Screening for Fabry disease in high-risk populations: a systematic review


Published in:
Journal of Medical Genetics

DOI:
10.1136/jmg.2009.072116

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Screening for Fabry disease in high-risk populations: a systematic review

G E Linthorst,¹ M G Bouwman,² F A Wijburg,² J M F G Aerts,³ B J H M Poorthuis,³ C E M Hollak¹

ABSTRACT
Introduction Fabry disease (FD) may present with left ventricular hypertrophy (LVH), renal insufficiency or stroke. Several studies investigated FD prevalence in populations expressing these symptoms. A systematic review was conducted to calculate the overall prevalence of FD in these cohorts.

Methods Online databases were searched for studies on screening for FD. Study population selection, screening methods and outcome of screening were recorded.

Results 20 studies were identified, 10 of which included both male and female patients. In all (n=19) studies with male and almost all (n=10) with female patients, α-galactosidase A (α-Gal A) activity was used as the screening method. In men on dialysis (10 studies), overall FD prevalence was 0.33% (95% CI 0.20% to 0.47%) and in women (6 studies) 0.10% (95% CI 0.0% to 0.19%). Combined prevalence of FD in patients with renal transplant was 0.38% in men (95% CI 0.07% to 0.69%) and 0% in women. In patients with LVH, selection of study population and differences in the method of screening hampered the calculation of an overall prevalence (ranging from 0.9% to 3.9% in men and 1.1% to 11.8% in women). In premature strokes (n=2 studies), overall FD prevalence was 4.2% (95% CI 2.4% to 6.0%) in men and 2.1% (95% CI 0.5% to 3.7%) in women.

Discussion The prevalence of FD in dialysis patients is 0.33% for men and 0.10% for women. The prevalence of FD in LVH is at least 1% for both genders. In women, most studies were performed with α-Gal A activity measurements as the screening tool, although this method fails to detect one third of female patients with FD, underestimating the overall prevalence in women.

Fabry disease (FD, Online Mendelian Inheritance in Man 301499) is an X-linked lysosomal storage disorder caused by a deficiency of the lysosomal hydrolase α-galactosidase A (α-Gal A). Glycosphingolipid accumulation (mainly globo- triaoseceramide, Gb-3) in different cell types ultimately results in organ dysfunction.¹² Historically, FD was believed to manifest primarily in men at puberty, with angiokeratoma, anhidrosis and acroparesthesias,³ followed by renal insufficiency, left ventricular hypertrophy (LVH) and strokes in the fourth or fifth decade of life. However, during the past decade, patients with α-Gal A deficiency with relatively few and isolated symptoms, such as isolated LVH or renal failure at later stages in life, had been described.⁴ Five initially, these patients were named atypical FD patients to distinguish them from patients with multiorgan symptoms at a young age, designated as classic FD patients. Currently, it is generally accepted that FD exhibits a vast phenotypic spectrum, lacking a clear genotype–phenotype correlation.⁶ This diversity in clinical presentation applies even more to carrier women. Some women with FD remain asymptomatic throughout their entire life, whereas others manifest symptoms with a severity comparable with male patients.⁷–⁹ The residual enzyme activity in women varies considerably, ranging from normal to nearly completely absent.

Clinical trials with intravenous administration of α-Gal A showed a beneficial response, with stabilisation of the disease, especially in less severely affected patients.¹⁰–¹³ In more advanced disease, documented by the presence of proteinuria or a glomerular filtration rate of less than 60 ml/min, renal function continues to deteriorate despite continued treatment.¹¹¹⁴ It is generally hypothesised that early treatment may be the best option to prevent organ damage, which is the subject of currently employed clinical trials. Unfortunately, the low prevalence of the disease, the variability in clinical expression and the paucity of specific clinical symptoms hamper easy recognition by physicians. Consequently, patients are often identified when irreversible organ damage is already present. More recently, it has been recognised that the prevalence is probably significantly higher than initially assumed. While data from the Dutch metabolic diagnostic centres resulted in an estimated birth prevalence of 1 in 238 000 in male newborns,¹⁵ a landmark study from Nakao et al.¹⁶ showed that 5% of Japanese men referred to a tertiary centre as having LVH are with α-Gal A deficiency. In addition, a pilot study on newborn screening for FD revealed a remarkably high prevalence.¹⁷ These findings, combined with the need for early diagnosis, have prompted several researchers to perform screening studies for FD in “high-risk” populations: patient populations that express a specific symptom that could be caused by FD, such as renal failure or LVH. In this review, we summarise the results of these studies. The specific issues around neonatal screening are not included in this review. In addition, we discuss currently applied methods of screening, their pitfalls and new techniques that may become of use in the near future.

