Directions for clinical practice improvement in *HFE* gene mutation testing

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ABSTRACT

Objective: To audit the clinical indications for *HFE* gene mutation testing in a consecutive series of requests.

Design: Retrospective audit of reasons prompting 187 *HFE* test requests received between June 2003 and June 2005, by examination of the request form, hospital notes (when available) and, when required, information from the referring doctor.

Setting: A tertiary care public teaching hospital laboratory, Perth, Western Australia.

Main outcome measures: Reasons prompting requests for *HFE* genotype testing and compliance with accepted clinical indications (biochemical evidence of iron overload on repeated samples, or a first-degree relative with either haemochromatosis or a C282Y mutation).

Results: Insufficient clinical details in requests prevented the inclusion of interpretive comments in *HFE* genotype reports in 70 of 187 cases (37%). Re-evaluation after collation of the missing details for all but seven requests revealed that 103 of the 180 auditable requests (57%) had been prompted for reasons other than biochemical evidence of iron accumulation or family history.

Conclusions: A substantial proportion of *HFE* genotype test requests are made for inappropriate reasons. Clinical practice could be improved by educating doctors on the practical utility of this genetic test and by laboratories taking steps to secure the clinical information needed to include appropriate interpretive comments in their reports.

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A number of inherited and acquired disorders may result in iron overload; hereditary haemochromatosis (HH) is the most frequent in populations of northern European descent. HH was previously regarded as a distinct clinical entity with a single genetic aetiology. The discovery of HH-causative mutations in the *HFE* gene[1] revealed that both these assumptions were wrong.

HH is usually associated with homozygosity for the C282Y mutation in the *HFE* gene.[2] However, one in eight healthy Australians are heterozygous carriers of C282Y[3] and one in four are carriers of an H63D mutation,[4] which must be considered when interpreting *HFE* genotype results in patients with iron overload. Although homozygosity for the C282Y mutation was thought to predispose people to severe organ damage from excessive iron accumulation, a number of studies have shown that only in a minority of cases do people develop severe clinical disease or organ damage.[3,5-9]

From a clinical perspective, it is now evident that *HFE* mutations are not responsible for all cases of HH; other genes contributing to iron homeostasis have been identified,[2] although homozygosity for C282Y predominates among Australian HH patients, most of whom have western European ancestry.[10]

Biochemical testing for iron overload and genetic testing for *HFE* status are handled differently. The former will confirm or rule out current iron overload, while *HFE* testing, depending on the prior risk, will give a post-test probability of the patient developing iron overload. Clinical interpretation of *HFE* genotypes found in patients being investigated for iron overload and interpretation of *HFE* results in healthy first-degree relatives of a newly diagnosed patient can therefore vary substantially.

In our laboratory, we seek to offer appropriate guidance by including interpretive comments with our *HFE* genotype test results. However, in the absence of clinical details on the request form (a not uncommon occurrence) and relevant biochemical details, this is not always possible. With the objective of improving the quality of our *HFE* genotype reports, we retrospectively audited the clinical indications for a consecutive series of requests.

METHODS

We reviewed all requests for *HFE* genotyping received by the Biochemical Genetics Laboratory at Royal Perth Hospital (RPH) over a 25-month period (June 2003 – June 2005). The audit was registered with the Quality Unit in the RPH Division of Laboratory Medicine. Samples were forwarded either by doctors within RPH or from doctors (predominantly general practitioners) external to the hospital. When the clinical reason for the test had not been provided, the notes of hospital cases were reviewed, and external referring doctors were contacted directly.

Reasons prompting the requests for *HFE* genotyping were then audited for compliance with the indications specified in the Australian Medicare Benefits Schedule (MBS),[11] which are:

- elevated transferrin saturation or serum ferritin level on testing of repeated specimens;
- a first-degree relative with haemochromatosis; or
- a first-degree relative with homozygosity for the C282Y mutation or compound heterozygosity for recognised genetic mutations associated with raised susceptibility to iron overload.

RESULTS

We reviewed 105 requests from doctors within RPH and 82 requests from external doctors. Seventy of the 187 requests (37%) did not have sufficient accompanying clinical details to permit inclusion of a suitable interpretive comment with the result. Relevant details could not be retrospectively ascertained for seven of the 82 external requests. Re-evaluation of the remaining 180 requests after missing clinical details had been collated revealed that 103 (57%) were made for reasons outside the defined criteria for genotyping (Box). These included: problems possibly attributable to iron overload (ie, arthritis, diabetes, cardiomyopathy, porphyria cutanea tarda and hypogonadism), but without biochemical confirmation of iron overload; abnormal liver function test results, again in the absence of evidence of iron overload; the discovery of an isolated increase in serum ferritin; or having an HH-

Reasons for requesting *HFE* genotyping

Reason	Hospital requests	External requests	Total
Compliant with MBS indications	*38*	*39*	*77*
Elevated transferrin saturation or serum ferritin level on testing of repeated specimens	33	12	45
Family history (first-degree relative with either haemochromatosis or a C282Y mutation)	5	27	32
Not compliant with MBS indications	*67*	*36*	*103*
Abnormal liver function test results with unconfirmed biochemical evidence of iron overload	22	7	29
Abnormal liver function test results only	39	4	43
Unconfirmed biochemical evidence of iron overload only	2	13	15
Symptoms possibly attributable to iron overload in the absence of known iron or liver function test abnormalities	0	7	7
Affected partner or family history (not first-degree relative)	4	5	9
Unable to obtain patient details retrospectively	0	7	7
Total number of cases	105	82	187

MBS = Medicare Benefits Schedule.[11] ◆

affected partner or kin more distant than a first-degree relationship.

