Lymphoblastoid Cell Lines for Diagnosis of Peroxisome Biogenesis Disorders

Sabine Grønborg, Ralph Krätzner, Hendrik Rosewich, and Jutta Gärtner

Abstract Peroxisome biogenesis disorders (PBDs) are a group of autosomal-recessive developmental and progressive metabolic diseases leading to the Zellweger spectrum (ZS) phenotype in most instances. Diagnosis of clinically suspected cases can be difficult because of extensive genetic heterogeneity and large spectrum of disease severity. Furthermore, a second group of peroxisomal diseases caused by deficiencies of single peroxisomal enzymes can show an indistinguishable clinical phenotype. The diagnosis of these peroxisomal disorders relies on the clinical presentation, the biochemical parameters in plasma and erythrocyte membranes, and genetic testing as the final step. Analysis of patients' cells is frequently required during the diagnostic process, e.g., for complementation analysis to identify the affected gene before sequencing. In the cases with unclear clinical or biochemical presentation, patients' cells are analyzed to prove PBD or to demonstrate biochemical abnormalities that might be elusive in plasma. Cell lines from skin fibroblast that are usually generated for diagnostic workup are not available in all instances, mainly because the required skin biopsy is invasive and sometimes denied by parents. An alternative cellular system has not been analyzed sufficiently. In this study, we evaluated the alternative use of lymphoblastoid cell lines (LCLs), derived from a peripheral blood sample, in the diagnostic process for PBD. LCLs were suitable for immunofluorescence visualization of peroxisomal enzymes, complementation analysis, and the biochemical analysis to differentiate between control and PBD LCL. LCLs are therefore an easily obtainable alternative cellular system for a detailed PBD diagnostic workup with a reliability of diagnostic results equal to those of skin fibroblasts.

Keywords Diagnosis · D-bifunctional protein deficiency · LCL · Peroxisome biogenesis disorder · Zellweger syndrome

Introduction

Due to their clinical and pathogenetic heterogeneity, inherited disorders of peroxisomal metabolism pose a diagnostic challenge. Peroxisomal diseases can be subdivided into two groups: PBD or group I peroxisomal diseases lead to a loss of function of most or all peroxisomal metabolic activities. Group II comprises diseases that are caused by deficiencies of single peroxisomal proteins only. More recently, a combined defect of peroxisomal and mitochondrial fission caused by mutation of a fission factor (DLP1) common to both organelles has added an inherited peroxisomal disease (Waterham et al. 2007). Different reports have emphasized mild and variant manifestations, especially of PBD, that are likely to elude diagnostic screening or even consideration as peroxisomal disease (Dursun et al. 2009; Raas-Rothschild et al. 2002; Zeharia et al. 2007).

Clinically, PBD patients present with Zellweger syndrome spectrum (ZSS) or, in 20% of cases, with rhizomelic chondrodysplasia punctata. Typical symptoms of ZSS include facial dysmorphism with high forehead, broad nasal bridge, external ear deformities, and large fontanelles, as well as severe neurological impairments such as muscular hypotonia, failure to thrive, seizures, and developmental deficiencies. Disease severity varies and life expectancy ranges from under 1 year in patients with classical Zellweger syndrome to survival into adulthood (Weller et al. 2003). Peroxisomal single protein deficiencies as of D-bifunctional protein (DBPD) or acyl-CoA oxidase show phenotypes resembling ZSS or have a distinct clinical picture as X-linked adrenoleukodystrophy (XALD), the most common peroxisomal disease (Wanders and Waterham 2006).

