

1 **TITLE**

2 **New *GJA8* variants and phenotypes highlight its critical role in a broad spectrum of eye**  
3 **anomalies**

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51 **Acknowledgements**

52 We would like to thank the patients and their families for their participation in our study. We  
53 are grateful to Suzanne Broadgate for assisting with the coordination of the project. We thank  
54 the Centrum Menselijke Erfelijkheid laboratory, Leuven, Belgium, for the diagnostic *PAX2*  
55 testing. We also thank Dr Jean-Philippe Bault for the ultrasound examinations performed on  
56 family 2. This work was supported by grants from Baillie Gifford, Visually Impaired  
57 Children Taking Action (VICTA) (<http://www.victa.org.uk/>), Microphthalmia,  
58 Anophthalmia, Coloboma Support (MACS) ([www.macs.org.uk](http://www.macs.org.uk)), Oxford Brookes University  
59 Central Research Fund, Retina France, Fondation Maladies Rares, Spanish Institute of Health  
60 Carlos III (CP12/03256), Spanish Ministry of Economy and Competitiveness (SAF2013-  
61 46943-R), Mutua Madrileña Foundation and Cátedra de Patrocinio UAM-IIS-FJD of  
62 Genomic Medicine (University Autónoma of Madrid). The DDD study presents independent  
63 research commissioned by the Health Innovation Challenge Fund [grant number HICF-1009-  
64 003], see [www.ddduk.org/access.html](http://www.ddduk.org/access.html) for full acknowledgement.

65 **ABSTRACT**

66 *GJA8* encodes connexin 50 (Cx50), a transmembrane protein involved in the formation of  
67 lens gap junctions. *GJA8* mutations have been linked to early onset cataracts in humans and  
68 animal models. In mice, missense mutations and homozygous *Gja8* deletions lead to smaller  
69 lenses and microphthalmia in addition to cataract, suggesting *Gja8* may play a role in both  
70 lens development and ocular growth.

71 Following screening of *GJA8* in a cohort of 426 individuals with severe congenital eye  
72 anomalies, primarily anophthalmia, microphthalmia and coloboma, we identified four known  
73 (p.(Thr39Arg), p.(Trp45Leu), p.(Asp51Asn) and p.(Gly94Arg)) and two novel (p.(Phe70Leu)  
74 and p.(Val97Gly)) likely pathogenic variants in seven families. Five of these co-segregated  
75 with cataracts and microphthalmia, whereas the variant p.(Gly94Arg) was identified in an  
76 individual with congenital aphakia, sclerocornea, microphthalmia and coloboma. Four  
77 missense variants of unknown or unlikely clinical significance were also identified.  
78 Furthermore, the screening of *GJA8* structural variants in a subgroup of 188 individuals  
79 identified heterozygous 1q21 microdeletions in five families with coloboma and other ocular  
80 and/or extraocular findings. However, the exact genotype-phenotype correlation of these  
81 structural variants remains to be established.

82 Our data expand the spectrum of *GJA8* variants and associated phenotypes, confirming the  
83 importance of this gene in early eye development.

84 **Key words: Cataract, microphthalmia, coloboma, congenital aphakia, *GJA8*, Cx50**

85

## 86 **INTRODUCTION**

87 Anophthalmia (absent eye), microphthalmia (small eye) and coloboma (optic fissure closure  
88 defects), collectively referred to as AMC, form a spectrum of developmental eye disorders,  
89 with an overall estimated incidence of 6-13 per 100,000 births (Shah et al., 2011; Skalicky et  
90 al., 2013). AMC can occur alone or in combination with other ocular anomalies, such as early  
91 onset cataract and anterior segment dysgenesis (ASD). They are associated with extraocular  
92 features in just over half of cases (Shah et al., 2012) and can form part of a syndrome  
93 (Slavotinek, 2011). The etiology of AMC is characterised by marked genetic heterogeneity.  
94 This reflects the complexity underlying eye morphogenesis, a conserved process that requires  
95 a series of highly coordinated events, both at the molecular and the structural level, and is  
96 tightly regulated by a network of transcription factors, extracellular signaling molecules, cell-  
97 cycle regulators and adhesion proteins (Reis and Semina, 2015).

98 Connexins (Cxs) are a homogeneous family of transmembrane proteins with a crucial role in  
99 intercellular communication. They present a conserved topology, which consists of four  
100 transmembrane  $\alpha$ -helices (TM1-TM4) joined by two extracellular loops (ECL1 and ECL2)  
101 and one cytoplasmic loop (ICL), flanked by a short cytoplasmic N-terminal domain (NT) and  
102 a long cytoplasmic and less conserved C-terminal domain (CT). Cxs oligomerise in  
103 hexameric complexes called connexons, and allow the transmembrane passage of ions and  
104 small solutes ( $\leq 1$  kDa). Connexons can function independently as hemichannels (HCs) or  
105 they can dock with their counterparts on the juxtaposed cell to form a gap junction channel  
106 (GJC), enabling the direct exchange of small molecules. Given their role in cell-cell  
107 communication and tissue homeostasis, Cxs have been implicated in a variety of biological  
108 and pathological processes (Pfenniger et al., 2011; García et al., 2016), including myelin-  
109 related diseases (Cx32 and Cx47), heart malformations and arrhythmia (Cx40), hearing loss

110 and skin disorders (Cx26, Cx30, Cx30.3 and Cx31), oculodentodigital dysplasia, a syndrome  
111 also involving microphthalmia, microcornea, cataract and/or spherophakia (Cx43), and early  
112 onset cataract (Cx46 and Cx50).

113 As with AMC, developmental or early onset cataracts are a clinically heterogeneous group of  
114 disorders, presenting as isolated anomalies or part of a syndrome. More than 110 genes have  
115 been implicated in congenital cataracts (Gillespie et al., 2014), with mutations in Cxs  
116 accounting for around 16% of cases with a known genetic cause (Shiels and Hejtmancik,  
117 2017). Since the lens does not have any blood supply, it strongly depends on an extensive  
118 network of GJCs for the intercellular communications that are critical for its development and  
119 the maintenance of its transparency. The most abundant Cxs in the lens are Cx46 and Cx50,  
120 which can also form mixed hexamers. Cx46, encoded by *GJA3*, is expressed only in fiber  
121 cells, whereas Cx50, encoded by *GJA8*, is present throughout the lens.

