

# Guidelines for the genetic diagnosis of hereditary recurrent fevers

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► Additional material is published online only. To view these files please visit the journal online (<http://dx.doi.org/10.1136/annrheumdis-2011-201271>).

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Accepted 8 April 2012

## ABSTRACT

Hereditary recurrent fevers (HRFs) are a group of monogenic autoinflammatory diseases characterised by recurrent bouts of fever and serosal inflammation that are caused by pathogenic variants in genes important for the regulation of innate immunity. Discovery of the molecular defects responsible for these diseases has initiated genetic diagnostics in many countries around the world, including the Middle East, Europe, USA, Japan and Australia. However, diverse testing methods and reporting practices are employed and there is a clear need for consensus guidelines for HRF genetic testing.

Draft guidelines were prepared based on current practice deduced from previous HRF external quality assurance schemes and data from the literature. The draft document was disseminated through the European Molecular Genetics Quality Network for broader consultation and amendment. A workshop was held in Bruges (Belgium) on 18 and 19 September 2011 to ratify the draft and obtain a final consensus document. An agreed set of best practice guidelines was proposed for genetic diagnostic testing of HRFs, for reporting the genetic results and for defining their clinical significance.

## INTRODUCTION

Patients with hereditary recurrent fevers (HRFs) present with recurrent bouts of fever and inflammatory symptoms involving, in particular, the abdomen, joints and skin.<sup>1,2</sup> The causative genes for HRFs encode proteins involved in the regulation of innate immunity, mainly by affecting proinflammatory cytokines and apoptosis pathways. While familial Mediterranean fever (FMF) is relatively common in several Mediterranean and Middle Eastern populations,<sup>3</sup> most HRFs are rare diseases. The best-characterised HRFs are two recessively inherited diseases: (FMF, gene *MEFV*, MIM 608107) and mevalonate kinase deficiency (MKD, gene *MVK*, MIM 251170) and two dominantly inherited diseases: tumour necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS, gene *TNFRSF1A*, MIM 191190) and cryopyrin-associated periodic syndrome (CAPS, gene *NLRP3*, MIM 606416). Patients with HRF often display similar inflammatory symptoms with variable intensity and localisation of symptoms, making their clinical diagnosis difficult.

Since the discovery of these four HRF genes, almost 700 nucleotide variants have been identified and recorded in Infevers, a database dedicated to autoinflammatory sequence variants (<http://fmf.igh.cnrs.fr/ISSAID/infevers/>).<sup>4-6</sup> Some of these variants are clearly pathogenic, but most are unconfirmed or seemingly non-pathogenic variants. A significant number of patients clinically diagnosed with recessive HRFs have been found to carry only one disease-associated mutation in the respective genes<sup>7</sup> despite extensive searching for a second pathogenic mutation in the coding region,<sup>8,9</sup> and continuing search for mutations affecting regulatory sequences or transcript splicing that would affect gene expression.<sup>10,11</sup>

Genetic testing for HRF is a logical and feasible way to corroborate clinical diagnosis.<sup>12,13</sup> Five-year experience of external quality assessment and proficiency testing (PT) (external quality assurance/PT) conducted between 2006 and 2010 showed that although there has been an impressive improvement in the quality of HRF testing and reporting, many issues still remain to be addressed and standardised.<sup>14</sup> Guidelines using the standard definition by Field and Lohr<sup>15</sup> are now proposed to provide a framework for best laboratory practice and reporting on the genetic diagnosis of HRFs as agreed by an international consortium of experts in the field. They are intended to be used primarily by molecular geneticists and by other health professionals involved in the care of these patients.

## METHODOLOGY

A draft report was written by the organisers and assessors of the European Molecular Genetics Quality Network for HRFs with reference to relevant literature, reviews of reports issued during the five previous international HRF meetings, web-based resources relating to the subject and examples of guidelines for other hereditary diseases (eg, haemochromatosis,<sup>16</sup> cystic fibrosis<sup>17</sup> and von Willebrand<sup>18</sup> diseases). The draft was disseminated to molecular geneticists and clinicians working in the field of HRFs and discussed with them during a best practice workshop held in Bruges (Belgium) on 18 and 19 September 2011. In the light of feedback of the participants, amendments were made and a second draft was disseminated by email, after which, the final document was ratified.