A common approach to the diagnosis of peroxisomal disorders is the measurement of peroxisomal metabolites, which

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accumulate or deplete in plasma or red blood cell membranes in case of a peroxisomal disease. Very long chain fatty acids (VLCFA) are measured as a screening tool for peroxisomal diseases in general, but may be normal in spite of the presence of peroxisomal disease (Ferdinandusse et al. 2006; Rosewich et al. 2006). A more detailed biochemical analysis and measurements in cultured patient cells, typically fibroblasts, can avoid this pitfall. Bile acid intermediates, phytanic and pristanic acid as well as plasmalogen measurements, help to differentiate between the two groups of peroxisomal diseases and direct suspicion to certain forms in the case of single protein deficiencies. Visualization of peroxisomes and the cellular distribution of peroxisomal matrix enzymes in patients' skin fibroblast cell lines is needed to establish the diagnosis of PBD and is included in a recent diagnostic flowchart for ZSS (Krause et al. 2009). It is also a prerequisite to diagnose the peroxisomal disorder with altered peroxisomal morphology due to a fission factor deficiency (Waterham et al. 2007) and possible further, related pathologies. The genetic heterogeneity of PBD – at least 13 different human genes are involved in peroxisome biogenesis and can cause the disease if mutant – makes complementation analysis in patient cell lines a rational tool to identify the underlying genetic defect (Krause et al. 2009).

In one patient referred to our center with suspected peroxisomal disease but inconclusive biochemical parameters in plasma (Grønberg et al. 2010), the patient's parents declined skin biopsy to establish a fibroblast cell line. With the parents' consent, we turned toward a lymphoblastoid cell line (LCL) that could be derived from a peripheral blood sample without any further burden to the patient. In one previous report, LCL from a PBD patient and a control individual were evaluated regarding cellular catalase distribution, VLCFA levels, and plasmalogen synthesis (Santos et al. 1993). To establish the usefulness of LCL for the diagnosis of peroxisomal disorders and PBD in particular, we studied immunofluorescence staining of peroxisomal membrane and matrix proteins, exemplified complementation analysis in PBD, and analyzed the biochemical profile of VLCFA, phytanic acid, and plasmalogens in LCL from three patients with peroxisomal disease and 19 control individuals. A clear-cut diagnosis could be confirmed for two patients with PBD relying on the biochemical profile, immunofluorescence, and complementation analysis in LCL.

Materials and Methods

Culture of LCLs

Lymphocytes from patients and controls were immortalized by standard Epstein–Barr virus transformation techniques using 5 ml of heparinized blood per patient (Neitzel 1986). LCLs were cultured in RPMI1640 medium supplemented

with 25% FCS, 2 mM L-glutamine, 1% phythemagglutinin, 100 units/ml penicillin, and 100 µg/ml streptomycin at 37°C and 5% CO₂ following standard protocols.

Immunofluorescence Staining of LCL

Immunofluorescence staining was performed as described previously (Grønberg et al. 2010). Primary antibodies were rabbit anti-catalase (1:2,000), rabbit anti-PEX14 (1:1,000), and mouse anti-myc antibody (1:500) (Oxis International Inc., Foster City, CA, USA; Protein Tech Group, Chicago, IL, USA; BD Bioscience, Franklin Lakes, NJ, USA). Secondary antibodies were Alexa Fluor 488 goat anti-rabbit IgG (1:300), Texas Red goat anti-rabbit IgG (1:200), and Alexa Fluor 488 goat anti-mouse IgG (1:2,000) (Molecular Probes, Carlsbad, CA, USA). Subsequently, cells were washed and mounted with ProLong Gold Antifade Reagent with DAPI (Invitrogen GmbH, Karlsruhe, Germany) and analyzed under an Axio Imager M1 fluorescence microscope (Carl Zeiss MicroImaging GmbH, Göttingen, Germany).

Complementation Analysis

Transfections of LCL were conducted by electroporation using the Nucleofector Technology (Lonza, Basel, Switzerland); 1×10^6 LCLs were transfected with 2 µg total DNA per transfection, essentially following the manufacturers protocol. As a marker for peroxisomal protein import, the plasmid mycPECI for expression of peroxisomal $\Delta 3$, $\Delta 2$ -enoyl-CoA isomerase (Geisbrecht et al. 1999) was transfected together with empty expression plasmid (pcDNA3.1; Invitrogen, Carlsbad, USA) or a plasmid for the expression of human PEX6 (pTY3) (Yahraus et al. 1996), respectively. PECI is a peroxisomal matrix protein with the conserved C-terminal peroxisomal import signal –SKL (–serine-lysine-leucine). Twenty-four hours after transfection, cells were processed for immunofluorescence as described above. The localization of mycPECI in the cytosol or in peroxisomes was determined by microscopy to prove complementation.