122 Genetic studies in mice have demonstrated that the homozygous knockout of either *Gja3* or  
123 *Gja8* leads to cataracts, but with important phenotypic differences. The deletion of *Gja3*  
124 causes severe progressive nuclear cataracts, but does not alter ocular growth (Gong et al.,  
125 1997). In contrast, *Gja8*-null mice develop milder nuclear cataracts at an early postnatal age  
126 and exhibit significantly smaller lenses and microphthalmia (White et al., 1998; Rong et al.,  
127 2002), indicating that the two Cxs have overlapping, but distinct functions. In addition, the  
128 targeted replacement of *Gja8* with *Gja3* (Cx50KI46 knockin mice) prevents the loss of  
129 crystalline solubility, but not the postnatal growth defect resulting from the *Gja8* deletion  
130 (White, 2002), confirming the functional diversity of the two proteins and the involvement of  
131 *Gja8* in the control of normal ocular growth. This is also supported by mouse lines carrying  
132 missense mutations in *Gja8* (Steele et al., 1998; Graw et al., 2001; Chang et al., 2002; Xia et  
133 al., 2012; Berthoud et al., 2013) and by rabbit models with CRISPR-Cas9 mediated *GJA8*  
134 knockout (Yuan et al., 2016): both develop cataracts, microphthalmia and smaller lenses.

135 Moreover, severe cataracts and small lenses have also been observed in transgenic mice  
136 overexpressing *Gja8* (Chung et al., 2007), indicating that any significant dysregulation of  
137 *Gja8* could be deleterious for eye development.

138 In humans, missense and frameshift mutations in *GJA8* (OMIM 600897) have been  
139 associated with cataracts (Beyer et al., 2013; Yu et al., 2016). Rarely, the phenotype also  
140 includes additional ocular abnormalities, mainly microcornea and iris hypoplasia (Devi and  
141 Vijayalakshmi, 2006; Hansen et al., 2007; Hu et al., 2010; Sun et al., 2011; Prokudin et al.,  
142 2014; Ma et al., 2016), but in a few cases also microphthalmia (Ma et al., 2016) and  
143 sclerocornea (Ma et al., 2018). Interestingly, defects in the formation of the lens have also  
144 been observed (Ma et al., 2018). The cataracts described in these individuals vary in both  
145 their location (e.g., nuclear, zonular, lamellar or total) and appearance (e.g., total, pulverulent  
146 or dense). The mutations are predominantly heterozygous and only few homozygous variants  
147 have been reported, all in consanguineous families (Ponnam et al., 2007; Schmidt et al., 2008  
148 Ponnam et al., 2013; Ma et al., 2016). These pathogenic variants lead to amino acid  
149 alterations distributed throughout the protein (Yu et al., 2016), although mostly localised  
150 between the domains TM1 and TM2. They are predicted to affect protein function through  
151 various mechanisms, such as by inducing misfolding and/or mislocalisation or by altering  
152 channel properties (Beyer et al., 2013).

153 Copy number variants (CNVs) in the distal region of chromosome 1q21 and including *GJA8*  
154 are rare in the general population, but have recurrently been identified in individuals with a  
155 broad range of different clinical diagnoses (Brunetti-Pierri et al., 2008; Mefford et al., 2008;  
156 Stefansson et al., 2008 Bernier et al., 2016). These primarily include developmental delay,  
157 microcephaly and psychiatric disorders, although the enrichment of 1q21 CNVs in  
158 individuals with these disorders could partly be related to ascertainment bias. However, some  
159 cases also have eye anomalies, such as cataracts (Brunetti-Pierri et al., 2008; Mefford et al.,

160 2008; Rosenfeld et al., 2012; Bernier et al., 2016; Ha et al., 2016), microphthalmia (Mefford  
161 et al., 2008) and coloboma (Brunetti-Pierri et al., 2008). Among the genes affected by these  
162 recurrent microdeletions/microduplications, *GJA8* represents a strong candidate for the ocular  
163 anomalies described in some of the 1q21 CNV carriers.

164 To investigate further the importance of *GJA8* in human eye morphogenesis and provide a  
165 better understanding of the range of developmental ocular anomalies associated with  
166 mutations in this gene, we screened *GJA8* in a cohort of 426 unrelated patients (304 UK, 121  
167 Spanish and 1 large French pedigree) with congenital eye anomalies in the AMC spectrum.  
168 Two novel and four known likely pathogenic sequence variants were identified in seven  
169 families, with one variant being present in two unrelated families. This expands the catalogue  
170 of *GJA8* variants likely to be contributing to eye anomalies and the spectrum of phenotypes  
171 associated with this gene. Moreover, we also identified heterozygous 1q21 microdeletions  
172 including the gene *GJA8* in five additional families, although the pathogenicity of these  
173 variants remains to be established.



174 **MATERIALS AND METHODS**

175 **Cohort description.**

176 A cohort of UK, Spanish and French families with AMC (Supplementary Table 1) was  
177 analysed for *GJA8* variants. The UK families (n=304) were recruited as part of a national  
178 ‘Genetics of Eye and Brain anomalies’ study, approved by the Cambridge East Ethics  
179 Committee (04/Q0104/129) and had not received a genetic diagnosis. Family 5 was also  
180 recruited into the Deciphering Developmental Disorders (DDD) Study, which has UK  
181 Research Ethics Committee approval (10/H0305/83, granted by the Cambridge South REC,  
182 and GEN/284/12 granted by the Republic of Ireland REC). The UK families consisted of 55  
183 probands with anophthalmia, 205 with microphthalmia and 44 with other anomalies within  
184 the AMC spectrum; 168 individuals were bilaterally affected and 160 had extra-ocular  
185 anomalies. The Spanish families (n=121) consisted of 6 individuals with anophthalmia, 42  
186 with microphthalmia and 73 with other anomalies within the AMC spectrum; 100 individuals  
187 were bilaterally affected and 41 had extra-ocular anomalies. They were consented for genetic  
188 studies approved by the Ethics Committee of the University Hospital Fundación Jiménez  
189 Díaz (FJD, Madrid, Spain) and according to the tenets of the Declaration of Helsinki. The  
190 four-generation French pedigree consisted of 15 individuals with congenital cataracts and  
191 microphthalmia and consented for the study during their clinical treatment.