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## INDICATIONS FOR HRF TESTING

### Minimal requirements for the genetic test

We suggest that the following minimum set of requirements should be obtained: patient name, date of birth, gender, ethnicity/origin, written informed consent (depending on country-specific law), referring doctor's name and contact details of the person who will receive the results.

### Symptomatic patients

The main indication for genetic testing of HRFs is in the case of a patient with a clinical symptom pattern consistent with one or more of the syndromes. Thus, clinical data that justify the choice of one or more HRFs genetic tests are required. It is not unusual that when overlapping, partial or atypical clinical symptoms impede an accurate clinical diagnosis, screening of several HRF-responsible genes gives the correct diagnosis.

The clinical HRF referral usually includes the frequency of attacks, duration, sites affected, acute phase reactants levels, biomarkers for mevalonic aciduria or amyloidosis and a letter by an expert clinician. An example of a clinical chart is provided in online supplementary figure S1. It has been established in France by GenMAI, the national network for genetic diagnosis of auto-inflammatory diseases, in conjunction with the clinical reference centres. Decision trees for genetic diagnosis in atypical patients and patients with sporadic disease have been proposed.<sup>13–19</sup> In addition, a diagnostic score for children with periodic fever has been elaborated in Italy.<sup>20</sup>

### Presymptomatic diagnosis and carrier status

In general, presymptomatic diagnosis is not advisable, as its interpretation is inconclusive, may be complicated for mutations with incomplete penetrance and it usually does not call for medical intervention. Presymptomatic testing may be recommended after genetic counselling for asymptomatic family members when a severe genotype has been found in relatives with an overt disease, or if there is a family history of amyloidosis. Follow-up of people at risk may avoid occurrence of this life-threatening complication. However, whether such cases should be given prophylactic treatment remains controversial. Evaluation of carrier status could be recommended in healthy relatives to phase two known disease-associated or new mutations, when identified in an affected patient.

### Prenatal diagnosis and preimplantation genetic diagnosis

Generally prenatal diagnosis (PND) and preimplantation genetic diagnosis (PGD) are not considered appropriate for HRF as most of these conditions are treatable and symptoms usually decrease over time. However, it may be appropriate to discuss PND or PGD in families affected by a particularly severe form of MKD or chronic infantile neurological, cutaneous and articular syndrome (CINCA) as these disorders can be associated with debilitating complications: blindness, deafness, mental retardation, ataxia and bone deformation. However, most severe CINCA-associated mutations occur *de novo* and this should be considered before offering PND and PGD. If PND is planned, it should be performed after genetic counselling.

## DIAGNOSTIC STRATEGY

### Diagnostic laboratories and expert structures

Genetic diagnosis for HRFs is now widely available. There are 99 laboratories providing FMF testing in Europe (source Orphanet at [www.orpha.net](http://www.orpha.net)).<sup>21</sup> A registry of sequence variants was developed online at <http://fmf.igh.cnrs.fr/ISSAID/infevers> to assist the

molecular geneticist. It provides a comprehensive and updated list of gene variants and a reference database for the mutation nomenclature, but an accurate phenotype–genotype correlation is not available. In addition, clinical reference centres were formally nominated or are recognised in several countries (France, Italy, Spain, UK, Germany, Turkey, Israel, USA...). As HRFs are rare diseases mostly caused by single-nucleotide substitutions, genetic testing should be referred to specialised laboratories to ensure that pertinent tests are performed and proper information is reported to clinicians, particularly those inexperienced with HRFs. These laboratories should work within a comprehensive quality management system (accreditation), use validated methods, participate annually in interlaboratory comparisons such as external quality assessment and proficiency testing for HRF and/or the relevant techniques (eg, DNA sequencing) and define a typical turn-around time.

