

CLINICAL UTILITY GENE CARD

Clinical utility gene card for: Hereditary thrombocythemia

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European Journal of Human Genetics advance online publication, 5 June 2013; doi:10.1038/ejhg.2013.117

1. DISEASE CHARACTERISTICS

1.1 Name of the disease (synonyms)

Hereditary thrombocythemia (congenital or familial thrombocytosis or essential thrombocythemia).

1.2 OMIM# of the disease

187950 (Thrombocythemia 1/THCYT1, autosomal dominant); 601977 (thrombocythemia 2/THCYT2, autosomal dominant and autosomal recessive); 614521 (thrombocythemia 3/THCYT3, autosomal dominant with incomplete penetrance); 300331 (thrombocythemia, X-linked, THCYTX, autosomal recessive).

Note that the OMIM designation of the thrombocythemia types have been changed in 2012 following 'erythrocytosis, familial 1–4 (ECYT1–4)': 187950 was formerly 'essential thrombocythemia' (now THCYT1) and 601977 was formerly 'benign familial microcytic thrombocytosis' (now THCYT2).

1.3 Name of the analyzed genes or DNA/chromosome segments

Thrombopoietin (*THPO*)/3q27; myeloproliferative leukemia virus oncogene (*MPL*, synonym thrombopoietin receptor)/1p34; Janus kinase 2 (*JAK2*)/9q24.

1.4 OMIM# of the gene(s)

600044 (*THPO*); 159530 (*MPL*); 147796 (*JAK2*).

1.5 Mutational spectrum

THPO (THCYT1): IVS3, G–C, +1 (G to C transversion at position +1 of intron 3).^{1–3} 1-BP DEL, 3252G (1-bp G deletion in the 5'-untranslated region at nucleotide position 3252 and localization of deleted G 47 bases upstream of the authentic initiation codon).^{4,5} G516T of the 5'-untranslated region.^{6,7} Mutations of *THPO* signalling-associated factors other than *MPL* and *JAK2* (eg, SH2B adaptor protein 3/SH2B3) have not yet been reported in hereditary cases.

MPL (THCYT2): G1238T/K39N (germline alteration, 'MPL Baltimore').⁸ G317T/P106L (germline alteration).⁹ G1073A/S505N (germline alteration).^{10–12} Codon W515 mutations have not yet been reported in hereditary cases but in sporadic essential thrombocythemia (ET).¹³

JAK2 (THCYT3): G1894T/V617F (somatic mutation in THCYT3 and sporadic ET).^{14,15} G1894A/V617I (germline mutation in THCYT3).¹⁶ The molecular pathology of THCYTX¹⁷ and benign familial microcytic thrombocytosis is not known.¹⁸

1.6 Analytical methods

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

Restriction analyses:

THPO IVS3, G–C, +1: the mutation abolishes a *Bsr*I restriction endonuclease recognition site.² *MPL* G1238T mutation: the mutation creates a *Tsp*509I restriction endonuclease cleavage site.⁸ *MPL* G317T mutation: the mutation abolishes an *Aci*I restriction endonuclease recognition site.⁹ *JAK2* G1894T mutation: the mutation abolishes a *Bsa*XI restriction endonuclease recognition site.¹⁹

1.7 Analytical validation

Bidirectional sequencing, double measurements.

Control samples: mutated patient sample (if available), unmutated patient sample and/or cell lines (eg, *JAK2* G1894T-positive leukemia cell lines are HEL, MB-02, MUTZ-8, UKE-1 and SET2). As yet, *THPO* or *MPL*-mutated cell lines are not known.

1.8 Estimated frequency of the disease

(incidence at birth ('birth prevalence') or population prevalence)

Population prevalence: < 1:100.000.^{20,21}

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

The exact overall prevalence is not known, but likely < 1:100.000.^{20,21}

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In principle, individuals from any ethnic group can develop THCYT/hereditary ET.

Hereditary thrombocythemia cases with associated *THPO* alterations (THCYT1) have been described in Japan,^{4,6,7} Italy,⁵ the Netherlands^{1,2} and Poland.³

In an affected Italian family with *THPO* 1-BP DEL, 3252G distal unilateral limb defects were present; presumably secondary defects (grandfather without limb defects; father with absence of right forearm and hand and absence of right foot, one of three male children with absence of the right foot, the calcaneus and astragalus; a second child with absence of the last phalange of 2nd digit and last 2 phalanges of 3rd to 5th digits; the third child had no limb defects).⁵

Hereditary thrombocythemia cases with associated *MPL* alterations (THCYT2) have been described in Japan,¹⁰ Italy^{11,12} North America (African Americans)⁸ and Arabia.⁹

Regional/ethnic association is known for *MPL* K39N (~7% of African Americans are heterozygous for *MPL* K39N, $n = 12/161$) and *MPL* P106L (~5% in Arab population, $n = 11/213$; ~1% are homozygous and ~4% are heterozygous for *MPL* P106L). The frequency of *MPL* K39N in other American ethnics is 0% (Caucasian, $n = 0/250$; Hispanic, $n = 0/40$; Asian, $n = 0/39$). The frequency of *MPL* P106L in German individuals is 0% ($n = 0/193$).^{8,9} As yet, *MPL* S505N has not been systematically evaluated regarding regional/ethnic association.