192 **Identification of sequence and structural variants in *GJA8*.**

193 The human gene *GJA8* presents one isoform (NM\_005267.4), comprising of two exons, with  
194 the coding sequence (CDS) entirely contained within exon 2. Sequence variants in the CDS  
195 were detected using a combination of Next-Generation Sequencing (NGS) methods and  
196 direct sequencing: 35 patients were screened by whole exome sequencing (WES), 207  
197 patients using different targeted NGS panels of eye development genes including *GJA8*, and  
198 184 patients by Sanger sequencing, which was also used to validate NGS findings and check

199 family segregation. Additionally, CNV data was available for 188 of these patients: 151  
200 individuals (96 UK and 55 Spanish) had been assessed by array-based Comparative Genomic  
201 Hybridization (aCGH), with resolutions ranging from 44 kb to 244 kb, whereas for 37  
202 Spanish individuals, CNVs were detected from NGS data using a read depth comparison  
203 approach. A detailed description of the different methods can be found in the Supplementary  
204 Materials and Supplementary Table 1. The genomic coordinates of the sequence and  
205 structural variants are reported according to Build GRCh37/hg19. The allelic frequencies of  
206 the sequence mutations were obtained from the Genome Aggregation Database (gnomAD,  
207 <http://gnomad.broadinstitute.org/>) (Lek et al., 2016). For each variant of interest, amino acid  
208 conservation across species was visually inspected using the Vertebrate Multiz Alignment &  
209 Conservation (100 Species) track from the UCSC Genome Browser. Three conservation  
210 scores were annotated using the database dbNSFP v.3.3 (Liu et al., 2016), specifically the  
211 GERP++ Rejected Substitutions (RS) score (Davydov et al., 2010), phyloP  
212 100way\_vertbrate score (Siepel et al., 2006) and phastCons 100way\_vertbrate score (Siepel  
213 et al., 2005). Putative functional effects of amino acid substitutions were evaluated with the  
214 *in silico* tools SIFT (Kumar et al., 2009) and PolyPhen-2 (Adzhubei et al., 2010).

### 215 **Validation of mosaicisms and CNVs.**

216 In order to assess potential mosaicism and independently validate aCGH findings, we  
217 developed Digital Droplet PCR (ddPCR) assays for the sequence variant in family 1 and three  
218 of the *GJA8* microdeletions identified (families 12, 13 and 14) (Supplementary Materials).  
219 ddPCR assays were performed using a ddPCR QX200 System (Bio-Rad Laboratories).  
220 Primers and Taqman probes were specifically designed for the *GJA8* variant p.(Thr39Arg)  
221 using a custom Applied Biosystems TaqMan SNP Genotyping Assay (Thermo Fisher  
222 Scientific). For CNV analysis, commercial Taqman Copy Number assays (Thermo Fisher  
223 Scientific) were used for exon 2 of *GJA8* and a reference gene (human RNase P gene).

## 224 **RESULTS**

### 225 **Point mutations identified in *GJA8*.**

226 Screening of the *GJA8* coding region in our cohort of 426 individuals with AMC detected 10  
227 missense variants in 11 unrelated families (Tables 1 and 2). For each missense variant, the  
228 amino acid conservation across species is shown in the Supplementary Fig. 1. Taking into  
229 account the segregation patterns, the frequency of the variants in public databases of  
230 unaffected individuals (Table 2), *in silico* predictions of functional effects and previous  
231 reports from the literature (Table 2), as suggested by (Richards et al., 2015), six of these  
232 variants (p.(Thr39Arg), p.(Trp45Leu), p.(Asp51Asn), p.(Phe70Leu), p.(Gly94Arg) and  
233 p.(Val97Gly)) were considered likely causative, giving a frequency of 1.6% of independent  
234 individuals with AMC conditions (7/426) carrying likely pathogenic *GJA8* sequence variants.

235 In family 1 (Fig. 1a), the heterozygous variant p.(Thr39Arg) (NM\_005267.4:c.116C>G) was  
236 identified in the male proband (III:1), who presented with bilateral microphthalmia,  
237 sclerocornea, cataracts and nystagmus, left secondary glaucoma and a grossly cupped  
238 atrophic disc (Fig. 1b). Extraocular anomalies were not observed. The Sanger sequencing  
239 profile was suggestive of mosaicism in his mother (II:2), who was diagnosed with early onset  
240 cataracts and right exotropia. Mosaicism was confirmed and quantified by ddPCR in blood  
241 samples, with an estimated fractional abundance of 25% for the mutated allele (Fig. 1c). The  
242 variant was absent in the maternal grandparents, suggesting that it arose as a *de novo* post-  
243 zygotic event in the mother. The substitution of threonine 39, located in the TM1 domain, is  
244 predicted deleterious by Polyphen-2 and SIFT. Interestingly, the change p.(Thr39Arg) is  
245 absent in dbSNP147 and gnomAD, but has been previously described in a family with  
246 congenital cataracts, microcornea and iris hypoplasia (Sun et al., 2011).

247 In family 2 (Fig. 1d), a four-generation pedigree with autosomal dominant congenital  
248 cataracts, we initially identified the variant p.(Trp45Leu) (NM\_005267.4:c.134G>T) in the

249 proband III:2, who had a diagnosis of dense congenital cataracts, microphthalmia and  
250 nystagmus. Sanger sequencing was performed on five additional family members (four  
251 affected with the same clinical diagnosis and one unaffected) and showed that the variant co-  
252 segregated with the ocular phenotype. The same amino acid substitution, predicted  
253 deleterious by Polyphen-2 and SIFT, has been previously described in another multi-  
254 generation family including eleven individuals with autosomal dominant congenital cataracts  
255 (Mohebi et al., 2017). Moreover, a different missense variant affecting the same amino acid,  
256 p.(Trp45Ser) (NM\_005267.4:c.134G>C, rs864309688), has been reported in a three  
257 generation family with bilateral congenital cataracts and microcornea (Vanita et al., 2008), in  
258 a sporadic case with bilateral anterior cortical/nuclear cataracts (Ma et al., 2016) and in a  
259 three generation family with paediatric cataracts (Javadiyan et al., 2017). Functional  
260 experiments showed that p.(Trp45Ser) inhibited the formation of functional intercellular  
261 channels or hemichannels and decreased the junctional conductance induced by wild-type  
262 Cx50 and Cx46, acting as dominant negative inhibitor (Tong et al., 2011). Tryptophan 45 is  
263 an evolutionary conserved residue located in the TM1 domain and its substitution with  
264 leucine or serine has not been observed in controls (gnomAD).

