Short Report

Regional differences in the frequency of the c.985A>G ACADM mutation: findings from a meta-regression of genotyping and screening studies


Several countries include medium-chain acyl-CoA dehydrogenase (MCAD) deficiency, a rare autosomal recessive disease, in their newborn screening programmes despite prevalence uncertainty. We estimated the frequency of its most common mutation, c.985A>G, tested for regional differences and compared screening and genotype frequencies. We identified 43 studies reporting the frequency of c.985A>G over 10 million individuals, and pooled frequency data using a novel Bayesian approach. We found significant variation in the frequency of the mutation across regions supporting a reported founder effect. The proportion of c.985A>G homozygotes was highest in Western Europe with 4.1 (95%CI: 2.8–5.6) per 100,000 individuals, then the New World (3.2, 95%CI: 2.0–4.7), Southern (1.2, 95%CI: 0.6–2.0) and Eastern European regions (0.9, 95%CI: 0.5–1.7). No cases with the mutation were identified in Asian and Middle Eastern regions. Significant differences were found in some countries between the genotype and screening allele frequency of c.985A>G. Our predictions could inform the frequency of the mutation by region and our approach could apply to other genetic conditions.

Conflict of interest

The authors report no conflict of interests.

Medium-chain acyl-CoA dehydrogenase deficiency (MCAD deficiency, MIM ID#201450) is a recessively inherited genetic disorder caused by mutations in the ACADM gene (HGNC:89). Most symptomatic MCAD deficiency cases present early with 20–25% of children dying at first presentation and survivors facing an average 6% risk of developing severe neurocognitive impairments (1). Thirteen European countries, Australia, Canada, New Zealand, and the United States include MCAD deficiency in their newborn screening programmes (2–6).

The most common mutation replaces the amino acid lysine with the amino acid glutamic acid at position 304 in the MCAD enzyme (c.985A>G ). Almost 80% of clinically identified individuals are homozygous for c.985A>G, 18% are heteroallelic and heterozygous for c.985A>G, and 2% have the two ACADM alleles with rare gene variations associated with the disease (7). Frequency estimates of c.985A>G mutation are available directly from DNA samples in genotyping studies and indirectly, following biochemical presentation, from newborn screening programmes using tandem mass spectrometry (MS/MS).

We combined genotyping and screening surveys systematically to estimate c.985A>G allele and homozygote frequency across several regions and explore the
Materials and methods

Literature search

We conducted a literature search and identified 24 studies reporting the genotype frequency of c.985A>G mutation and 19 studies reporting the frequency of c.985A>G homozygotes from MCAD deficiency newborn screening studies (Appendix S1, Tables S1 and S2). Two studies (North Carolina and Paris) reported the frequency of c.985A>G in Caucasian populations (8, 9). Asian (Japanese, Chinese, and South Korean), Middle Eastern and African American newborn populations were represented in eight studies (8, 10–16). However, detailed and comparable ethnic information was difficult to find in other studies. For example, only three of the remaining MS/MS screening studies provided the ethnicity of the screened populations and c.985A>G homozygotes (17–19). Therefore, we assumed these populations to be mostly White, given location (Europe, North America and Australia) where most births are of White ethnicity, and that these data could be pooled to obtain more precise frequency estimates. We grouped countries into four regional categories: New World, Southern Europe, Western Europe and Eastern Europe (Table S4); based on previous genetic mapping (20).

Statistical methods

Meta-regression to estimate frequency by world region

Following Hardy-Weinberg (HW) principles (21), we used a Bayesian meta-regression to pool genotype and MS/MS screening data and estimate the allele frequencies of c.985A>G using regional location to explain heterogeneity (Appendix S1). Two logistic models were considered:

- M1: Fixed Effects within region – allele frequencies depend on geographical region but are identical within region;
- M2: Random Effects within region – allele frequencies vary randomly around regional means;

We used the models to synthesize 52 regional observations representing 8,177,253 individuals (63,621 in genotype data), of which 315 were homozygotes (4 in genotype data). As there were no carriers of c.985A>G mutation in Asian and Middle Eastern population studies, these data were not synthesized. The frequency of c.985A>G mutation is reported separately for African Americans.