### Testing strategy

Most laboratories focus the molecular analysis on mutational hot-spot regions in various genes. Recommendations on the reference sequence to be used for analysis and for the extent of the initial mutation screening are provided in table 1. The minimum diagnostic screen should include variants that are clearly shown to be pathogenic and that are frequently identified in patients. Although this screening recommendation is valid worldwide, the ethnic background of the patient needs to be considered. For instance, the four clearly pathogenic *MEFV* variants are almost exclusively found in Mediterranean populations, while the frequency of the debated p.Glu148Gln (NM\_000243.2:c.442G→C) variant is as high as 20% in Asiatic countries.<sup>22</sup> The p.Phe479Leu (NM\_000243.2:c.1437C→G) is especially relevant in Greek and Iranian patients.<sup>22</sup> The p.Pro75Leu (NM\_001065.3:c.224C→T) (usual name P46L) of *TNFRSF1A* is commonly found in Arabic and African populations.<sup>23</sup> Accordingly, many laboratories have adopted a two-step strategy—that is, an initial search for the most common pathogenic variants followed, if necessary, by an extended search spanning the complete coding sequence of the various genes. For the *MEFV* gene, there is limited utility in searching for rare variants for patients with clinically established FMF and no mutations in exons 2, 3, 5 and 10.

### Techniques

A variety of techniques are used to identify HRFs gene sequence variations but direct mutation analysis by DNA sequencing is the method employed by most laboratories. Other methods include PCR with restriction enzyme digest, allele-specific PCR, PCR-single-strand conformation polymorphism and primer extension and reverse hybridisation-based kits. Commercially available kits should specify whether they are CE-IVD (in vitro diagnostics) marked or Food and Drug Administration approved. General guidelines<sup>18</sup> should be followed. No standard primer set is recommended for amplification of the essential regions in HRF genes, but:

1. Primer sequences should be regularly checked for underlying single-nucleotide polymorphisms (SNPs) particularly for highly polymorphic exons of the HRF genes (eg, *MEFV* exons 2 or 5).
2. PCR primer design should avoid possible amplification of sequences from homologous genes (eg, *NLRP* genes).

## INTERPRETATION

### Classification and validation of HRF sequence variants

The Human Genome Variation Society (HGVS) has recommended avoiding the use of 'mutation' and 'polymorphism' as



LABORATORY identifiers and contact information

**GENETIC DIAGNOSIS OF [ACRONYME HRF]**  
(*Extended name HRF*)

Name of referring health care professional :  
Date of receipt of the sample :

**Subject:** [*Family name, given name patient*]

Date of birth: --/--/----  
Patient ID: [*internal ID*]  
Type of sample: [*DNA/blood*]

**Indication for testing:**

**Method:** Method (sequencing, kit etc...) and extent of the screening (exons ---) of the [*HRF*] gene (ref seq: NM\_XXXX.X). This allows identification of XX% of the known mutations.

**Results:** Two [type of the variants] were found  
*Mutations and genotype to be given using HGVS nomenclature*

Mutations	DNA level	Protein level
1.	c.---	p.---
2.	c.---	p.---

Genotype:

p.[---];[---]	<b>if phased not allelic</b>
p.[---];---	<b>if phased allelic</b>
p.[---(;)-]	<b>if not phased</b>

**Interpretation:**  
[To be reported here as suggested in table 2]

**Recommendations:** Genetic counseling is advised.

**Date of report**  
**Name of the molecular geneticist(s)**

**Figure 1** A proposed model for reporting the genetic diagnosis of hereditary recurrent fevers (HRFs). This is an example of a recessive disease, showing the minimal items to be reported. A report should ideally highlight the major findings and not exceed one page. HGVS, Human Genome Variation Society.

p.Ser52Asn (NM\_000431.1:c.155G→A, MAF=0.09) of *MVK* and p.Gln703Lys (NM\_001243133.1:c.2107C→A, MAF=0.02) of *NLRP3*.<sup>27</sup> We believe that reporting these SNPs in the context of genetic diagnosis of HRF might mislead the report recipient.

