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**Article:**

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1  
2  
3  
4 **Cover Letter for European Journal of Medical Genetics Revision Submission**  
5

6 **Submission to: Prof Verloes, Editor-in-chief, Clinical Genetics**  
7

8  
9 **Title of paper: *MAN1B1* causing a congenital disorder of glycosylation with a**  
10 **distinct phenotype**  
11

12  
13  
14 **Date submitted: 31.5.2018**  
15

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17

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31

32  
33  
34 Dear Prof. Verloes,  
35

36  
37 Attached is the revised manuscript detailing two families with recessive variants in  
38 *MAN1B1* known to cause a form of CDG-type II. We have expanded the phenotype  
39 on this rare form of congenital disorder of glycosylation. I hope you are able to  
40 consider this submission favourably.  
41  
42  
43

44 Thank you.  
45

46 Yours sincerely,  
47  
48  
49

50  
51 Meena Balasubramanian  
52  
53  
54  
55  
56  
57  
58  
59

1  
2  
3 Ref: EJMG\_2018\_212

4 Title: MAN1B1 causing a congenital disorder of glycosylation with a distinct phenotype  
5 Journal: European Journal of Medical Genetics  
6

7  
8 Professor Verloes

9  
10 Editor-in-Chief

11  
12 European Journal of Medical Genetics

13  
14 Dear Professor Verloes,

15  
16 Thank you for considering my manuscript submitted to European Journal of Medical  
17 Genetics. Please see attached an in-depth revision of the work which is much more concise as  
18 suggested by the reviewers. I hope this revision meets your approval and you are able to  
19 consider our work favourably.  
20

21 Your's sincerely,

22  
23 Meena Balasubramanian

24  
25  
26 **Response to comments from the editors and reviewers:**

27  
28 **Response to Editor's comment: declaration to a public database appears missing**

29  
30 Dissemination of the information about published genetic and genomic variants is important.  
31 As requested in the Guidelines for Authors, at this stage, **you have to submit new and rarely**  
32 **(less than 5 times) reported DNA variants or CNV mentioned in this article to a public**  
33 **database** such as ClinVar (<http://www.ncbi.nlm.nih.gov/clinvar>) or to any other suitable  
34 public reference database (LOVD, DECIPHER...) before your publication could be  
35 definitively accepted. Please mention the database and quote accession number(s) in the  
36 manuscript, in the result section.  
37

38  
39 *Response: Apologies, this has now been added.*

40  
41 **-Response to Reviewer 1**

42  
43 This manuscript reports on three novel MAN1B1-CDG patients with three novel variants.  
44 The language needs attention. The text should be made more concise and avoid redundancy  
45 and self-evident statements. I propose to summarize the reported and present patients in a  
46 table, so that the discussion can be shortened by referring to this table.  
47

48  
49 *Response: Thank you, we have taken your well thought out suggestions on board and made*  
50 *the manuscript concise as suggested below.*

51  
52 The following are corrections, and suggestions for improvement.

53  
54 TITLE: should tell what is new; therefore I propose the following: "MAN1B1-CDG: three  
55 novel variants"  
56  
57  
58  
59

60  
61  
62 ABSTRACT: L 47-57: are a group of genetic diseases due to .....It comprises a high ..... and  
63 a wide range of clinical phenotypes (drop the last sentence; is self-evident)  
64

65 L 62-64: two families each with two siblings with ID  
66

67 L 69-72: included isoelectrofocusing (IEF) of serum transferrin.  
68

69 L 74-77: Results: The four patients were found to have three novel variants in  
70 MAN1B1 Inherited from their parents. Serum transferrin IEF showed a type 2 pattern.  
71

72 L 79-90: Discussion: The present patients showed the phenotype previously  
73 reported in MAN1B1-CDG: ID, a characteristic facial dysmorphism, hypotonia, truncal  
74 obesity, and, in some,  
75 behavioural problems.  
76

77 L 92-101: Conclusion: In unexplained ID, serum transferrin should be included in  
78 the first-line screening.  
79

80 INTRODUCTION: L 111-114: lipids. There are over 100 known CDG.  
81

82 L 121-130: to be dropped.  
83

84 L 130-136: CDG due to an N-glycosylation defect are divided into CDG-I  
85 (glycan assembly defects in cytosol and ER) and CDG-II (glycan remodelling defects in  
86 Golgi).  
87

