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CLINICAL UTILITY GENE CARD

Clinical utility gene card for: Zellweger syndrome spectrum

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1. DISEASE CHARACTERISTICS

1.1 Name of the disease (synonyms)

Zellweger syndrome spectrum (ZSS).

1.2 OMIM# of the disease

214100.

1.3 Name of the analysed genes or DNA/chromosome segments

PEX1 (7q21-q22), PEX2 (8q21.1), PEX3 (6q24.2), PEX5 (12p13.31), PEX6 (6p21.1), PEX10 (1p36.32), PEX11β (1q21), PEX12 (17q21.1), PEX13 (2p15), PEX14 (1p36.2), PEX16 (11p12-p11.2), PEX19 (1q22), PEX26 (22q11.21).

1.4 OMIM# of the gene(s)

*602136 (PEX1), *170993 (PEX2), *603164 (PEX3), *600414 (PEX5), *601498 (PEX6), *602859 (PEX10), *603867 (PEX11β), *601758 (PEX12), *601789 (PEX13), *601791 (PEX14), *603360 (PEX16), *600279 (PEX19),*608666 (PEX26).

1.5 Mutational spectrum

The Zellweger syndrome spectrum (ZSS) comprises three overlapping clinical phenotypes defined before the identification of their common biochemical and genetic causes. First described by Bowen and Zellweger in 1964[1] the ZS, or cerebrohepatorenal syndrome, marks the severe end of the disease spectrum with severe neurologic impairments (muscular hypotonia, failure to thrive and seizures), typical dysmorphic features (large fontanel, wide sutures, high forehead, hypertelorism, broad nasal bridge and external ear deformity) and inner organ impairment including hepatomegaly with elevated serum concentrations of liver enzymes and renal cysts. Neonatal adrenoleukodystrophy (NALD) shows a similar, but less severe clinical phenotype and infantile Refsum disease (IRD) depicts the mild end of this clinical continuum. A growing number of patients with even milder or isolated phenotypes different from these classical phenotypes are reported. These are patients with a prolonged survival,[2,3] six patients with an unusual mild variant with progressive spastic paraparesis and ataxia due to five different *PEX16* mutations,[4] patients with autosomal-recessive ataxia as the only neurological symptom due to mutations in the *PEX2* and *PEX10* gene,[5,6] patients with only sensory deficits without intellectual disability at diagnosis in adulthood,[7,8] as well as the first patient identified with a mutation in the *PEX11beta* gene. This patient presented with congenital cataracts, mild intellectual disability, progressive hearing loss, sensory nerve involvement, gastrointestinal problems, as well as recurrent migraine-like episodes.[9] All peroxisome biogenesis disorders leading to ZSS are inherited in a autosomal-recessive manner and can be caused by mutations in any of the 13 human *PEX* genes mentioned above (see 1.3).[10] *PEX* genes encode peroxins. These are proteins required for proper peroxisomal assembly including protein targeting and protein import.

The standard reference sequence indicating reported variants (ENSG00000127980, ENSG00000164751, ENSG00000034693, ENSG00000139197, ENSG00000124587, ENSG00000157911, ENSG00000131779, ENSG00000108733, ENSG00000162928, ENSG00000142655, ENSG00000121680, ENSG00000162735, ENSG00000215193) can be found at http://www.ensemble.org.

Gene variants in all 13 known human PEX genes can be obtained in the following public gene variant databases: http://www.dbpex.org; http://www.hgmd.cf.ac.uk/ac/index.php; http://www.ncbi.nlm.nih.gov/snp/.