METHODS We searched PubMed, Medline and Embase databases using the keywords “Fabry disease AND prevalence OR Screening” up until 2009. Additional studies were found by crosschecking references.
Only studies that screened a well-defined cohort for α-Gal A deficiency were selected. Small studies in which family members were screened after an initial diagnosis of FD in an index case were not included. We recorded selection of subjects, screening method, methods of confirmation of FD and outcome of screening. We also recorded the number of false positives. We separately analysed and discussed the outcome for the screening of male and female subjects. Patients already known to have FD but who were included in the studies were included in the prevalence calculation. Patients in whom no DNA confirmation was performed after a positive screening result for α-Gal A activity were excluded. The present study was evaluated in accordance with the Standards for Reporting of Diagnostic Accuracy principle.

RESULTS

Search results

Our search revealed 26 studies. Two studies on newborn screening were excluded.17 18 Two studies were excluded since they were not performed in recognised high-risk populations.19 20 Two studies were only published as an abstract and were excluded as well.21 22 Table 1 summarises the results of the screening studies. Of the 20 remaining studies, 9 were performed in male subjects only.51 62 3e29 One study was performed exclusively in women.30 The remainder (n=10) was done in a combined male and female cohort.31 40

In total, two patients who had a positive screening but in whom no DNA mutation analysis was performed were excluded: one patient from Utsumi et al’s study31 and one patient from Porsch et al’s study.27 In the latter study, the other identified FD patient had a mutation in the α-Gal A gene (Porsch, personal communication 2008), but it was not mentioned in the original publication. This patient was included in the analysis.

Dialysis and transplantation

Most screening studies have been carried out in patients on renal dialysis (n=12 of which four were carried out in Japan). In three studies, patients on chronic ambulatory peritoneal dialysis were also included,23 26 31 all other were performed in patients on haemodialysis only. A total of 7182 men were screened in 12 studies and 24 patients were found to have FD, corresponding with a mean prevalence of 0.33% (95% CI 0.20% to 0.47%) (see also figure 1). The detection rate of FD in the six studies comprising a total of 4179 female dialysis patients revealed four female patients diagnosed as having FD, corresponding with a prevalence of 0.10% (95% CI 0.0% to 0.19%) (see figure 2).

Two studies were performed in patients who had received renal transplantation in which 0.38% (6/1584, 95% CI 0.07% to 0.69%) of the studied men and 0% (0/395, 95% CI could not be calculated) of the studied women were shown to have FD. Most of the studies in dialysis and renal transplantation did not exclude patients and evaluated an unselected cohort. In four studies, a selection of patients eligible for screening was performed: in one study, patients were excluded with a prior diagnosis of FD24; in three other studies, those with a biopsy proven other cause of renal failure were excluded.27 36 37