88 L 136-147: to be dropped.  
89

90 L 149-158: recessive disorder characterized by variable  
91 intellectual/developmental disability, a characteristic facial dysmorphism, truncal obesity and  
92 hypotonia. The facial  
93 dysmorphism comprises prominent  
94

95 L 164-173: have been reported . Serum transferrin IEF shows a type 2  
96 pattern.  
97

98 CLINICAL REPORT: L 186: aged 8 years, first  
99

100 L 188-190: 'There are .... Well' is not relevant.  
101

102 L 192-197: At the age of 6 months he was noted to be hypotonic and  
103 showed a delayed development. He sat at 14 months  
104

105 L 207-211: (Figure 1a). Weight was consistently ....., height between the  
106 50th and 75th centiles and head  
107

108 L 218: Brain MRI at 3.5 years  
109

110 L 227-229: At 8 years of age, he was .....support, and was  
111  
112  
113  
114  
115  
116  
117  
118

119  
120  
121 L 231: He was able to speak in  
122

123 L 241-253: There was marked truncal obesity although he had a normal  
124 ..... Behaviour. He had a similar facial appearance as before (...). Weight was 60.6 .....  
125 centile), ....132  
126 cm ...circumference 54 cm. Transferrin IEF, following ..., showed  
127 a type 2 pattern.  
128  
129

130 L 257-261: He showed delayed development with cruising ..... age, and  
131 walking at 3 years  
132

133 L 272-278: Weight was 13.9 kg .... centile), height 89 cm .... and ..... 54 cm  
134 ...of age. Brain MRI at 2 years  
135

136 L 285: At 6 years of age, he was ... school; he  
137

138 L 291: by the family  
139

140 L 298: but able to walk  
141

142 L 302: (Fig. 2c). Weight was  
143  
144

145 L 307: showed a type 2 pattern.  
146

147 L 318-322: noted. She walked at 19 months of age and her first ..... 18  
148 months  
149

150 L 336: back. Weight was .... (between 91st and 98th centiles) ... 126 cm ...  
151 centiles ..... cm  
152

153 L 345-360: support. Weight was ..... cm ..... showed a type 2 pattern.  
154

155 L 362: is the younger ... of patient 3... At 6 years, she was  
156

157 RESULTS: L 402, 403: identified in patient 1 a  
158

159 L 407-409: reported before. In silico  
160  
161

162 L 416, 417: was found .... variant as his brother. These  
163

164 DISCUSSION: L 438-442: ( ....) has 13 coding exons and encodes a Golgi mannosidase. This  
165 enzyme is involved in the N-glycan remodeling.  
166

167 L 445-495: I suggest to remove this part because it is not really relevant for this  
168 case report.  
169

170 FIGURE LEGENDS: L 804, 811 and 818: patient  
171

172 L 806 and 813: Brain MRI shows  
173  
174  
175  
176  
177

178  
179  
180 *Response: Thank you, I have incorporated all the above changes in keeping with the style of*  
181 *written text.*  
182

183  
184  
185 **-Response to Reviewer 2**  
186

187 The authors presented two families with MAN1B1-CDG. MAN1B1-CDG is very  
188 rare. But undiagnosed patients with MAN1B1-CDG may exist. Clinical report is well written.  
189 Figures for molecular studies are not enough.  
190

- 191 1. The authors should show the results of transferrin glycoforms by IEF.
- 192
- 193 2. The authors should show the family trees.
- 194
- 195 3. All reported patients with MAN1B1-CDG should be summarized in a Table.
- 196
- 197 4. Screening for CDG should include O-linked type abnormalities.
- 198

199 **Minor points**  
200

201 In page 5, golgi should be Golgi.  
202

203 Line 519, then should be there.  
204

205 *Response: Thank you for your excellent suggestions which have now been incorporated.*  
206

207 *I hope you are able to consider this revised manuscript favourably.*  
208

209 *Yours sincerely,*  
210

211 *Meena Balasubramanian*  
212  
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236

**MAN1B-CDG: novel variants with a distinct phenotype and review of literature**

**Running Title:** *MAN1B* recessive variants causing CDG- type II

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**Keywords:** glycosylation, intellectual disability, transferrins, obesity, syndromal

**ABSTRACT**

Background: Congenital disorders of glycosylation (CDG) are a group of rare metabolic diseases due to impaired lipid and protein glycosylation. It comprises a characteristic high frequency of intellectual disability (ID) and a wide range of clinical phenotypes.

