

CLINICAL UTILITY GENE CARD

Clinical utility gene card for: Alagille Syndrome (ALGS)

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1. DISEASE CHARACTERISTICS

1.1 Name of the disease (synonyms)

Alagille syndrome (ALGS); Alagille syndrome 1 (ALGS1); Alagille syndrome 2 (ALGS2). Other synonyms include Alagille–Watson Syndrome (AWS); cholestasis with peripheral pulmonary stenosis; arteriohepatic dysplasia (AHD); hepatic ductular hypoplasia, syndrome; Miller–Watson syndrome.^{1–4}

1.2 OMIM# of the disease

ALGS1: 118450; ALGS2: 610205.

1.3 Name of the analysed genes or DNA/chromosome segments

JAG1 (*Jagged1* gene; locus 20p12.2; disease ALGS1); *NOTCH2* (*Notch2* gene; locus 1p12-p11; disease ALGS2).

1.4 OMIM# of the gene(s)

JAG1 (601920); *NOTCH2* (600275).

1.5 Mutational spectrum

JAG1 mutations are identified in roughly 94% of patients with clinically diagnosed Alagille syndrome (ALGS) and *NOTCH2* mutations are identified in about 1.5% of patients with clinically diagnosed ALGS. The distribution of mutation types is shown below for *JAG1* (Figure 1) and *NOTCH2* (Figure 2).^{5,6,7}

1.6 Analytical methods

Genomic sequencing of *Jagged1* coding regions and *Jagged1* multiplex ligation-dependent probe amplification (MLPA) assays are utilized to detect mutations or deletions/duplications within the gene. Gene-sequencing panels including *Jagged1* have also recently become available. Clinical testing for *NOTCH2* is currently offered in Germany, Austria and England. For a full list of laboratories that offer *JAG1* or *NOTCH2* testing, please refer to GeneTests.⁸

1.7 Analytical validation

Possible mutations detected during initial bidirectional Sanger sequencing can be re-sequenced in normal, ethnically matched controls to exclude possible polymorphisms. Due to the variable expressivity of Alagille syndrome, it is not recommended that unaffected family members be used as controls. Family members carrying the mutation may present with mild or subclinical disease features.^{9,10}

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

In 1977, the incidence of ALGS was estimated to be 1 in 70 000 live births.¹¹ However, given that not all patients with ALGS present with neonatal cholestasis, as the original estimate assumes, this figure is thought to be an underestimate. Based on the work by Kamath *et al.*¹⁰ in 2003 we estimate that the incidence of ALGS is 1 in 30 000–50 000 live births, but due to the variable phenotype it is likely to remain underdiagnosed.

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

Not applicable.

1.10 Diagnostic setting

	Yes	No
A. (Differential) diagnostics	<input checked="" type="checkbox"/>	<input type="checkbox"/>
B. Predictive testing	<input checked="" type="checkbox"/>	<input type="checkbox"/>
C. Risk assessment in relatives	<input checked="" type="checkbox"/>	<input type="checkbox"/>
D. Prenatal	<input checked="" type="checkbox"/>	<input type="checkbox"/>

Comment:

Mutation testing for ALGS is used mainly to confirm a suspected clinical diagnosis or to carry out screening in the relatives of an affected individual. A list of laboratories that perform clinical and research testing of the ALGS genes can be found on the GeneTests website.⁸ Prenatal testing is available for families with known mutations.

