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CLINICAL UTILITY GENE CARD

Clinical utility gene card for: haemophilia B

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1. DISEASE CHARACTERISTICS

1.1 Name of the disease (synonyms)
Haemophilia B, Hemophilia B, (Christmas disease; heritable Factor IX deficiency).

1.2 OMIM# of the disease
306900.

1.3 Name of the analysed genes or DNA/chromosome segments
Factor IX (F9).

1.4 OMIM# of the gene(s)
300746.

1.5 Mutational spectrum
Haemophilia B results from the deficiency of blood coagulation factor IX (FIX). All heritable cases of haemophilia B are due to mutations in or near the *factor IX* gene (*F9*). The gene located on Xq27.1–27.2 is ~36 kb long and comprises eight exons. Severe haemophilia (FIX < 1% of normal) is caused by a wide spectrum of mutations including nonsense, missense, splice site mutations and less commonly by large and small indels. Moderate and mild haemophilia (FIX 1–4%, and 5–40%, respectively) are generally caused by missense mutations or less commonly splice-site alterations. A locus-specific database for haemophilia B mutations is available at http://www.kcl.ac.uk/ip/petergreen/haemBdatabase.html.

1.6 Analytical methods
Typically standard PCR of genomic DNA and direct resequencing of essential coding and flanking regions is performed.[1,2] Mutation screening methods have been described.[3–5] Multiplex ligation-dependent probe amplification can be applied for determination of large insertions or deletions.[6]

1.7 Analytical validation
A guideline for recommended practice in the molecular analysis of haemophilia B is available.[1] This discusses analytical design, mutation validation procedures, and analytical pitfalls. External quality assurance (EQA) should be carried out where available. An EQA scheme has been established for genetic investigation of haemophilia, details of this scheme are available from UK NEQAS for Blood Coagulation (http://www.ukneqasbc.org).[7] Use of internal controls, especially in the analysis of extended family members is recommended.

1.8 Estimated frequency of the disease (incidence at birth ('birth prevalence') or population prevalence)
1.1–4.3 per 100 000 males.[8]

1.9 If applicable, prevalence in the ethnic group of investigated person
No known ethnic variation in prevalence, however, there is variation in detection and diagnosis.[8]

1.10 Diagnostic setting

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

Comment: Haemophilia B is an X-linked disorder. Diagnosis of affected males is by laboratory measurement of functional factor IX levels (FIX:C). Possible carriers are definitively diagnosed by analysis of the mutation site once the underlying mutation has been determined in a related affected male index. Molecular analysis can aid differential diagnosis by linkage of mild FIX deficiency to the *F9* gene.

Predictive testing in general does not apply as males with a clinically significant F9 mutation will be affected as shown by a low FIX:C level. In certain rare cases molecular testing can predict amelioration of the disease with increasing age (see 2.4 below).

Prenatal diagnosis is available, preimplantation genetic diagnosis is possible for haemophilia B.[9] Knowledge of carrier status can inform clinical management of antenatal delivery.

2. TEST CHARACTERISTICS

	Genotype or disease		A: True positives B: False positives	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)

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2.1 Analytical sensitivity
(proportion of positive tests if the genotype is present)
>99%.

2.2 Analytical specificity
(proportion of negative tests if the genotype is not present)
>99%.

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.

Sensitivity of >99% for patients with severe haemophilia B (FIX:C <1%) and ~98% for patients with mild or moderate haemophilia (FIX:C 1–40%). Issues that affect the sensitivity are the correct diagnosis of the disorder and sensitivity of the genetic screening technique.[1] Acquired (non-congenital) haemophilia B is extremely rare and usually secondary to other underlying disorders. There is no published data regarding the prevalence of acquired haemophilia B, however, it is unlikely to affect the analytical specificity or clinical sensitivity to a significant degree.

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.

Prior diagnosis of haemophilia B by demonstration of low or absent FIX:C is the primary indication for genetic screening of an affected male. Symptoms generally develop at an early age and are associated with a lifelong risk of bleeding. A notable exception is the phenotype Haemophilia B 'Leyden' or Factor IX 'Leyden', which exhibits epigenetic modification of the disease. Mutations associated with this variant occur in the *F9* promoter. The *F9* promoter region contains an androgen response element and with the onset of puberty the effects of the mutation are bypassed. FIX:C levels increase in patients with this variant type rising over a prolonged period to near normal or normal levels.[10–12]

Heterozygous female carriers do not generally exhibit haemophilia B although approximately 10% of carriers with low FIX levels may exhibit mild bleeding symptoms. On occasion genetic screening for identification of the familial mutation is performed on an obligate carrier female where a sample from an affected male in the kindred is not available.[13]

2.5 Positive clinical predictive value
(lifetime risk of developing the disease if the test is positive).

Not applicable, the condition is present from birth, and diagnosis is usually by laboratory measurement of FIX:C.

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.

Index case in that family had been tested:

Not applicable.

Index case in that family had not been tested:

Not applicable.

3. CLINICAL UTILITY

3.1 (Differential) diagnosis: 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).

No	☐ (continue with 3.1.4)
Yes	☒
Clinically	☒
Imaging	☐
Endoscopy	☐
Biochemistry	☒
Electrophysiology	☐
Other (please describe)	☒

Definitive diagnosis is made by measuring the circulating FIX:C level in affected males by standard coagulation assays. Carrier status of females cannot be definitively diagnosed by coagulation testing.

3.1.2 Describe the burden of alternative diagnostic methods to the patient.
Low for affected patients, diagnosis is based on circulating FIX:C levels measured from a peripheral blood sample.

3.1.3 How is the cost effectiveness of alternative diagnostic methods to be judged?
Primary diagnosis of affected males is performed by measurement of circulating FIX:C level using standard laboratory assays. Identification of the causative *F9* mutation in index males allows diagnosis of carrier females as no definitive phenotypic assay is available for diagnosis in heterozygous females. No alternative technique exists for prenatal diagnosis (PND) except for cord blood sampling for FIX level determination.

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

No	☒
Yes	☐
Therapy (please describe)	
Prognosis (please describe)	
Management (please describe)	

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 result is negative (please describe):

The test result will not have an influence on lifestyle of affected males. A definitive diagnosis of female carrier status may influence reproductive choices. A positive carrier result allows subsequent PND, and may influence management of delivery. Exclusion as a carrier of the affected *F9* gene provides reassurance to the individual and removes concerns with regard to family planning.

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).
Lifestyle for an affected male is unaffected by knowledge of the genetic lesion.Reproductive decisions for females may be more cautious if genetic testing has not been undertaken.

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, providing the familial mutation has been identified.

3.3.2 Can a genetic test in the index patient save genetic or other tests in family members?
Identification of the causative mutation in an index defines the mutation in the family and may avoid testing of other affected male family members. It does not preclude screening for the familial mutation in possible carrier females wishing to definitively know their carrier status.

3.3.3 Does a positive genetic test result in the index patient enable a predictive test in a family member?
Yes, however, affected males are generally diagnosed by FIX:C measurement.

3.4 Prenatal diagnosis
(To be answered if in 2.10 'D' was marked)

3.4.1 Does a positive genetic test result in the index patient enable a prenatal diagnostic?
Yes. PND, if requested, is only performed when the foetus is male and generally only in families with severe haemophilia B.

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 analysis is is often requested by possible carriers in order to define their carrier status due to the psychosocial consequences of haemophilia to the patient, carrier and kindred. Knowing the carrier status in females who are pregnant with a male foetus may influence the degree of planned medical intervention during delivery.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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