Modelling was carried out using a Bayesian Markov Monte Carlo (MCMC) simulation via WinBUGS v.1.4.3 (22). Model selection was based on the posterior corrected mean deviance (Dbar), and the Deviance Information Criterion (DIC) (23).

Results

Frequency by world region

Table 2 reports the logistic models results. M1 (fixed effects) had a lower overall goodness of fit than M2 (random effects), which showed a good fit (Dbar similar to the number of data points, 89). The data with the poorest fit were the observations from Bulgaria and Spain, where homozygotes were reported despite the low allele frequency of c.985A>G in samples.

Southern and Eastern European countries had a significantly lower frequency of c.985A>G compared to the Western European group, with odds ratios of 0.52 (95% CI: 0.37–0.74) and 0.47 (95% CI: 0.32–0.68), respectively. Observations from the New World were also associated with a lower mutation frequency, but not significantly (OR: 0.87, 95% CI: 0.67–1.16).

Figure 1 shows the posterior estimates of the frequency of homozygotes for c.985A>G by region and the regional fixed effects and random effects means. Table 3 reports the posterior allele frequency of c.985A>G mutation by region with the expected number of homozygotes and carriers obtained from M2. The allele frequency of c.985A>G in the African American and Asian/Middle East populations was estimated at 1.1 (95% CI: 0.1–3.5) and 0 per 1000 alleles, respectively. The expected number of homozygotes and heterozygotes for the c.985A>G mutation amongst African Americans was estimated at 0.2 (95% CI: 0.0–1.2) and 218 (95% CI: 21–704) per 100,000 individuals, respectively.

Comparing screening and genotype frequencies

Table 4 compares the allele frequency of c.985A>G mutation in the MS/MS screening and genotyping studies. The allele frequency in MS/MS studies was significantly higher in Germany (OR: 1.7, 95% CI: 1.1–2.6), the Netherlands (OR: 1.5, 95% CI: 1.0–2.2) and Spain (OR: 2.2, 95% CI: 1.2–4.0) compared to genotyping data. Overall, our models showed good overall fit to the data, (Dbar close to number of data points) with the exception of genotype data from Spain, which showed poor fit to the HW principles.
Regional differences in the frequency of the c.985A>G ACADM mutation.

**Western Europe**
- BE [2]
- DK [8]
- FR [13]
- FR [14]
- FR [15]
- DE [17]
- NL [21]
- SE [26]
- CH [27]
- UK [30]
- UK [31]
- UK [32]
- UK [33]
- UK [34]
- DK [35]
- UK [44]
- UK [55]
- NL [66]

**Eastern Europe**
- CZ [6]
- EE [10]
- FI [11]
- FI [12]
- HU [18]
- HU [19]
- PL [22]
- RU [23]

**Southern Europe**
- BG [3]
- IT [20]
- ES [24]
- ES [25]
- TR [26]
- TR [27]
- GR [s7]
- IT [s8]
- ES [s10]
- ES [s11]

**New World**
- AU [1]
- CA [4]
- CA [5]
- US [35]
- US [36]
- US [37]
- AU [s1]
- CA [s2]
- US [s12]
- US [s13]
- US [s14]
- US [s15]

**Fig. 1.** Forest plot of posterior estimates of the frequency of homozygotes for the c.985A>G mutation. Information in brackets refers to the study number in Tables S1 and S2. FE: Fixed effects mean and 95% CI; RE: random effects mean and 95% CI; PR: predicted frequency within region if new study was to be performed (mean and 95% CI). The predictive prevalence can be interpreted as a predictive distribution of the birth prevalence we might expect in a new study in that region. Country names were coded as follows: AU: Australia, BE: Belgium; BG: Bulgaria; CA: Canada; CH: Switzerland; CZ: Czech Rep; DE: Germany; DK: Denmark; EE: Estonia; ES: Spain; FI: Finland; FR: France; GR: Greece; HU: Hungary; IT: Italy; NL: Netherlands; PL: Poland; RU: Russia; SE: Sweden; TR: Turkey; UK: United Kingdom; US: United States.
Table 1. Allele frequency of the c.985A>G mutation in countries with both genotype and biochemical newborn screening data