Biochemistry

For biochemical analysis, cells were pelleted, washed with medium, and resuspended at a concentration of 1×10^7 cells per 0.3 ml in NaCl 0.9%. For biochemical analysis, 100 µl of cell suspension was used. Each analysis was performed in replicate. Selected control and patients' samples were measured in two to three independently collected samples with two technical replicates each. For quantification of VLCFA and phytanic acid, we used the method of Hunnemann and

Hanefeld (1988). Briefly, VLCFA and phytanic acid were derivatized with acetylchloride as methylesters and calibrated via GC/MS against internal deuterated standards. Plasmalogens were determined accordingly, but 50 µl of cell suspension were used and levels were quantified relatively to C16:0 and C18:0 (Duran and Wanders 2008).

Patient and Control Cell Lines

Nineteen LCLs were used as control cell lines in total. Control cell lines were derived from healthy individuals and from patients with apparent nonmetabolic diseases. Patient LCLs were derived from two patients with peroxisomal biogenesis disorders (PBD1 and PBD2) and one patient with D-bifunctional protein deficiency (DBPD). The clinical phenotype and peroxisomal plasma biochemistry of the DBPD patient are described in a recent case report (Grønborg et al. 2010). This patient's peroxisomal parameters in plasma were only discretely conspicuous. PBD1 showed the symptoms of classical Zellweger syndrome and died at the age of 7 months. PBD2 has a milder phenotype and is currently 8 years old. Blood biochemistry from these patients pointed unambiguously toward a PBD with elevated VLCFA and reduced plasmalogen levels in erythrocyte lipids (C22:0: PBD1 12.1, PBD2 14.4, normal 15–113 nmol/ml; C24:0: PBD1 27.5, PBD2 17.9, normal 12–94 nmol/ml; C26:0: PBD1 8.8, PBD2 3.45, normal 0.2–1.6 nmol/ml; C24:0/C22:0: PBD1 2.27, PBD2 1.25, normal 0.55–1.05; C26:0/C22:0: PBD1 0.727, PBD2 0.24, normal 0.005–0.029; phytanic acid: PBD1 1.7, PBD2 34.7, normal 0.3–9 nmol/ml; pristanic acid: PBD1 0.6, PBD2 19.9, normal 0–2 nmol/ml; C16:0 plasmalogens × 100/C16 fatty acids: PBD1 0.3, PBD2 4.4, normal 6.8–11.9; C18:0 plasmalogens × 100/C18 fatty acids: PBD1 0.2, PBD2 7.9, normal 10.6–24.9). Both PBD patients had previously been diagnosed on the molecular level by sequencing of the *PEX1* gene [PBD2: p.G843D/R872X; (Rosewich et al. 2005)] or by cDNA complementation analysis in fibroblasts and sequencing of the *PEX6* gene (PBD1: p.Asp865_Phe890del, unpublished) and were used in this study to examine the diagnostic possibilities in LCL.

