Clinical utility gene card for: Holoprosencephaly.

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Disease characteristics

Name of the Disease (Synonyms)

Holoprosencephaly (HPE)

A mild subtype of HPE is called Middle InterhemisphericVariant (MIHF) or syntelencephaly.

OMIM# of the Disease

236100

Name of the Analysed Genes or DNA/Chromosome Segments

Major genes : SHH, 7q36 (HPE3); ZIC2, 13q32 (HPE5); SIX3, 2p21 (HPE2); TGIF, 18p11.3 (HPE4)

Minor genes : GLI2, 2q14 (HPE9); PATCHED-1, 9q22 (HPE7); DISP1, 1q42; FOXH1, 8q24.3; NODAL, 10q22.1 and others

OMIM# of the Gene(s)

SHH #600725; ZIC2 #603073; SIX3 #603714; TGIF #602630

GLI2 #165230; PATCHED-1 #601309; DISP1 #607502; FOXH1 #603621; NODAL #601265

Mutational Spectrum

The following percentages are referred to patients with non-chromosomic, non-syndromic HPE.

- Point mutations and microrearrangements in the four main genes in ~27% of isolated HPE cases (SHH ~12%, ZIC2 ~9%, SIX3 ~5%, TGIF ~1%)

- Alterations in minor genes <1%. These minor genes with low mutation frequency rates are tested only in selected cases: for example, GLI2 is tested when specific abnormalities occur in the development of the pituitary gland, in the context of variable brain and craniofacial anomalies consistent with the broad spectrum of HPE (Pineda-Alvarez et al 2010).

Analytical Methods

- Search for point mutations:

D-HPLC (Denaturing High Performance Liquid Chromatography) or HRM (High Resolution DNA Melting) with confirmation by sequencing, or direct bi-directional sequencing

- Search for microrearrangements:

MLPA (Multiplex Ligation-dependent Probe Amplification) with SALSA Kit P187 Holoprosencephaly (MRC-Holland, Amsterdam, Netherlands) or FISH (Fluorescence In Situ Hybridization)

Analytical Validation

Parallel analysis of positive and negative controls, depending on analytical method

Estimated Frequency of the Disease

(Incidence at birth (“birth prevalence”) or population prevalence):

1:10–16000 live births; 1:250 conceptuses

If applicable, prevalence in the ethnic group of investigated person

Ethnic variations in birth prevalence rates could occur in HPE, but the available data are not convincing. Higher rates were generally observed among less favored minorities probably because of a lower prenatal detection rate of HPE, and consequently less terminations of pregnancy (Orioli and Castilla, 2010).

Diagnostic Setting

	Yes.	No.
A. (Differential) diagnosis	□	□
B. Predictive Testing	□	□
C. Risk assessment in Relatives	□	□
D. Prenatal	□	□

Comment: Prenatal diagnosis is based primarily on fetal imaging, but “molecular” prenatal diagnosis can be performed if a mutation or a microrearrangement has been previously identified in a proband. Interpretations of molecular diagnosis must be given with caution, given the lack of strict genotype-phenotype correlation, and should be offered in addition to fetal imaging, using ultrasound followed by fetal RMI.

Test characteristics

		genotype or disease	
		present	**absent**
test	pos.	A	B
	neg.	C	D

A: true positives, B: false positives, C: false negatives, D: true negatives
sensitivity : A/(A+C), specificity : D/(D+B), pos. predict. value : A/(A+B), neg. predict. value : D/(C+D)

Analytical Sensitivity

(proportion of positive tests if the genotype is present)

D-HPLC and HRM : >95% for heterozygous variants

Bi-directional sequencing : close to 100%

MLPA : not yet validated

Analytical Specificity

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

D-HPLC and HRM : >95% for heterozygous variants

Bi-directional sequencing : close to 100%

MLPA : not yet validated

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 a quantification can only be made case by case.

By testing the four main genes, mutations are identified in ~27% of isolated HPE cases.

- Mutations in SHH are identified in ~12% of propositi (10–30% de novo). The presence of structural brain anomalies in patients with SHH mutations is estimated to be ~45%, while the penetrance of any manfestations (including microform HPE) is estimated to be ~90%.

- Mutations in ZIC2 are estimated to occur in up to 9% of propositi (72% de novo). Almost 90% of patients with mutations in ZIC2 have structural brain anomalies, and it is rare that a parent with a mutation will not show clear signs of cognitive impairment.

