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CLINICAL UTILITY GENE CARD

Clinical utility gene card for: familial polycythaemia vera

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1. DISEASE CHARACTERISTICS

1.1 Name of the disease (synonyms)

Polycythaemia vera (PV; polycythaemia rubra vera, Osler-Vasquez-syndrome), entity of Philadelphia chromosome-negative myeloproliferative neoplasms (MPN).

1.2 OMIM# of the disease

263300.

1.3 Name of the analysed genes or DNA/chromosome segments

Janus kinase 2 (JAK2)/chromosome segment 9p24.
Other genes that can be associated with familial PV:
Ten-eleven translocation 2 (TET2)/4q24.
Egl nine homologue 1 (*C. elegans*) (EGLN1, synonym HIF prolyl hydroxylase 2, PHD2)/1q42.

1.4 OMIM# of the gene(s)

147796 (JAK2).
Other genes that can be associated with familial PV:
612839 (*TET2*).
606425 (*EGLN1*).

1.5 Mutational spectrum

Somatic JAK2 exon 14 G1849T/V617F (80–100% of all familial and sporadic PV patients).[1–4]

Multiple somatic JAK2 exon 12 (non-V617F) mutations (<10% of familial and sporadic PV patients).[5–8]

Germ-line JAK2 polymorphism rs10974944.[9–12]

Multiple somatic TET2 exons 3–12 mutations (10–15% of V617F-positive familial and sporadic PV patients).[12–14]

Germ-line EGLN1 G471C/G147H (JAK2 V617F-positive familial PV case).[15]

Recurrent somatic cytogenetic aberrations in 20–30% of familial PV and sporadic PV (+8, +9, del(9p), del(13q) and del(20q)).[16,17]

1.6 Analytical methods

Sequencing of peripheral blood or bone marrow leukocytes (DNA and/or RNA/cDNA).

Restriction analysis for JAK2 G1849T mutation: the mutation abolishes a *Bsa*X I restriction endonuclease recognition site. Required mutation analysis: JAK2 gene. Optional mutation analysis: TET2, EGLN1.

Cytogenetics.

1.7 Analytical validation

Bidirectional sequencing, double measurements.

Control samples: mutated cell lines (JAK2 V617F+/+ HEL, MB-02, MUTZ-8 and UKE-1, JAK2 V617F+/- SET-2[18]), mutated patient sample, unmutated patient sample or unmutated cell lines (eg, HL60).

1.8 Estimated frequency of the disease

The exact population prevalence and incidence for familial PV is not known but likely <1/100 000 (sporadic PV: population prevalence 25/100 000; incidence: 0.7–2.6/100 000).[19,20]

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

The exact prevalence in different ethnic groups is not known, but likely <1:100 000 for familial PV. In principle, individuals from any ethnic group can develop familial and sporadic PV. An ethnic association is known for Jewish descent, particularly Ashkenazi (5.8% PV cases *versus* 3% in a reference population in France).[21]

1.10 Diagnostic setting

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

Comment:

Diagnosis of familial and sporadic PV requires JAK2 mutation analysis.[16]

Prenatal testing is not indicated in familial PV (JAK2 V617F is a somatic mutation that occurs with disease manifestation after birth, and detection of non-V617F JAK2 germ-line polymorphisms or germ-line EGLN1 mutations have no consequence during pregnancy). Prenatal JAK2 V617F PV is extremely rare and is not associated with familial MPN.[22]

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2. TEST CHARACTERISTICS

	Genotype or disease		A: True positive B: False positive	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)
>99% (false-negative <1%).

2.2 Analytical specificity
(proportion of negative tests if the genotype is not present)
>99% (false-positive rate <1%).

2.3 Clinical sensitivity
(proportion of positive tests if the disease is present)
The clinical sensitivity is undetermined but likely >50% of cases with PV and positive family history are positive for JAK2 V617F.

2.4 Clinical specificity
(proportion of negative tests if the disease is not present)
The clinical specificity is undetermined but likely >99% of cases with no PV are negative for JAK2 V617F.

2.5 Positive clinical predictive value
(life-time risk of developing the disease if the test is positive)
The general risk to develop a familial PV is increased if JAK2 V617F is detected, but JAK2 V617F can be associated with other MPN entities. The risk to develop a familial PV is undetermined if EGLN1 is found to be mutated.[15] TET2 and cytogenetic aberrations alone are too unspecific (occur also in other myeloid neoplasms) for determination of a positive clinical predictive value.

JAK2 polymorphism rs10974944 predisposes to the development of PV and other entities of MPN[9–12] but is not a genetic indicator of familial PV/MPN.[12]

2.6 Negative clinical predictive value
(probability of not developing the disease if the test is negative)
The negative predictive value is undetermined but likely low (JAK2 mutation-negative PV <10%).