265 In family 3 (Fig. 1e) and family 4 (Fig. 1f), we identified the variant p.(Asp51Asn)  
266 (NM\_005267.4:c.151G>A; rs864309703), which affects a highly conserved amino acid  
267 located in the ECL1 domain. This change, predicted deleterious by Polyphen-2 and SIFT, has  
268 been previously reported in a patient with bilateral microphthalmia, congenital cataracts and  
269 sclerocornea (Ma et al., 2016; Ma et al., 2018). In family 3, the mutation occurred as a *de*  
270 *novo* event in the male proband, who presented with bilateral microphthalmia with associated  
271 cataracts, anterior segment dysgenesis and persistent pupillary membranes. Extraocular  
272 anomalies were not observed. In the three-generation family 4, the heterozygous variant was  
273 identified in both the proband (III:1) and her affected father (II:4). Head axial computed

274 tomography scanning of the proband at 29 years old showed borderline bilateral  
275 microphthalmia and enophthalmos (posterior displacement of the eye), although her ocular  
276 globes had a size of 20mm (right eye) and 18mm (left eye). At 32 years of age, the proband  
277 had no light perception on the right and light perception on the left. The right eye was  
278 phthisical, with no discernible anterior segment structures; the left eye had a corneal  
279 leukoma, cataract and corectopia. The father was diagnosed with bilateral microphthalmia  
280 and congenital cataracts. The paternal grandfather (I:1) and one of the paternal uncles (II:3),  
281 now deceased, were also affected. The mother (II:5) was affected by congenital glaucoma.  
282 However, the proband did not carry a mutation in any known congenital glaucoma-associated  
283 genes included in a custom targeted NGS panel containing 121 eye developmental genes, 9 of  
284 which are associated with congenital glaucoma. No extraocular anomalies were observed.

285 In family 5 ([Fig. 1g](#)), the novel variant p.(Phe70Leu) (NM\_005267.4:c.208T>C) was  
286 identified in the proband III:2, diagnosed with bilateral microphthalmia, congenital cataracts  
287 and secondary glaucoma. Segregation analysis showed that the mutation was a *de novo* event  
288 in the affected mother (II:2), who also had microphthalmia and cataracts. Phenylalanine 70 is  
289 a conserved amino acid located in the ECL1 domain, and its substitution is predicted to be  
290 deleterious by SIFT and Polyphen-2.

291 In family 6 ([Fig. 1h](#)), we identified a missense variant p.(Gly94Arg)  
292 (NM\_005267.4:c.280G>A) in a male proband of Chinese ethnicity (II:1) presenting with  
293 bilateral congenital aphakia (absence of the lens), corneal opacity, bilateral microphthalmia  
294 with iris and optic disc coloboma, and bilateral primary glaucoma. No extraocular anomalies  
295 were observed. No details of parental phenotype or DNA were available. Interestingly this  
296 change, predicted deleterious by SIFT and Polyphen-2 and located in the TM2 domain, is  
297 absent in gnomAD, but has been previously identified as a *de novo* event in a child with  
298 bilateral corneal opacification and microcornea, bilateral rudimentary lenses and bilateral

299 glaucoma (Ma et al., 2018).

300 In family 7 (Fig. 1i), the female proband (II:1) carried a *de novo* variant p.(Val97Gly)  
301 (NM\_005267.4:c.290T>G), predicted deleterious by SIFT and Polyphen-2 and located in the  
302 TM2 domain. This previously undescribed variant was identified by the DDD study  
303 (DECIPHER ID: 259194) and confirmed with Sanger sequencing. She had bilateral  
304 microphthalmia, anterior segment dysgenesis and dense cataracts, treated with lensectomies,  
305 and right secondary glaucoma, with no extraocular features.

306 The significance of the other four variants identified in the screening (Supplementary Fig. 2)  
307 was considered ‘uncertain’ (p.(Leu292Gln)) or ‘unlikely to be pathogenic’ (p.(Leu7Met),  
308 p.(Asn220Asp) and p.(Gly333Arg)).

309 A novel amino acid change, p.(Leu292Gln) (NM\_005267.4:c.875T>A) was identified in a  
310 proband with bilateral mild cataracts and optic nerve coloboma associated with nystagmus,  
311 photophobia and small kidneys (family 8). The substitution of leucine 292, located in the CT  
312 domain, is predicted benign by SIFT, but deleterious by Polyphen-2. Sanger sequencing of  
313 *PAX2* revealed that the proband II:3 also carried a novel heterozygous frameshift variant in  
314 this gene (NM\_003987.2:c.529\_530ins13, p.(Ala177Glyfs\*8)), which introduces a premature  
315 stop codon in exon 5. Sanger sequencing excluded the maternal inheritance of both the *GJA8*  
316 and the *PAX2* variants; paternal DNA was unavailable for segregation analysis.

317 The *GJA8* variant p.(Leu7Met) (NM\_005267.4:c.19C>A; rs150441169), located in the N-  
318 terminal domain and predicted deleterious by SIFT and Polyphen-2, was detected in a patient  
319 (II:1) with syndromic unilateral microphthalmia, and was inherited from his unaffected father  
320 (family 9). The family is of African ethnicity and the minor allele frequency (MAF) for the  
321 African/African-American population in gnomAD is 0.28%. Different substitutions of this  
322 amino acid have been described before as disease-causative mutations: p.(Leu7Pro)  
323 (NM\_005267.4:c.20T>C) was identified in a family with inherited cataracts (Mackay et al.,

2014) and p.(Leu7Gln) (NM\_153465.1:c.20T>A) in a rat model with nuclear pulverulent cataracts and, in the case of homozygous rat mutants, microphthalmia with hypoplastic lens (Liska et al., 2008). However, in contrast with these previously reported variants, the frequency of the p.(Leu7Met) variant in unaffected individuals, in particular of African/Afro-American ethnicity, suggests that the substitution with a methionine might be tolerated.

The variant p.(Asn220Asp) (NM\_005267.4:c.658A>G; rs138140155, gnomAD total-MAF=0.24%) was identified in an individual with bilateral microphthalmia and chorioretinal colobomas involving the optic disc, as well as microcephaly associated with normal development and faltering growth (family 10) and was inherited from her unaffected father. This substitution of asparagine 220, located in the TM4 domain and predicted deleterious by SIFT and Polyphen-2, has been reported before in a proband with congenital cataract and microcornea (Ma et al., 2016) and in a three generation family with congenital cataracts and aphakic glaucoma (Kuo et al., 2017). However, in those families it did not co-segregate with the phenotype and therefore was classified as benign. This was also supported by functional experiments showing that this rare polymorphism did not abolish intercellular channel function (Kuo et al., 2017).

The variant p.(Gly333Arg) (NM\_005267.4:c.997G>C; rs587600450, gnomAD total-MAF=0.009%) was observed in a proband with unilateral microphthalmia and chorioretinal coloboma involving the optic disc, and was inherited from her unaffected father (family 11). This heterozygous change occurs in the CT domain and is predicted as tolerated by both SIFT and Polyphen-2.