<table>
<thead>
<tr>
<th>ID</th>
<th>Type</th>
<th>Country</th>
<th>Region</th>
<th>c.985A&gt;G allele frequency per 1000a (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>G</td>
<td>Australia</td>
<td>Melbourne</td>
<td>7.0 (2.2–14.4)</td>
</tr>
<tr>
<td>s1</td>
<td>S</td>
<td>New South Wales</td>
<td></td>
<td>5.3 (4.1–6.6)</td>
</tr>
<tr>
<td>4</td>
<td>G</td>
<td>Canada</td>
<td>Province of Manitoba</td>
<td>3.2 (1.8–5.2)</td>
</tr>
<tr>
<td>5</td>
<td>G</td>
<td>Canada</td>
<td>Quebec city</td>
<td>7.1 (5.8–8.5)</td>
</tr>
<tr>
<td>s2</td>
<td>S</td>
<td>Canada</td>
<td>Province of British Canada</td>
<td>7.4 (4.8–10.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Yukon</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>G</td>
<td>Denmark</td>
<td>Copenhagen</td>
<td>5.0 (2.3–8.6)</td>
</tr>
<tr>
<td>9</td>
<td>G</td>
<td>Denmark</td>
<td>Whole country</td>
<td>4.9 (3.0–7.3)</td>
</tr>
<tr>
<td>s3</td>
<td>S</td>
<td>Denmark</td>
<td>Whole country</td>
<td>6.9 (5.6–8.3)</td>
</tr>
<tr>
<td>17</td>
<td>G</td>
<td>Germany</td>
<td>Bavaria, Schleswig-Holstein</td>
<td>4.3 (2.8–6.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Westfalen</td>
<td></td>
</tr>
<tr>
<td>s6</td>
<td>S</td>
<td>Germany</td>
<td>Bavaria</td>
<td>7.2 (5.9–8.5)</td>
</tr>
<tr>
<td>20</td>
<td>G</td>
<td>Italy</td>
<td>Piedmont</td>
<td>1.5 (0.3–3.6)</td>
</tr>
<tr>
<td>s8</td>
<td>S</td>
<td>Italy</td>
<td>Provinces of Florence, Prato, Pistoia and Tuscany</td>
<td>4.2 (2.0–6.8)</td>
</tr>
<tr>
<td>21</td>
<td>G</td>
<td>Netherlands</td>
<td>Not reported</td>
<td>8.0 (6.5–9.7)</td>
</tr>
<tr>
<td>s9</td>
<td>S</td>
<td>Netherlands</td>
<td>Northern part</td>
<td>12.2 (8.6–16.2)</td>
</tr>
<tr>
<td>24</td>
<td>G</td>
<td>Spain</td>
<td>Catalonia</td>
<td>3.5 (1.4–6.5)</td>
</tr>
<tr>
<td>25</td>
<td>G</td>
<td>Spain</td>
<td>Whole country</td>
<td>2.5 (1.2–4.2)</td>
</tr>
<tr>
<td>s10</td>
<td>S</td>
<td>Spain</td>
<td>Murcia</td>
<td>6.2 (2.9–10.0)</td>
</tr>
<tr>
<td>s11</td>
<td>S</td>
<td>Spain</td>
<td>Galicia</td>
<td>5.8 (3.7–8.1)</td>
</tr>
<tr>
<td>31</td>
<td>G</td>
<td>England</td>
<td>Trent</td>
<td>7.3 (2.7–14.3)</td>
</tr>
<tr>
<td>32</td>
<td>G</td>
<td>England</td>
<td>Trent</td>
<td>6.0 (4.6–7.6)</td>
</tr>
<tr>
<td>33</td>
<td>G</td>
<td>England</td>
<td>West Midlands</td>
<td>12.6 (6.5–20.5)</td>
</tr>
<tr>
<td>34</td>
<td>G</td>
<td>England</td>
<td>West Midlands</td>
<td>9.6 (7.8–11.5)</td>
</tr>
<tr>
<td>s4</td>
<td>S</td>
<td>England</td>
<td>Six regions</td>
<td>7.0 (6.3–7.8)</td>
</tr>
<tr>
<td>s5</td>
<td>S</td>
<td>England</td>
<td>Northern region</td>
<td>8.2 (5.3–11.4)</td>
</tr>
<tr>
<td>35</td>
<td>G</td>
<td>USA</td>
<td>Monroe County, New York</td>
<td>7.1 (0.2–26.3)</td>
</tr>
<tr>
<td>37</td>
<td>G</td>
<td>USA</td>
<td>North Carolina</td>
<td>5.9 (4.0–8.2)</td>
</tr>
<tr>
<td>36</td>
<td>G</td>
<td>USA</td>
<td>Houston, Texas</td>
<td>1.0 (0.1–2.9)</td>
</tr>
<tr>
<td>s13</td>
<td>S</td>
<td>USA</td>
<td>New York state</td>
<td>3.6 (2.9–4.4)</td>
</tr>
<tr>
<td>s14</td>
<td>S</td>
<td>USA</td>
<td>North Carolina</td>
<td>6.9 (5.9–7.9)</td>
</tr>
<tr>
<td>s15</td>
<td>S</td>
<td>USA</td>
<td>New England</td>
<td>5.3 (4.1–6.5)</td>
</tr>
<tr>
<td>s12</td>
<td>S</td>
<td>USA</td>
<td>Pennsylvania, Ohio, New Jersey, Illinois, Florida, and North Carolina</td>
<td>5.8 (3.7–8.1)</td>
</tr>
</tbody>
</table>