60  
61  
62 Objective(s): To identify the underlying diagnosis in two families each with two siblings with  
63  
64 variable level of ID through trio whole exome sequencing.  
65

66  
67 Methods: Both the families were recruited to the Deciphering Developmental Disorders  
68  
69 (DDD) study to identify the aetiology for their ID. Further work-up included isoelectric  
70  
71 focusing (IEF) of serum transferrin done to add evidence to the molecular diagnosis.  
72

73  
74 Results: The four patients were found to have three novel variants in *MAN1B1* inherited from  
75  
76 their healthy parents. Serum transferrin IEF showed a type 2 pattern.  
77

78  
79 Discussion: *MAN1B1* variants were initially described in association with non-syndromic ID;  
80  
81 subsequent literature suggested that variants in *MAN1B1* resulted in a CDG-type II syndrome.  
82  
83 However, there remains a paucity of literature on detailed clinical phenotyping and it still  
84  
85 remains a rare form of CDG. The present patients showed the phenotype previously reported  
86  
87 in *MAN1B1*-CDG: a characteristic facial dysmorphism, hypotonia, truncal obesity and in  
88  
89 some, behavioural problems.  
90

91  
92 Conclusions: In unexplained ID, serum transferrin should be included in the first-line  
93  
94 screening. With advances in genomic medicine, it is important to diagnose CDG as this has  
95  
96 implications for management and recurrence risk counselling.  
97  
98  
99

## 100 101 102 **INTRODUCTION**

103  
104  
105 Congenital disorders of glycosylation (CDG) are a rapidly growing group of inborn errors of  
106  
107 metabolism with abnormal glycosylation of proteins and lipids. There are over 100 known  
108  
109 CDG. CDG due to an N-glycosylation defect are divided into CDG-I (glycan assembly  
110  
111 defects in cytosol and endoplasmic reticulum (ER)) and CDG-II (glycan remodelling defects  
112  
113 in Golgi). CDG-type I rarely present with isolated ID; in contrast, CDG-type II has a highly  
114  
115  
116  
117  
118

119 heterogeneous clinical presentation with lack of specific clinical clues to suggest an  
120  
121  
122  
123 underlying diagnosis. This is especially true for MAN1B1-CDG<sup>(1)</sup>.  
124

125  
126 MAN1B1-CDG is an autosomal recessive disorder characterized by variable  
127  
128 intellectual/developmental disability, a characteristic facial dysmorphism, truncal obesity and  
129  
130 hypotonia. The facial dysmorphism comprises prominent eyebrows with lateral thinning,  
131  
132 downward-slanting palpebral fissures, bulbous tip of the nose, large ears and thin upper lip.  
133  
134 Behavioural problems including overeating, verbal and physical aggression have also been  
135  
136 reported in some cases. Serum transferrin IEF shows a type 2 pattern <sup>(2)</sup>.  
137  
138  
139  
140  
141

## 142 **CLINICAL REPORT**

143  
144  
145 **Patient 1:** Patient 1 is the older sibling in Family 1 aged 8-years, first child of healthy, non-  
146  
147 consanguineous White European parents with no significant family history (Figure 1a). He  
148  
149 was born following a normal pregnancy at term with a birthweight of 3.37 kilograms (25<sup>th</sup>  
150  
151 centile). At the age of 6 months, he was noted to be hypotonic and delayed with his  
152  
153 development. He sat at 14-months, crawled at 19-months and started walking at over 2 years  
154  
155 but remained unsteady on his feet with a wide-based gait. He had no speech and  
156  
157 communicated using Makaton. He was reported to have occasional night tremors but no overt  
158  
159 seizures. He was reviewed in the Genetics clinic from 2-years and noted to be dysmorphic  
160  
161 with anterior hair whorl, frontal bossing, hypertelorism, downward-slanting palpebral fissures  
162  
163 and a mild pectus carinatum (Figure 1b). Weight was consistently above the 91<sup>st</sup> centile,  
164  
165 height between the 50<sup>th</sup>-75<sup>th</sup> centiles with head circumference on 50<sup>th</sup> centile. Investigations  
166  
167 at the time included normal 60K arrayCGH, chromosome breakage studies and metabolic  
168  
169 work-up (including plasma and urinary amino acids and organic acids, CK, serum lactate,  
170  
171 renal and liver function tests and bone profile). MRI-brain at 3.5-years was structurally  
172  
173  
174  
175  
176  
177