DISCUSSION

Good clinical laboratory practice extends to the interpretation of raw data,[12] which is difficult in the absence of clinical details. This is true for the interpretation of *HFE* genotype results and for offering guidance about the significance of the results for first-degree relatives. Previously reported experience with integrating clinical indications for *HFE* testing with genotype results has revealed the high utility of *HFE* test results for patients in whom testing is clinically justifiable.[13] As a prelude to integrating prior-risk data obtained from clinical details into *HFE* genotype reports, we retrospectively audited the clinical rationale for a consecutive series of samples forwarded for testing to our pathology service. Unexpectedly, we found that more than half the requests were for reasons not specified in the Australian MBS guidelines.[11]

This audit finding reveals that our laboratory's current practice of accepting and testing all samples forwarded for *HFE* genotyping is suboptimal. As well as receiving samples without accompanying clinical details, we encountered requests to genotype samples from patients suspected to have HH, but without biochemical confirmation of excessive body iron stores. A small proportion of requests received were for samples forwarded from distant relatives and healthy partners of HH-affected people.

Although not included in the MBS-defined indications, *HFE* testing of partners of HH-affected people has been demonstrated to be a cost-effective investigation that frequently eliminates the need for testing of children.[14] Our findings prompted us to consider both the validity of the indications defined in the MBS for *HFE* testing and the laboratory's expectation that clinicians fully appreciate the clinical utility and limitations of *HFE* genotype tests that they order. Comparison of the Australian MBS guidelines for *HFE* genotyping with the recommended guidelines in the United States[15] and United Kingdom,[16] which are also formulated for populations that originate predominantly from western Europe where *HFE* mutations are responsible for over 90% of HH, reveals a consistent approach across these jurisdictions.

Our findings complement a recent Dutch study, which evaluated the impact of a clinical guideline for targeted early detection and treatment of HH aided by *HFE* gene mutation testing and found that one in five *HFE* gene mutation test results had been interpreted incorrectly.[17] This is perhaps not surprising, particularly as HH is a relatively uncommon disorder, and only a minority of clinicians will have broad experience with the practical issues surrounding diagnosis in an index case and follow-up of high-risk first-degree relatives. To add to this difficulty, new information continues to emerge about the clinical significance of *HFE* mutations.[18]

These findings suggest a need for ongoing postgraduate education on the practical utility of genetic testing for HH, bearing in mind the differing needs of hospital specialists and GPs who serve patients with very different illness patterns. Our results support the findings of a survey of over 2500 American physicians, which concluded that many physicians have inadequate knowledge about haemochromatosis diagnosis.[19] A physician education program on detection and management of HH in the US resulted in earlier case detection and treatment of iron overload.[20]

The key question is how best to deliver the required education, particularly in the Australian context of an MBS-listed genetic test. A physician survey of an innovative laboratory medicine interpretive service at Massachusetts General Hospital in Boston,[21] which integrates clinical details and other relevant test results into the interpretation, revealed a high level of physician satisfaction with the service. The laboratory interpretive service involved a daily sign-out round, with a pathologist and a trainee "resident" reviewing each case and integrating the laboratory results with relevant clinical details to provide a clinically relevant, patient-specific interpretation of each patient's laboratory test results. A notable strength of the service was the delivery of test-ordering education to clinicians in the context of their daily workflow, which resulted in saved time and improvements to the diagnostic process. A similar approach to genetic testing for HH could be effective, particularly as a vehicle for targeted postgraduate education. The strategy could also be extended to include other genetic tests, where enhanced clinical care or reduced levels of inappropriate testing may be achieved by closer clinical laboratory liaison.

Some may question whether it is the laboratory's role to add interpretive comments to reports, particularly those of gene mutation analysis. It is uncommon in clinical biochemistry for individualised interpretive comments to be included on reports such as liver function tests, urea and electrolyte profiles. The high volume of such tests, as well as the frequent lack of clinical details, usually precludes this.[22] However, recent improvements in information technology are reducing the size of these barriers.[23] For more specialised and recently introduced investigations, particularly genetic tests such as *HFE* genotyping, our findings reveal opportunities for health cost savings and clinical practice improvement

that could be realised by targeted input at the critical points of assessing clinical indications for testing and preparing appropriate interpretive comments for genotype results.

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COMPETING INTERESTS

None identified.

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