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CLINICAL UTILITY GENE CARD

Clinical utility gene card for: Alveolar rhabdomyosarcoma

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1. DISEASE CHARACTERISTICS

1.1 Name of the disease (synonyms)

Alveolar rhabdomyosarcoma (ARMS).

1.2 OMIM# of the disease

268220.

1.3 Name of the analysed genes or DNA/chromosome segments

FORKHEAD BOX O1A, FOXO1A (formerly FORKHEAD IN *RHABDOMYOSARCOMA, FKHR*), gene locus: chromosome 13q14.1
PAIRED BOX GENE 3, *PAX3*, gene locus: chromosome 2q35
PAIRED BOX GENE 7, *PAX7*, gene locus: chromosome 1p36
ALL1-FUSED GENE FROM X CHROMOSOME, AFX1 (MYELOID/LYMPHOID OR MIXED LINEAGE LEUKAEMIA, TRANSLOCATED TO, 7, MLLT7; FORKHEAD BOX O4, FOXO4), gene locus: chromosome Xq13.1
NUCLEAR RECEPTOR COACTIVATOR 1, NCOA1, gene locus: chromosome 2p23.

1.4 OMIM# of the gene(s)

136533, 606597, 167410, 300033, 602691.

1.5 Mutational spectrum

Recurrent reciprocal translocations and insertions that form chimeric fusion genes.

- t(2;13)(q35;q14) that forms fusion gene *PAX3–FOXO1A*; identified in 75% of fusion-positive ARMS cases.[1,2]
- t(1;13)(p36;q14) that forms fusion gene *PAX7–FOXO1A*; identified in 25% of fusion-positive ARMS cases.[3,4]
- t(2;X)(q35;q13) that forms fusion gene *PAX3–AFX1*; identified in $<1\%$ of ARMS cases.[5]
- t(2;2)(q35;p23) that forms fusion gene *PAX3–NCOA1*; identified in $<1\%$ of ARMS cases.[6,7]

Tetraploidy is common in ARMS and occurs in 77% of ARMS demonstrated in one of the studies.[8]
PAX7–FOXO1A fusions are commonly amplified.[9]
Amplification of chromosome 2p24 region including *MYCN* gene occurs in 13% of cases of fusion-positive ARMS but has no significant association with clinical outcome.[10]
Amplification of chromosome 12q13-14 region including *CDK4* gene occurs in 12% of cases of fusion-positive ARMS (majority are *PAX3–FOXO1A* fusion) and is associated with worse outcome.[10]
TFAP2 β (6p24), *CDH3* (16q22.1) and *CNR1* (6q14-q15) are highly expressed in fusion-positive ARMS, irrespective of tumour histology.[11]
Approximately 20–30% of ARMS have no *PAX–FOXO1A* fusion (ie, fusion negative ARMS). Oligonucleotide microarrays have demonstrated that fusion-negative ARMS has a distinctive gene expression profile different from fusion-positive ARMS. Some gene expression studies show that fusion-negative ARMS constitutes a heterogeneous group that overlaps with embryonal rhabdomyosarcoma (ERMS), with frequent whole-chromosome copy number changes, notably gain of chromosome 8 with associated high levels of expression of genes from this chromosome.[11–13]

1.6 Analytical methods

Routine cytogenetic karyotyping on fresh, unfixed tissue.
Reverse transcriptase (RT)-PCR on fresh, frozen or formalin-fixed, paraffin-embedded tissues.
Fluorescence *in situ* hybridisation (FISH) on either cytologic touch preparations or formalin-fixed, paraffin-embedded tissue.
Microarray for gene or protein expression analysis is currently only used in research field, but may be used as a clinical test in the future.

1.7 Analytical validation

Although the subclassification of rhabdomyosarcoma has traditionally relied on histological analysis, cytogenetic and molecular genetic analytic methods are increasingly being used as standard confirmatory tests. All testing should be validated based on histological criteria, but future treatment protocols may rely on fusion status rather than histology.

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

The incidence for overall rhabdomyosarcomas is 4.5 cases per million children/adolescents (age 0–19) per year in the United States between 1975 and 2005, of which ARMS account for 23%.[14]

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

In the United States between 1975 and 2005, African-American children had slightly higher rates of ARMS than Caucasian children (1.3 of 1 000 000 *vs* 1.0 of 1 000 000, respectively).[14]

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1.10 Diagnostic setting

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

Comment: Patients with fusion-positive ARMS have a significantly worse outcome than those with fusion-negative lesions having similar histology.[15] Some studies suggest that tumours with *PAX7* fusions have a better prognosis than other ARMS.[13,16]

2. TEST CHARACTERISTICS

	Genotype or disease		A: True positives B: False positives	C: False negatives D: True negatives
	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)
Routine cytogenetic karyotyping: 28%[17]
RT-PCR: 25–86%[17,18]
FISH: 38–88%.[18,19]

Comment: Depends on the technique and methods used in each laboratory, the sensitivity may vary. False negative results with routine cytogenetic method may be associated with normal cellular components overgrowing tumour cells and low-level gene expression may cause false negative results associated with RT-PCR.