1.6 Analytical methods

The clinical diagnosis is based on the presence of clinical signs and symptoms mentioned above (see 1.5). Patients suspected of ZSS are first screened by measurement of the concentration of very long chain fatty acids (VLCFAs), phytanic and pristanic acid in serum/plasma and of plasmalogens in erythrocytes. If the concentration of VLCFAs, phytanic and pristanic acid (depending on the diet) are elevated and concentrations of plasmalogens are decreased the diagnosis of a ZSS is established biochemically.[11] If concentrations of VLCFAs are within the normal range and biosynthesis of plasmalogens in erythrocytes is also within the normal range or is only slightly decreased, but the clinical presentation strongly points to a PBD, we recommend a skin biopsy to culture primary human fibroblasts (PHFs). Measurement of the concentration of VLCFAs, as well as biosynthesis of plasmalogens, should be repeated in these PHFs. In addition, immunohistochemistry with anti-catalase antibody can be performed to determine if peroxisome biogenesis is impaired. Because mutations in any of the 13 currently known human *PEX* genes can be the primary genetic cause for ZSS, three different methods are presently used to identify causative gene defects as the next step:

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1. The PEX gene screen reported in the study by Steinberg *et al*[12] in which the most frequently affected exons of the different *PEX* genes are analysed in a seven-step algorithm.
2. The rational diagnostic strategy for ZSS patients reported by Krause *et al.*[13] in which the two most common mutations in the most frequently affected *PEX* gene (*PEX1*) are analysed first, followed, when negative, by *PEX* cDNA transfection complementation assays of patient´s cells and mutation analysis of the *PEX* gene thus identified, or a variation thereof including consecutive PEX cDNA complementation testing.[14]
3. Next generation panel sequencing of all *PEX* genes mentioned above with confirmation of the identified mutations by Sanger sequencing.

1.7 Analytical validation

Measurement of peroxisomal metabolites (VLCFAs, pristanic acid, phytanic acid and plasmalogens in erythrocytes) is complex and their interpretation in aggregate is challenging and requires expert knowledge. Selection of an expertise laboratory for this analysis is crucial. For mutation analysis Sanger sequencing of both DNA strands of the respective exons with flanking intronic regions of the different *PEX* genes is currently the gold standard. Mutations should be found in *PEX* genes demonstrated to be defective by genetic complementation assays when these have been performed. Missense mutations should be in evolutionary conserved regions and/or should be predicted by prediction software to be at least probably pathogenic. For splice-site mutations, the consequence on mRNA and protein should be analysed. We recommend confirming the segregation of mutations in the parents.

1.8 Estimated frequency of the disease (incidence at birth ('birth prevalence') or population prevalence.

(Incidence at birth ('birth prevalence') or population prevalence. If known to be variable between ethnic groups, please report

Despite a worldwide occurrence of ZSS, the prevalence can be quite variable among populations. Even though the general prevalence of ZSS is estimated at 1:50.000[15] the main diagnostic centre for peroxisomal diseases in Japan reported only 31 Japanese individuals over a 20-year period with an estimated birth prevalence of 1:500.000.[16,17]

1.9 Diagnostic setting:

	Yes	No
A. (Differential) diagnostics	☒	☐
B. Predictive testing	☒	☐
C. Risk assessment in relatives	☒	☐
D. Prenatal	☒	☐

Comment:

The main diagnostic signs of ZSS are severe muscular hypotonia, dysmorphic features mentioned above (see 1.5) and hepatomegaly with hepatic dysfunction. Patients with a phenotype similar to ZS with elevated levels of VLCFAs, pristanic and phytanic acid, but normal concentrations of plasmalogens, might suffer from D-bifunctional enzyme deficiency (DBP, *HSD17B4*),[18] a single peroxisomal β-oxidation defect. Patients with a slightly milder phenotype (NALD/IRD) might have clinical signs less characteristic and can be misdiagnosed with other conditions, resulting in severe muscular hypotonia (congenital myopathies, spinal muscular atrophy, congenital myotonic dystrophy type 1, chromosomal abnormalities, Down- and Prader–Willi syndrome and acyl-CoA oxidase deficiency (single peroxisomal β-oxidation defect)[19]). Patients with a mild phenotype might come to attention later in life with sensorineural hearing loss, retinal abnormalities, developmental delay with muscular hypotonia and liver dysfunction or even isolated ataxia. These patients can be assumed to suffer from other disorders with sensorineural hearing loss and retinal abnormalities like Leber congenital amaurosis, congenital infections and Cockayne syndrome. Prenatal diagnostic can be performed on samples from chorionic villus sampling or amniocentesis.

