

1 **Are we ready for genetic testing for primary open-angle glaucoma?**

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9 The authors declare that they have no conflict of interest.

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21 **Abstract**

22 Following a dramatic reduction in the cost of genotyping technology in recent years, there  
23 have been significant advances in the understanding of the genetic basis of glaucoma.  
24 Glaucoma patients represent around a quarter of all outpatient activity in the UK hospital eye  
25 service and are a huge burden for the National Health Service. A potential benefit of genetic  
26 testing is personalised glaucoma management, allowing direction of our limited healthcare  
27 resources to the glaucoma patients who most need it. Our review aims to summarise recent  
28 discoveries in the field of glaucoma genetics and to discuss their potential clinical utility.

29 While genome-wide association studies have now identified over ten genes associated with  
30 primary open-angle glaucoma (POAG), individually, variants in these genes are not predictive  
31 of POAG in populations. There are data suggesting some of these POAG variants are  
32 associated with conversion from ocular hypertension to POAG and visual field progression  
33 among POAG patients. However, these studies have not been replicated yet and such genetic  
34 testing is not currently justified in clinical care. In contrast, genetic testing for inherited early-  
35 onset disease in relatives of POAG patients with a known genetic mutation is of clear benefit;  
36 this can support either regular review to commence early treatment when the disease  
37 develops, or discharge from ophthalmology services of relatives who do not carry the  
38 mutation. Genetic testing for POAG at a population level is not currently justified.

39

## 40 **Introduction**

41 Glaucoma remains the second commonest cause of certifiable visual loss in England and  
42 Wales.<sup>1</sup> Given the chronic nature of glaucoma, lifelong follow-up is generally required.  
43 Therefore, glaucoma patients form a large proportion of outpatient activity in the UK hospital  
44 eye service (an estimated 23% of all follow-up attendances) with over 1 million glaucoma-  
45 related visits per year.<sup>2,3</sup> This represents a huge burden for the National Health Service (NHS)  
46 which is likely to grow further given the projected increase in the number of people with  
47 glaucoma.<sup>4</sup> Genetic testing offers the promise of personalised glaucoma management and  
48 directing limited healthcare resources to the patients that need it most.

49 There is strong evidence for a genetic contribution to the commonest form of glaucoma,  
50 primary open-angle glaucoma (POAG). One twin study estimated the heritability of POAG to  
51 be 13%, though this is a likely underestimate given glaucoma case ascertainment was via  
52 prescribing registries and that a considerable proportion of glaucoma in a population is  
53 undiagnosed.<sup>5</sup> First-degree relatives of POAG patients were shown to have a 9-fold increased  
54 risk of developing glaucoma in their lifetime compared to relatives of controls in the  
55 population-based Rotterdam Study.<sup>6</sup> Most convincingly, with the advent of affordable high-  
56 throughput DNA genotyping, there have now been multiple genes identified as contributing  
57 to susceptibility for POAG.<sup>7</sup>

58 What is the future potential of genetic testing in the management of POAG? For patients  
59 already diagnosed with POAG, genetic testing may offer prognostic information which may  
60 guide the intensity of their treatment and follow-up strategy. Genetic testing may also guide  
61 which treatments are most suitable for individual patients, predicting the most efficacious  
62 treatment and the treatment least likely to induce side effects. Within families with  
63 hereditary glaucoma, identifying the genetic cause will allow testing of offspring to determine  
64 who requires close monitoring and early treatment. The potential benefits are clear to see,  
65 but is our knowledge sufficient or our tools accurate and affordable enough that we are now  
66 ready for genetic testing in glaucoma management? Our review aims to answer these  
67 questions while giving a conceptual overview of POAG genetics and an update on recent  
68 advances in the field. The role of genetic testing in congenital glaucomas<sup>8</sup> is established and  
69 beyond the scope of this review.

70

## 71 **Search strategy**

72 We conducted the following Medline search: "Genetic Testing"[Mesh] AND  
73 "Glaucoma"[Mesh]. We further considered studies that were referenced in the articles  
74 identified in the initial search.