2. TEST CHARACTERISTICS

Test	Genotype or disease		A: true positives	C: false negative
	Present	Absent	B: false positives	D: true negative
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)

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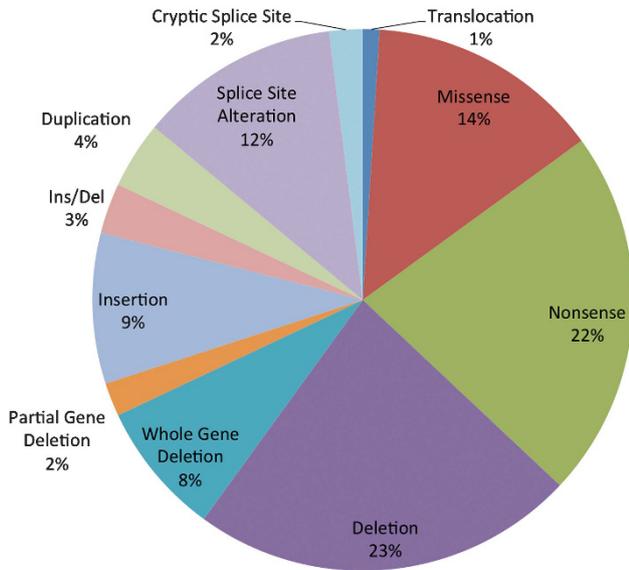


Figure 1 Prevalence of *JAG1* mutations by mutation type ($n=422$ unrelated probands).⁵

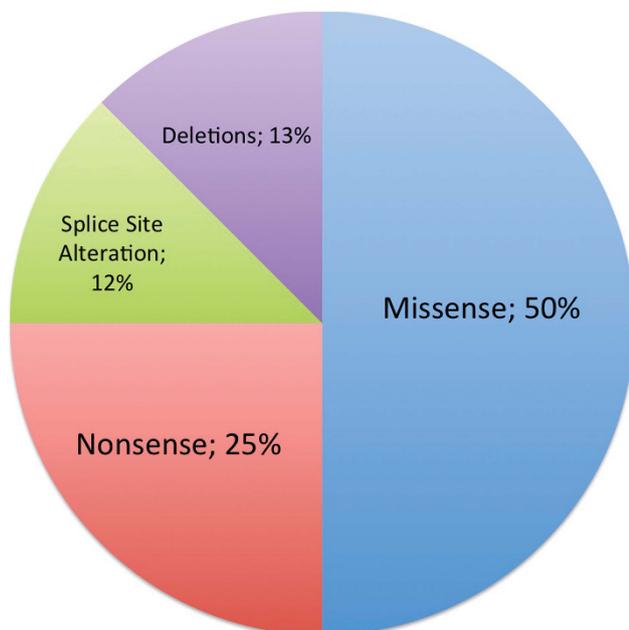


Figure 2 Prevalence of *NOTCH2* mutations by mutation type ($n=8$ unrelated probands).^{5,6}

2.1 Analytical sensitivity

(proportion of positive tests if the genotype is present)

The sensitivity for genomic sequencing approaches 100% for mutation detection. Deletions of exons or whole gene deletions are detected by MLPA and may be cross-checked with homozygous polymorphisms in deleted regions via Sanger sequencing.^{5,6,9–11} Additionally, mutations outside coding exons in promoters or enhancers are likely to be missed.⁷

**When screening parents for their child's known mutation, it is important to note that a negative result does not rule out the possibility of germline mosaicism.¹²

2.2 Analytical specificity

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

Analytical specificity is nearly 100%. False positives are rare. In some cases, missense mutations may be reported as disease-causing or likely disease-causing by protein prediction software but later shown to be benign polymorphisms based on functional protein assays.

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.

In patients clinically consistent with ALGS, *JAG1* mutations are identified in ~94%, *NOTCH2* mutations in ~1.5% and no mutation in ~4.5% of patients. Therefore, the clinical sensitivity is ~95% and is estimated to increase as we learn more about the cause of disease in the 4.5% of patients who are clinically consistent with ALGS but have no identifiable mutation in Jagged1 or Notch2.⁵

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.

