European Journal of Human Genetics (2013) 21, doi:10.1038/ejhg.2012.227
© 2013 Macmillan Publishers Limited All rights reserved 1018-4813/13
www.nature.com/ejhg

CLINICAL UTILITY GENE CARD

Clinical Utility Gene Card for: incontinentia pigmenti

Francesca Fusco[1], Alessandra Pescatore[1], Julie Steffann[2], Ghislaine Royer[2], Jean-Paul Bonnefont[2] and Matilde Valeria Ursini*[,1]

European Journal of Human Genetics (2013) **21**, doi:10.1038/ejhg.2012.227; published online 10 October 2012

1. DISEASE CHARACTERISTICS

1.1 Name of the disease (synonyms)

Incontinentia pigmenti (IP); familial male-lethal type, Bloch–Sulzberger syndrome; IP TYPE II; IP2.

1.2 OMIM# of the disease

308300.

1.3 Name of the analysed genes or DNA/chromosome segments

NEMO/IKBKG, X Chomosome, Xq28.

1.4 OMIM# of the gene(s)

300248.

1.5 Mutational spectrum

IP is a rare X-linked genodermatosis, characterized by typical skin alterations, the hallmarks of the disease, and, in addition, by other neuroectodermal defects affecting the eyes, the nails, the hair, the teeth, and the central nervous system (CNS).

The clinical diagnosis of IP is based on the presence of dermatological lesions that develop in four successive, sometimes overlapping, characteristic stages that start shortly after birth with an inflammatory vesicular rash (stage1), followed by verrucous lesions (stage2). The third stage is marked by the appearance of a skin area displaying hyperpigmentation that at the fourth stage becomes patches of atrophic hypopigmented skin. In addition, IP females have heterogeneous and often severe clinical signs including ophthalmological (strabismus, cataracts, optic atrophy, retinal vascular pigmentary abnormalities, microphthalmia), odontological, (partial anodontia, delayed dentition, cone/peg-shaped teeth, impactions) and neurological defects (seizures, spastic paralysis, motor, and mental retardation, microcephaly).[1]

IP is a genomic disorder inherited as an X-linked dominant trait. IP is generally lethal in males while heterozygous females survive owing to functional mosaicism.[1] All cases of IP are due to mutations in *NEMO* (nuclear-factor-kappa-B essential modulator)/*IKBKG* gene located in Xq28 region, and the mutation detection rate in IP is around 80%. *IKBKG*, encodes the regulatory subunit of the IkB kinase complex required for nuclear factor-kB (NF-kB) activation.[2,3]

Mutations in different domains of protein may produce different effects on NF-kB activation by reducing or abolishing the response after stimulations. Noteworthy, some *IKBKG* hypomorphic mutations, affecting the zinc finger (ZF) domain of the NEMO protein and reducing but do not eliminating NF-kB activation, were found in surviving male patients. These males are affected by a different disease, named hypohidrotic ectodermal dysplasia-associated with severe immunodeficiency (EDA-ID HED-ID OMIM#300291) or occasionally associated with osteopetrosis and lymphoedema (OL-EDA-ID).[4,5]

The most frequent mutation in IP (70%) is a recurrent exon 4_10 deletion (*NEMOexon4_10del*) due to non allelic homologous recombination that occurs between two repeats (*MER67B*) located in intron 3 and downstream exon 10, causing the removal of the entire genomic region from exon4 to 10.[6,7]

Missense, nonsense, deletions, and insertions have been reported in addition to gross rearrangements.[8] With the exception of a tract of cytosines in exon 10 that appears to be prone to mutations in IP/HED-ID, no mutational hotspots or common point mutations are seen.

To date, 53 different mutations (from large deletions to single amino-acid substitutions) affecting *IKBKG* have been reported: 7 gross deletions, 27 frameshift, 11 nonsense, four missense, one is an in-frame deletion of one codon, two are splice-site mutations, and one is a nonstop mutation.[6–8]

No evident genotype–phenotype correlation is apparent from comparison of patients with different loss-of-function mutations. The majority of mutations are 'private' to specific families. The rate of *de novo* mutations is about 65%.