75

## 76 **Mendelian versus complex disease**

77 Mendelian disorders are conceptually the simplest demonstration of how genes can be  
78 responsible for disease. A single genetic defect alone causes a disease and if this is passed on  
79 by parents, their children will potentially inherit the disease. Common forms of inheritance  
80 of Mendelian disorders include autosomal dominant, autosomal recessive and X-linked  
81 recessive. If the genetic defect responsible for the disease in the family is identified, it is  
82 possible to screen offspring to determine their risk of disease and potentially take  
83 preventative action. For example, Angelina Jolie famously underwent bilateral mastectomy  
84 to prevent breast cancer knowing she had inherited the *BRCA1* gene mutation that had  
85 caused breast cancer in her family.<sup>9</sup>

86 A complex disease is generally not caused by a single genetic defect; multiple genetic and/or  
87 environmental factors combine to collectively result in disease. Conceptually, it can be  
88 considered that each individual risk factor is insufficient to cause disease on its own and that  
89 each risk factor may not be present in all cases of disease (**Figure 1a**). The fact that the risk  
90 factor may not be present in all cases and yet present in some controls makes identifying each  
91 individual risk factor challenging in complex disease. Large sample sizes are required to  
92 provide adequate power to identify each risk factor. An alternative way of conceptualising  
93 complex disease is shown in **Figure 1(b-d)**. It may be that a single genetic factor is sufficient  
94 to cause disease, and that another single genetic factor is also sufficient to cause the disease,  
95 and these two different 'flavours' of the disease are indistinguishable or have not been  
96 separated during analyses. Again, in this situation also, important risk factors may not be  
97 present in all cases of the disease, posing a challenge for their identification. Similarly, large  
98 sample sizes can help identify each risk factor. Additionally, in this conceptual model,

99 accurate phenotyping and separating cases into biologically meaningful subgroups can help  
100 improve power for detection of risk factors.

101

## 102 **Genetic mutations versus genetic variants**

103 As stated above, a Mendelian disease is caused by a single genetic alteration which is usually  
104 rare and is alone sufficient to cause a gene to malfunction and result in disease. Such genetic  
105 alterations are termed ‘mutations’. ‘Variants’, on the other hand, are points in the genome  
106 (DNA code) at which we vary from one another. The human genome is greater than 3 billion  
107 nucleotides long, but we vary at less than 1% of these. The commonest form of variation is a  
108 nucleotide substitution at a single point in the genome and this is referred to as a single  
109 nucleotide polymorphism (SNP). A genetic variant alone does not *cause* disease, but may be  
110 *associated* with disease. Possessing a variant may increase or decrease the risk of disease,  
111 but alone is insufficient to cause disease and is unlikely to be predictive of who will develop  
112 disease (*cf* arrows in **Figure 1a**). Complex diseases may have many associated genetic  
113 variants. It is the cumulative contribution of these associations, or potentially interactions  
114 between them, that ultimately result in disease (**Figure 1a**).

115

## 116 **Approaches to discovering genes that contribute to POAG**

117 Identifying a new gene for POAG in a hypothesis-independent manner requires methodology  
118 that looks for association across the whole genome. Until recently, it was not feasible to  
119 examine all independently inherited SNPs genome-wide. However, this was not necessary if  
120 examining genetic factors that segregate with disease in large families with inherited POAG.  
121 This approach is called linkage analysis and requires only around 400 markers to cover the  
122 whole genome. Linkage studies have identified several genes associated with glaucoma, such  
123 as myocilin (*MYOC*),<sup>10</sup> optineurin (*OPTN*)<sup>11</sup> and WD repeat domain 36 (*WDR36*).<sup>12</sup> Mutations  
124 in these genes have been reported to cause autosomal dominant Mendelian POAG in the  
125 studied families. Further details on the roles of these genes in POAG have been previously  
126 reviewed.<sup>7,13,14</sup> While a mutation in one of these genes may completely explain the  
127 development of POAG in some families, collectively, mutations in these genes contribute to

128 only around 6% of POAG cases in the general population.<sup>15-17</sup> More recently, family studies  
129 have identified TANK binding kinase 1 (*TBK1*) as another cause of Mendelian POAG.<sup>18</sup> Rather  
130 than a mutation within the gene, it is duplication of the gene and the resultant increase in  
131 function that appears to be causing the glaucomatous process.