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Selected Test</th>
<th>Confirmation</th>
<th>Fabry/n</th>
<th>False positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dialysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Utsumi 2000</td>
<td>HD+CAPD</td>
<td>No Plasma DNA</td>
<td>1/440 0/282</td>
<td>0 0</td>
<td></td>
</tr>
<tr>
<td>Nakao 2003</td>
<td>HD No Plasma</td>
<td>L DNA</td>
<td>6/514 *</td>
<td>0 *</td>
<td></td>
</tr>
<tr>
<td>Linthorst 2003</td>
<td>HD+CAPD</td>
<td>No Full blood DNA</td>
<td>1/508 *</td>
<td>0 *</td>
<td></td>
</tr>
<tr>
<td>Kotanko 2004</td>
<td>HD No DBS</td>
<td>L DNA</td>
<td>4/1516 0/964</td>
<td>49 32</td>
<td></td>
</tr>
<tr>
<td>Beki 2005</td>
<td>HD No L DNA</td>
<td>1/59 0/47</td>
<td>2 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ichinose 2005</td>
<td>HD No Plasma</td>
<td>L DNA</td>
<td>1/450 *</td>
<td>2 *</td>
<td></td>
</tr>
<tr>
<td>Malsaukien 2007</td>
<td>HD No DBS</td>
<td>L DNA</td>
<td>0/536 *</td>
<td>0 *</td>
<td></td>
</tr>
<tr>
<td>Tanaka 2005</td>
<td>HD No Plasma</td>
<td>L DNA</td>
<td>4/401 1/295</td>
<td>10 0</td>
<td></td>
</tr>
<tr>
<td>Andrade 2007</td>
<td>HD+CAPD No Plasma</td>
<td>L DNA</td>
<td>0/499 *</td>
<td>0 *</td>
<td></td>
</tr>
<tr>
<td>Merta 2007</td>
<td>HD No DBS P, L DNA</td>
<td>4/1521 1/1849</td>
<td>54 58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Terryn 2008</td>
<td>HD Yes DBS</td>
<td>L DNA</td>
<td>1/180 2/742</td>
<td>NA NA</td>
<td></td>
</tr>
<tr>
<td>Porsch 2008</td>
<td>HD Yes DBS DNA</td>
<td>1/558 *</td>
<td>24 *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>24 4</td>
<td></td>
</tr>
<tr>
<td>Renal transplantation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schoenmakere 2008</td>
<td>Renal Tx Yes DBS</td>
<td>L DNA</td>
<td>1/278 0/395</td>
<td>1 9</td>
<td></td>
</tr>
<tr>
<td>Kleinert 2008</td>
<td>Renal Tx No DBS, L</td>
<td>L DNA</td>
<td>5/1306 *</td>
<td>157 *</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>6 0</td>
<td></td>
</tr>
<tr>
<td>Hyperthropic cardiomyopathy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nakao 1995</td>
<td>LVH No Plasma</td>
<td>L DNA</td>
<td>7/230*</td>
<td>0 *</td>
<td></td>
</tr>
<tr>
<td>Sachdev 2002</td>
<td>HCMF Yes Plasma</td>
<td>DNA</td>
<td>6/153 *</td>
<td>0 *</td>
<td></td>
</tr>
<tr>
<td>Chimenti 2004</td>
<td>LVH Yes Tissue</td>
<td>DNA</td>
<td>* 4/034 *</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Monserrat 2007</td>
<td>HCMF No Plasma</td>
<td>DNA</td>
<td>3/328 2/180</td>
<td>3 7</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>16 6</td>
<td></td>
</tr>
<tr>
<td>Cryptogenic stroke</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rolfs 2005</td>
<td>Stroke Yes Plasma</td>
<td>DNA</td>
<td>21/432 7/289</td>
<td>1 3</td>
<td></td>
</tr>
<tr>
<td>Brouns 2007</td>
<td>Stroke Yes DBS</td>
<td>DNA</td>
<td>0/64 0/39</td>
<td>1 2</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>21 7</td>
<td></td>
</tr>
</tbody>
</table>

CAPD, chronic ambulatory peritoneal dialysis; DBS, dried blood spot; HCMF, hypertrophic cardiomyopathy; HD, hemodialysis; L, leukocytes; LVH, left ventricular hypertrophy; P, plasma; Tx, transplantation.