Hereditary thrombocythemia cases with associated *JAK2* alterations (THCYT3) have been described in a cohort of 66 European and 6 African families with somatic *JAK2* V617F mutation¹⁵ and germline *JAK2* V617I was detected in a family from Great Britain.¹⁶

Data from Sweden revealed that relatives of ET patients had elevated risk for any myeloproliferative neoplasm (MPN; RR = 6.8; 95% CI, 3.4–13.5), ET (RR = 8.8; 95% CI, 2.6–24.5), Polycythemia vera (RR = 5.4; 95% CI, 2.2–13.1) and unclassifiable MPN (RR = 16.1; 95% CI, 1.9–146.1) but no significantly increased risk for primary myelofibrosis (PMF; RR = 4.0; 95% CI, 0.2–61.7).²²

THCYTX has been described in an Arab family from Saudi Arabia.¹⁷

Benign familial microcytic thrombocytosis has been described in a Jewish family (mother with disease phenotype emigrated from India).¹⁸

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 type="checkbox"/>	<input checked="" type="checkbox"/>

Comment:

Prenatal testing is not indicated in THCYT1–3 (even if limb defects are sonographically present) because the result of a genetic test has no consequence during pregnancy (detection of an associated mutation is no indication for induced abortion); the child of an affected mother can be tested after birth.

2. TEST CHARACTERISTICS

	Genotype or disease		A: True positives	C: False negative
	Present	Absent	B: False positives	D: True negative
Test				
Positive	A	B	Sensitivity:	A/(A + C)
			Specificity:	D/(D + B)
Negative	C	D	Positive predict. value:	A/(A + B)
			Negative predict. value:	D/(C + D)

2.1 Analytical sensitivity

(proportion of positive tests if the genotype is present)

More than 99% (False-negative rate < 1%).

2.2 Analytical specificity

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

More than 99% (false-positive rate < 1%).

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.

The clinical sensitivity is undetermined but likely < 50% of cases with thrombocythemia and positive family anamnesis are positive for one of the associated mutations.

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.

The clinical specificity is undetermined but likely > 99% of cases with no thrombocythemia are negative for associated mutations.

2.5 Positive clinical predictive value

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

Autosomal recessive *MPL* P106L-positive THCYT2: < 25% in heterozygously mutated cases (only a small subfraction of heterozygous carriers develop thrombocythemia) and 100% in homozygously mutated cases.⁹

Autosomal-dominant THCYT1, non-*MPL* P106L THCYT2 and THCYT3: 100% in heterozygously and homozygously mutated cases.^{20,21}

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.

The negative clinical predictive value is undetermined, because > 50% of cases with thrombocythemia and positive family anamnesis are negative for one of the associated mutations.

Index case in that family had been tested:

If the index patient is carrying a mutation, the risk of mutation-negative first-degree relatives to develop the disease is 0%.

Index case in that family had not been tested:

If the index patient has not been tested for thrombocythemia-associated mutations, the risk of non-affected first-degree relatives to

develop the disease depends on recessive or dominant transmission of the disease:

Autosomal recessive MPL P106L-positive THCYT2, risk to develop the disease: <25% if only one parent is a heterozygous carrier and the other parent is not (only a small subfraction of heterozygous carriers develop thrombocythemia), 25% if both parents are heterozygous carriers, 50% if one parent is a heterozygous carrier and one parent is a homozygous patient, 100% if both parents are homozygous patients.²

Autosomal-dominant THCYT1, non-MPL P106L THCYT2 and THCYT3, risk to develop the disease: 50% if one parent is a heterozygous patient and the other parent is not, 75% if both parents are heterozygous patients, 100% if one parent is a heterozygous patient and one parent is a homozygous patient, 100% if both parents are homozygous patients.^{20,21}

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 type="checkbox"/>
	Endoscopy <input type="checkbox"/>
	Biochemistry <input checked="" type="checkbox"/>
	Electrophysiology <input type="checkbox"/>
	Other: <input checked="" type="checkbox"/>
	Anamnesis.
	Peripheral blood analysis: evaluation of hematological parameters.