132 The cost of genome-wide genotyping has fallen dramatically in recent years, at a rate much  
133 faster than Moore's Law. This has resulted in affordable high-throughput technologies that  
134 can measure all common independently inherited genetic variation across the whole genome  
135 in individuals. Therefore, it has become possible to investigate genetic associations with  
136 POAG, hypothesis independent and genome-wide, without the need for families. Instead,  
137 unrelated POAG cases are collected and compared with unrelated controls at several million  
138 genetic markers (some directly measured and others imputed based on reference data). This  
139 approach is called a genome-wide association study (GWAS). GWAS identifies common  
140 variants (with a frequency of over 5% in the general population) associated with disease.  
141 Given the number of genetic variants examined, there is a multiple testing statistical issue.  
142 For this reason, associations are only considered significant and valid if the *P*-value is very  
143 small (a 'hit' is considered to be 'genome-wide significant' if  $P < 5 \times 10^{-8}$ ) and there is evidence  
144 for the same association in an independent cohort. The first glaucoma GWAS discovery was  
145 the *LOXL1* locus for exfoliation glaucoma.<sup>19</sup> The first replicated GWAS discovery for POAG  
146 was in an Icelandic population which identified a significant locus near *CAV1* and *CAV2* (both  
147 of which are expressed in retinal ganglion cells and trabecular meshwork).<sup>20</sup> Further GWAS  
148 of European populations have identified other significant POAG loci in discovery cohorts from  
149 the United States<sup>21,22</sup> (near or at *SIX1/SIX6*, *TXNRD2*, *ATXN2* and *FOXC1*) and Australia<sup>23,24</sup>  
150 (*TMCO1*, *CDKN2B-AS1*, *ABCA1*, *AFAP1* and *GMD5*). A POAG GWAS in people of Chinese  
151 descent identified a significant locus in *PMM2*.<sup>25</sup> Despite these identified variants being  
152 common, the effect of each one is small, and collectively they explain only a small fraction  
153 (<5%) of POAG heritability. It is anticipated many more loci will be identified as the statistical  
154 power improves with a larger sample of POAG cases. The first glaucoma GWAS success was  
155 for pseudoexfoliation glaucoma in 2007 which identified common variants in lysyl oxidase-  
156 like protein 1 (*LOXL1*) as strongly associated with disease.<sup>19</sup> Following this, the combination  
157 of cases from a large international consortium has identified further pseudoexfoliation  
158 glaucoma loci at *CACNA1A*,<sup>26</sup> *POMP*, *TMEM136*, *AGPAT1*, *RBMS3* and *SEMA6A*.<sup>27</sup> There has also

159 been some GWAS success for primary angle-closure glaucoma, with eight genetic loci  
160 identified to date (near or at *PLEKHA7*, *COL11A1*, *EPDR1*, *PCMTD1–ST18EPDR1*, *CHAT*, *GLIS3*,  
161 *FERMT2*, and *DPM2–FAM102A*).<sup>28,29</sup>

162 There have also been multiple GWAS hits for heritable quantitative traits related to glaucoma  
163 (endophenotypes), such as intraocular pressure (IOP), and optic cup-disc ratio (CDR). A large  
164 IOP GWAS from the International Glaucoma Genetics Consortium (IGGC) identified *GAS7* as a  
165 significant locus for both IOP and POAG.<sup>30</sup> There have been over 40 genetic loci identified for  
166 CDR in the largest published GWAS meta-analysis from the IGGC.<sup>31</sup> However, it remains  
167 unclear what role these loci have in disease, as the majority do not demonstrate association  
168 with POAG when tested in the available cohorts. It is possible these variants are related to  
169 developmental processes and associated with optic disc anatomy and not the pathological  
170 glaucomatous cupping process. Alternatively, these variants are associated with POAG  
171 aetiological processes, but there is insufficient power in the currently available POAG case-  
172 control datasets to confirm the associations.

173

#### 174 **Evidence for clinical utility of genetic testing in POAG**

175 Learning which genes contribute to POAG can inform us about previously unknown biological  
176 pathways that are important in disease aetiology and progression. In the longer term, these  
177 discoveries can prompt further research into these pathways and potentially lead to new  
178 treatments. In the shorter term, it is possible that genetic markers are of predictive value and  
179 can help personalise glaucoma management.