178  
179  
180 normal (Figure 1c) and MR spectroscopy was essentially normal. He was subsequently  
181  
182 enrolled to the DDD study and saliva samples taken for trio WES (Decipher ID: 272692).  
183  
184

185 At recent review aged 8-years, he was in a mainstream school with additional support, was  
186  
187 able to read and write. He remained unsteady on his feet with supportive footwear. He was  
188  
189 able to speak in short sentences. He had a happy, friendly personality and there were no  
190  
191 behavioural concerns. He had been toilet-trained since 6-years of age. There was marked  
192  
193 central obesity although he had normal appetite and no food-seeking behaviour. He had a  
194  
195 similar facial appearance to before (Figure 1d-e); weight~60.6 kilograms (75<sup>th</sup>-91<sup>st</sup> centile);  
196  
197 height~132 cms (75<sup>th</sup> centile) and head circumference of 54 cms (50<sup>th</sup> centile). Transferrin  
198  
199 IEF, following identification of *MAN1B1* variants, showed a type 2 pattern.  
200  
201

202  
203 **Patient 2:** Patient 2 is the younger sibling of Patient 1 (Family 1) aged 6-years. He was born  
204  
205 following a normal pregnancy at term with a birth weight of 4.22 kilograms (91<sup>st</sup> centile). He  
206  
207 was well immediately after birth but again was noted to be delayed with his development  
208  
209 with cruising around furniture at 14-months, walking at 3-years but had no speech and unlike  
210  
211 his brother, was not communicating by sign language. He was also noted to be hypotonic and  
212  
213 wears glasses for hypermetropia. He was noted to be aggressive on occasions and had a  
214  
215 quieter personality than his older sibling. He was initially reviewed in the Genetics clinic at  
216  
217 2.5-years of age and noted to be dysmorphic with similar facial appearance to his brother  
218  
219 (Figure 2a). He also had mild 2-3 toe syndactyly, frontal bossing; weight~13.9 kilograms  
220  
221 (50<sup>th</sup>-75<sup>th</sup> centile); height~89 cms (25<sup>th</sup>-50<sup>th</sup> centile) and head circumference~54 cms (98<sup>th</sup>  
222  
223 centile) at 2.5-years of age. MRI-brain at 2-years of age identified bilateral periventricular  
224  
225 heterotopia with overlying cortical dysplasia which was thought to account for his more  
226  
227 severe developmental impairment (Figure 2b).  
228  
229  
230  
231  
232  
233  
234  
235  
236

237  
238  
239 At recent review aged 6-years, he was in a special needs school, he still remained in nappies.  
240  
241 His sleep and appetite were reported to be normal. He wore glasses and a back brace for  
242  
243 correction of scoliosis. He had no speech and was noted by the family to have occasional  
244  
245 aggressive outbursts. He was in a wheelchair for long distances, but able to walk  
246  
247 independently for short distances. There was less evidence of truncal obesity. On  
248  
249 examination, he was noted to have similar facial dysmorphism as his brother (Figure 2c);  
250  
251 weight~28.5 kilograms (98<sup>th</sup> centile); height~119 cms (75<sup>th</sup> centile) and head circumference  
252  
253 of 57 cms (98<sup>th</sup> centile). Transferrin IEF, following identification of *MAN1B1* variants,  
254  
255 showed a type 2 pattern.  
256  
257

258  
259 **Patient 3:** This patient is the older sibling in Family 2, aged 10-years and is the first child of  
260  
261 healthy, non-consanguineous White European parents with no significant family history  
262  
263 (Figure 3a). There were no concerns in the pregnancy and she was born at term+2 weeks  
264  
265 gestation with a birth weight of 4.30 kilograms (98<sup>th</sup> centile) by forceps delivery. She was in  
266  
267 a good condition immediately after birth and there were no feeding problems noted. She  
268  
269 walked at 19-months of age and her first words were at 18-months of age. She was in  
270  
271 mainstream school with additional 1:1 support. She was also noted to have occasional  
272  
273 outburst of aggressive behaviour.  
274  
275

276  
277 This patient was initially reviewed in the Genetics clinic aged 7-years following a referral by  
278  
279 the Community paediatric team in view of her developmental impairment. On examination,  
280  
281 she was noted to be dysmorphic with downward-slanting palpebral fissures, hypertelorism  
282  
283 with epicanthic folds, full lips (Figure 3b) and a café-au-lait patch on her right lower back;  
284  
285 weight~31.5 kilograms (91<sup>st</sup>-98<sup>th</sup> centiles); height~126 cms (75<sup>th</sup>-91<sup>st</sup> centiles) and head  
286  
287 circumference~54.3 cms (75<sup>th</sup> centile). She was recruited to the DDD study and saliva  
288  
289 samples obtained for trio WES (Decipher ID: 294436).  
290  
291  
292  
293  
294  
295