Comment:

A thrombocythemia is present when the platelet count is chronically elevated $>450 \times 10^9/l$. By definition, it is congenital if it is present from birth. In such persons the thrombocythemia is usually detected at a young age. There can be a family history in keeping with the inherited nature of the defect. However, sporadic cases without positive family history can occur.

Hereditary thrombocythemia can be primary or secondary.^{20,21}

A primary thrombocythemia in terms of THCYT2 and THCYT3 occurs when there is an intrinsic defect in the megakaryocytic cells in the bone marrow (MPL and/or JAK2 mutations). In these cases, THPO levels will be normal (which would be inappropriate for a raised platelet count) or below normal.

A secondary thrombocythemia is present when THPO production in the liver (main source of THPO production), kidney and bone marrow stromal cells is increased. In case of THCYT1, mutation in the 5'-untranslated region of THPO leads to disturbed regulation of translation with increased THPO production.

Hereditary thrombocythemia can be non-neoplastic (THCYT1 and THCYT2) or neoplastic (THCYT3):

THCYT1 and THCYT2 have no elevated risk to develop acute leukemia; these cases are positive for THPO mutations or MPL mutations K39N or S505N and negative for MPL G1544T/W515L and MPL T1543A-G1544A/W515K (MPL W515L/K mutations can be found in sporadic ET), JAK2 V617F and TET2 mutations.^{11,20,22,23} Sporadic ET but not hereditary thrombocythemia with MPL codon W515 mutations have not been described so far.¹³

Adult and elderly patients, but not children with MPL S505N-positive THCYT2, have frequently developed myelofibrosis.¹¹ These cases are indistinguishable from other BCR-ABL-negative PMF. Neoplastic THCYT3/hereditary ET have a low but nevertheless elevated risk to develop myelofibrosis and/or acute leukemia.^{20,21} THCYT3 can be positive for somatic JAK2 V617F¹⁵ or germline JAK2 V617I (reported so far in one family).¹⁶

Note that: MPL W515L/K mutations have been described in sporadic ET but as yet not in THCYT2. Without a positive family anamnesis THCYT3/hereditary ET and sporadic ET are indistinguishable. No detectable JAK2 mutation does not exclude hereditary ET because ~50% of cases can be JAK2 V617F-negative and JAK2 V617I has been reported so far in only one family.^{15,16} Diagnosis of THCYT3/hereditary ET in an index patient does not necessarily mean that other related patients must have an THCYT3/ET phenotype; it is known that different entities of MPN might present in individual patients of a family (hereditary MPN with individual ET manifestation).^{13,15,20,21} Diagnosis of THCYTX requires exclusion of all other THCYT subtypes. THPO IVS3, G-C, +1 mutation has been excluded in THCYTX but not other THPO mutations or mutations of MPL and JAK2. The serum THPO level has not been determined.¹⁷ Cytogenetic aberrations are thought to be rare in sporadic ET (<10% of cases) and, if present, unspecific. Recurrent cytogenetic aberrations have not been reported in THCYT subtypes.

Consider screening those with (Figure 1): True thrombocythemia (>6 months and $>450 \times 10^9/l$ platelets), no identifiable secondary cause, young patients and/or positive family history.

Diagnosis of hereditary thrombocythemia:^{19,20}

Anamnesis: positive family anamnesis and thromboembolic complications.

Physical examination and exclusion of secondary (reactive) cause of thrombocythemia: exclusion of acute/chronic infection (~50% of thrombocythemia cases are associated with infection), iron deficiency, acute blood loss, hemolysis, asplenia, paraneoplastic/malignancies. Spleen and liver status (splenomegaly and/or hepatomegaly are usually absent but can be present in neoplastic THCYT3/ET; asplenia causes thrombocythemia itself).

Laboratory testing (non-phlebotomized patients): isolated increased platelet counts $>450 \times 10^9/l$ (white blood cell counts and red cell parameters are usually normal). THPO level for discrimination of primary/secondary thrombocythemia and determination of mean platelet volume (MPV) for exclusion of benign familial microcytic thrombocythemia.

Mutation analysis: THPO; MPL; JAK2 (in somatic V617F-negative cases consider germline V617I mutation).

The home page of MPN&MPNr-EuroNet (COST Action BM0902) provides addresses of European laboratories which perform mutation analysis for hereditary thrombocythemia: <http://www.mpneuro.net/>.

3.1.2 Describe the burden of alternative diagnostic methods to the patient

Low: blood collection is required for mutation analyses but also for evaluation of platelet counts.