1.6 Analytical methods

Different strategies for *IKBKG* mutation screening procedures are currently applied on genomic DNA extracted from peripheral blood:

1. Long-range PCR using two specific primers able to detect the pathogenical *IKBKG* deletion (*IKBKGexon4_10del*) in the gene and able to discriminate it from the non pathogenical pseudogene deletion (*PIKBKGexon4_10del*).[9,10] Indeed, a non-functional copy of the *IKBKG* gene is located (99% identity with the gene) in the IP *locus*.[11] No evidence of an involvement of the *PIKBKG* pseudogene in human diseases has so far been reported. The exon 4_10 deletion of *PIKBKG* is a benign variant in the control population.[12] Those cases support the need to discriminate between deletions occurring in gene or in pseudogene to perform a correct molecular diagnosis of IP.[9] A possible way to discriminate between *IKBKG* and *PIKBKG* deletions consists in testing the X inactivation pattern in white blood cells from female carriers. Conversely to *PIKBKG* deletions, *IKBKG* deletions are

[1]Institute of Genetics and Biophysics 'Adriano Buzzati-Traverso', IGB-CNR, Naples, Italy; [2]Department of Genetics, Necker Enfants Malades Hospital Paris, Paris Descartes University, Paris, France
*Correspondence: Dr MV Ursini, Institute of Genetics and Biophysics 'Adriano Buzzati-Traverso', IGB-CNR, Via P. Castellino, 111, 80131 Naples, Italy. Tel: +39 081 6132262; Fax: +39 081 6132706; E-mail: ursini@igb.cnr.it

almost consistently associated with a full X inactivation skewing, at least after one year of age. This test usually relies on the amplification of either a polymorphic CAG repeat in the Androgen receptor gene[13] or a polymorphic CGG repeat in the *FMR1* gene.[14] It additionally enables to determine the parental origin of the rearranged chromosome, an useful information for genetic counselling purpose in apparently sporadic cases.

2. When no large deletion is identified in the gene, while both clinical picture and X inactivation skewing are highly suggestive of a *IKBKG* anomaly, a microrearrangement can be searched for, using direct sequencing of the coding regions with flanking intronic sequences of the *IKBKG* gene. If an intragenic mutation is detected, it is necessary to verify that the mutation has occurred in the gene and not in the pseudogene. This requires additional testing by long-range PCR amplification able to generate gene-specific and/or pseudogene-specific products, followed by PCR nested method and sequence analysis.[6]
3. When no point mutation is identified, a search for *IKBKG* genomic rearrangements different from the prevalent exon 4_10 deletion is performed by MLPA or quantitative real- time PCR using oligo primers distributed along the IP *locus*. A total of 26 primers for quantitative PCR, sequencing of breakpoint junction are used. Using such qPCR seven genomic deletions (35%) were found in study of 20 patients who met diagnostic criteria for IP disease molecularly unsolved.[8] This test is currently only used in research field, but may be used as a clinical test in the future. If genomic rearrangements, outside the IP locus, are suspected, high-density array CGH can be performed.

1.7 Analytical validation

Parallel analysis of negative and positive controls. Direct sequencing of both DNA strands is performed. All mutations identified should be confirmed by a second, independent test (long-range PCR, quantitative real-time PCR, sequencing). Sequencing results are confirmed by re-sequencing using different sets of primers. It is recommended to confirm the segregation of the mutation in the parents. For potential missense mutations, protein assays and stability may be performed on a research basis using recombinant NEMO protein containing the mutation. Moreover, missense mutations should be in evolutionary conserved regions and they should be predicted by applicable software to be considered probably pathogenic.

1.8 Estimated frequency of the disease
(incidence at birth ('birth prevalence') or population prevalence. If known to be variable between ethnic groups, please report)

Birth prevalence: 1:10000–1:20000.

1.9 Diagnostic setting

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

Comment:
Predictive testing in IKBKG should be considered on an individual case basis only, as long as no preventive treatment is available.

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)

2.1 Analytical sensitivity
(proportion of positive tests if the genotype is present)

Long-range PCR: 100% (only heterozygous *IKBKG*exon 4_10 deletion).

IKBKG sequencing: > 80% (heterozigous for *IKBKG* point mutation).

Quantitative PCR: 60% heterozygous *IKBKG* deletion.

Comment: Quantitative PCR does not detect the point mutations in the gene nor other genomic alterations outside the IP locus. Depending on the technique and methods used in each laboratory, the sensitivity may vary.

It is recommended to scan SNP data bases periodically, to check for the identification of novel SNPs, prone to interfere with primer hybridization (http://www.ncbi.nlm.nih.gov/).

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

Long-range PCR: 100%, provided that the PCR specifically targets the *IKBKG* gene.

IKBKG sequencing: > 90%. The main concern is the occasional detection of exonic variants of uncertain significance, of which the responsibility for the disease is often difficult to demonstrate.

Quantitative PCR: > 90% for heterozygous *IKBKG* deletions.