Results

Immunofluorescence Staining of Peroxisomal Markers

Immunofluorescence staining of peroxisomal structures can visualize a protein import defect in patients with PBD and is frequently required to confirm or exclude the diagnosis (Krause et al. 2009). In the case of PBD, catalase or other

peroxisomal matrix enzymes are not detected in punctate peroxisomal structures but evenly distributed in the cytosol. Peroxisomal membrane proteins (PMPs) can be found correctly localized or completely absent. Cells with group II peroxisomal disorders do not show mislocalization of peroxisomal proteins but might show fewer and enlarged peroxisomes (Huyghe et al. 2006). Peroxisomal morphology can be judged and a peroxisomal fission defect can be excluded. In LCL derived from a healthy control individual, a patient with D-bifunctional protein deficiency and a patient with PBD, immunofluorescence staining against PEX14, a PMP, revealed a punctate staining pattern in all three cell lines (Fig. 1, upper row). Staining against catalase, a peroxisomal matrix enzyme, clearly highlighted the patient with PBD in which anti-catalase staining pattern is distributed evenly throughout the cytosol in contrast to punctate staining in control and DBPD LCL (Fig. 1, lower row). No clear difference of peroxisome size and number could be detected comparing control LCL with LCL from the two patients.

Complementation of Peroxisome Biogenesis in a PBD Cell Line

Complementation analysis is suitable to determine which of the 12 known *PEX* genes accounts for ZSS and should be analyzed by direct sequencing (Krause et al. 2009). Figure 2 exemplifies complementation of peroxisomal protein import in an LCL derived from a patient with PBD: Cell line PBD1 was transfected with an expression construct for mycPECI (Geisbrecht et al. 1999), which is a peroxisomal matrix enzyme and serves as reporter for intact peroxisome biogenesis. Cotransfection of mycPECI with empty cDNA expression vector results in a cytosolic distribution of the protein within the cell (a); while cotransfection with *PEX6* cDNA expression vector rescued the import defect, mycPECI is localized in punctate structures colocalizing with PEX14 (b), indicating complementation of the defective gene. Panels (c) and (d) show LCL from a control individual (d) and a patient with D-bifunctional protein deficiency (c) after transfection with mycPECI expression construct. Immunofluorescence analysis reveals colocalization of mycPECI and PEX14 as shown by the yellow color in the merged picture (right column).

Measurement of Peroxisomal Metabolites in LCL

Table 1 summarizes the concentrations of VLCFA (C22:0, C24:0, C26:0, C26:0/C22:0, C24:0/C22:0), phytanic acid, and plasmalogens in LCL of controls and patients with peroxisomal disorders. In 19 or 16 control individuals

Fig. 1 Immunofluorescence staining of peroxisomal markers. LCL from a control person (*left*), a patient with D-bifunctional protein deficiency (DBPD, *middle*), and a patient with PBD (PBD1, *right*) were stained against the peroxisomal membrane protein PEX14 (*top*) and the peroxisomal matrix protein catalase (*bottom*) individually, using indirect immunofluorescence staining. PEX14 staining results in a punctate, peroxisomal staining pattern in all three cell lines, whereas catalase staining reveals a cytoplasmic distribution of the protein in the cell line with deficiency of peroxisome biogenesis only

Fig. 2 Complementation of the peroxisome biogenesis defect. LCLs were transfected with a myc-tagged reporter construct for peroxisomal matrix protein import, mycPECI ($\Delta 3$, $\Delta 2$ -enoyl-CoA-isomerase) (a–d) in addition to a vector expressing human PEX6 cDNA (b) or empty vector (a). Immunofluorescence staining visualizes the distribution of mycPECI in green (*left column*) and the peroxisomal membrane protein PEX14 in red (*middle column*). The *right column* shows the merged pictures to determine colocalization of the markers resulting in yellow color. Cells from a patient with PBD (PBD1) do not import mycPECI into peroxisomes resulting in a diffuse cytosolic distribution of the protein (a). After cotransfection of PBD1 cells with a vector expressing human PEX6 cDNA, mycPECI is imported into peroxisomes resulting in a punctate staining pattern of the protein and colocalization with PEX14 (b), confirming *PEX6* as the mutated gene in this cell line. Panels (c) and (d) show colocalization of peroxisomal matrix protein mycPECI and membrane protein PEX14 in LCLs from a patient with D-bifunctional protein deficiency and a control person