2. TEST CHARACTERISTICS

	Genotype or disease		A: True positives B: False positives	C: False negative D: True negative
	Present	Absent		
Test				
Positive	A	B	Sensitivity:	A/(A+C)
			Specificity:	D/(D+B)
Negative	C	D	Positive predictive value:	A/(A+B)
			Negative predictive value:	D/(C+D)

2.1 Analytical sensitivity
(proportion of positive tests if the genotype is present)

The sensitivity for Sanger sequencing of genomic DNA is >95% for mutation detection when all exons and flanking intronic regions of *PEX* genes are analysed. Deletions due to cryptic splice-site activation may be missed. Larger deletions and insertions spanning more than one exon can be missed. Mutations in gene expression influencing regions (promoters, enhancers and so on) are not detected and errors can be made because of polymorphisms causing allele dropout.

2.2 Analytical specificiy
(proportion of negative tests if the genotype is not present)

>95%.

2.3 Clinical sensitivity
(proportion of positive tests if the disease is present)

The clinical sensitivity can be dependent on variable factors such as age or family history. In such cases a general statement should be given, even if quantification can only be made case by case.

Close to 100%.

2.4 Clinical specificity
(proportion of negative tests if the disease is not present)

The clinical specificity can be dependent on variable factors such as age or family history. In such cases a general statement should be given, even if quantification can only be made case by case.

Close to 100%.

2.5 Positive clinical predictive value
(life time risk to develop the disease if the test is positive).

Close to 100%.

2.6 Negative clinical predictive value
(Probability not to develop the disease if the test is negative)
Assume an increased risk based on family history for a non-affected person. Allelic and locus heterogeneity may need to be considered.
Index case in that family had been tested:
Close to 100%.
Index case in that family had not been tested:
Close to 100%.

3. CLINICAL UTILITY

3.1 (Differential) diagnostics: The tested person is clinically affected
(To be answered if in 1.9 'A' was marked)

3.1.1 Can a diagnosis be made other than through a genetic test?

No	☐ (continue with 3.1.4)
Yes	☒
	Clinically ☐
	Imaging ☐
	Endoscopy ☐
	Biochemistry ☒
	Electrophysiology ☐
	Other (please describe)

3.1.2 Describe the burden of alternative diagnostic methods to the patient
There is no burden for the patient using alternative diagnostic methods. Blood samples are needed for both biochemical analyses and genetic testing. Nevertheless, if the diagnosis is based on the biochemical parameters mentioned in section 1.7 special problems and pitfalls have to be considered: (1) The finding of a suggestive but unproven biochemical profile (eg, elevated concentrations of VLCFAs, elevated concentrations of pristanic and phytanic acid but normal levels of plasmalogens) should lead to further evaluation and may require repetitive blood sampling. (2) There are pitfalls in the metabolic screening, because patients with proven *PEX* gene mutation but normal or near normal concentrations of VLCFAs have been reported. (3) The degree of peroxisomal metabolite alterations does not correlate with the severity of the disease (no chemotype–phenotype correlation), although the knowledge of the mutation (type and coding effect) in most cases allows estimation of the clinical phenotype (existing genotype–phenotype correlation). (4) Mutation analysis is the only possibility for carrier testing, because heterozygous carriers almost always display normal peroxisomal metabolites. (5) For prenatal diagnosis biochemical and immunohistochemical testing options in cultured chorionic villous samples or cultured amniocytes do exist and are used routinely. The results can be confirmed by mutation analyses in the affected *PEX* gene, if the mutation of an index patient and family history are known.