296  
297  
298 At recent review aged 10-years, she was noted to be in a mainstream school with support. She  
299  
300 was noted to have similar facial dysmorphism as before (Figure 3c); weight~48.2 kilograms  
301  
302 (91<sup>st</sup>-98<sup>th</sup> centiles) and height~140.6 cms (75<sup>th</sup> centile). Transferrin IEF, following  
303  
304 identification of *MAN1B1* variants, showed a type 2 pattern.  
305  
306

307 **Patient 4:** This patient in the younger sibling of Patient 3. Aged 6-years, she was referred to  
308  
309 Genetics following identification of a *MAN1B1*-CDG in the older sibling. There were initial  
310  
311 concerns in the first year of life regarding dairy intolerance but this settled. She was  
312  
313 subsequently referred to Ophthalmology for hypermetropia needing glasses. She was noted to  
314  
315 have non-specific mild global developmental delay and referred to the Genetics clinic  
316  
317 following the diagnosis in her older sibling.  
318  
319  
320  
321  
322

## 323 MATERIALS AND METHODS

324  
325

326 Both families 1 and 2 were recruited to the Deciphering Developmental Disorders (DDD)  
327  
328 study. Trio-based exome sequencing was performed on the affected individual and their  
329  
330 parents, as previously described<sup>(3)</sup>. Each affected individual also had a high-resolution  
331  
332 analysis for copy number abnormalities using array-based comparative genomic  
333  
334 hybridization (aCGH). Putative *de novo* mutations were identified from exome data using  
335  
336 DeNovoGear software<sup>(4)</sup> and were validated using targeted Sanger sequencing.  
337  
338  
339  
340  
341

## 342 RESULTS

343  
344

### 345 Genetic analysis:

346  
347

348 Trio WES through DDD study identified in Patient 1 a homozygous c.1311del,p.Leu438fs  
349  
350 likely pathogenic variant in *MAN1B1* (NM\_016219.4- HGVS nomenclature) (Figure 1f).  
351  
352  
353  
354

355  
356  
357 This variant has not been reported before; *in silico* analysis supports its likely pathogenicity  
358  
359 confirming the diagnosis of MAN1B1-CDG. Patient 2 was found to carry the same  
360  
361 homozygous variants as seen in his brother. These variants were biparentally inherited. This  
362  
363 result was confirmed by Sanger sequencing.  
364  
365

366  
367 In Family 2, trio WES in Patient 3 identified compound heterozygous *MAN1B1* variants: a  
368  
369 likely pathogenic c.761\_764del,p.Ile254Thrfs\*20 frameshift variant and a missense  
370  
371 c.1000C>T,p.Arg334Cys in *MAN1B1* (NM\_016219.4- HGVS nomenclature) which was  
372  
373 biparentally inherited (Figure 3d and e). *In silico* analysis supports its likely pathogenicity  
374  
375 confirming the diagnosis of MAN1B1-CDG. Both the variants are publicly accessible via the  
376  
377 Decipher website (<https://decipher.sanger.ac.uk>) using their Decipher ID numbers.  
378  
379  
380  
381

## 382 DISCUSSION

383  
384  
385 *MAN1B1* (OMIM 604346) which is situated on chromosome 9q34.3, has 13 coding exons  
386  
387 and encodes a Golgi mannosidase. These enzymes are involved in N-glycan remodelling.  
388  
389 These enzymes also contribute to the timing and disposal of misfolded glycoproteins through  
390  
391 the ER associated degradation (ERAD) pathway. ERManI cleaves the terminal mannose from  
392  
393 the middle branch of Man9GlcNAc<sub>2</sub>, producing a Man8GlcNAc<sub>2</sub> isomer B. This is believed  
394  
395 to play a critical role in glycoprotein quality control by targeting terminally misfolded  
396  
397 proteins in ERAD. MAN1B1 was initially predicted to act as an ER-resident protein<sup>(5)</sup> but  
398  
399 recent studies have shown that MAN1B1 localises to the Golgi apparatus in mammalian  
400  
401 cells<sup>(6)</sup>, further reinforcing the fact that quality control is not confined to ER alone but extends  
402  
403 through the secretory pathway<sup>(1)</sup>. This provides further evidence that MAN1B1 operates as a  
404  
405 check-point within the Golgi apparatus recycling misfolded proteins that escaped ERAD back  
406  
407 to ER by interacting with the COP-I machinery resulting in retrograde transport of these  
408  
409  
410  
411  
412  
413