Table 1 Peroxisomal metabolites in lymphoblastoid cell lines

A						
	C22	C24	C26	Phytanic acid	C24/C22	C26/C22
Controls ($n=19$)	7.19 (2.0/4.23–11.18)	10.84 (3.63/5.1–19.17)	1.89 (0.94/0.51–3.94)	1.21 (0.37/0.75–2.2)	1.49 (0.18/1.21–1.85)	0.26 (0.11/0.11–0.47)
DBPD	7.17 (0.39)	11.08 (0.41)	1.68 (0.09)	1.29 (0.21)	1.55 (0.03)	0.23 (0.01)
PBD1	6.63 (0.53)	13.12 (0.48)	7.94 (0.52)	1.61 (0.16)	1.98 (0.1)	1.21 (0.15)
PBD2	3.90 (0.25)	8.19 (1.11)	5.45 (1.01)	0.85 (0.06)	2.09 (0.16)	1.39 (0.18)
B						
	C16 plasmalogens		C18 plasmalogens			
Controls ($n=16$)	7.99 (2.23/4.15–11.35)		4.25 (1.1/2.25–5.6)			
DBPD	6.38 (1.57)		3.0 (0.61)			
PBD1	1.17 (0.12)		0.87 (0.15)			
PBD2	1.88 (0.21)		1.13 (0.05)			

Concentrations of C22:0, C24:0, and C26:0 fatty acids and phytanic acid in LCLs of controls ($n=19$) and three patients with DBPD and PBD ($2\times$) (A). Values (nmol/ 1.67×10^7 cells) of controls are given as means with the standard deviation and the range of values in parentheses. Values of patients are given as means; the standard deviation is added in parentheses. Each control sample was measured in two to four replicates, selected control samples and patient PBD2 were measured in two to three independent biological samples, two replicates each.

C16 and C18 plasmalogen content in LCLs of controls ($n=16$) and three patients with DBPD and PBD ($2\times$), respectively (B). The values are presented as described for A and are expressed as the ratio of C16 or 18 plasmalogens to C16 or 18 fatty acids, respectively, multiplied by 100. The values for each control sample were determined in replicate; selected control samples and all patient samples were measured in two to three independent biological samples, two replicates each.

C24/C22 and C26/C22 ratios clearly reveal the peroxisomal β -oxidation defect of VLCFAs in LCLs with peroxisome biogenesis disorders (PBD1 and PBD2) but not in a patient with DBP deficiency. Remarkably, this patient had only discretely conspicuous peroxisomal parameters in plasma (see text; Grønborg et al. 2010). Whether patients with DBPD and clear plasma biochemical phenotype will show pathological C24/C22 and C26/C22 ratios has yet to be shown. C16 and C18 plasmalogens are clearly reduced in patients with peroxisome biogenesis disorders (PBD1 and PBD2).

(for fatty acids or plasmalogens, respectively), these parameters were determined to define a normal range of values for the analyses in our laboratory (Table 1A, B). These can be contrasted to the results in LCL from one patient with DBPD and two patients with PBD (PBD1, PBD2). C22:0 values from patients DBPD and PBD1 lie well in the range of values for controls (4.23–11.18 nmol/ml). PBD2 has a slightly decreased value compared to controls (3.9 nmol/l). All three patients' cell lines have C24:0 values in the range of controls (5.1–19.17 nmol/l). C26:0 values from both patients with PBD (7.94 and 5.45 nmol/l, respectively) are outside the normal range (0.51–3.94 nmol/ml). The same is true for the values from C24/C22 and C26/C22 for PBD1 and PBD2. The deviation is more pronounced for C26/22 where the normal range of values is 0.11–0.47, while the PBD patients' values are 1.21 and 1.39, respectively. The DBPD patient does not have values outside the range determined for control individuals for any of the VLCFA analyses. It is important to note that the DBPD patient also had a mild clinical and plasma biochemical phenotype that led to diagnostic difficulties (Grønborg et al. 2010).

