. To evaluate a possible structural defect of the ST6Gal I gene, RT-PCR was carried out and the ST6Gal I gene was sequenced and expressed in COS cells. The expressed enzyme activity was detected in the clones without any or with conservative substitutions. However, clones with non-conservative substitutions were also obtained. These clones showed no enzymatic activity. The analysis of these substitutions is currently under investigation.

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Different phenotypes in 3-methylglutaconic (3-MGC) aciduria

Patient 1, male, was hypotonic since birth and presented with progressive cardiomyopathy, lactic acidosis and died at 18 months of age; urinary organic acids showed 3-MGC (80 mmol/mol creat.), 3-methyl-glutaric and 2-ethylidracrilic acid. A sister had similar clinical features but no 3-MGC in urine. Both presented a deficiency of mitochondrial adenosine-triphosphate syntase (Complex V). Patient 2, male, presented since birth with failure to thrive and severe neurological symptoms (microcephaly, seizures, bilateral optic atrophy); brain MRI revealed cerebellar hypoplasia and white matter leukodystrophy. He died suddenly at home at age of 2 years following a crisis of apnea. Lactic acidosis was always present and organic acid analysis revealed only excretion of 3-MGA (75 mmol/mol creat.). In patient 3, male, the clinical history and neuroimaging findings led to a diagnosis of Leigh syndrome; metabolic investigations revealed 3-MGC in urine (80 mmol/mol creat.). Patient 4, female, was referred for metabolic investigations at the age of 2 months with a history of seizures (West syndrome), developmental delay and dysmor-

phic features; brain MRI showed cerebellar hypoplasia and delayed myelination; 3-MGC in urine was 140 mmol/mol creat. In all the patients different regimes of treatment (vitamins, coencyme Q and diet) did not influence the urinary excretion of 3-MGC acid.

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Serum transferrin isoelectric focusing as a diagnostic tool

The heterogeneity of human transferrin results from (i) differences in iron content (ii) genetic polymorphism and (iii) differences in the carbohydrate moiety. This lecture primarily deals with the last phenomenon, the microheterogeneity of human transferrin. Owing to the comparatively simple carbohydrate structure of human transferrin in the high resolving powder of isoelectric focusing in immobilized pH gradients, microheterogeneous forms of transferrin can be separated. Differences between samples can be quantitated by crossed immunoelectrophoresis. Examples of the differences between the microheterogeneity patterns of transferrin in several biological fluids and the changes that can be observed in diseases such as rheumatoid arthritis, idiopathic hemochromatosis and Kahler's disease are presented. Special attention has been focused on changes occurring during pregnancy.

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Peculiar biochemical findings in two patients with a disorder of peroxisome biogenesis

We present two infants, a girl (patient 1) and a boy (patient 2) (2–3 months of age), affected by a generalized peroxisomal disorder, type Zellweger. Both manifested disturbances in the (mitochondrial) metabolism of the branched chain amino acids, especially of valine, as was detected by organic acid analysis of cerebrospinal fluid (CSF) (Table 1). This finding is another proof that mitochondrial dysfunction exists in patients with peroxisomal disorders, and is not confined to the respiratory chain. In patient 2, neurotransmitter analysis in CSF revealed elevated levels of HVA, 5-HIAA and MHPG compared to controls. There seems to be a high flux through the metabolic pathways of catecholamines and serotonine (Table 2).

These findings need further investigation as they can lead to new insights in the pathophysiology of peroxisomal disorders.

Table 1

	Patient 1	Patient 2	Normal values
2-OH-isovaleric acid	104 µMol/L	74 µMol/L	undet.–18
2-OH-methyl-valeric acid	absent	36 µMol/L	undet.–8
2-OH-isocaproic acid	trace	13 µMol/L	undet.–9.4
3-OH-isobutyric acid	53 µMol/L	100 µMol/L	trace –38

Table 2

	Patient 1	Patient 2	Normal values
HVA	–	715 nMol/L	200–450
5-HIAA	–	297 nMol/L	75–175
HVA/5-HIAA ratio	–	2.4	1.9–4.0
MHPG	–	101	28–64

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Serum glycoproteins and their liver precursors in patients with carbohydrate-deficient glycoprotein syndrome type 1: deficiency of serum clusterin