2.3 Clinical sensitivity
(proportion of positive tests if the disease is present)

The clinical sensitivity depends on variable factors such as age or family history. Moreover, IP patients have heterogeneous clinical presentation. Indeed, while they have always-typical linear skin lesions (starting at birth and spontaneously evolving in four overlapping dermatological stages), they inconsistently exhibit ophthalmologic (strabismus, cataracts, optic atrophy, retinal vascular pigmentary abnormalities, microphthalmia), odontological (partial anodontia, delayed dentition, cone/peg-shaped teeth, impactions), and neurological defects (seizures, spastic paralysis, motor and mental retardation, microcephaly). The severity of these additional clinical signs is variable.[1,6,15]

2.4 Clinical specificity
(proportion of negative tests if the disease is not present)

Clinical specificity is around 100%. The only (rare) pitfall consists in detecting the *PIKBKG* deletion, erroneously interpreted as the *IKBKG* deletion. The clinical specificity can be dependent on variable factors such as age or family history. In most cases, a detailed clinical assessment and skin biopsy will have been performed before genetic testing; therefore, presence of typically skin alterations represents not

only a prerequisite to start genetic testing but also for the interpretation of *IKBKG* variations of uncertain significance.

2.5 Positive clinical predictive value
(life-time risk to develop the disease if the test is positive).
On the basis of the studies of large pedigrees, most, if not all, patients appear to develop symptoms. Skin lesions are almost consistently found, tooth and eye anomalies are found in more than 50% cases, and a CNS involvement is present in 10–30% cases.[1,6,15] For patients who are tested, and result positive for mutations, genetic counselling should be provided.

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:
Undetermined. We estimate that it is close to 100%.
Index case in that family had not been tested:
Undetermined. We estimate that it is >95%.

It is notewhorty to mention that there is a low level risk for somatic mutations in *IKBKG* that could cause IP-like skin features, escaping classical molecular diagnosis.

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)	Highly experienced dermatologist may be able to diagnose the IP, and the information from skin biopsy can help to complete the diagnosis.

3.1.2 Describe the burden of alternative diagnostic methods to the patient
IP disorder can be diagnosed clinically, but not solely, using the revised criteria for classification of IP which established that the affected females have a history of perinatal blistering and at least one of the other stages of skin lesions.

Clinical diagnosis may include: nervous system exam for seizures, spastic paresis, motor and mental retardation, microcephaly, ocular defects, dental defects, hair defects, and nail defects taking a family pedigree and clinical assessment by a clinical geneticist or other physician familiar.

Skin biopsy is painful and will not specify the underlying genetic defect.

3.1.3 How is the cost effectiveness of alternative diagnostic methods to be judged?
Low. In our experience, considering a IP diagnosis ineluctably results in request for genetic testing.

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

No	☐	
Yes	☒	The genetic resolution of clinical diagnosis will help to devise multidisciplinary management, therapeutic interventions and follow-up as outlined below.
	Therapy (please describe)	There are no specific pharmacological agents currently proved to be effective in IP patients. Only symptomatic treatments are available.
	Prognosis (please describe)	Confirmation of genetic defect in an IP patient contributes to the definite resolution of IP patients that have not entirely specific clinical features.
	Management (please describe)	The genetic result will help to focus multidisciplinary clinical follow-up and treatments, including regular assessments of function: nervous system, ocular system, dental, hair and nail systems.

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)

Identification of a *IKBKG* mutation allows carriers to make informed reproductive decisions, which take into account the risk of having an IP-affected child.

A woman with a mutation may decrease her risk of having an IP child affected with IP by taking advantage of prenatal diagnosis oocyte donation, adoption, and so on. Preimplantation genetic diagnosis (PGD) is possible but particular technical difficulties exist for IP.

If the test result is negative (please describe)

Determining that a female patient is not a carrier can relieve the anxiety related to genetic risk and allow for confident 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)?
No special options; prevention is not possible.

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, it confirms the mode of inheritance and is the prerequisite for genetic risk assessment in relatives.

3.3.2 Can a genetic test in the index patient save genetic or other tests in family members?
Yes, when the IKBKG mutation is shown to have occurred *de novo* in the proband.

3.3.3 Does a positive genetic test result in the index patient enable a predictive test in a family member?
Yes, a positive test in a female allows identifying her mother as a carrier if the mutation is not a *de novo* mutation. We personally recommend to test also for asymptomatic parents and other female relatives, both to determine the precise inheritance pattern in the

family but also to advise the parents about their potential IP risk. Indeed, even if a *IKBKG* mutation is usually 'fully' penetrant, IP signs can sometimes be underdiagnosed when clinical picture is restricted to scars of skin lesions.

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?
All females with a IKBKG mutation can be offered a prenatal diagnosis.

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)

Yes. It is advised to confirm IP carrier status in affected mother.

Although there is no cure for IP, the diagnosis helps to follow appropriate physical, cognitive, and behavioural management of the affected individual.