3.1.3 How is the cost effectiveness of alternative diagnostic methods to be judged?
Measurement of concentrations of peroxisomal metabolites is very cost-effective. The genetic heterogeneity of ZSS was a burden for genetic testing, but the algorithms mentioned in section 1.6 as a combination of cell and molecular biology methods have made genetic testing a cost-effective approach. Furthermore, diagnostic panel sequencing of all *PEX* genes might revolutionise PBDs and also other characteristic clinical phenotypes with diverse primary genetic defects (see 1.3).

3.1.4 Will disease management be influenced by the result of a genetic test?

No	☐	
Yes	☒	
	Therapy (please describe)	Treatment of peroxisome biogenesis disorders (Zellweger syndrome spectrum) is only symptomatic and will not depend on the test result.
	Prognosis (please describe)	There is a limited genotype–phenotype correlation for patients with mutations in the *PEX1* gene that allows the prediction of the clinical course of the patient to a certain extend. *PEX1* mutations can be divided into two classes: class I mutations lead to residual PEX1 protein level and function and a milder phenotype; class II mutations almost abolish PEX1 protein level and function, resulting in a severe phenotype. Compound heterozygote patients for a class I and class II mutation display an intermediate phenotype.[20]
	Management (please describe)	Peroxisome biogenesis disorders are multi-organ diseases, and therefore the involvement of a multidisciplinary team is essential for optimal management of these patients.

3.2 Predictive setting: The tested person is clinically unaffected but carries an increased risk based on family history
(To be answered if in 1.9 'B' was marked)

3.2.1 Will the result of a genetic test influence lifestyle and prevention?
If the test result is positive (please describe) Might be of interest in consanguineous relationships. Definitive biochemical and molecular diagnosis of ZSS in a patient can establish future reproductive risks for the parents.

If the test result is negative (please describe) Might be of interest in consanguineous relationships. If there is an index patient in the family a negative test can inform about the potential risk for the couple in a genetic counselling session.

3.2.2 Which options in view of lifestyle and prevention does a person at-risk have if no genetic test has been done (please describe)?
All relatives of ZSS patients are at-risk being a carrier of a *PEX* gene mutation. If no genetic test has been done the carrier status and the reproductive risk cannot be established. If a relative of a ZSS patient will be identified as a carrier the partner would require molecular testing to assess their reproductive risk.

3.3 Genetic risk assessment in family members of a diseased person
(To be answered if in 1.9 'C' was marked)

3.3.1 Does the result of a genetic test resolve the genetic situation in that family?
Genetic testing of family members in a family with an index patient (with a peroxisome biogenesis disorder) can resolve the genetic situation by carrier testing.

3.3.2 Can a genetic test in the index patient save genetic or other tests in family members?
No, if the index patient has two pathogenic *PEX* gene mutations, the patients' parents should be tested to confirm homo or heterozygosity.

3.3.3 Does a positive genetic test result in the index patient enable a predictive test in a family member?

A positive genetic test result in the index patient enables a predictive test in a family member.

3.4 Prenatal diagnosis

(To be answered if in 1.9 'D' was marked)

3.4.1 Does a positive genetic test result in the index patient enable a prenatal diagnosis?

A positive genetic test allows for accurate carrier testing and prenatal diagnosis through chorionic villus sampling (between the 10 and 11 week of gestation) or amniocenteses (between the 15 and 16 week of gestation)

4. IF APPLICABLE, FURTHER CONSEQUENCES OF TESTING

Please assume that the result of a genetic test has no immediate medical consequences. Is there any evidence that a genetic test is nevertheless useful for the patient or his/her relatives? (Please describe)

See 3.4.1: a positive genetic test result in an index patient should lead to carrier testing in the patients´ parents to confirm homo or heterozygosity. This allows for carrier testing and the possibility of accurate prenatal diagnosis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DISCLAIMER

The Laboratory Genetic Metabolic Diseases and the Department of Pediatrics and Adolescent Medicine offers biochemical and genetic diagnostic testing on a non-commercial basis for these defects (academic setting).

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