#### **1q21 copy number variants overlapping with *GJA8*.**

*GJA8* is part of a complex genomic locus, 1q21.1-q21.2, characterised by the presence of numerous segmental duplications (SDs), which make the region susceptible to recurrent rearrangements. To investigate whether structural variants affecting *GJA8* were present in our

349 cohort of families with AMC, we examined a subset of 188 unrelated individuals for whom  
350 copy number information was available from aCGH and/or NGS data. As such, the samples  
351 for which CNV data were generated were not chosen according to any selection criteria  
352 applied across the total cohort, and therefore they effectively represented a randomly-selected  
353 subset of independent AMC cases. This resulted in the identification of 1q21 microdeletions  
354 in five families (Fig. 2, Table 1).

355 The first microdeletion was identified in a proband (family 12, Fig. 2b) with bilateral  
356 coloboma of the iris and choroid, mild dysmorphic features (broad forehead, narrow  
357 palpebral fissures, depressed nasal root and low set ears), scoliosis, *genu valgum* and  
358 gastroesophageal reflux. She had normal developmental milestones. This CNV, detected  
359 from the screening of a custom NGS panel of 121 eye development genes, was further  
360 confirmed by both aCGH and ddPCR (Supplementary Fig. 3). It spans approximately 2 Mb  
361 (chr1:145388977-147395401, Build GRCh37/hg19) and affects 40 RefSeq genes.  
362 Segregation analysis revealed that this structural variant arose as a *de novo* event in the  
363 proband.

364 The second microdeletion was identified in a female proband with unilateral chorioretinal  
365 coloboma involving the optic disc, band keratopathy, cataract and secondary glaucoma  
366 without extraocular anomalies, and was inherited from her unaffected father (family 13, Fig.  
367 2b). The minimal deleted region (chr1:146155983-147824178, Build GRCh37/hg19) spans  
368 approximately 1.67 Mb and affects 24 RefSeq genes. To validate the microdeletion and test  
369 the hypothesis that the unaffected status of the father could be due to mosaicism, we  
370 performed a ddPCR assay. However, this experiment confirmed the full heterozygous status  
371 of the microdeletion in both individuals (Supplementary Fig. 3b).

372 The third microdeletion was found in a female proband with extreme microphthalmia in the  
373 right eye and iris, chorioretinal coloboma in the left eye, cleft lip and palate, and neonatal



374 seizures (family 14, [Fig. 2b](#)). The minimal deleted region (chr1:146564743-147735011, Build  
375 GRCh37/hg19) spans approximately 1.17 Mb and affects 17 RefSeq genes. The presence of  
376 the CNV in the mother was excluded by ddPCR (Supplementary Fig. 3b). The father and  
377 other family members were unavailable for phenotypic and segregation analysis.

378 The fourth microdeletion (chr1:146497694-147825519, Build GRCh37/hg19), spanning  
379 approximately 1.33 Mb and affecting 20 RefSeq genes, was identified in two independent  
380 families (families 15 and 16, [Fig. 2b](#)). In family 15, it occurred as a *de novo* event in a  
381 proband with bilateral iris and chorioretinal coloboma involving disc, and nystagmus, without  
382 extraocular anomalies. The presence of the CNV in the parents was excluded by aCGH.  
383 Clinical re-assessment of the family revealed that the father presented blue dot lens opacities  
384 and cavernous disc anomalies with a pit in the right eye and mild cavernous disc anomaly or  
385 pronounced optic cup in the left eye. In family 16, a three-generation pedigree with  
386 coloboma, the microdeletion was detected by aCGH in the proband (III:6), who showed  
387 bilateral chorioretinal coloboma and microphthalmia in the right eye associated with  
388 microcephaly and normal development, and in the affected father (II:5), who presented with  
389 microphthalmia and coloboma in the right eye. The cousin III:2 was also affected with  
390 unilateral iris and chorioretinal coloboma. However, segregation analysis could not be  
391 performed on this individual.

392 The predicted boundaries of these CNVs indicated that these rearrangements belonged to  
393 different classes of 1q21 microdeletions. Recurrent 1q21 CNVs occur at four breakpoint  
394 regions (BP1-BP4), each corresponding to a large block of highly homologous SDs (Mefford  
395 et al., 2008). Further, the locus can be divided into two distinct regions: a proximal region  
396 included between BP2 and BP3 and a distal region, flanked by BP3 and BP4, which mediate  
397 the most recurrent CNVs of the 1q21 locus. While the microdeletions found in families 13-16  
398 were distal rearrangements occurring between BP3 and BP4 (class I), the microdeletion

399 detected in family 12 was flanked by the breakpoints BP2 and BP4 and extended from the  
400 proximal through to the distal region (class II). Despite their rarity in the general population,  
401 both types of 1q21 microdeletions appeared to be enriched in our AMC cohort. We compared  
402 the frequency of these CNVs in our cases with control individuals previously reported in the  
403 literature (Rosenfeld et al., 2012): BP3-BP4 microdeletions occurred in 4 out of 188  
404 individuals with AMC *versus* 12 out of 65282 controls (2.13% *versus* 0.02%, Fisher's exact  
405 test  $p=1.17 \times 10^{-7}$ ), whereas BP2-BP4 microdeletions occurred in 1 out of 188 individuals  
406 with AMC *versus* 1 out of 65927 controls (0.532% *versus* 0.002%, Fisher's exact test  
407  $p=0.0057$ ).

408 Taking into account all the 1q21 microdeletions identified in our cohort, the minimally  
409 deleted region spans approximately 830 kb (chr1:146564743-147395401, Build  
410 GRCh37/hg19) and includes 11 genes (*NBPF19*, *NBPF13P*, *PRKAB2*, *CHD1L*, *PDIA3P1*,  
411 *FMO5*, *LINC00624*, *BCL9*, *ACP6*, *GJA5*, *GJA8*). In addition to *Gja8*, a role in eye  
412 development has been shown also for *Bcl9*, a co-activator for  $\beta$ -catenin-mediated  
413 transcription in Wnt signaling (Bienz, 2005). A recent study has demonstrated that *Bcl9* is  
414 also part of the Pax6-dependent regulatory circuit and contributes to mouse lens formation  
415 (Cantù et al., 2014). No other genes known to be relevant in eye development are present in  
416 the region.

417 Sequence analysis of the coding region of *GJA8* in the probands carrying 1q21  
418 microdeletions did not reveal any variant on the remaining allele. In family 12, no additional  
419 pathogenic variants were identified from the targeted NGS screening of 121 eye  
420 developmental genes. In family 13, the NGS targeted sequencing of 351 diagnostic genes for  
421 eye developmental anomalies in proband II:2 identified an in-frame deletion of 6bp in  
422 *FOXC1* (NM\_001453.2:c.1338\_1343del, p.(Gly447\_Gly448del)), maternally inherited. This  
423 rare variant (gnomAD total-MAF=0.06%) is reported as a multi-allelic SNP (rs572346201),

424 which occurs in a region coding for a poly-Glycine stretch. Although its clinical significance  
425 is unknown, due to the repetitive nature of this region, it is likely to represent a natural  
426 polymorphism.