Although clinical specificity cannot be predicted, it is not 100%. Due to highly variable expressivity, many individuals who do not meet the full diagnostic criteria for ALGS or have mild features of the disease may still carry mutations in Jagged1 or Notch2. Family members of affected individuals may carry the same genetic mutation but have mild clinical manifestations such as slightly elevated liver enzymes.^{9,10}

2.5 Positive clinical predictive value

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

ALGS is an autosomal dominant disorder and therefore a single mutation on one allele is sufficient to cause disease. Although non-penetrance is rare, ALGS has variable expressivity and severity of disease cannot be predicted based on the presence of a mutation. Both mild and severe ALGS have been described and there is no apparent link between mutation type or location and severity of disease.^{13, 14–16}

2.6 Negative clinical predictive value

(probability of not developing 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:

Nearly 100%.

Index case in that family had not been tested:

Genetic heterogeneity with undiscovered genes means that ~5% of individuals who test negative for both Jagged1 and Notch2 genes may still have the disease.¹⁴

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	<input type="checkbox"/> (continue with 3.1.4)
Yes	<input checked="" type="checkbox"/>
	Clinically <input checked="" type="checkbox"/>
	Imaging <input checked="" type="checkbox"/>
	Endoscopy <input type="checkbox"/>
	Biochemistry <input checked="" type="checkbox"/>
	Electrophysiology <input type="checkbox"/>
	Other (please describe): liver biopsy

The five primary diagnostic criteria are cholestatic liver disease (characterized by bile duct paucity), cardiac disease, skeletal abnormalities (butterfly vertebrae), eye abnormalities (posterior embryotoxon) and characteristic facial features. Secondary diagnostic criteria include renal and vascular abnormalities. The presence of three of the five primary diagnostic criteria is sufficient for diagnosis of ALGS.^{10,17–19}

3.1.2 Describe the burden of alternative diagnostic methods to the patient

The majority of clinical diagnostic tests (biochemical blood tests) are performed as a part of routine clinical care and therefore do not add additional risk or burden. A liver biopsy is not always necessary for the diagnosis of ALGS if initial evaluation of liver function tests is sufficient. Genetic evaluations of affected individuals and their parents have the largest impact if the family wishes to address the chances of future children being affected with Alagille syndrome.

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

Although an accurate clinical assessment can confirm the diagnosis of ALGS and therefore establish the need for appropriate monitoring and management, genetic testing remains useful for genetic counseling and prenatal testing.

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

No	<input type="checkbox"/>
Yes	<input checked="" type="checkbox"/>
Therapy (please describe)	
Prognosis (please describe)	Due to the phenotypic variability among patients with known JAG1 and NOTCH2 mutations, genetic testing does not determine prognosis.
Management (please describe)	A genetic diagnosis of ALGS will encourage the treating physician to refer the patient to other sub-specialities including ophthalmology, nephrology, gastroenterology and cardiology.

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

(To be answered if in 1.10 '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 a clinically unaffected person undergoes genetic testing and is found to have a disease causing mutation, he/she should be screened

for clinical features of the disease and then referred to the appropriate specialists.

If the test result is negative (please describe):

If a clinically unaffected person undergoes genetic testing and does not have a disease-causing mutation identified, the likelihood of developing the disease approaches 0% and therefore there will be no changes in lifestyle.

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)?

We recommend that individuals who are at risk (have a significant family history of ALGS) undergo genetic testing and follow the guidelines in section 3.2.1.

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.

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

No—if testing is positive for ALGS in the index patient, it can reduce the need for testing for other genetic conditions in family members by providing a diagnosis. However, testing for ALGS may still be suggested in clinically affected or unaffected family members.

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

Yes. Given the variable expressivity of ALGS, a positive genetic test result in the index patient provides a molecular diagnosis of ALGS that can inform the diagnosis of other mildly affected family members.^{9,10}

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. Prenatal diagnosis can be performed if the index patient has a positive genetic test result. Testing for the known familial mutation can be performed on fetal DNA.

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 tests can provide families knowledge about the genetics and inheritance pattern of ALGS. Genetic test results may also address questions about the risk of future children inheriting the disease.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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