Serum glycoproteins were studied in 18 patients with carbohydrate-deficient glycoprotein syndrome type 1 (CDGS type 1) by two-dimensional gel electrophoresis (2-DE). The silver-stain pattern of serum glycoproteins in all the patients showed the near absence of clusterin (Apolipoprotein J) isoforms as compared to controls. Quantitation of serum clusterin using monoclonal antibodies directed against the human form of the glycoprotein confirmed the apparent clusterin deficiency in patients with CDGS type 1. The 2-DE pattern of immunodetected precursors of clusterin in liver cells from 1 patient with CDGS type 1 showed the accumulation of abnormal low-mass precursors. These abnormal precursors are identified as precursors with an intermediate form of protein-bound oligosaccharides without added sialic acid at their surfaces. These results suggest that abnormal clusterin precursors accumulate during the early oligosaccharide processing of the nascent protein-bound oligosaccharides, and that they are poorly secreted by hepatocytes from patients with CDGS type 1. In conclusion, one can envisage that the primary defect in CDGS type 1 not only alters the N-glycosylation of proteins but subsequently deranges the intracellular transport and turnover of glycoproteins.

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Looking at brain glycosylation in carbohydrate-deficient glycoconjugate (CDG) syndromes by isoelectrofocusing of cerebrospinal fluid (CSF) β-trace protein

CDG syndromes are genetic multisystem diseases with different clinical presentations and mostly comprising nervous system involvement. Five types have been reported; in type 1a and 2 the basic defects have been identified (resp. phosphomannomutase [PMM] and N-acetylglucosaminyltransferase 2 deficiency). Beta-trace protein is a major intrathecally synthesized polypeptide in human CSF that is almost quantitatively modified with biantennary complex-type N-linked oligosaccharides with „brain-type" glycosylation characteristics [Hoffmann A et al (1994) J Neurochem 63: 2285–2291]. It has been shown to have a similar glycosylation defect than serum transferrin in PMM as well as N-acetylglucosaminyltransferase 2 deficiency [Pohl S et al (1997) Glycobiology, in press]. Several patients are known with a CDG syndrome type 1 with normal or much better psychomotor development than that seen in PMM deficiency. The question arises whether these patients have a defect limited to organs outside the brain. Since it is unfeasible to measure enzyme activities in brain, isoelectrofocusing of CSF β-trace protein, a brain-derived glycoprotein, provides an ideal tool to answer this question. On the other hand, analysis of this CSF glycoprotein is an elegant way to determine whether some patients with an unexplained „pure" brain disease (psychomotor retardation, epilepsy, olivopontocerebellar hypoplasia, Dandy Walker variants, etc.) might have a glycosylation defect limited to brain tissue.

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Mannose therapy in carbohydrate-deficient glycoprotein syndrome type 1 – first results of the German multicenter study

Addition of mannose to the culture medium of fibroblasts from patients with CDG syndrome type I was shown to correct the consequences of phosphomannomutase deficiency. In order to study the effects of mannose treatment in vivo, a german multicenter study was initiated. More than 10 patients with CDG syndrome type 1 have been treated with mannose.

Mannose was given orally in a dose of 100 mg/kg BW five times daily for the first 4 weeks. Peak serum mannose levels reached more than 200 µM. After 4 weeks the dosage was increased to 150 mg/kg BW five times daily.

Phosphomannomutase activity was determined from leukocyte samples in all patients and their parents. Serum mannose levels before and during therapy were closely monitored. Isoelectric focussing patterns before, during and after mannose therapy were analysed.

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Segregation of early onset glutaric aciduria type 1 and congenital nephrotic syndrome of the diffuse mesangial sclerosis type

Glutaric aciduria type 1 is an autosomal recessively inherited inborn error of lysine, hydroxylysine and tryptophan metabolism caused by a deficiency of glutaryl-coenzyme A dehydrogenase. We report on a male Turkish patient which presented with an unusual early and severe clinical manifestation of glutaric aciduria type 1 already during the neonatal period in association with congenital nephrotic syndrome. Glutaric acid was found to be elevated in urine (7870 mmol/mol of creatinine), plasma (12 µmol/L) and CSF (43 µmol/L). Additionally, 3-hydroxyglutaric acid was increased in urine (590 mmol/mol of creatinine), plasma (1.8 µmol/L) and CSF (2.2 µmol/L). Glutaryl-coenzyme A dehydrogenase activity in cultured skin fibroblasts was found to be < 1%. In the course of the disease the patient developed therapy-resistant seizures, choreoathetosis and dystonia. MRI showed bilateral frontotemporal atrophy, subependymal pseudocysts and basal ganglia atrophy. The disease was associated with the severe clinical picture of congenital nephrotic syndrome of the diffuse mesangial sclerosis type which led to early death at 15 weeks of life due to end-stage renal failure. This report expands the spectrum of the age of onset and phenotype variability in glutaric aciduria type 1 and illustrates an hitherto unknown segregation with diffuse mesangial sclerosis.