## 427 **DISCUSSION**

428 Mutations in Cx50, encoded by *GJA8*, have been primarily linked to congenital and early  
429 onset cataract in humans and also animal models. However, recently in a small number of  
430 cases *GJA8* mutations have also been associated with a broader phenotype which can include  
431 microphthalmia, sclerocornea and lens abnormalities (Ma et al., 2018).

432 In this study, we have investigated the role of *GJA8* in a cohort of 426 individuals with a  
433 wide range of developmental eye anomalies, and identified 16 families with AMC carrying  
434 genetic alterations of *GJA8*. These included six likely pathogenic sequence variants  
435 (p.(Thr39Arg), p.(Trp45Leu), p.(Asp51Asn), p.(Phe70Leu), p.(Gly94Arg) and p.(Val97Gly))  
436 detected in seven unrelated families, four missense variants (p.(Leu7Met), p.(Asn220Asp),  
437 p.(Leu292Gln) and p.(Gly333Arg)) with uncertain or unlikely clinical significance and four  
438 heterozygous 1q21 microdeletions involving *GJA8* detected in five unrelated families of  
439 uncertain significance.

440 Segregation analyses were possible for five out of six likely pathogenic sequence variants and  
441 showed that these occurred either *de novo* or co-segregated with the disease in an autosomal  
442 dominant fashion. These variants were bioinformatically predicted damaging and have not  
443 been reported in unaffected individuals according to public databases. Interestingly, three of  
444 these changes (p.(Thr39Arg), p.(Trp45Leu) and p.(Asp51Asn)) have been previously  
445 described in families with cataracts (Sun et al., 2011; Ma et al., 2016; Javadiyan et al., 2017;  
446 Mohebi et al., 2017; Ma et al., 2018) and a fourth (p.(Gly94Arg)) in a proband with  
447 sclerocornea and lens abnormalities (Ma et al., 2018). Given the rarity of these variants, the  
448 identification of the same missense changes in unrelated affected individuals strongly

449 supports a causative relationship between these variants and eye developmental disorders. In  
450 particular, p.(Asp51Asn) had been reported as a *de novo* mutation in a patient with bilateral  
451 microphthalmia, congenital cataracts and sclerocornea (Ma et al., 2016). In the present study,  
452 the same variant was detected in two independent families with a similar phenotype,  
453 including microphthalmia, cataracts and other anterior chamber eye anomalies. This  
454 emerging genotype-phenotype correlation suggests that this amino acid substitution might  
455 have a severe effect on GJA8 function and supports the involvement of this protein in a  
456 broader range of eye developmental anomalies.

457 Our identification of the variant p.(Gly94Arg) in another patient also aids genotype-  
458 phenotype correlation for amino acid substitutions of this highly conserved residue. In our  
459 cohort, the change was identified in a case with bilateral corneal opacification, congenital  
460 aphakia and microphthalmia with iris and optic disc coloboma. The same variant has been  
461 previously reported as a *de novo* event in an individual diagnosed with bilateral corneal  
462 opacification, glaucoma, and rudimentary lenses (Ma et al., 2018). Interestingly, Ma et al.  
463 (2018) also described another variant affecting the same amino acid, p.(Gly94Glu), in a  
464 proband with total sclerocornea and cataractous disc-like lenses with microcornea. Mice  
465 models expressing heterozygous missense mutations (e.g. Cx50D47A, Cx50S50P,  
466 Cx50V64A and Cx50R205G) (Graw et al., 2001; Xia et al., 2006; Xia et al., 2012; Berthoud  
467 et al., 2013) or with complete *Gja8* knockout (White et al., 1998; Rong et al., 2002) have  
468 shown that Gja8 is important for lens development. Therefore, the identification of glycine 94  
469 mutations in three individuals with lens abnormalities supports the hypothesis that this amino  
470 acid is particularly important for GJA8 to perform this role in eye development. Interestingly,  
471 the phenotype of bilateral aphakia associated with sclerocornea overlaps with that of  
472 individuals with biallelic mutations in *FOXE3* (Iseri et al., 2009). Therefore, when screening  
473 patients with this phenotype, it is important to screen for variants in both *FOXE3* and *GJA8*.

474 Multiple sequence alignment indicated that all likely pathogenic sequence variants identified  
475 in our cohort affected conserved residues (Supplementary Fig. 1) and were located within the  
476 N-terminal region of the protein (Fig. 3). Our findings are consistent with previous studies,  
477 since mutations associated with cataracts tend to cluster between TM1 and TM2 (Yu et al.,  
478 2016). The transmembrane domains are thought to play an important role in oligomerisation  
479 and pore formation, while the ECL1 domain is important in the docking of two opposing HCs  
480 to form the GJCs. While these domains are evolutionarily conserved and present high  
481 homology among the members of the Cx family, the CT region is the most isotype-specific  
482 domain and contains motifs for regulatory kinases (Liu et al., 2011; Wang et al., 2013). In  
483 this region, we identified a novel missense change, p.(Leu292Gln), of unknown clinical  
484 significance. The variant was found in a proband (family 8) who also carried an insertion of  
485 13bp in *PAX2* (NM\_003987.2:c.529\_530ins13, p.(Ala177Glyfs\*8)). Heterozygous variants  
486 of *PAX2* (MIM 167409) are identified in approximately half of the cases presenting with  
487 renal coloboma syndrome (Bower et al., 2012), also known as papillorenal syndrome (OMIM  
488 120330). Therefore, this novel *PAX2* variant is likely to be responsible for optic nerve  
489 coloboma and kidney anomalies observed in the patient, but it is possible that the *GJA8*  
490 variant might lead to a subtle effect on the protein function and contribute to his mild cataract  
491 phenotype.

492 By contrast, the three additional heterozygous variants p.(Leu7Met) (family 9),  
493 p.(Asn220Asp) (family 10) and p.(Gly333Arg) (family 11) were considered as likely benign.  
494 These were identified in individuals with AMC, but without cataracts, in unaffected parents  
495 either in this or previous studies (Ma et al., 2016; Kuo et al., 2017) and in controls  
496 (gnomAD).