**Genetic confirmation of the clinical diagnosis**

As for any Mendelian conditions, the definitive genetic diagnosis of HRFs is based on the finding of unambiguous mutations in the causative genes. Theoretically, identification of one single pathogenic variant in dominant diseases, homozygosity or compound heterozygosity (confirmed by studying the parental

alleles) in recessive disorders, should be enough to confirm the diagnosis. Of note, finding a single *MEFV* pathogenic variant in patients of Mediterranean origin does not exclude the possibility of disease-causing mutations in other HRF genes.<sup>28</sup> However, interpretation of a result should always take into account the sensitivity of the molecular screening strategy. Failure to identify a causal mutation in a given gene can almost never exclude the diagnosis. Indeed, the entire gene is not sequenced in most routine settings and even complete gene sequencing based on PCR technology could miss a pathogenic variant (primer variants, inversion of exons, duplication of exons...) Likewise mosaicism will be most likely missed by standard sequencing.

**Table 2** Guidelines for reporting and interpreting genetic results in the four main HRFs

a	Report of results	Interpretation
Patients with symptoms		
Recessive diseases (FMF or MKD)		
Two clearly pathogenic variants		
Phased	The patient is (homozygote or compound heterozygote) for two clearly pathogenic variants in the ( <i>MEFV/MVK</i> ) gene	This genotype confirms clinical diagnosis of (FMF/MKD). <i>If relevant add:</i> and is generally associated with a (mild or severe) phenotype
Not phased	Two clearly pathogenic variants were identified in the ( <i>MEFV/MVK</i> ) gene. They have (never/already) been reported in cis (complex allele)	This genetic result is consistent with clinical diagnosis of (FMF/MKD). Parental testing should resolve the issue of complex allele. <i>If relevant add:</i> proven homozygosity or compound heterozygosity with these two variants is generally associated with a (mild or severe) phenotype
One clearly pathogenic and one VUS		
Phased	The patient is compound heterozygote for one clearly pathogenic variant and one variant of uncertain clinical significance in the ( <i>MEFV/MVK</i> ) gene	This genotype could be consistent with clinical diagnosis of (FMF/MKD). <i>If relevant add:</i> and is generally associated with a (mild or severe) phenotype
Not phased	One clearly pathogenic mutation and one variant of uncertain clinical significance were identified in the ( <i>MEFV/MVK</i> ) gene. They have (never/already) been reported in cis (complex allele)	This genetic result could be consistent with clinical diagnosis of (FMF/MKD). Parental testing should resolve the issue of complex allele. <i>If relevant add:</i> proven homozygosity or compound heterozygosity with these two variants is generally associated with a (mild or severe) phenotype
Two VUS		
Phased	The patient is (homozygote or compound heterozygote) for two variants of uncertain clinical significance in the ( <i>MEFV/MVK</i> ) gene	Diagnosis relies on clinical judgement or criteria. <i>If relevant add:</i> possible association with a mild phenotype
Not phased	Two variants of uncertain clinical significance were identified in the ( <i>MEFV/MVK</i> ) gene	Diagnosis relies on clinical judgement or criteria. Parental testing should resolve the issue of complex allele. <i>If relevant add:</i> possible association with a mild phenotype
One clearly pathogenic variant	One clearly pathogenic variant was identified in the ( <i>MEFV/MVK</i> ) gene	Rare undetected variants may exist. Diagnosis relies on clinical judgement or criteria
One VUS or no variant	No pathogenic or one variant of uncertain clinical significance was identified in the ( <i>MEFV/MVK</i> ) gene	Rare undetected variants may exist. Diagnosis relies on clinical judgement or criteria. Refer to an expert clinician to consider other HRFs
Dominant diseases (TRAPS or CAPS)		
One clearly pathogenic variant	One clearly pathogenic variant was identified in the ( <i>TNFR1SF1A/NLRP3</i> ) gene	This genotype confirms clinical diagnosis of (TRAPS/CAPS). <i>If relevant add:</i> and is generally associated with a (mild/severe) phenotype
One VUS	One variant of uncertain clinical significance was identified in the ( <i>TNFR1SF1A/NLRP3</i> ) gene.	Diagnosis relies on clinical judgement or criteria. Refer to an expert clinician to consider other HRFs. <i>If rare or new add:</i> (parental testing/familial segregation) may help understanding the clinical significance of this variant
No variant	No pathogenic variant was identified in the ( <i>TNFRSF1A/NLRP3</i> ) gene	Rare undetected variants may exist. Diagnosis relies on clinical judgement or criteria. Refer to an expert clinician to consider other HRFs
Asymptomatic individuals		
Genotype consistent with HRF	Adapt from above	The individual is at risk of developing symptoms of HRF. <i>If relevant add:</i> or inaugural renal amyloidosis. It is recommended that acute phase reactants (CRP, SAA) and the kidney function (urine analysis) be regularly monitored
Recessive diseases (FMF or MKD)		
One sequence variant	The individual is a carrier for a (clearly pathogenic variant/variant of uncertain significance) in the ( <i>MEFV/MVK</i> ) gene	This individual is not likely to develop (MKD/FMF)