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Detection of DNA mutations in German patients with hereditary fructose intolerance

Hereditary fructose intolerance (HFI) is an autosomal recessive disease that results from a deficiency of liver aldolase (EC 4.1.2.13). Affected patients suffer from abdominal pain, vomiting, and hypoglycemia after ingestion of fructose or related sugars and are at high risk for severe metabolic disturbance at weaning. Episodic hypoglycemia, liver disease, renal tubular acidosis, and growth retardation follow exposure to fructose in later life, occasionally with fatal results. For an effective dietary management of this disease, an early and prompt diagnosis is therefore important. In order to prevent invasive procedures such as intravenous fructose test and/or assay of aldolase activity in liver biopsy specimen a molecular biological approach for the diagnosis of hereditary fructose intolerance was established.

The analysis of the molecular basis of HFI was done in 37 patients (32 pedigrees, 64 apparently independent mutant alleles of liver aldolase) by direct analysis of liver aldolase genes amplified by means of PCR and characterized by restriction fragment- and SSCP-analysis or direct sequencing. The mutation A149P was found in 79.7% and the mutation A174D in 15.6% of the investigated alleles. The mutation N334K was observed in 3 alleles (3.2%) Only in one allele another mutation (37Δ4) was detected. Because of the prevalence of these three point mutations among German patients more than 98% of HFI patients will be susceptible to genetic diagnosis.

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Carbohydrate-deficient glycoprotein syndrome type 2. An autosomal recessive disease due to point mutations in the N-acetylglucosaminyltransferase 2 gene

Carbohydrate-deficient glycoprotein syndrome (CDGS) type 2 is a multisystemic congenital disease with severe involvement of the nervous system associated with over 98% reduction in UDP-GlcNAc:α-6-D-mannoside β-1,2-*N*-acetylglucosaminyltransferase 2 (GnT 2) activities in fibroblast and mononuclear cell extracts from two CDGS 2 patients. GNT 2 is essential for biosynthesis of complex Asn-linked glycans. The gene for human GnT 2 has been cloned and is located on chromosome band 14q21. Genomic DNA was isolated from mononuclear cells and single stranded DNA from the GnT 2 coding region was prepared using PCR with biotin-labeled oligonucleotide primers followed by purification with magnetic beads. DNA was sequenced using fluorescent oligonucleotide primers and an automated system. Two point mutations in the catalytic domain of GNT 2 (His to Arg and Ser to Phe) were detected in two unrelated CDGS type 2 patients (JV from Belgium and MB from Iran, respectively). DNA sequencing showed that the father, mother and brother of JV each carry one allele with the same mutation as JV. Restriction endonuclease methods for detecting these two mutations have been developed and were used to show that 13 of 23 blood relatives of JV were heterozygotes; the other relatives and 21 unrelated donors were normal homozygotes. All heterozygotes showed a significant reduction (33 to 68%) in mononuclear cell GnT 2 activity. Both mutations caused decreased expression of enzyme protein in a baculovirus/insect cell system and inactivation of enzyme

activity. The data indicate that CDGS type 2 is an autosomal recessive disease and that complex Asn-linked glycans are essential for normal neurological development.

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Mutations in a phosphomannomutase gene, *PMM2*, on chromosome 16 are a cause of carbohydrate-deficient glycoprotein type 1 syndrome (Jaeken syndrome)

Carbohydrate-deficient glycoprotein syndrome type 1 (CDG1 or Jaeken syndrome) is an autosomal recessive glycosylation disorder. Most patients show a deficiency of phosphomannomutase (PMM), an enzyme necessary for the synthesis of GDP-mannose. CDG1 is genetically heterogeneous and the major locus is on chromosome 16p13. We have previously cloned the *PMM1* gene, which is on chromosome 22q13. We now report the identification of a second PMM gene, *PMM2*, encoding a protein with 66% identity to *PMM1*. We mapped *PMM2* to the CDG1 candidate region on 16p13. A plethora of mutations in *PMM2* were found in CDG1 patients from different geographical origin and with a documented PMM deficiency. Our results give conclusive support to the biochemical finding that the phosphomannomutase deficiency is the basis for CDG1.