180

#### 181 *Diagnosis in hereditary POAG*

182 There are situations when genetic testing can be helpful for managing families with inherited  
183 POAG. For example, a young member of a family with severe, early onset, autosomal  
184 dominant POAG may benefit from knowing their likelihood of developing the disease.<sup>32</sup> If the  
185 mutation causing POAG in that family is identified (e.g. by testing for myocilin mutations in  
186 affected family members), then the individual concerned can be tested for that mutation  
187 (cascade genetic testing). If they do not carry the myocilin mutation, then their risk of

188 developing POAG will be similar to the risk in the general population, and this would allow  
189 discharge from routine ophthalmic examinations.<sup>32</sup> Such information may even inform life  
190 choices such as occupation, especially if the disease is of early onset. Conversely, if they do  
191 carry the mutation, this would warrant regular follow-up for early signs of raised IOP and  
192 permit early treatment.

193 More general screening of relatives for an identified disease-causing mutation is termed  
194 cascade genetic testing. There is some evidence that early diagnosis and treatment of  
195 myocilin-related POAG following cascade genetic testing may result in a better clinical  
196 outcome. In a retrospective study, glaucoma severity parameters were compared between  
197 patients who were identified by cascade genetic testing (Genetic cases) and patients who  
198 presented through normal clinical pathways and were subsequently found to have a myocilin  
199 mutation (Clinical cases).<sup>33</sup> Clinical cases had significantly higher maximum IOP, larger CDR  
200 and worse visual field mean deviation than Genetic cases.<sup>33</sup>

201 It has been suggested there may be benefit in screening patients with advanced POAG for  
202 myocilin mutations if they meet certain criteria (young age of onset, high maximum IOP and  
203 strong family history).<sup>34</sup> The prevalence of myocilin mutations in this phenotypically selected  
204 group ranged from 16% to 40% depending on the cut-off thresholds. Identification of a  
205 myocilin mutation could then prompt cascade genetic testing and early treatment of family  
206 members at high risk.<sup>34</sup>

207 Deciding whether to test patients or family members for myocilin mutations may not be  
208 straight-forward and genetic counselling should be offered.<sup>35</sup> This may involve referral to a  
209 clinical genetics service. Information provided should include details about the condition and  
210 its prognosis, its inheritance pattern, and risk to children or other family members.  
211 Counselling for at-risk but currently unaffected family members should explore the underlying  
212 motivation for genetic testing, and explain the testing process and potential impact of the  
213 test result.<sup>35</sup> Accredited testing for myocilin mutations is currently available to NHS clinicians  
214 via the UK Genetic Testing Network.<sup>36</sup> At the time of writing, sequencing the entire myocilin  
215 gene to look for any mutation cost £305, whereas testing for one known mutation in a family  
216 member cost £180.<sup>36</sup> There is currently regional variation on whether commissioners will  
217 cover the cost of myocilin genetic testing.



218

219 *Predicting conversion from ocular hypertension (OHT) to POAG*

220 A subset of participants of the Ocular Hypertension Treatment Study (OHTS) were genotyped  
221 for variants previously associated with POAG and these variants tested for association with  
222 subsequent conversion from OHT to POAG.<sup>37</sup> Among the largest ethnic group in cohort, non-  
223 Hispanic Whites, a SNP in *TMCO1* was significantly associated with the development of POAG.  
224 *TMCO1* has been strongly associated with IOP<sup>30,38</sup> and it is assumed that the variant mediates  
225 its increased risk of POAG by raised IOP. Remarkably, the association between the *TMCO1*  
226 variant and POAG conversion remained highly significant even after adjustment for all  
227 parameters in the previously published risk calculator,<sup>39</sup> including baseline IOP; the hazard  
228 ratio was 1.7 per risk allele (95% confidence interval 1.3 – 2.3,  $P = 0.0004$ ).<sup>37</sup> This equates to  
229 a 3-fold increased risk of POAG in people with two risk alleles compared to people with no  
230 risk alleles, an effect size that is comparable to other established risk factors such as age. It  
231 is perhaps surprising that the *TMCO1* effect remains significant even after adjustment for a  
232 direct measurement of IOP. This suggests that the *TMCO1* variant provides information  
233 regarding the cumulative level of true IOP over and above that provided by a single  
234 measurement at baseline. While this is an exciting finding that offers hope for the potential  
235 of genetic testing in the management of OHT, replication of this finding in an independent  
236 study would provide stronger evidence. It should also be noted that there was no discernible  
237 association between the *TMCO1* variant and conversion to POAG in the Black subgroup.<sup>37</sup>