*Not relevant.
Other risk populations

Four studies were performed in cohorts of patients with cardiac hypertrophy. These studies differed in patient selection and method. Three studies were performed in male patients. These patients were selected either because of LVH on echocardiography, a diagnosis of hypertrophic cardiomyopathy or not otherwise explained LVH. The outcome of these studies in men, which were all performed by means of enzyme activity analysis, varied from 7/203 (3.4%) to 3/328 (0.9%) in unselected cohorts, and 6/155 (3.9%) in a selected cohort. The differences in selection criteria between these cohorts precluded the calculation of an overall prevalence. It should be noted that although Nakao and coworkers initially could not identify a mutation in the α-Gal A gene in five of seven patients, additional investigations revealed a mutation in this gene in all patients.

For the two studies that investigated female patients, a difference in screening method was noted as well. One study investigated 34 women with LVH with unknown cause using endomyocardial biopsies as a screening tool. Four patients were found to have FD (12%). In the only other study in a female cohort, enzyme activity analysis was applied as the screening method in an unselected population of women with hypertrophic cardiomyopathy. Of these patients, 2/180 (1.1%) were found to have FD. Again, the difference in patient selection and screening method precludes an overall estimation of prevalence in female patients with LVH/ hypertrophic cardiomyopathy.

Two studies reported on the prevalence of FD in patients with cryptogenic stroke. In both cohorts, other known causes of stroke were excluded. In these two studies, a prevalence of 4.9% (21/432) and 0% (0/64) was demonstrated, respectively, which corresponds to an overall prevalence of 4.2% (95% CI 2.4% to 6.0%) in men and 2.1% (95% CI 0.5% to 3.7%) in women.

Methods of screening

In all studies concerning male patients, α-Gal A activity analysis was used as the primary screening method. In the case of women, all studies but one used α-Gal A activity analysis. Sources for α-Gal A activity analysis were plasma (9/20, 45%), bloodspots (8/20, 40%), leucocytes (5/20, 15%) and whole blood (1/20, 5%). In a single study, two different methods for screening were applied: partly leucocytes and partly bloodspots. Histological analysis of myocardial biopsies was employed in one study. In all 20 studies, confirmation of positive screening was performed by mutation analysis.

The α-Gal A activity level serving as a threshold for positive screening varied widely in the different studies and ranged from <60% to <10%. In most studies in which both men and women were investigated, the same cut-off value was used for both sexes. The number of false positives in the entire cohort (data available from 18 studies in which enzyme activity analysis was used, thus comprising 19 methods in total) was 1.54% (0.50% to 3.38%) in men and 2.5 % (0.96% to 4.06%) in women. Bloodspots as screening tool yielded more false-positive results (4.2%, 95% CI 1.2% to 7.1%), when compared to plasma (1.0%, 95% CI –0.2% to 2.0%, p<0.01) or leucocytes (1.3%, 95% CI –0.4% to 3%, p=0.06). There was a statistically significant correlation between the percentage of false positives and the cut-off value of a positive test (Spearman’s r 0.44, p=0.04). Thus, setting a higher threshold of residual enzyme activity results in more false negatives. The exact number of false negatives cannot be retrieved from the publications included in this study.

DISCUSSION

We analysed 20 studies in which high-risk populations were screened for the presence of FD. These studies varied with respect to the type of organ involvement (renal, cardiac or neurologic), included patients (men or women or both) and the method of screening. The confirmation of diagnosis in all studies was done by DNA mutation analyses in men and women, ensuring a proper diagnosis and allowing pooling of the individual studies. One important limitation of combining the results is that the selection of patients was not identical for all studies: exclusion of other causes of disease was not always performed in the same way, which can influence the outcome. The prevalence of FD in the dialysis population has been studied most extensively and was shown to be 0.33% for men and 0.10% for women. Only a few studies have been performed in cohorts of patients with LVH. In two of these studies, other causes of LVH were excluded, causing a selection bias in comparison with two other studies in which these remained included. This bias most likely explains the large difference in prevalence observed in these studies (varying from 0.9% to 3.9% in men and 1.1% to 11.3% in women). These confounders preclude the calculation of a combined overall prevalence of FD in patients with LVH or hypertrophic cardiomyopathy. Two studies were carried out in patients with premature strokes, resulting in a combined prevalence of 4.2% in men and 2.1% in women. This is entirely due to the results of one study because in the other (smaller) study, not a single patient with FD was detected. Also, the detection of potential polymorphisms (see below) that may add to the high prevalence found in one study cannot be excluded since this report did not provide the mutations that were found in the identified FD patients. Patients found in this study seem to have less Fabry-related symptoms than those who...
are included in the available disease registries; for instance, cornea verticillata is seen in >70% in patients in one FD registry (Fabry Outcome Survey) and only ~25% of subjects in the study of Rolfs et al. This could be caused by the inclusion of patients with mutations that cause less severe disease, or polymorphisms. Given their limited number, and the differences in inclusion criteria, additional studies in renal transplantation, LVH and premature cerebrovascular accident are needed to allow a better calculation of the prevalence of FD in these groups.