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Biochemical and molecular biological analysis in a patient with succinate dehydrogenase deficiency

Biochemical analysis in a skeletal muscle biopsy of a patient with generalized hypotonia, muscle weakness, delayed mental and motor milestones and normal clinical chemistry revealed a partial, isolated, succinate dehydrogenase deficiency (0.04 mU/mU CS; reference values 0.07–0.18). Immunoblot analysis revealed a decrease in the levels of both the Fp and Ip subunits. Probing the blot with a COX IV antibody demonstrated a comparable loading of protein onto the gel (D. M. Turnbull and R. W. Taylor, Newcastle upon Tyne, UK). Total RNA was extracted from the patient's cultured fibroblasts and reversed transcribed to cDNA. PCR amplification of both the Fp and Ip subunits was followed by direct DNA sequence analysis of the generated fragments.

Recently, the first mutation in the Fp subunit (chromosome 5) was found by Bourgeron et al. (Nature Genetics 11, 144–149, 1995). Besides two so far unknown polymorphisms, no mutation in the Fp subunit of our patient were found. We mapped the Ip subunit to chromosome 1. Mutation detection in the Ip subunit in the patient and his relatives is in progress and the results will be discussed.

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Secondary CDG syndromes

The primary carbohydrate-deficient glycoprotein (CDG) syndromes are a new group of genetic metabolic diseases with multisystemic clinical features. Incomplete carbohydrate chains in serum glycoproteins are pathognomonic for the CDG syndromes. The diagnosis is based on isoelectric focusing (IEF) of serum transferrin or other glycoproteins showing bands shifted towards the cathode. This is due to the decreased negatively charged sialic acid content of the glycoproteins. In controls carbohydrate-deficient transferrins (CDT) both visualized by IEF, and quantified by the commercially available CTD-test (CD-Tect®) are only present in very low amounts. However, at birth and during the first weeks of life a considerable fraction of serum transferrin is present in hypoglycosylated form; a CDG-like pattern. The phenomenon of hypoglycosylation is also found in some inborn errors of carbohydrate metabolism, galactosemia due to galactose-1-phosphate uridyl transferase or UDP-galactose-4-epimerase deficiency and hereditary fructose intolerance due to fructose-1-phosphate aldolase deficiency. The aberrant isoelectric focusing pattern of sialotransferrins in these metabolic diseases found in untreated stage, which are similar to those in the CDG syndrome type 1, appear to normalize within a few weeks of treatment. The underlying mechanisms of hypoglycosylation in these secondary CDG syndromes probably are related to the primary metabolic defect. An acquired disturbance in glycosylation of serium transferrin is seen in chronic alcoholism.

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Carbohydrate-deficient glycoprotein syndrome type I: the Italian experience

Clinical findings from 13 Italian patients (11 males and 2 females) suffering from Carbohydrate-deficient glycoprotein syndrome type 1 (CDG1) and diagnosed since 1993 are reported (Table 1). In 9 patients the diagnosis was achieved by isoelectric focusing of serum transferrin with immunofixation, in 3 patients by high-resolution 2-dimensional electrophoresis analysis of serum glycoproteins, in 1 patient by determination of carbohydrate deficient transferrin in serum. The age at diagnosis ranged 6 months – 23 years. The 6 older patients (11–23 years) belong to 3 sets of sibs. The retrospective analysis showed that some neurologic or visceral symptoms were already present during the first months of life. Involvement of the nervous system in CDG1 syndrome was confirmed in all cases.

Table 1. CDG syndrome type 1: clinical presentation at diagnosis of 13 Italian patients

Neurologic signs		*Dysmorphic signs*	
psychomotor delay	13/13	facial dysmorphism	7/13
cerebellar hypoplasia	12/13	inverted nipples	7/13
ataxia	12/13	skeletal deformities	3/13
hypotonia	11/13	lipodystrophy	5/13
strabismus	11/13		
retinopathy	7/13		
neuropathy	6/13		
stroke-like episodes	4/13		
epilepsy	3/13		
Visceral signs			
hepatopathy	7/13		
failure to thrive	5/13		
coagulopathy	6/13		
hypogonadism	1/13		

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Carbohydrate deficient glycoprotein (CDG) syndrome type 1: a Portuguese case

We report a female patient, one of the two cases of CDG syndrome recognised in Portugal. She is the first child of healthy and unrelated parents and she was born at term by caesarean section. Her birth weight was 2500 g, length 47.5 cm, and head circumference 34 cm.

Since the neonatal period she has presented hypotonia, failure to thrive, feeding difficulties, diarrhoea, seizures and convergent strabismus. Since the first weeks she has suffered from frequent urinary infections.