414  
415  
416 proteins<sup>(7)</sup>. It is also said to act as a lectin retrieving these proteins back to the ER prior to  
417  
418 degradation<sup>(1)</sup>.  
419

420  
421 The likely explanation for MAN1B1 deficiency resulting in a multi-system disorder is due to  
422  
423 the fact that there is defective quality control as a result of *MAN1B1* genetic defects, resulting  
424  
425 in defective check-point unable to minimise the level and toxicity of misfolded proteins  
426  
427 within the cell. Interestingly, compared to most CDG phenotypes, MAN1B1-CDG  
428  
429 considering how important MAN1B1 activity is within the cell, only presents with a milder  
430  
431 phenotype. This supports the hypothesis that there may be more check-points within the ER-  
432  
433 Golgi machinery. Further work on the secretory pathway and its regulation in various body  
434  
435 systems will provide further insight into the phenotypic contribution of specific forms of  
436  
437 CDG.  
438  
439

440  
441 Recessive variants in *MAN1B1* were first identified in non-syndromic mental retardation-15  
442  
443 (MRT15; OMIM 614202) in four consanguineous families by Rafiq *et al.*, 2011<sup>(8)</sup>. By  
444  
445 undertaking WES and homozygosity mapping in these families with several affected siblings  
446  
447 in one generation, they were able to identify several candidate genes narrowing it down to  
448  
449 variants in *MAN1B1* as being causal<sup>8</sup>. The authors characterised the phenotype as being  
450  
451 consistent with non-syndromic AR ID except in one family (MR43) with a nonsense variant  
452  
453 where clear dysmorphism was identified, no photographs of this family were however  
454  
455 published. Description of the facial features is however very similar to the facial phenotype  
456  
457 we describe in our families. The authors concluded that ERAD is a new disease associated  
458  
459 pathway and disruption of other ERAD pathway candidates may result in a similar clinical  
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461 phenotype.  
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465 Since then, there have been very few cases of MAN1B1-CDG reported so far and matchmaker  
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467 repositories such as Genematcher, Phenome central and Decipher do not produce any  
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475 matches suggesting this remains a rare, potentially undiagnosed form of CDG. This is likely  
476  
477 because of the varying presentation and what is initially thought to be a non-specific  
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479 presentation as evidenced by our families. However, by including transferrin IEF in initial  
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481 screening of global developmental delay, along with other routine investigations such as  
482  
483 urinary organic acids, plasma amino acids will help pick this up early. Screening for CDG  
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485 should also include O-linked type abnormalities. However, interestingly, the CDG-type II  
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487 pattern seen may also indicate sample degradation requiring a repeat sample for confirmation  
488  
489 which in this cohort may not always be possible. With further genomic advances (and  
490  
491 resultant cost-benefit), use of first-line WES/ WGS in clinical practice for diagnostic work-up  
492  
493 of children with developmental delay should hopefully address this issue. It is important,  
494  
495 however, to ensure that WES/ targeted gene panels thus generated for developmental delay  
496  
497 include genes associated with CDG.  
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500  
501 Table 1 summarises all the patients reported so far with *MAN1B1*-CDG in comparison to our  
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503 cohort. Rymen *et al.*, 2013 reported seven patients with recessive variants in *MAN1B1* from  
504  
505 their cohort of unsolved CDG-type II and were able to provide further functional evidence of  
506  
507 *MAN1B1* role in protein quality at the Golgi apparatus<sup>(1)</sup>. All patients had hypotonia, variable  
508  
509 degree of ID, truncal obesity but with normal MRI-brain in all but one patient who also  
510  
511 presented with epilepsy. Behavioural problems do not appear to be a major component of the  
512  
513 phenotype unlike some other CDG identified so far.  
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516  
517 Van Scherpenzeel *et al.*, 2014 reported twelve patients with *MAN1B1* recessive variants from  
518  
519 their cohort of molecularly undiagnosed CDG-type II patients<sup>(9)</sup>. Patients in this study  
520  
521 showed a predominant neurological phenotype with moderate ID. They were also noted to  
522  
523 have macrocephaly, truncal obesity, early hypotonia and characteristic facial dysmorphism  
524  
525 including an oval face, bulbous nasal tip with thin upper lip. However, many of the patients  
526  
527 did not have the classic CDG-type II features including inverted nipples, ataxia, abnormal fat  
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534 distribution and cutis laxa. They did have a variable phenotype including intra-familial  
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distribution and cutis laxa. They did have a variable phenotype including intra-familial  
variability as is apparent in the families we report here.