Yes, genetic testing is the gold standard for confirmation of the diagnosis and the mode of inheritance, helps to avoid unnecessary and invasive diagnostic procedures. It allows prognostic evaluations and is the prerequisite for prenatal testing, PGD, and genetic risk estimation of relatives.

Molecular confirmation of the diagnosis will limit unnecessary further aetiological investigations, which can often be invasive and unpleasant.

Many parents feel guilty, and may be relieved after a genetic diagnosis is obtained. Parents also find encouragement and support in dealing with daily anxieties and difficulties by becoming members of clubs and associations that welcome affected families.

A molecular diagnosis enables a female carrier of mutation to make informed reproductive decisions.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by Telethon (grant#GGP08125) to MVU and by Fondazione 'Roma-Terzo Settore' to MVU. AP is supported by a Postdoctoral fellowship from the Association Incontinentia Pigmenti France (IPF). The authors wish to thank the patients, their families and patient associations: Italian I.P.ASS.I. Onlus (www.incontinentiapigmenti.it), the Incontinentia Pigmenti International Foundation (IPIF) (www.ipif.org), and the Incontinentia Pigmenti France (http://www.incontinentiapigmenti.fr).

1 Scheuerle A, Ursini MV: Incontinentia Pigmenti; in Pagon RA, Bird TD, Dolan CR, Stephens K (eds) *GeneReviews*. University of Washington: Seattle (WA), 2010; pp 1993–1999.
2 Hayden MS, Ghosh S: Signaling to NF-kappaB. *Genes Dev* 2004; **18**: 2195–2224.
3 Nelson DL: NEMO, NFkappaB signaling and Incontinentia Pigmenti. *Curr Opin Genet Dev* 2006; **16**: 282–288.
4 Doffinger R, Smahi A, Bessia C *et al*: X-linked anhidrotic ectodermal dysplasia with immunodeficiency is caused by impaired NF-kappa-B signaling. *Nat Genet* 2001; **27**: 277–285.
5 Zonana J, Elder ME, Schneider LC *et al*: A novel X-linked disorder of immune deficiency and hypohidrotic ectodermal dysplasia is allelic to incontinentia pigmenti and due to mutations in IKK-gamma (NEMO). *Am J Hum Genet* 2000; **67**: 1555–1562.
6 Fusco F, Bardaro T, Fimiani G *et al*: Molecular analysis of the genetic defect in a large cohort of IP patients and identification of novel NEMO mutations interfering with NF-kappaB activation. *Hum Mol Genet* 2004; **13**: 1763–1773.
7 Fusco F, Pescatore A, Bal E *et al*: Alterations of the IKBKG locus and diseases: an update and a report of 13 novel mutations. *Hum Mutat* 2008; **29**: 595–604.
8 Fusco F, Paciolla M, Napolitano F *et al*: Genomic architecture at the Incontinentia Pigmenti locus favours de novo pathological alleles through different mechanisms. *Hum Mol Genet* 2012; **21**: 1260–1271.
9 Bardaro T, Falco G, Sparago A *et al*: Two cases of misinterpretation of molecular results in incontinentia pigmenti, and a PCR-based method to discriminate NEMO/IKKgamma gene deletion. *Hum Mutat* 2003; **21**: 8–11.
10 Steffann J, Raclin V, Smahi A *et al*: A novel PCR approach for prenatal detection of the common NEMO rearrangement in incontinentia pigmenti. *Prenat Diagn* 2004; **24**: 384–388.
11 Aradhya S, Bardaro T, Galgoczy P *et al*: Multiple pathogenic and benign genomic rearrangements occur at a 35 kb duplication involving the NEMO and LAGE2 genes. *Hum Mol Genet* 2001a; **10**: 2557–2567.
12 Fusco F, Paciolla M, Pescatore A *et al*: Microdeletion/duplication at the Xq28 IP locus causes a de novo IKBKG/ NEMO/IKKgamma exon4_10 deletion in families with Incontinentia Pigmenti. *Hum Mutat* 2009; **30**: 1284–1291.
13 Allen RC, Zoghbi HY, Moseley AB *et al*: Methylation of HpaII and HhaI sites near the polymorphic CAG repeat in the human androgen-receptor gene correlates with X chromosome inactivation. *Am J Hum Genet* 1992; **51**: 1229–1239.
14 Houdayer C, Lemonnier A, Gerard M *et al*: Improved fluorescent PCR-based assay for sizing CGG repeats at the FRAXA locus. *Clin Chem Lab Med* 1999; **37**: 397–402.
15 Hadj-Rabia S, Froidevaux D, Bodak N *et al*: Clinical study of 40 cases of incontinentia pigmenti. *Arch Dermatol* 2003; **139**: 1163–1170.