Phytanic acid measurement did not show abnormal results in both PBD patients. As phytanic acid is not an endogenous metabolite, its concentration in cells will depend on the phytanic acid content of the growth media. In fibroblasts systematically challenged with phytanic acid in the growth media, cells from PBD patients could be

distinguished from control cells by indirectly measuring phytanic acid metabolism (Skjeldal et al. 1986).

C16 and C18 plasmalogen values from both PBD patients lie clearly outside the normal range, while the DBPD patient shows no deviation from the normal values, as expected (Table 1B).

Discussion

According to a recent diagnostic flowchart for the diagnosis of PBD, the evaluation of cultured cells becomes necessary when neither of the two most common mutations in the most frequently affected gene for PBD, namely G843D and c.2097-2098insT in *PEX1*, can be detected in the first diagnostic step (Krause et al. 2009). This applies only for those patients who present with typical biochemical changes in plasma (i.e., elevated VLCFA and decreased plasmalogens), otherwise immunofluorescence analysis in a fibroblast cell culture is required before the diagnostic flowchart is entered. Likewise, Steinberg et al. (2004) include patients into their diagnostic process only if the biochemical changes are clearly indicative of PBD, or if catalase immunofluorescence staining proved PBD and excluded a group II peroxisomal disease. Skin biopsy to obtain fibroblast cell culture might, although generally well

accepted, be declined by parents because of its invasiveness or their beliefs as in one of our cases (Grønberg et al. 2010). In these cases and also in general, LCLs have the advantage to be easily obtained by transformation of blood lymphocytes sparing the patient a skin biopsy. Moreover, LCLs are immortal in contrast to primary fibroblast cell lines. A possible limitation is the required blood volume of 5 ml, especially in critically sick newborns and infants. LCL should thus represent an excellent alternative to fibroblasts in the diagnostic procedure for PBD that has so far not been studied in detail. We therefore evaluated the use of LCL in the diagnostic procedure for PBD.

Immunofluorescence staining clearly distinguished PBD patient LCL from LCL derived from a control individual and a patient with DBPD (Fig. 1), showing that clear-cut identification of PBD cells required for the initialization of different diagnostic procedures is possible in LCL with standard immunofluorescence staining. Previously, immunostaining of a peroxisomal matrix and membrane protein has been shown in LCL from one PBD patient and control (Santos et al. 1993). In addition to distinguishing between peroxisomal diseases of groups I and II, the quality and resolution of the immunostaining here should allow to demonstrate an altered peroxisomal morphology as present in patients with defects of peroxisomes and mitochondria caused by *DLPI* mutations (Waterham et al. 2007). As a screening tool, immunofluorescence analysis of peroxisomes in LCLs could be used to examine collections of LCL from patients with unclear dysmorphic and/or neurodegenerative syndromes with the potential to reveal further patients with abnormal peroxisomal morphology. Successful complementation analysis in LCL, exemplified for PBD1 and the complementation of the cellular phenotype with PEX6 cDNA (Fig. 2), makes LCLs a well-suited alternative starting material for the complete diagnostic program for PBD aiming at the identification of the genetic defect.

Plasma peroxisomal parameters can be normal in spite of the presence of peroxisomal disease, more frequently so in group II than in group I disorders (Ferdinandusse et al. 2006; Rosewich et al. 2006; Soorani-Lunsing et al. 2005; Wanders and Waterham 2006). Measurements of peroxisomal metabolites in cultivated cells, namely fibroblasts, have in some of these cases helped to elucidate the diagnosis of peroxisomal disease. Biochemical analysis of LCL in this report established that the profile of peroxisomal metabolites of PBD LCL can be diagnostic. C26:0, C24:0/C22:0, C26:0/C22:0, as well as C16 and C18 plasmalogens offered a clear diagnostic discrimination between values of controls and PBD patients 1 and 2 (Table 1). Whether LCL analysis of patients with ambiguous plasma values could lead to diagnosis has yet to be established. This was not the case for the DBPD patient in this study in whom plasma peroxisomal parameters had been discretely conspicuous

only after repeated measurements (Grønberg et al. 2010). All values determined in LCL from this patient were within the normal range of values from control samples (Table 1). Further group II patients have to be studied to prove the reliability of peroxisomal metabolite measurements in LCL also for these patients. Ferdinandusse and colleagues describe DBPD patients with normal plasma C26:0 levels and C26/C22 ratio; however, in these cases elevated C26:0 levels were found in fibroblasts (Ferdinandusse et al. 2006).