497 Human *GJA8* maps to a structurally complex locus on chromosome 1q21.1-q21.2, with at  
498 least four large blocks of highly homologous SDs, which make it prone to nonallelic

499 homologous recombination (NAHR) (Mefford et al., 2008). As with other genomic loci  
500 subject to recurrent rearrangements (such as 15q11, 15q13, 16p11.2, 16p12.1, 16p13.11,  
501 17q12, 22q11.2) (Girirajan and Eichler, 2010; Stankiewicz and Lupski, 2010), 1q21 CNVs  
502 have been associated with a wide range of phenotypes including dysmorphic features,  
503 developmental delay, neuropsychiatric disorders, and cardiac and eye anomalies. The  
504 reported eye anomalies include cataracts (Brunetti-Pierri et al., 2008; Mefford et al., 2008;  
505 Rosenfeld et al., 2012; Bernier et al., 2016; Ha et al., 2016) and in a minority of cases more  
506 severe defects such as microphthalmia (Mefford et al., 2008) and coloboma (Brunetti-Pierri et  
507 al., 2008).

508 The most common 1q21 CNVs occur between the breakpoints BP3 and BP4 (Fig. 2a),  
509 spanning ~1.35 Mb (Mefford et al., 2008). This region contains only ~800 kb of unique (i.e.  
510 nonduplicated) DNA sequence (Bernier et al., 2016) and includes at least 11 genes (*NBPF19*,  
511 *NBPF13P*, *PRKAB2*, *CHD1L*, *PDIA3P1*, *FMO5*, *LINC00624*, *BCL9*, *ACP6*, *GJA5* and  
512 *GJA8*), which might contribute to different aspects of the disease manifestations observed.

513 Alternatively, 1q21 CNVs can involve only the proximal region (BP2-BP3) or both the  
514 proximal and the distal region (BP1/BP2-BP4). Microdeletions of the proximal region have  
515 been reported to be a predisposing factor for Thrombocytopenia-absent radius (TAR)  
516 syndrome (Klopocki et al., 2007), together with sequence variants in the *RBM8A* gene.

517 Within the distal region, a potential role in eye development has been shown for two of the  
518 genes, *GJA8* and *BCL9*. A recent study has demonstrated that *Bcl9* is a downstream effector  
519 of *Pax6* during mouse lens development (Cantù et al., 2014). However, the role of *BCL9* in  
520 human eye development has not yet been established. Given the involvement of *GJA8* in both  
521 cataractogenesis and ocular growth, as previously described, this gene seems to be a good  
522 candidate for the ocular anomalies observed in some of the 1q21 CNV carriers.

523 In most cases, the 1q21 rearrangements are inherited. Their presence in unaffected parents  
524 has brought into question their pathogenic significance, but the analysis of large clinical and  
525 population cohorts has shown that 1q21 microdeletions/microduplications occur at  
526 significantly higher frequency in individuals with clinical diagnoses compared with controls  
527 (Brunetti-Pierri et al., 2008; Mefford et al., 2008; Rosenfeld et al., 2012; Bernier et al., 2016).  
528 In particular, the comparison of a large cohort of individuals with developmental delay,  
529 intellectual disability, dysmorphic features and congenital anomalies with previously  
530 published control cohorts showed that the frequency of BP2-BP4 deletions was 0.024% in  
531 cases (11/45744) *versus* 0.002% in controls (1/65927), whereas the frequency of BP3-BP4  
532 deletions was 0.285% in cases (86/30215) *versus* 0.018% in controls (12/65282) (Rosenfeld  
533 et al., 2012). This enrichment suggests that these CNVs might increase susceptibility to  
534 developmental anomalies with variable expressivity and incomplete penetrance, although the  
535 factors underlying their heterogeneous phenotypes remain unexplained. In this study, we  
536 identified four microdeletions in five families, three overlapping with the  
537 microdeletions/microduplications recurrently found between breakpoints BP3 and BP4 and  
538 one larger BP2-BP4 microdeletion encompassing both the proximal and the distal region.  
539 Given the rarity of these rearrangements, the presence of 1q21 microdeletions in our AMC  
540 cohort, with a frequency of 2.13% in AMC cases for BP3-BP4 microdeletions ( $p=1.17 \times 10^{-7}$ )  
541 and a frequency of 0.53% in AMC cases for BP2-BP4 microdeletions ( $p=0.0057$ ), seems to  
542 support their role as a risk factor for developmental disorders, including eye anomalies.  
543 However, consistent with previous studies, the segregation pattern in families 13 and 15  
544 indicates that other genetic and/or environmental modifiers are likely to be important for the  
545 phenotypic outcome. Therefore, the exact genotype-phenotype correlation remains to be  
546 established.

547 Mouse models have shown that *Gja8* copy number losses and point mutations act through  
548 different mechanisms and modes of inheritance. Deletions of the entire coding region of the  
549 gene cause cataracts and microphthalmia only when homozygous, indicating a recessive  
550 mode of inheritance (White et al., 1998; Rong et al., 2002). In contrast, mouse strains  
551 carrying pathogenic *Gja8* missense mutations develop microphthalmia and cataracts in a  
552 dominant or semi-dominant fashion (Steele et al., 1998; Graw et al., 2001; Chang et al., 2002;  
553 Liska et al., 2008; Xia et al., 2012). Since Cxs function in hexameric complexes which can be  
554 homo- or heteromeric, it is possible that the impact of single amino acid substitutions may be  
555 more severe than the loss of one functional allele. Mutant Cx subunits can interfere with  
556 correct formation of the oligomeric complexes in a dominant negative manner and, since  
557 GJCs can be formed by different types of Cx subunits, this effect can also extend to the  
558 function of other Cxs. Functional and cellular studies have shown that point mutations can  
559 alter the activity of the human GJA8 protein in various ways (Beyer et al., 2013). For  
560 instance, pathogenic variants can cause misfolding, improper oligomerisation and/or  
561 trafficking defects, leading to a reduced number of functional channels on the membrane.  
562 Alternatively, the pathogenic variants could alter some physiological properties of the  
563 channels, such as permeability or conductance, or lead to the formation of HCs with new and  
564 aberrant functions. Therefore, a single base mutation can affect several aspects of the Cx  
565 function. This complexity may explain the phenotypic heterogeneity observed among the  
566 carriers of *GJA8* variants, and also the difference in penetrance between sequence and copy  
567 number variations.