Refers to study number in Tables S1 and S2.

aG, Genotype study; S, MS/MS newborn screening study.

In genotyping studies, allele frequency was estimated as number of c.985A>G alleles divided by total number of alleles in the population (two times the sample size). In biochemical newborn screening studies, allele frequency was estimated using the HW equation, that is squared root of the frequency of c.985A>G homozygotes.

Discussion

Our meta-regression approach to explore the frequency of c.985A>G mutation across regions supports previous hypotheses that this mutation is of predominantly Northern European origin (8). No alleles with c.A985A>G mutation were identified in studies involving Asian (Japanese/Chinese/South Korean) and Middle Eastern populations, whereas Western and Northern European regions were associated with the highest frequency of c.985A>G mutation. Furthermore, the proportion of c.985A>G homozygotes in the Western European region was estimated as 4.1 (95%CI: 2.8–5.6) per 100,000 individuals. Although some studies may have frequencies of the c.985A>G mutation that appear different from their regional means, these differences are explained by the variation within region.

We also identified significant differences in the allele frequency of c.985A>G from genotyping and MS/MS screening studies in Germany, the Netherlands and Spain. The frequency of c.985A>G reported in MS/MS screening programmes, was almost twofold than that in corresponding genotyping studies. For this, we used
Regional differences in the frequency of the c.985A>G ACADM mutation

Table 2. Mean odds ratios (95%CI) for the c.985A>G allele frequencies in the different regions relative to the Western European region

<table>
<thead>
<tr>
<th>Variables</th>
<th>M1: Fixed effects within region</th>
<th>M2: Random effects within region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio (95%CI)</td>
<td>Odds ratio (95%CI)</td>
</tr>
<tr>
<td>Reference group: Western Europe</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>New World</td>
<td>0.82 (0.74–0.90)</td>
<td>0.87 (0.67–1.16)</td>
</tr>
<tr>
<td>Eastern Europe</td>
<td>0.41 (0.32–0.53)</td>
<td>0.47 (0.32–0.68)</td>
</tr>
<tr>
<td>Southern Europe</td>
<td>0.51 (0.40–0.64)</td>
<td>0.52 (0.37–0.74)</td>
</tr>
<tr>
<td>Interobservation SD (τ)</td>
<td>–</td>
<td>0.10 (0.04–0.20)</td>
</tr>
<tr>
<td>DIC</td>
<td>183.6</td>
<td>125.6</td>
</tr>
<tr>
<td>pD</td>
<td>5.7</td>
<td>41.4</td>
</tr>
<tr>
<td>Dbar</td>
<td>177.9</td>
<td>84.1</td>
</tr>
</tbody>
</table>

DIC, deviance information criterion; CI, Bayesian confidence interval; SD, standard deviation.