238

239 *Predicting progression of POAG*

240 Examining risk factors for susceptibility to progression of POAG in treated cohorts is  
241 challenging, not least because intensity of treatment is difficult to quantify and account for.  
242 A study of 469 Singaporean Chinese POAG patients with 5 or more visual fields showed that  
243 only one of ten POAG loci tested was associated with visual field progression (ascertained by  
244 pointwise linear regression criteria).<sup>40</sup> This locus was in the *TGFBR3-CDC7* region and was  
245 associated with a 6.7 (95% CI 1.9 - 23.7,  $P = 0.003$ ) times increased chance of visual field  
246 progression. The wide confidence interval suggests uncertainty of this effect estimate and  
247 there is a possibility this is a chance finding. Replication in an independent cohort is required

248 before firm conclusions can be made. Unfortunately, data for the *TMCO1* variant that was  
249 examined in OHTS were not available for this study.

250

### 251 *Predicting response to treatment*

252 There is good evidence that, in general, there may be a genetic basis for effectiveness of  
253 treatment in different individuals, as well as for the development of side effects for  
254 treatment.<sup>41</sup> However, pharmacogenomic studies for glaucoma treatments have been small  
255 and with conflicting results. For example, variants in the prostaglandin F2 $\alpha$  gene have been  
256 associated with response to prostaglandin analogues in Japanese studies<sup>42,43</sup> but not in a  
257 North American study.<sup>44</sup> A variant in *ADRB2* has been associated with response to timolol  
258 drops, but this finding remains unreplicated.<sup>45</sup> Currently, there is no convincing evidence for  
259 genetic testing to support the choice of treatment for POAG.

260

### 261 **Targeted therapy for Mendelian POAG**

262 Identifying the disease-causing mutation in Mendelian POAG offers the potential for targeted  
263 therapy to fix the specific molecular defect caused by the mutation. It has been suggested  
264 that myocilin mutations result in misfolded MYOC protein accumulating in trabecular  
265 meshwork cells resulting in an adverse effect.<sup>46</sup> Phenylbutyrate, a chemical chaperone that  
266 aids proteins folding into their correct conformations, appears to cure myocilin-caused  
267 glaucoma in transgenic mice when administered orally or as an eyedrop.<sup>46,47</sup> While  
268 phenylbutyrate has not been tested in humans, this may serve as a proof of concept for  
269 targeting treatment to the underlying pathology caused by a specific genetic defect.

270 More recently, clustered regularly interspaced short palindromic repeats (CRISPR)-mediated  
271 genome editing was used to disrupt the mutant myocilin gene in a mouse model, resulting in  
272 reduced endoplasmic reticulum stress, lower IOP, and prevention of further glaucomatous  
273 damage.<sup>48</sup> Additionally, the investigators demonstrated the potential feasibility of human  
274 genome editing in the eye using an ex vivo human organ culture system.<sup>48</sup>

275

276 **Conclusions**

277 The pace of new genetic discoveries for glaucoma has increased significantly in recent years  
278 due to the exponential drop in cost of high-throughput genome-wide genotyping platforms.  
279 While there is some evidence supporting the clinical utility of this new knowledge, such as  
280 *TMCO1* variation being predictive of conversion from OHT to POAG, such studies are small  
281 and not replicated to date. Genetic testing for glaucoma is clearly helpful in some specific  
282 situations, such as screening of family members in autosomal dominant POAG of early onset.  
283 POAG pharmacogenomics is an understudied area that warrants further work in the GWAS-  
284 era. However, genetic testing for POAG at a population level is not currently justified. We  
285 look forward to further genetic discoveries for glaucoma as statistical power increases, from  
286 large cohorts such as the UK Biobank and from global collaborations such as the IGGC. Time  
287 will tell if these discoveries will help us manage our patients better, or at least help direct  
288 resources to those who need them most.