Low/medium: physical examinations (low) and additional examinations, for example, sonography/radiology (low/medium).

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

Analysis of platelet counts, MPV and THPO levels: low costs, low/medium sensitivity and specificity.

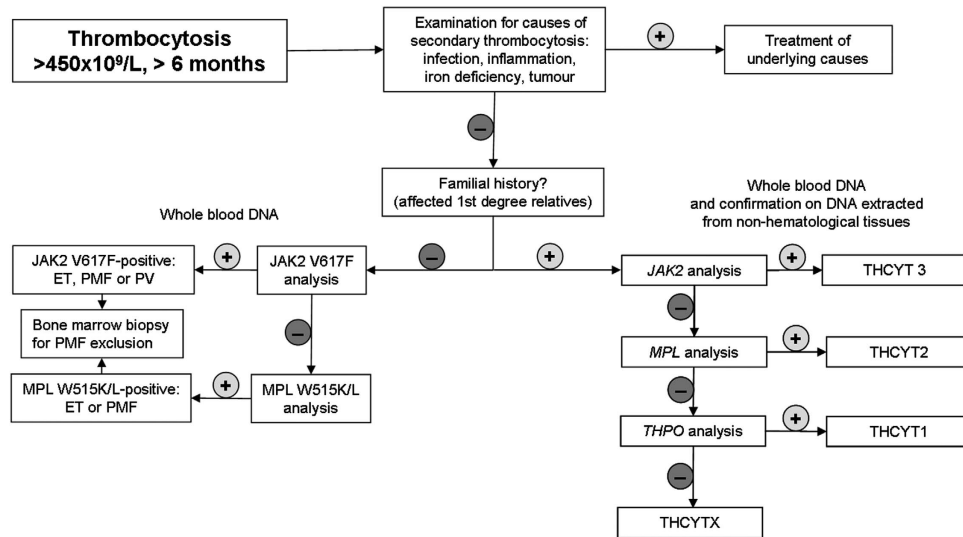


Figure 1 Proposed algorithm for evaluation of suspected THCYT.

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 (please describe)	Control (watch-and-wait), acetylsalicylic acid (if symptomatic).
Prognosis (please describe)	Good prognosis, > 10 years survival.
Management (please describe)	Possible complications: thrombosis, thromboembolism and hemorrhage. No increased risk for progression to acute leukemia or myelofibrosis in THCYT1. Increased risk for progression to myelofibrosis in MPL S505N-positive THCYT2. No increased risk for progression to acute leukemia in THCYT2. Low risk for progression to myelofibrosis and/or acute leukemia in THCYT3/hereditary ET; similar risk as in sporadic ET ^{11,20,21} . Provide information for the patient, drug therapy for prevention of complications and control of disease status. Patients with the diagnosis of THCYT2 (in particular MPL S505N-positive cases) and THCYT3 should be monitored carefully and bone marrow biopsy as well as cytogenetic analysis should be performed if progression of the disease is suspected.

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?

Yes, particularly detection of JAK2 V617F or MPL S505N, because the cases might develop myelofibrosis (JAK2 V617F and MPL S505N) or acute leukemia (JAK2 V617F).¹¹

Simultaneous factor VIII:C or von Willebrand factor antigen abnormalities with increased risk of bleeding diathesis might be present, in particular in THCYT1 and THCYT3.^{6,24} Compared with ET patients, THPO-mutated patients (THCYT1) have a similarly higher prothrombotic risk.⁸

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

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?

Yes, if germline mutations can be detected (JAK2 V617F, MPL K39N, MPL P106, MPL S505N and THPO mutations).

No, if somatic (secondary) mutations are detected (JAK2 V617F and MPL mutations). Note that JAK2 and MPL mutations can be present in B and T lymphocytes without necessarily indicating germline distribution (non-myeloid differentiation of the neoplastic clone).²⁵ Use non-hematopoietic tissue for germline analysis, for example, oral epithelia or others.²⁶

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

Depends on the platelet counts of other family members, but genetic testing should be performed, particularly if the index patient carries a thrombocytosis-associated mutation.

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

Yes, detection of associated mutations in a family member other than the index patient is associated with an increased risk of having/developing thrombocytosis.

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?

Advantage of genetic testing:

Mutation analysis is helpful to establish diagnosis and sub-classification of the disease. Mutation analysis helps to determine the risk regarding homozygous and heterozygous mutation-carrying individuals.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by EuroGentest, an EU-FP6 supported NoE, contract number 512148 (EuroGentest Unit 3: 'Clinical genetics, community genetics and public health', Workpackage 3.2).

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