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CLINICAL UTILITY GENE CARDS

Clinical utility gene card for: Smith-Lemli-Opitz Syndrome [SLOS]

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1. DISEASE CHARACTERISTICS

1.1 Name of the disease (synonyms)

Smith-Lemli-Opitz Syndrome.
SLOS.
SLO Syndrome.
RSH Syndrome.
Rutledge Lethal Multiple Congenital Anomaly Syndrome.
Polydactyly, Sex Reversal, Renal Hypoplasia, and Unilobar Lung.
Lethal Acrodysgenital Syndrome.

1.2 OMIM# of the disease

270400.

1.3 Name of the analysed genes or DNA/chromosome segments

DHCR7.

1.4 OMIM# of the gene(s)

602858.

1.5 Mutational spectrum

The Smith-Lemli-Opitz syndrome, an autosomal recessive metabolic malformation/mental retardation syndrome is caused by mutations in the *DHCR7* gene, which encodes the 3β-hydroxysteroid-Δ7-reductase (DHCR7, E.C.1.3.1.21).[1] More than one-hundred SLOS causal mutations have been described so far (database in HGVS: http://lovd.i-med.ac.at/home.php, HGMD: www.hgmd.org/). The estimated frequency of the disease in European populations is 1:10 000–1:40 000.[2] The syndrome is characterized by facial dysmorphism, palatal clefting, 2,3-toe syndactyly, postaxial polydactyly, malformations of heart, kidney, genitalia, and lungs; occasional holoprosencephaly and other CNS malformations, as well as severe to profound mental retardation, and failure to thrive (Smith *et al.*[3]). Later in life, most patients have behavioural abnormalities such as aggressive and self-injurious behaviour and autism like characteristics.

Characterisation of the mutational spectrum of the *DHCR7* gene was possible after studying >100 SLOS patients (Witsch-Baumgartner M *et al*[4,5] Correa-Cerro *et al*[6]). Until now >100 different mutations: nonsense (eg, p.Trp151*), deletions (eg, c.720–735del and c.385–412IVS5 + 1–5del, HGVS: c.385_412 + 5del), splice site mutations (eg, c.964–1G > C) and missense mutations have been described. The most frequent class of mutations are missense mutations. Approximately half of them involve conserved amino acids. They are located in or near the transmembrane domains, in the fourth cytoplasmic loop or in the C terminal region of the DHCR7 protein.[4] More than 95% SLOS patients are homozygous or compound heterozygous for *DHCR7* point mutations.

A correlation of the genotype with the SLOS phenotype[7] demonstrates that patients carrying homozygous or compound heterozygous functional null *DHCR7* alleles have the most severe phenotypes. Missense mutations may be associated with residual activity and hence milder phenotypes. Unfortunately even with a degree of genotype–phenotype correlation, it is not possible to predict the phenotype by knowing the genotype and vice versa. Other factors appear to influence the phenotype including maternal apolipoprotein E genotype (Witsch-Baumgartner M *et al*[8]).

1.6 Analytical methods

Detection of homozygous/heterozygous mutations in the *DHCR7* gene by amplification of coding exon sequences (exons 3–9) with bordering intron sequences by PCR of genomic DNA and complete sequencing of these fragments.

Depending on ethnic background, it might be reasonable to start analysis specifically for frequent mutations for instance in British patients for the frequent mutation c.964–1G > C or in Polish patients for the p.Trp151* mutation. This might be done by mutation-specific PCR methods or by sequencing the corresponding exon.

Targeted sequence analysis referrals for carrier testing or prenatal diagnosis for familial mutations.

1.7 Analytical validation

Obtained PCR fragments have been analyzed in ~50 DNA samples by sequencing. All variants had been tested for frequency in 50–100 DNA samples of corresponding population background. If possible, in each case with homozygosity or compound heterozygosity for mutations they are tested in parental DNAs.

External quality assurance (EQA) should be carried out for DNA sequencing (URL: http://www.emqn.org/).

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

The reported birth prevalence varies depending on geographic region. In middle Europe, it is approx. 1:20 000–1:60 000 (Kelley and

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Hennekam 2000[9] and Ryan *et al.* 1998[10]). In the US, it is 1:60 000. In Eastern European population, prevalence might be as high as 1:16.000. In African or Asian populations, SLOS is nearly unknown. The spectrum of phenotype in Smith-Lemli-Opitz syndrome is wide and cases may be underdiagnosed. Clinical incidence does not appear to be as high as predicted from carrier frequency possibly because of underascertainment of mild cases, prenatal loss, or neonatal mortality before diagnosis.

1.9 If applicable, prevalence in the ethnic group of investigated person

Birth prevalence is significantly increased in populations of Eastern European origin: 1:16 000.

Birth prevalence is significantly low in African and Asian populations.

1.10 Diagnostic setting

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

Comment:
Predictive diagnostics are not offered because the disease already manifests at birth. *DHCR7* mutation analysis for risk assessment in possible carriers of the disease is important as biochemical carrier testing is challenging and specialized.