To make a reliable comparison of studies, it should be defined which criteria result in a reliable diagnosis. Both α-Gal A activity or DNA mutation analysis have limitations as a criterion for diagnosing FD. Since we expect that the need for early screening for FD will increase in light of therapeutic developments, these limitations deserve further discussion.

**Enzyme activity analysis as a method of screening**

We found that the vast majority (91%) of the screening studies in women were performed using α-Gal A activity analysis as the primary screening method. It is known that enzyme analysis is not suitable for detection of female patients with α-Gal A deficiency, because of the random X inactivation. Recent studies again confirmed that irrespective of the source used (either bloodspot, plasma or leukocytes), up to one third of female FD patients is not identified by enzyme activity analysis (false-negative screening result). If one would extrapolate this to the entire cohort summarised in this article, with 19 women detected, nine patients could have been missed. Previously, it was thought that a high residual enzymatic activity might protect against symptoms. If this were true, missing women with high enzyme activity would be of no clinical significance in the context of screening for FD patients. However, recent studies revealed that there is no correlation between residual α-Gal A activity in plasma and severity of the disease in heterozygotes for FD. This is illustrated by four Dutch female FD patients with significant LVH who have high residual enzyme activity in plasma and leukocytes. These patients will not be detected in (LVH) screening studies that employ a cut-off value of <50%. Therefore, we believe that enzyme activity should not be used for screening for FD in women.

Enzyme activity analysis is usually reliable for screening male patients. Disease-causing mutations in the α-Gal A gene result in significant reduction of enzyme activity in cell extracts and plasma specimens as can be detected with artificial substrates. However, the finding of a reduced enzyme activity in such in vitro tests does not always render a firm diagnosis of FD. The D313Y substitution in the α-Gal A gene serves as example. While in male D313Y carriers, the plasma enzyme activity towards the artificial substrate is significantly reduced, additional studies demonstrated high residual enzyme activity and no abundant excretion of urinary Gb-3. As a result, the D313Y substitution is now generally considered to be a so-called pseudo-deficiency. The D313Y substitution is a quite common polymorphism that is present in at least 1% of the normal control population. In addition, in a recent report by Aerts et al., a family with the R112H mutation in the α-Gal A gene was found to exhibit no classic Fabry symptoms, near normal to normal levels of both plasma urinary GL-3 as well as the newly discovered lysoGL-3, despite a reduced leukocyte enzyme activity in the male subjects. Additional studies are under way to establish whether R112H is a true obligate disease-causing mutation. In conclusion, not all individuals with significantly reduced enzyme activity in in vitro tests develop symptomatic FD.