Since these publications of two large cohort of patients with MAN1B1-CDG, there have only  
been couple more reports of this in literature<sup>(10,11)</sup>. Hoffjan et al., 2014 reported a Turkish  
consanguineous family with three affected siblings and recessive variants in *MAN1B1* with a  
similar phenotype as previously reported patients<sup>(10)</sup>. Gupta et al., 2016 reported two patients  
with digenic inheritance: homozygous variants in two recessive genes, *SEC23A* which is  
associated with Cranio-lenticulo-sutural dysplasia (CLSD) and *MAN1B1*<sup>(11)</sup>. The authors  
suggest a composite phenotype with variants in both these genes contributing to their clinical  
presentation.

So far, there seems to be a combination of missense, nonsense, deletion and splicing variants  
reported and there is no clear emerging genotype-phenotype correlation. However, the  
number of reported cases is still small, so with further cases being reported this may provide  
us with further clues to clarify phenotypic variability. Although there is no treatment or  
curative therapy for MAN1B1-CDG and management is purely symptomatic, it is likely that  
with advances in precision medicine, identifying the underlying genetic aetiology early in  
patients with CDG-type II may have an impact on outcomes.

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591  
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610

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613  
614  
615 All authors recruited their respective patients to the DDD study and provided data regarding  
616  
617 their patients; DDD study provided trio exome sequencing data. MB planned the study; MB  
618  
619 recruited Patient 1 to DDD; wrote manuscript; DSJ recruited Patient 3 to DDD; all authors  
620  
621 reviewed and contributed to the manuscript.  
622

623  
624 **D. Competing Interest:** None to declare for all authors.  
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## 746 747 **FIGURE LEGENDS**