2.2 Analytical specificity
(proportion of negative tests if the genotype is not present)
Routine cytogenetic karyotyping: 100%[17]
RT-PCR: 93–100%[17,18]
FISH 100%.[18]

Comment: Depends on the technique and methods used in each laboratory, the specificity may vary.

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 a quantification can only be made case by case.
Approximately 70–80%.
Around 70–80% ARMS (so called fusion-positive ARMS) possess either *PAX3-FOXO1A* or *PAX7-FOXO1A* translocations. Approximately 25% of cases have classic ARMS histology, but do not contain a fusion gene (so called fusion-negative ARMS). Gene expression arrays indicate that fusion-negative ARMS constitute a heterogeneous group that overlaps with ERMS. Although *PAX3-FOXO1A* tumours comprise a molecularly homogeneous entity with a uniformly poor prognosis, *PAX7-FOXO1A*-positive tumours exhibit gene amplification rather than overexpression. This subset may have a better prognosis than other alveolar genetic subtypes.[13,16]

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 a quantification can only be made case by case.
Approximately 100%.

2.5 Positive clinical predictive value
(life-time risk to develop the disease if the test is positive)
Routine cytogenetic karyotyping: 100%[17]
RT-PCR: 90–100%[17,18]
FISH: 100%.[18]

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.

Routine cytogenetic karyotyping: no reference available (or 0% based on limited data from Ref. 18).
RT-PCR: 26–90%.[17,18]
FISH: 38%.[18]

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)	Histology, myogenin expression

Comment: The diagnosis of ARMS is currently based on routine histology, but some strongly feel that it should be supplanted by genetic studies.[11,13,16,18,20,21] The current gold standard for the diagnosis of ARMS is the combination of classic or solid 'alveolar' histological features and strong reactivity to myogenin by immunohistochemistry.[21–25] New study has shown that fusion-positive ARMS may be detected by using a set of immunohistochemical markers, AP2β and P-cadherin, with a specificity of 98% and a sensitivity of 64%.[26] However, at present time, cytogenetic testing is still a key ancillary test when the tumours do not have classic ARMS histological features or strong expression of myogenin or myoD1.

3.1.2 Describe the burden of alternative diagnostic methods to the patient.
Rhabdomyosarcoma is the most common soft tissue tumour in paediatric population. It has been subclassified into two major categories: ERMS and ARMS. In general, ARMS carries an unfavourable prognosis with an aggressive clinical behaviour and a poor response to chemotherapy; thus low-stage disease requires aggressive

treatment. Because of significant differences in survival and treatment strategies, distinction between ARMS, ERMS and other small round blue cell neoplasms of childhood are of clinical importance. However, diagnosis of ARMS based solely on histology can be very challenging, as histologic features can overlap – especially in solid ARMS and ARMS with mixed alveolar and embryonal features. Because ERMS and other paediatric small blue cell tumours only very rarely display a *FOXO1A* mutation, detection of a positive *FOXO1A* mutation has great value in confirming the diagnosis of ARMS.[21–23] Without cytogenetic confirmation, a misdiagnosis or misclassification may occur if the histology or/and immunohistochemistry is atypical. Consequently, the patient may receive less optimal treatment.

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

The cost of histological analysis (routine staining) plus immunohistochemical stains can be expensive if more than a minimum number of immunohistochemical stains are used. Depending on the experience of the histopathologist, the number of immunohistochemical stains can range from two to twenty.

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

No	☐	
Yes	☒	
	Therapy (please describe)	If the translocation is present, the patient will receive aggressive therapy.
	Prognosis (please describe)	The outcome for patients with rhabdomyosarcoma depends on clinical and biologic features including primary site, tumour histology (ERMS vs ARMS), clinical group (extent of resection), and stage (size of primary tumour, lymph node metastases, and distant metastases).[27,28] ARMS patients in general have an unfavourable prognosis, with more aggressive tumour behaviour and worse outcome. The overall 5-year failure-free survival (FFS) rate is about 65% for patients with ARMS compared with 82% for patients with ERMS.[28]
	Management (please describe)	The treatment is based on assessment of risk factors and tumour site. In general, ARMS patients belong to the intermediate- to high-risk groups (depending on stage, age and group) and are not treated as low-risk patients. Studies have shown that patients with ARMS have greater 10-year FFS rates and overall survival rates, compared with patients who receive no radiation therapy, when they receive radiation therapy in addition to adjuvant multiagent chemotherapy and local resection.[29,30]

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 the test is positive, patients with low-risk features will receive more aggressive therapy and may have improved FFS and lifespan.

If the test result is negative (please describe): If the test is negative but histological and immunohistochemical features indicate ARMS, patients with low stage, localised tumours currently are still not eligible for low-risk therapy and receive more aggressive treatment than they would otherwise, but this approach has been questioned[20] and may be revised in future protocols. There is currently no fusion-specific therapy.[31]

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

If a correct diagnosis can be made based on classic alveolar histology and immunohistochemical stain, there will be probably no significant adverse effect on the patient's disease management. Conversely, if the patients are misclassified as ERMS, they may receive suboptimal, less aggressive treatment and may have disease progression.

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?

Not applicable.

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

Not applicable.

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

Not applicable.

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?

Not applicable.

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).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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