CRP, C reactive protein; SAA, serum amyloid A; VUS, variant of uncertain clinical significance (includes debated variants and rare and novel variants with no relevant information).

## REPORTING

We recommend that genetic test results should be sent to the doctor(s) designated by the patient and not directly to the patient. The referring doctor should be invited to contact the laboratory if they have not fully understood the clinical significance of the test result. In the case of family studies, genetic results should not be communicated to other relatives without their consent. We recommend that a patient's information on genetic testing results should be given by a doctor skilled in the field of HRFs or by an expert geneticist, if available.

We strongly suggest that the reports include all items recommended by the OECD quality assurance in genetic testing (<http://www.oecd.org/dataoecd/43/6/38839788.pdf>) and match the international standard ISO-15189. The laboratory report should be limited to a single page, with the genetic

results and interpretation highlighted and the rest in smaller characters or presented in footnotes. A model report is proposed (figure 1).

## Genetic results

1. Variants should be described at both the protein and nucleotide level and should comply with the latest version of HGVS nomenclature (<http://www.hgvs.org/mutnomen/>). It is preferable to use the three-letter amino acid code.
2. Wherever the sequence variant name has changed owing to renumbering of the start codon, both the standard and HGVS nomenclature should be reported, to allow the referring clinician access to the relevant literature. Examples are p.Arg121Gln (NM\_001065.3:c.362G→A) (common name R92Q) of *TNFRSF1A* and p.Val198Met

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(NM\_001243133.1:c.592G→A) (also known as V200M) of *NLRP3*.

3. The number for the reference sequence used should be provided.
4. A brief description of the testing technique and its clinical and analytical sensitivities for the specific population being screened (ie, extent of mutation screening and coverage of disease-associated mutations, region and limits of detection) should be provided.

### Interpretative comment

Typical cases together with suggested clinical interpretation are listed in table 2. Reports should state, at a minimum, if the genotype is consistent or not with a diagnosis of HRF. Where the diagnosis is not confirmed genetically, the report should not state that a diagnosis of HRF is excluded. In this situation diagnosis relies on clinical judgement or criteria.

Additional comments in the report may refer to the presumed effect of the sequence variant on protein function and suggest further genetic testing or clinical management. Comments related to genotype–phenotype correlations are appropriate if there is enough evidence in the literature to support them. They are critical when discussing the risk of amyloidosis in patients with FMF with mutations affecting codons 680 or 694 of the *MEFV* gene, in patients with TRAPS with cysteine pathogenic variants and in familial cases of HRF with a history of amyloidosis. A comment on mild disease outcome can be considered for genotypes with mild or low penetrance sequence variants.