Table 3. Posterior mean estimates from the model with random-effects within region (M2)

<table>
<thead>
<tr>
<th>Region</th>
<th>Number of homozygotes per 100,000 individuals Mean (95% CI)</th>
<th>Number of heterozygotes per 100,000 individuals Mean (95% CI)</th>
<th>Allele frequency of c.985A&gt;G mutation in the population (×1000 alleles) Mean (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western Europe</td>
<td>4.1 (2.8–5.6)</td>
<td>1268 (1061–1485)</td>
<td>6.4 (5.3–7.5)</td>
</tr>
<tr>
<td>New World</td>
<td>3.2 (2.0–4.7)</td>
<td>1113 (890–1367)</td>
<td>5.6 (4.5–6.9)</td>
</tr>
<tr>
<td>Eastern Europe</td>
<td>0.9 (0.5–1.7)</td>
<td>601 (423–822)</td>
<td>3.0 (2.1–4.1)</td>
</tr>
<tr>
<td>Southern Europe</td>
<td>1.2 (0.6–2.0)</td>
<td>672 (487–890)</td>
<td>3.4 (2.4–4.5)</td>
</tr>
</tbody>
</table>

solely homozygote frequency to inform allele frequency in MS/MS screened populations. Heterozygotes identified by MS/MS are not representative of the c.985A>G carrier population as not all carriers will present biochemically at screening and, if they do, the lack of an agreed case definition for MCAD deficiency may result in considerable variation across jurisdictions. The reasons for the frequency discrepancies are unclear and could be due to misinterpretation of homozygosity in MS/MS screening studies, variation in cut-off levels, and/or failure to amplify c.A985A>G alleles in genotyping studies (24). Hence, it remains relevant to obtain direct measurements of c.985A>G homozygosity compared to indirect measurements from MS/MS newborn screening. Given the relatively low costs of targeted molecular analysis, it would be useful to investigate carrier frequency in newborn screened populations to obtain a more precise frequency of the c.985A>G mutation and estimate directly the accuracy of the MCAD deficiency screening programmes relative to it.

There are some limitations to our approach and data. The model assumed that the HW principles hold and predicted 304 homozygotes (95% CI: 272–339) compared to the observed 315 individuals across all regions. Unexpected numbers of homozygotes were reported in the Bulgarian and Spanish genotype samples that did not fit HW principles. One explanation for the high frequency of c.985A>G alleles in the Bulgarian sample may be non-assortative mating, especially among individuals of Roma origin who are at higher risk for MCAD deficiency (25). However, it is unclear whether the apparent deviation from HW is because HW does not hold, or data collection protocols may have selectively favoured including clinical cases of MCAD deficiency or repeat specimens. When data were re-analysed ignoring homozygotes in the genotype samples, the predicted number of homozygotes was comparable to the original model, suggesting that the results are robust regarding HW assumptions and data integrity.

The available data on genotyping and MS/MS screening frequency of c.985A>G homozygosity is limited. Only 4/24 genotyping studies we identified were published after the 1990s (24, 26–28) with most concerning European-based populations. Furthermore, genotype study sample sizes were small, hence detecting few homozygotes, and several MS/MS screening studies were excluded as no information was reported on the frequency of c.985A>G homozygosity or, when reported, it was incomplete as not all MCAD deficiency diagnosed cases underwent molecular diagnostic testing (Table S3).

In this study, we have confirmed that c.985A>G mutation is of a predominantly North European origin and further research is needed on the discrepancies in the homozygosity frequency between screening and genotyping studies. Our predictions could inform c.985A>G frequency by region and ethnic group,
support the adoption of MCAD Deficiency testing into newborn screening programmes, examine the penetrance of the mutation and inform natural history and cost-effectiveness models. Additionally, our metaregression approach could be applied to other genetic conditions.

Supporting Information

The following Supporting information is available for this article:

APPENDIX S1: Detailed information on the 24 genotyping studies and 19 screening studies identified in the literature search and details on the statistical framework used together with the respective WinBUGS code are given.

Table S1. Genotyping studies on the frequency of c.985A>G mutation.

Table S2. MS/MS screening studies on the frequency of c.985A>G mutation.

Table S3. Reasons for excluding MS/MS screening studies.

Table S4. Allocation of each country to four predefined world regional areas.

Additional Supporting information may be found in the online version of this article.

Acknowledgements

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References


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