289

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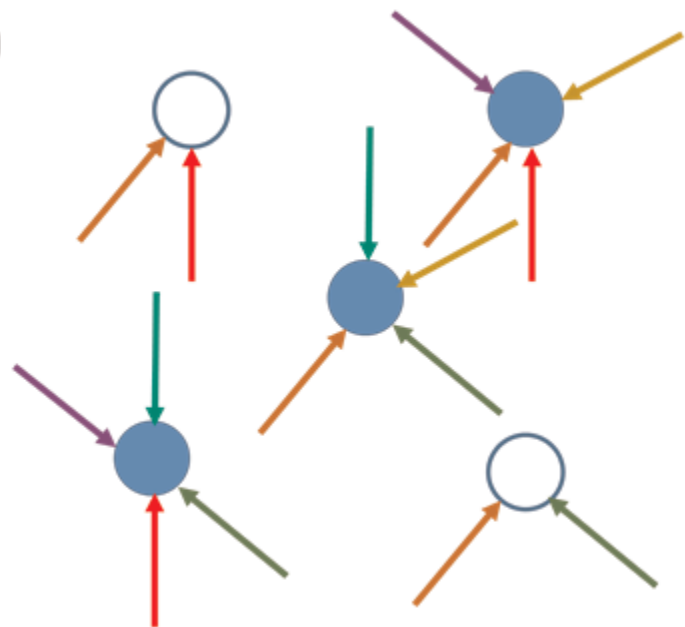
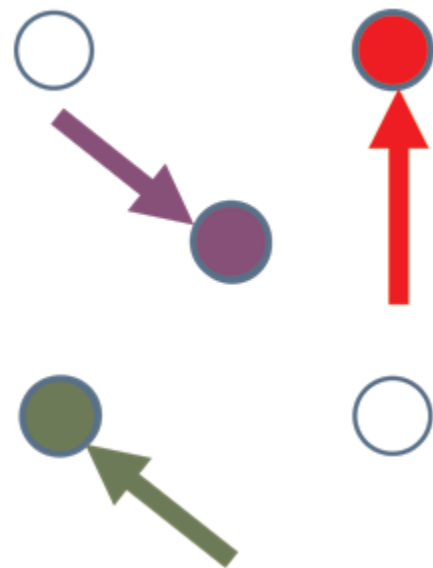
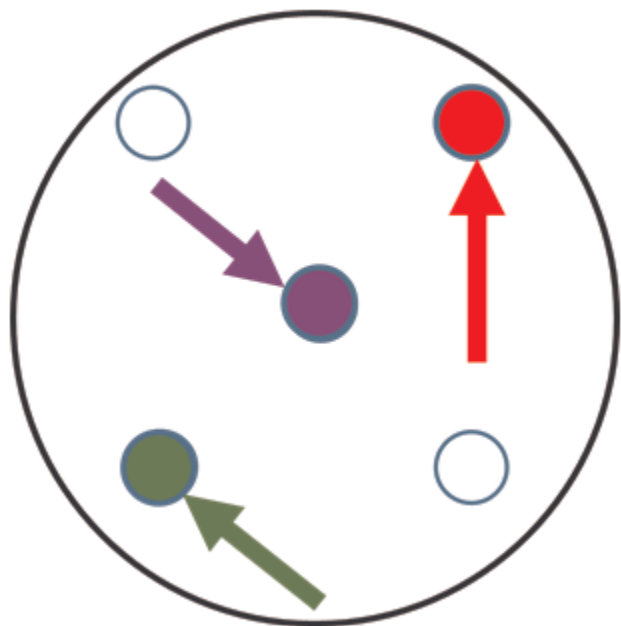
431 **Figure 1: Conceptual diagrams for complex disease.** Each circle represents an individual  
432 person; filled in circles are people affected by disease and hollow circles are unaffected  
433 controls. 1(a) and 1(b/c/d) are two different concepts for complex disease.

434 *First concept: 1(a)* - The arrows are risk factors (genetic or environmental); different colours  
435 represent different risk factors. It can be seen that none of the risk factors are present in all  
436 of the cases, and some of the risk factors that contribute to disease are present in controls.

437 *Second concept: 1(b)* – In this concept, each individual risk factor is sufficient to cause the  
438 disease. The different colours represent different subsets of disease which may or may not  
439 be clinically distinguishable. 1(c) – If all cases are examined together, identifying each risk  
440 factor can be challenging as they are present only in subset of cases. 1(d) – If the cases are  
441 subdivided in a biologically meaningful way, this can increase the power to identify risk factors  
442 despite the smaller sample size.

443

444

**(a)****(b)****(c)****(d)**