It is preferable to recommend referral to genetic counselling and/or to a clinical reference centre rather than to comment directly on treatment options or the predicted risk for the offspring or other family members. The clinical reference centres are better placed to fully discuss these pertinent issues. Testing should be offered to other symptomatic family members and to the parents where this can help with interpretation of the proband's results.

### Clerical information

The other important items suggested by the OECD are summarised below:

- More than one identifier that unequivocally links the report to the patient (name, date of birth, internal reference laboratory number).
- The name of the referring healthcare professional and contact information.
- The indication for testing and specific medical information where it is relevant.
- The date of receipt of the sample and of report issuing.
- The laboratory contact information and the identity of the individual approving the report.

### CONCLUSION

A consensus set of best practice guidelines has been developed for molecular genetic testing of HRFs based on feedback received from experts in this field. The guidelines described here are aimed at improving the quality of HRF molecular diagnostics and promoting harmonisation and standardisation of laboratory test reports. Understanding the molecular pathology of these diseases, their heterogeneity and genotype–phenotype correlations is steadily evolving as more data become available from large population cohorts of patients and healthy controls. A particular challenge for the genetic diagnosis of HRF will be in

the interpretation of clinical relevance of variants that are found at low, but >1%, frequency in various populations. These may function as susceptibility alleles to inflammation rather than disease-associated mutations and as such may give rise to an inflammatory phenotype when inherited through digenic inheritance (in the form of double heterozygous). In that context we feel that these guidelines may need to be regularly updated.

### Useful links

Infervers: Registry of autoinflammatory mutations: <http://fmf.igh.cnrs.fr/ISSAID/infervers/>  
 ISSAID: Website of the International Society of Systemic Autoinflammatory Diseases: <http://fmf.igh.cnrs.fr/ISSAID/>  
 Eurofever: Registry of autoinflammatory patients: <http://www.printo.it/eurofever/>  
 Orphanet: Reference portal for information on rare diseases and orphan drugs: <http://www.orpha.net/>  
 HGVS: Reference for the nomenclature for the description of sequence variants: <http://www.hgvs.org/mutnomen/>  
 EMQN: European Molecular Genetics Quality Network: <http://www.emqn.org/emqn/>  
 For FMF: The Centre of Arab Genomic Studies (<http://www.cags.org.ae>) and the Israeli National Genetic Database (<http://www.goldenhelix.org/server/israeli/>)

**Contributors** IT led the project, organised the meeting, wrote the first draft of the paper and was aided by YS, LO and IA. BB and HH edited the paper for English language. All authors participated in the development of the guidelines and contributed to the writing of the paper.

**Acknowledgements** This study was supported and performed within the framework of the EU projects EuroGentest (FP6-512148), Eurotraps (coordination theme 1 (health) of the European Community's FP7, grant agreement number HEALTH-F2-2008-200923 and la Région Languedoc-Roussillon) and Eurofever (EAHC, Project No2007332). It was also supported by the French Ministry of Health. The authors are indebted to Drs Catherine Dodé, Laurence Cuisset and Isabelle Jéru from the GenMAL network for the elaboration of the clinical questionnaire for the genetic diagnosis of HRFs. The authors thank Dr Robert Elles for reviewing the paper and Outi Kamarainen and Simon Patton for overseeing the HRF EQA scheme of EMQN and for the logistic support of the meeting.

**Collaborators** Hatem El Shanti, Marianne Jakobsen, Kostas Kostantopoulos, Davide Martorana, Maria Grazia Pomponi, Alison Bybee, Catherine Rydlewski

**Funding** EU.

**Note added in proof** While this manuscript was in press, a new article by Verma et al demonstrated a possible functional role of pQ703K (Q705K) in the regulation of inflammation. This variant should therefore be considered a VUS (should be in normal letters in table 1). The Q705K Polymorphism in NLRP3 Is a Gain-of-Function Alteration Leading to Excessive Interleukin-1 $\beta$  and IL-18 Production. Verma D, Särndahl E, Andersson H, et al. *PLoS One*. 2012;7:e34977. Epub 2012 Apr 17.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Correction notice** This article has been corrected since it was published Online First.

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