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)
Depending on the method used almost 100% for *DHCR7* mutations.

2.2 Analytical specificity
(proportion of negative tests if the genotype is not present)
Depending on the method used almost 100%.

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.

>95%.

SLOS patients have an increased serum concentration of 7-dehydrocholesterol (7-DHC) and 8-dehydrocholesterol (8-DHC), which is pathognomonic of the disease. If raised 7- and 8-DHC levels are present indicating SLOS, the clinical sensitivity is nearly 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.

>99.9%.

If 7- and 8-DHC levels determined by GC–MS analysis are within the normal range, the clinical specificity is nearly 100%. The plasma cholesterol concentration is decreased in most SLOS patients; however, ~10% of patients have normal cholesterol levels (Nowaczyk and Waye 2001[11], Kelley and Hennekam 2000[9]). Therefore routine measurement of cholesterol alone is not a suitable screening method.

2.5 Positive clinical predictive value
(life time risk to develop the disease if the test is positive)
Estimated >99% with homozygosity or compound heterozygosity for *DHCR7* mutations.

2.6 Negative clinical predictive value
(probability not to develop the disease if the test is negative)
Assume an increased risk on the basis of family history for a non-affected person. Allelic and locus heterogeneity may need to be considered.

Index case in that family had been tested:
>99% with homozygosity or compound heterozygosity for *DHCR7* mutations detected in the index case.
Index case in that family had not been tested:
>99%.

3. CLINICAL UTILITY

3.1 (Differential) diagnostics: The tested person is clinically affected
(To be answered if in 1.10 '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)	Yes, by quantification of 7- and 8-DHC in addition to cholesterol by GC–MS

3.1.2 Describe the burden of alternative diagnostic methods to the patient

Mildly affected patients may have 7-DHC concentrations in the upper normal range and only marginally elevated 8-DHC concentrations (Langius *et al.* 2003[12]). Very rarely, even patients with a classical phenotype have borderline sterol concentrations.[13] Therefore genetic testing should be done in all patients with elevated concentrations of 7- or 8-DHC, even if the elevation is only marginal.

3.1.3 How is the cost effectiveness of alternative diagnostic methods to be judged?

Genetic testing is significantly more expensive than GC–MS analysis. Therefore determination of sterols by GC–MS should be used as the primary screening method. In any case, genetic testing should be used whenever the diagnosis is problematic. In future in view of the decreasing cost of DNA analysis and of the more powerful diagnostic capacity of mutation analysis in SLOS genetic testing will be favored.

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

No	☐	
Yes	☒	
	Therapy	Dietary cholesterol supplementation results in improved growth and behavior in most patients. Photosensitivity and polyneuropathy improve significantly (Azurdia *et al*,[14] Starck *et al.* 1999[15]). Unfortunately, there is no effect of cholesterol supplementation on intrinsic cognitive abilities. Mildly affected patients may benefit from treatment with statins, whereas severely affected patients are prone to serious side effects. However, the differentiation between severely and mildly affected patients can be reliably determined by biochemical analysis (ratio of (7 + 8 −DHC)/cholesterol at time of diagnosis).[16]
	Prognosis	Life expectancy in SLOS is primarily determined by the degree of prenatally acquired internal malformations, which are most severe in patients with homozygosity or compound heterozygosity for two functional null mutations. Those patients usually die in the neonatal period. The individual course in patients with other mutations can be better related to the published experience and differentiated from other intellectual disabilities.
	Management	The result of genetic tests will influence the counseling of parents. If two functional null mutations are present, invasive interventions such as surgical correction of severe heart defects or liver transplantation in severe hepatopathy will not improve overall survival.

3.2 Predictive setting: The tested person is clinically unaffected but carries an increased risk on the basis of family history

(To be answered if in 1.10 '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)

If the test result is negative (please describe)

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)?

Not applicable.

3.3 Genetic risk assessment in family members of a diseased person

(To be answered if in 1.10 'C' was marked)

3.3.1 Does the result of a genetic test resolve the genetic situation in that family?

Yes, it confirms the genetic transmission and is prerequisite for genetic counseling of family members.

3.3.2 Can a genetic test in the index patient save genetic or other tests in family members?

No more tests are required in the patient to secure his diagnosis of SLOS unless sterol levels are equivocal. However, except for the parents, the risk of relatives is uncertain without individual genetic tests.

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

Yes (in most cases, however, a predictive test is only performed for diagnosing or excluding heterozygosity or prenatal analysis).

3.4 Prenatal diagnosis

(To be answered if in 1.10 'D' was marked)

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

Yes.

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).

Genetic diagnosis renders continuous diagnostics (clinical and others) unnecessary and confirms the mode of inheritance in a clinically and genetically heterogeneous group of intellectual disability with malformations and growth retardation. Heterozygote tests in relatives, prognostic statements in patients and prenatal diagnosis in pregnancies at risk become possible as a consequence.[17,18]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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