By the age of 2 years she was admitted at hospital due to severe developmental delay and malnutrition. At examination, she presented inverted nipples, protusion of the thorax and scoliosis, symmetrical fat accumulations, choreoatetotic movements and a cerebellar ataxia. There was a mild hepatomegaly. She is extrovert and has a happy appearance.

Renal ultrasound showed bilateral cortical cysts. Magnetic resonance imaging of the brain showed hypoplasia of the *vermis* and *cerebellum*–hemispheres.

Laboratory investigations revealed: moderate anaemia, increased serum aminotransferases, proteinuria, generalised aminoaciduria, and excessive excretion of adipate and suberate in the urine organic acid analysis. Carbohydrate-deficient transferrin (CDT) assay revealed a high value (124 U/L; normal < 26). Isoelectric focusing of serum transferrin, performed by *J. Jaeken* showed a typical type 1 pattern. The assay of phosphomannomutase in cultured fibroblasts showed a low activity.

The Joubert syndrome was the first diagnosis. The correct diagnosis was made at 4 years of age.

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Phytanyl-CoA hydroxylase deficiency: discovery of the enzyme defect in Refsum disease. Purification and characterization of the enzyme from rat liver

Heredopathia atactica polyneuritiformis was first described as a new familial neurologic syndrome by Sigvald Refsum in the 1940's, and is characterized by a constellation of clinical features including retinitis pigmentosa, peripheral polyneuropathy, cerebellar ataxia and elevated CSF protein without pleocytosis. Studies in the 1960's showed the accumulation of phytanic acid, an unusual branched chain fatty acid, in tissues and body fluids from Refsum disease patients. Subsequent studies revealed that this accumulation is due to a defect in the oxidation of phytanic acid. However, until now, the nature of this defect was not elucidated, despite many studies in the last 30 years. Recently, we have identified a new enzyme activity in rat liver peroxisomes catalyzing the conversion of phytanoyl-CoA to 2-hydroxyphytanoyl-CoA. This enzyme activity was one good candidate for the enzyme defect in Refsum disease, so we have now set up a method allowing phytanoyl-CoA hydroxylase activity measurement in human liver homogenates, using HPLC plus [1-^{14}C]phytanoyl-CoA to measure the formation of 2-hydroxyphytanoyl-CoA. The enzyme belongs to the family of dioxygenases and requires Fe^{2+}, ascorbate and 2-oxoglutarate for activity. We now report that phytanoyl-CoA hydroxylase activity is deficient in liver from a Refsum disease patient, thus showing that this is the enzymatic defect in Refsum disease. Furthermore we have purified and characterized phytanoyl-CoA hydroxylase from rat liver. We are now raising antibodies against the rat liver enzyme and will use these for further studies in Refsum disease.

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Molecular basis of rhizomelic chondrodysplasia punctata: discovery of the gene, PXR2, a peroxisome import receptor and identification of mutations in RCDP-patients

Rhizomelic chondrodysplasia punctata (RCDP) is an autosomal recessive disease characterized by a disproportionate stature, typical facial appearance, congenital contractures, eye abnormalities and severe growth- and mental retardation. Our earlier studies have revealed biochemical and genetic homogeneity among the majority of RCDP-patients. Indeed, they show a tetrad of biochemical abnormalities including a deficiency of dihydroxyacetonephosphate acyltransferase, alkyldihydroxyacetonephosphate synthase, phytanoyl-CoA hydroxylase and peroxisomal thiolase. Furthermore, all the patients belong to one single complementa-

tion group. We have previously demonstrated that RCDP cells are unable to import a reporter protein carrying the type 2 (but not type 1) Peroxisome Targeting Signal (PTS2). This phenotype is similar to that found in a *S. cerevisiae* mutant (pex7). The *S. cerevisiae* PTS2-receptor is a 375 AAs protein belonging to the β-transducin family. To learn which amino acid residues are important for PTS2 function, we first cloned a second yeast homologue *K. lactis* by functional complementation. This led to the identification of an EST showing high homology. An almost full-length human cDNA was constructed using RACE-PCR. The human ORF was screened for mutations by SSCP analysis after RT-PCR: three different alleles were found in 9 patients. Sequence analysis of SSCP fragments revealed a frequent L → STOP mutation at position 261 resulting in a 33 amino acid truncation of the protein. Expression studies showed that this truncated protein is indeed functionally inactive. In conclusion, we have resolved the molecular basis of RCDP which will be of great importance for pre- and postnatal diagnosis, carrier detection, etc.

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