- Mutations in SIX3 are estimated to occur in up to 5% of propositi (only 14% de novo). About 65% of patients with mutations in SIX3 have structural brain anomalies, most likely alobar rather than semilobar HPE.

- Of the four genes commonly tested in clinical laboratories, mutations in TGIF are the least common, occurring in ~1% of propositi. The role that alterations in TGIF play are not well understood.

Statistical analysis of combined results showed that propositi with structural brain anomalies who have either alobar or semilobar HPE are more likely to have a ZIC2 mutation, whereas SHH mutations seem to be responsible for most microform HPE (Solomon et al 2010).

In a research context, further analysis include subtelomeres exploration using MLPA, and array CGH.

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 a quantification can only be made case by case.

Close to 100% for SHH, ZIC2 and SIX3 alterations, if the biological meaning of variation has been ascertained by robust functional analyses.

The role of TGIF is less clear.

Positive clinical predictive value

(life time risk to develop the disease if the test is positive).

Non-chromosomal, non-syndromic HPE is classically considered an autosomal dominant condition with incomplete penetrance and highly variable expressivity. The spectrum of the effects of a single mutation within a single kindred is very wide.

Recent data point to a complex pattern of inheritance combining multiple interacting genetic and environmental factors (Solomon et al 2010). Therefore, because of this multihit hypothesis, not all carriers of a single deleterious mutation manifest clinically detectable symptoms. The identified mutation may be not sufficient to generate HPE, another event like an alteration in another gene (not yet identified) or an environmental factor being necessary.

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% if the biological meaning of the identified family variation has been ascertained by robust functional analyses.

Index case in that family had not been tested:

Not resolved.

Clinical Utility

(Differential) diagnosis: The tested person is clinically affected

(To be answered if in 1.10 “A” was marked)

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):	□

Describe the burden of alternative diagnostic methods to the patient

Brain magnetic resonance (MRI) imaging is essential for diagnosing holoprosencephaly. Modern high resolution MRI allows detailed analysis of the cortical, white matter and deep gray structural anomalies in HPE and leds to better classification of types of HPE (Hahn and Barnes, 2010).

In prenatal, ultrasound can detect central nervous system and facail abnormalities of severe HPE as early as the first trimester, but is less sensitive for detection of milder forms of HPE, such as lobar HPE. Fetal MRI provide better characterization of brain malformations, but only later in the third trimester of pregnancy (Mercier et al 2010).

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

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

No.	□
Yes.	□
Therapy (please describe)	Depending on clinical symptoms : cerebrospinal fluid shunt for treatment of hydrocephalus; antiepileptic medication; physical therapy, bracing and orthopedic surgery for motor impairment, anticholinergic drugs for dystonia; gastrostomy for oromotor dysfunction; tracheostomy for treatment of upper airway obstruction due to facial anomalies; H2 blockers or proton-pump inhibitors for gastrointestinal problems; modifying of the environment for hypothalamic dysfunction; hormones (Levey et al 2010).
Prognosis (please describe)	Higher mortality correlates with several factors, including the severity of brain malformation, the severity of facial malformation, the presence of a multiple congenital anomaly syndrome, and the presence of chromosomal abnormalities. Survival is associated with the severity of brain malformation (short for alobar, intermediate for semilobar and the best for lobar HPE and mild forms)(Levey et al 2010).
Management (please describe)	The results of genetic tests will influence genetic counselling by permitting “prenatal” diagnosis.

Predictive Setting: The tested person is clinically unaffected but carries an increased risk based on family history

(To be answered if in 1.10 “B” was marked)

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)

Which options in view of lifestyle and prevention does a person at-risk have if no genetic test has been done (please describe)?

Genetic risk assessment in family members of a diseased person

(To be answered if in 1.10 “C” was marked)

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

No

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

No

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

No

Prenatal diagnosis

(To be answered if in 1.10 “D” was marked)

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

Yes, but “molecular” prenatal diagnosis should be offered in addition to fetal imaging which takes precedence for interpretation of the results.

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)

For the parents, the result conveys clarity about an eventual cause of the disease. In case of an identified alteration, heterozygote tests in relatives and prenatal diagnosis in pregnancies at risk can be offered as a consequence.

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