3. CLINICAL UTILITY

3.1 (Differential) diagnosis: The tested person is clinically affected
(To be answered if in 1.10 'A' was marked)

The diagnosis of familial PV requires at least one other PV patient in the family or any other non-PV MPN (referred to as familial MPN).[23] The familial predisposition is consistent with autosomal dominant inheritance with incomplete penetrance. A positive family anamnesis of an effected PV patient increases the risk 3–7-fold of MPN manifestation in other family members in general but not necessarily a PV.[23,24]

Familial PV cases are genetically and phenotypically indistinguishable from sporadic PV. The disease onset is similar in familial and sporadic cases (median age at diagnosis ~60 years; in general juvenile PV cases are rare).[19,24,25] However, the second generation patients are often younger than those of the first generation.[20] Prenatal PV is extremely rare and has been described as a sporadic event.[22]

Consider screening those with:

- True erythrocytosis.
- No identifiable secondary cause.
- Young patients.
- Thromboembolic complications.
- Positive family history.

Diagnosis of familial and sporadic PV according to the 2008 classification of the World Health Organization[19] requires both major criteria and one minor criterion or, major criterion 1 and two of three minor criteria.

Major criteria:

1. Increased red blood cell parameters: haemoglobin >185 g/l (men)/ >165 g/l (women) or haemoglobin >170 g/l (men)/>150 g/l (women) if associated with a documented and sustained increase of at least 20 g/l from an individual's baseline value that cannot be attributed to correction of iron deficiency or haemoglobin/haematocrit >99th percentile of method-specific reference range for age, sex and altitude of residence or elevated red blood cell mass >25% above mean normal predicted value.
2. JAK2 exon 14 V617F mutation or other functionally similar JAK2 exon 12 mutations.

Minor criteria:

1. Bone marrow histology shows prominent trilineage proliferation of mature erythropoiesis, granulopoiesis and megakaryopoiesis. Megakaryocytes characteristically show pleomorphic cytoplasmic diameters, normal or hyperlobulated nuclei, loose clustered and paratrabecular distribution.
2. Serum erythropoietin levels below the reference range for normal.
3. Endogenous erythroid colony formation *in vitro*.

Differential diagnosis:

Detection of JAK2 and TET2 mutations allows the molecular discrimination of neoplastic proliferation in terms of MPN/PV from primary and reactive erythrocythosis. Non-neoplastic primary familal erythrocytosis typically exhibit mutations of the oxygen pathway and has no elevated risk of blastic transformation.[19,26] EGLN1 mutation is usually found in familial non-PV/non-neoplastic erythrocytosis but can also be present in familial JAK2 V617F PV.[15]

JAK2 and TET2 mutations are not specific for PV, neither in general nor for sporadic and familial cases. These mutations can be detected in other MPN (50–60% of essential thrombocythemia and primary myelofibrosis cases are JAK2 V617F-positive), myelodysplastic syndromes (particularly TET2 mutations), MPN, unclassifiable and myelodysplastic/myeloproliferative neoplasms (both JAK2 and TET2 mutations).[1–14]

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	☒
		Peripheral blood analysis: evaluation of haematological (red blood cell) parameters and EPO levels.
		Bone morrow histology.
		Endogenous erythroid colony formation assay.
		Anamnesis (the clinical presentation is mainly unspecific, eg B-symptoms and abdominal pain due to splenomegaly or thromboembolic complication-associated symptoms).

3.1.2 Describe the burden of alternative diagnostic methods to the patient

Low: blood collection is required not only for mutation analyses but also for evaluation of red cell parameters and EPO level.

Low/medium: bone marrow biopsy; infiltration of the periosteum with local anaesthetic can relieve the pain of a bone marrow biopsy.

Low/medium: physical examinations and additional examinations (eg, sonography/radiology) for the evaluation of splenomegaly, hepatomegaly and/or thromboembolic complications.

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

Analysis of red blood cell parameters and EPO levels: low costs, low/medium sensitivity and specificity.

Physical and additional examinations: depending on the examination medium to high costs, low/medium sensitivity and specificity.

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

No	☐	
Yes	☒	
	Therapy	Phlebotomy, anticoagulation, myelosuppressive agents. JAK2 inhibitors are under development.
	Prognosis	Good prognosis, 10 years survival >80%.[23]
		Possible complications: thrombosis, thromboembolism, haemorrhage.
		Disease progression (late phase of disease): post-PV myelofibrosis with increased splenomegaly, anaemia and B-symptoms, myelodysplasia and/or acute myeloid leukaemia.[19]
	Management	Provide information for the patient, control of disease status.

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?

No (independent of the positive/negative result of the test), because the result of the genetic test on its own has no implication on lifestyle and prevention.

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)?

Regular follow-up examinations.

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?

A JAK2 polymorphism can be associated with an elevated risk to develop a JAK2 V617F-positive MPN in affected families[9] but the presence of the polymorphism alone does not necessarily result in disease manifestation. Therefore, there is no established genetic test for genetic risk assessment.

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

No, because JAK2 V617F and TET2 mutations are somatic alterations and the disease is transmitted with an incomplete penetrance.[23]

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

No, somatic JAK2 V617F can be associated with another MPN entity.

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

Advantage of genetic testing:

(1) Clear-cut discrimination between neoplastic and non-neoplastic erythrocytosis.
(2) Quantification of JAK2 mutant alleles for monitoring therapy and disease status.
(3) In case of bone marrow transplantation after post-PV myelofibrosis, JAK2 V617F and other non-germ-line mutations can be used as molecular markers for minimal residual disease.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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