Earlier studies of peroxisomal metabolites in cultivated cells from PBD patients included various reports in fibroblasts (Dacremont et al. 1995; Molzer 1993; Santos et al. 1993; Schutgens et al. 1993) and one report in LCL (Santos et al. 1993). For XALD patients, additional cell types have been analyzed, including blood leukocytes (Molzer 1993; Schutgens et al. 1993; Unterberger et al. 2007) and LCL in one report (Uto et al. 2008). Santos and colleagues observed C26/C22 levels in a control LCL (0.31) that were well comparable to the mean value of our study (0.26) (Santos et al. 1993). Increase of C26/C22 in the PBD LCL in their study (0.65) was less pronounced than for our patients (1.21/1.39), although the authors noted a generally higher amount of C26:0 accumulation in lymphoblast membranes than in fibroblasts. Indeed, C26/C22 values in control fibroblasts are approximately tenfold lower than in LCL in our and Santos' study (Santos et al. 1993) in some reports (Schutgens et al. 1993; Valianpour et al. 2003). Other analyses in fibroblasts have C26/C22 levels in fibroblasts comparable to our findings in LCL (Dacremont et al. 1995; Santos et al. 1993). Differences between these cell types might represent differences in membrane composition and/or culture conditions. Nevertheless, the deviations between studies emphasize the impact of different protocols for extraction and analysis of fatty acids.

In summary, study of LCL represents a reliable cellular system for the diagnostic process that is mandatory to establish the diagnosis of PBD. All diagnostic steps (immunofluorescence visualization of peroxisomes and peroxisomal enzymes, biochemical analysis of peroxisomal parameters, complementation analyses) that might be required to reach a definite, genetic diagnosis in this heterogeneous disease group can be performed in LCL and make these cells equivalent to fibroblasts in the diagnostic process. Thus, LCLs are a useful alternative to cultured skin fibroblasts, especially in cases where these cells are not available.

Acknowledgments We thank M. Schniewind and J. Kaiser for expert technical assistance, S. Thoms for help with microscopy, and R. Steinfeld, M. Henneke, and C. Brendel for sharing control cell lines. We thank C. Krause and A. Ohlenbusch for mutation analyses, D. Kube for access to the Nucleofector apparatus, and L. Florin (Bonn) for establishing LCL. This work has been supported by the Deutsche Forschungsgemeinschaft (Ga354/7-1) and the Bundesministerium für

Bildung und Forschung (BMBF) through the German Leukodystrophy Network.

Synopsis

Lymphoblastoid cell lines are on par with skin fibroblast cell lines for the diagnosis of peroxisome biogenesis disorders requiring immunofluorescence staining of peroxisomal matrix proteins, measurements of peroxisomal metabolites, and complementation analysis.

Details of Contributions of Individual Authors

Planning of the study: S.G., R.K., J.G.

Conduct of the study: S.G., R.K., H.R.

Reporting of work: S.G., R.K., J.G.

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Competing Interest

All authors declare that the answers to all questions on the JIMD competing interest form are No, and therefore they have nothing to declare.

Details of Funding

This work has been supported by the Deutsche Forschungsgemeinschaft (Ga354/7-1) and the Bundesministerium für Bildung und Forschung (BMBF) through the German Leukodystrophy Network. The authors confirm independence from the sponsors; the content of this chapter has not been influenced by the sponsors.

Details of Ethics Approval

Ethics approval was not required for this study.

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