**DNA mutation analysis**

To reduce the chance of missing a diagnosis of FD in a female subject, screening for mutations on the α-Gal A gene can be performed. This is a laborious method since FD does not exhibit common mutations and full sequencing of the α-Gal A gene is required. Unless there is a male family member with the same mutation, absent α-Gal A activity and symptoms that are consistent with FD, predicting whether a mutation in the α-Gal A gene is pathogenic is difficult. This might even hold true if the same mutation has been described in other male patients with classic symptoms of FD or if the particular mutation has been described in male patients with classic FD symptoms. The aforementioned D313Y mutation may be found in screening patients for FD. Following its classification as a polymorphism causing pseudo-deficiency, some authors already excluded patients with this sequence in the screening studies. Thus, some presumed mutations in the α-Gal A gene may in fact be a polymorphism not causative of FD. It can be a priori not be excluded that there is no clear distinction between a mutation and polymorphism: in some individuals, a particular amino acid change in α-Gal A may be without consequences and in another individual this may be different. More insight into disease-causing mutations and possible pseudo-deficiencies or polymorphisms as well as the role of epigenetic factors is urgently needed, especially since introduction of FD in newborn screening programmes is currently being considered. In addition, a recent study revealed that a high number of male Taiwanese newborns had a reduced α-Gal A activity and a mutation: c.956+919G>A (IVS4+919G>A). It is at present unclear whether this mutation is associated with the clinical symptoms seen in FD. Given these considerations and the fact that long-term outcome of treatment in (asymptomatic) patients is currently unknown, newborn screening for FD does not yet meet the well-established criteria for efficacious screening.

**Alternative screening methods**

Other screening techniques were evaluated that have to be equally robust as α-Gal A activity in male patients, less laborious than DNA mutation analysis in female studies, but could be applicable for both genders. Candidates could be a combination of different lysosomal enzymes, the measurement of surrogate markers, the measurement of accumulating substrates or a combination of these. Meikle and colleagues showed that a ratio of saposin C to α-Gal A protein in blood spots could distinguish all four studied female FD patients from the control group. Their results have not yet been confirmed in a larger population. Assessment of Gb-3, the main accumulating glycosphingolipid in FD, as screening method for women with FD failed to give reliable results, with some of the heterozygotes not being detected. Another study using urine spots on filter paper could not detect atypical cardiac variants of FD in men and women, and this method does not seem suitable for use in children below the age of 6 months. Other study using urine spots on filter paper could not detect atypical cardiac variants of FD in men and women, and this method does not seem suitable for use in children below the age of 6 months. It is possible that FD patients do not express this enzyme activity in urine, or that the activity is too low to be detected. Therefore, urine analysis is not a suitable method for detecting FD in screening studies. Recently, a new plasma abnormality, lysoGb-3 (or lysoCTH) has been identified in FD. The sensitivity to detect symptomatic women by means of GL-3 measurement in bodily fluids remains to be established. To conclude, apart from enzyme activity analysis in men and α-Gal A gene analysis in men and women, there are currently no other well-established methods of screening for FD.
In conclusion, a considerable number of patients with one of the major symptoms of FD can be identified by screening. Since enzyme treatment is available and treatment should start pref-erably before end-organ damage is irreversibly present, FD should be considered in the differential diagnosis of patients with LVH, renal failure or stroke. In women, most studies were performed with α-Gal A activity measurements as screening tool, despite the knowledge that this method fails to detect one third of female patients with FD. This would imply that in addition to the observed 19 patients detected by screening in all studies combined, approximately nine may have been missed (being false negative). DNA mutation analysis as a screening method carries the risk of identifying polymorphisms of the α-Gal A gene that are sometimes mistakenly seen as obese disease-causing mutations (eg, the D513Y mutation).

Competing interests None to declare.

Contribution All authors took part in writing and correcting the manuscript.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES


Copyright © 2010 British Society for Genetic Medicine.


45. Linthorst GE, Poorthuis BJ, Hollak CE. Enzyme activity for determination of presence of Fabry disease in women results in 40% false-negative results. J Am Coll Cardiol 2008;51:2082; [author reply -3].


Screening for Fabry disease in high-risk populations: a systematic review

G E Linthorst, M G Bouwman, F A Wijburg, et al.

*J Med Genet* 2010 47: 217-222 originally published online September 24, 2009
doi: 10.1136/jmg.2009.072116

Updated information and services can be found at:
http://jmg.bmj.com/content/47/4/217.full.html

These include:

References
This article cites 54 articles, 19 of which can be accessed free at:
http://jmg.bmj.com/content/47/4/217.full.html#ref-list-1

Article cited in:
http://jmg.bmj.com/content/47/4/217.full.html#related-urls

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/