568 Intra-familial phenotypic variability was also observed for the sequence variant identified in  
569 family 1 (p.(Thr39Arg)) possibly related to mosaicism. While the proband carrying the  
570 heterozygous change presented with bilateral cataracts and microphthalmia, his mother, who  
571 was 25% mosaic for this variant, had a milder phenotype of early onset cataracts. Therefore,



572 we hypothesize that the somatic mosaicism detected in the mother may explain her milder  
573 phenotype and that lower doses of aberrant GJA8 protein during eye development might be  
574 responsible for less severe phenotypic outcomes. In support of this, a recent study has  
575 reported a correlation between the severity of developmental eye abnormalities and somatic  
576 mosaicisms of *Pax6* mutations in CRISPR/Cas9 genome-edited mouse embryos (Yasue et al.,  
577 2017).

578 In conclusion, this study expands our knowledge of the role of *GJA8* in eye development,  
579 highlighting how genetic alterations of this gene are likely to give rise not only to early onset  
580 cataracts, but also to other developmental eye anomalies. The screening of *GJA8* in 426  
581 individuals with AMC resulted in the identification of six likely pathogenic variants in seven  
582 families. In the six families where segregation analysis was possible, the variants co-  
583 segregated with both early onset cataracts and microphthalmia. In one singleton case with  
584 aphakia and corneal opacification where no segregation analysis was possible, we identified  
585 the variant p.(Gly94Arg). This finding, in combination with two previously reported patients  
586 with lens development abnormalities and with variants affecting the same amino acid,  
587 highlights the importance of this specific residue in the function of GJA8 and suggests that  
588 *GJA8* mutations can be responsible for phenotypes often associated with *FOXE3* variants.  
589 The role of *GJA8* microdeletions in AMC remains uncertain: the enrichment of rare 1q21  
590 microdeletions in our cohort seems to support their role as risk factors for developmental eye  
591 disorders. However, the incomplete segregation and the phenotypic variability of these  
592 variants indicate that other genetic and/or environmental factors might be of importance. In  
593 summary, these data expand the spectrum of human phenotypes associated with *GJA8*  
594 variants and the identification of specific mutations contributes to our understanding of their  
595 genotype-phenotype correlation. Therefore, this study demonstrates the importance of  
596 screening *GJA8* in individuals with developmental eye anomalies.

597 **Conflict of Interest:** On behalf of all authors, the corresponding author states that there is no  
598 conflict of interest.

599

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827

828 **FIGURE LEGENDS**

829 **Fig. 1 *GJA8* likely pathogenic sequence variants identified in 7 unrelated families with**  
830 **AMC. a.** Pedigree of family 1. Sanger sequencing results showing the segregation of the  
831 missense variant p.(Thr39Arg) are presented. The chromatogram of individual II:2 is  
832 suggestive of mosaicism. **b.** Photographs of the affected individuals of family 1 showing  
833 intra-familial phenotypic variability. The mother (II:2, top) presented with a milder  
834 phenotype, which included right exotropia and normal sized eyes with bilateral  
835 pseudophakia. The proband (III:1, bottom) presented with right microphthalmia and complete  
836 corneal opacification on the left. **c.** Absolute quantification of the allele abundance for the  
837 variant c.116C>G; p.(Thr39Arg) in family 1. Digital Droplet PCR (ddPCR) assays were  
838 performed using a Taqman FAM-labeled probe for genotyping the mutant allele and a VIC-  
839 labeled probe to detect the wild-type allele. On the left, 1-D fluorescence amplitude plot of  
840 droplets shows mutant allele detection in the FAM channel for the heterozygous carrier  
841 (III:1), the putative mosaic mother (II:2), a wild-type homozygous carrier (I:2) and no  
842 template control (NTC). FAM-positive droplets (blue), containing the mutant allele, exhibit  
843 increased fluorescence compared to negative droplets (grey). On the right, the fractional  
844 abundance of the mutated allele, represented in percentage, was calculated for the FAM-  
845 positive droplets versus VIC-positive droplets (wild type allele), confirming the mosaicism of  
846 this variant in individual II:2. **d.** Pedigree of family 2. On the left, a representative sequence  
847 chromatogram shows the heterozygous missense variant p.(Trp45Leu). The genotype of the  
848 six individuals tested for the variant is indicated below each symbol. **e-f.** Pedigree of families  
849 3 and 4, both carrying the missense variant p.(Asp51Asn). The sequence chromatograms  
850 show that the variant occurred *de novo* in family 3. N/A, genotype not available. In family 4,  
851 representative sequence chromatogram showing the p.(Asp51Asn) and pedigree indicating  
852 the inheritance of affected status and of the variant. For family 4, fully filled symbols

853 represent individuals affected with congenital cataracts and microphthalmia, but without  
854 glaucoma, quarter filled symbols represent individuals with congenital glaucoma. **g.** Pedigree  
855 of family 5 and sequence chromatograms showing the missense variant p.(Phe70Leu). **h.**  
856 Pedigree of family 6. The adopted child carries the missense variant p.(Gly94Arg). **i.**  
857 Pedigree of family 7. Sanger sequencing results show that the missense variant p.(Val97Gly)  
858 arose *de novo* in the child II:1

859 **Fig. 2 GJA8 structural variants identified in 5 unrelated families with AMC.** **a.** Modified  
860 schematic from the UCSC Genome Browser (NCBI Build GRCh37/hg19). Partial ideogram  
861 of the chromosome bands 1q21.1-q21.2 and the multiple blocks of highly homologous  
862 segmental duplications (SD) present in this region are shown. SD, reported under the UCSC  
863 track 'Duplications of >1000 Bases of Non-RepeatMasked Sequence', are stretches of DNA  
864 of at least 1 kb in length, sharing a sequence identity of at least 90% with another genomic  
865 region on the same or on a different chromosome (inter- or intra-chromosomal SD). The  
866 colours indicate different levels of similarity between duplications (grey: 90-98% similarity,  
867 yellow: 98-99% similarity, orange: greater than 99% similarity). The breakpoint regions  
868 (BP2, BP3 and BP4) overlapping with these SD clusters are represented by green bars. The  
869 genomic locations of the 1q21 deletions identified in this study are represented by red bars  
870 and indicated with family identifiers. RefSeq Genes are indicated by dark-blue rectangular  
871 bars. For genes with multiple isoforms, the bars represent the coordinates of the maximal  
872 region among the isoforms. **b.** Pedigrees of the families carrying heterozygous 1q21 deletions

873 **Fig. 3 GJA8 mutation spectrum.** Schematic of GJA8 showing the protein domains  
874 according to UniProt (entry ID: P48165). Above: previously published mutations are shown.  
875 Below: the missense variants identified in our cohort are indicated: red indicates likely  
876 pathogenic, blue, likely benign and grey, unknown clinical significance. NT, N-terminal

877 domain; TM, transmembrane domain; ECL, extracellular loop; ICL, cytoplasmic loop; CT,  
878 C-terminal domain