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749  
750 **Figure 1a-e:** Family pedigree and photographs of patient 1 at 3 and 8 years of age  
751 demonstrating frontal bossing, oval face, down-slanted palpebral fissures, thin upper lip;  
752 MRI-brain demonstrating nonspecific right frontal high signal but otherwise essentially  
753 normal.  
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759 **Figure 1f:** Trio WES identified in Patient 1 a homozygous c.1311del,p.Leu438fs likely  
760 pathogenic variant in *MAN1B1* (NM\_016219.4- HGVS nomenclature).  
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770 **Figure 2a-c:** Photographs of patient 1 at 3 and 6 years of age demonstrating similar facial  
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772 dysmorphism to his older sibling; MRI-brain demonstrating bilateral temporal heterotopia.  
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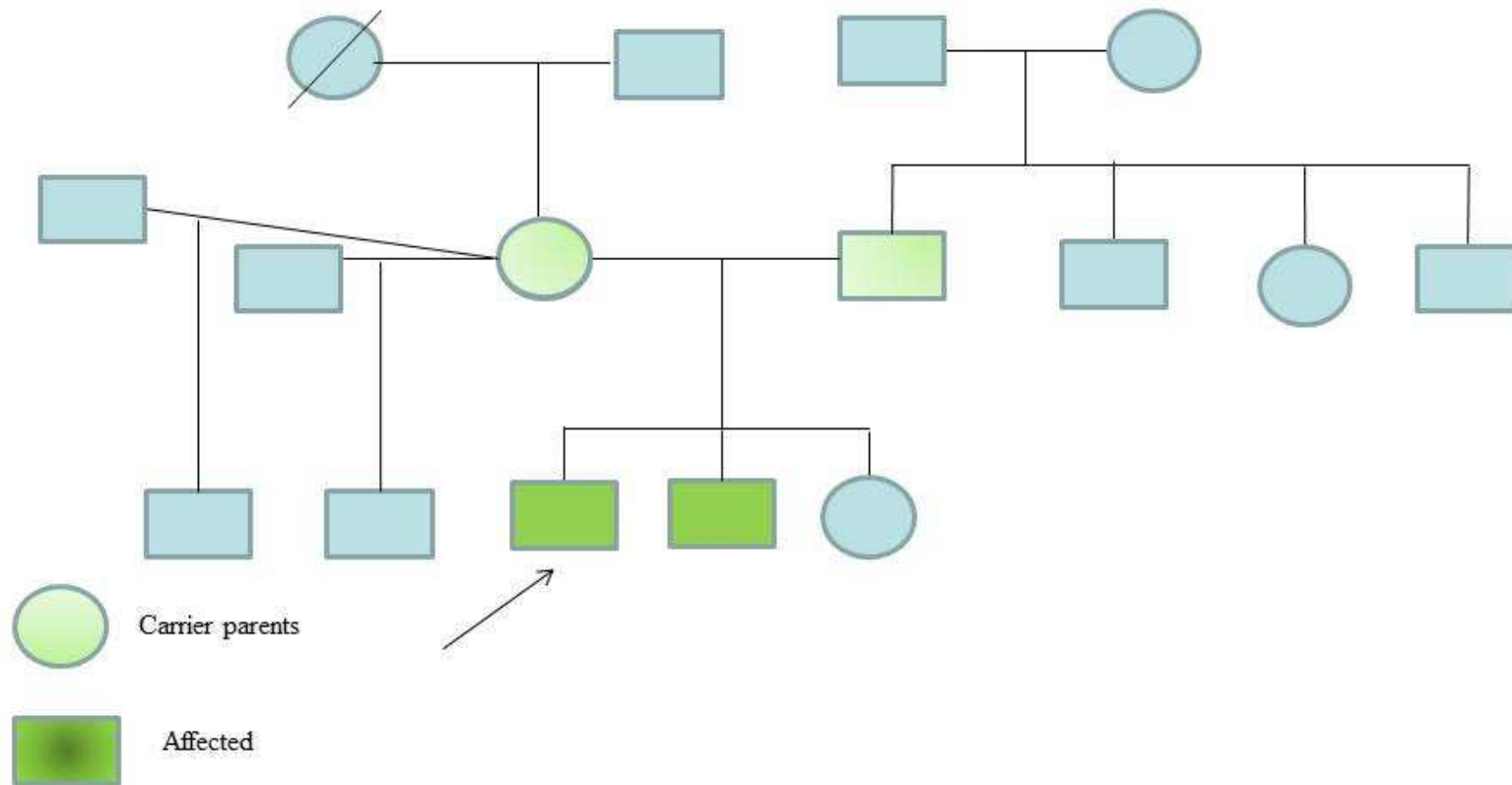
775 **Figure 3a-c:** Pedigree and photographs of patient 3 at 7-years and 10-years of age  
776  
777 demonstrating facial dysmorphism as previously described.  
778  
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780 **Figure 3d-e:** Trio WES in Patient 3 identified compound heterozygous *MAN1B1* variants: a  
781  
782 likely pathogenic c.761\_764del,p.Ile254Thrfs\*20 frameshift variant and a missense  
783  
784 c.1000C>T,p.Arg334Cys in *MAN1B1* (NM\_016219.4- HGVS nomenclature) which was  
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786 biparentally inherited.  
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**Table 1: Clinical features reported in patients with MAN1B1-CDG in comparison to our cohort**

<b>Clinical features</b>	<b>This study cohort</b>	<b>Rafiq et al.,2011</b>	<b>Rymen et al., 2013</b>	<b>Van Scherpenzeel et al.,2014</b>	<b>Hoffjan et al., 2014</b>	<b>Gupta et al., 2016</b>	<b>Total (of reported features)</b>
<b>Facial dysmorphism</b>	4/4	10/10	7/7	7/12	3/3	2/2	38/38 (100%)
<b>Seizures</b>	0/4	2/12	1/7	3/12	0/3	2/2	8/40 (20%)
<b>Hypotonia</b>	4/4	10/12	7/7	8/12	3/3	2/2	34/40(85%)
<b>Truncal obesity</b>	3/4	2/12	7/7	8/12	3/3	2/2	25/40 (62%)
<b>Delayed development</b>	4/4	12/12	7/7	12/12	3/3	2/2	40/40 (100%)
<b>Intellectual Disability</b>	4/4	12/12	7/7	12/12	3/3	2/2	40/40 (100%)
<b>Behavioural concerns</b>	2/4	2/12	2/7	3/12	0/3	0/2	9/40 (22%)
<b>Abnormal MRI-brain</b>	1/4	1/1	2/7	2/10	1/1	1/2	8/25 (32%)

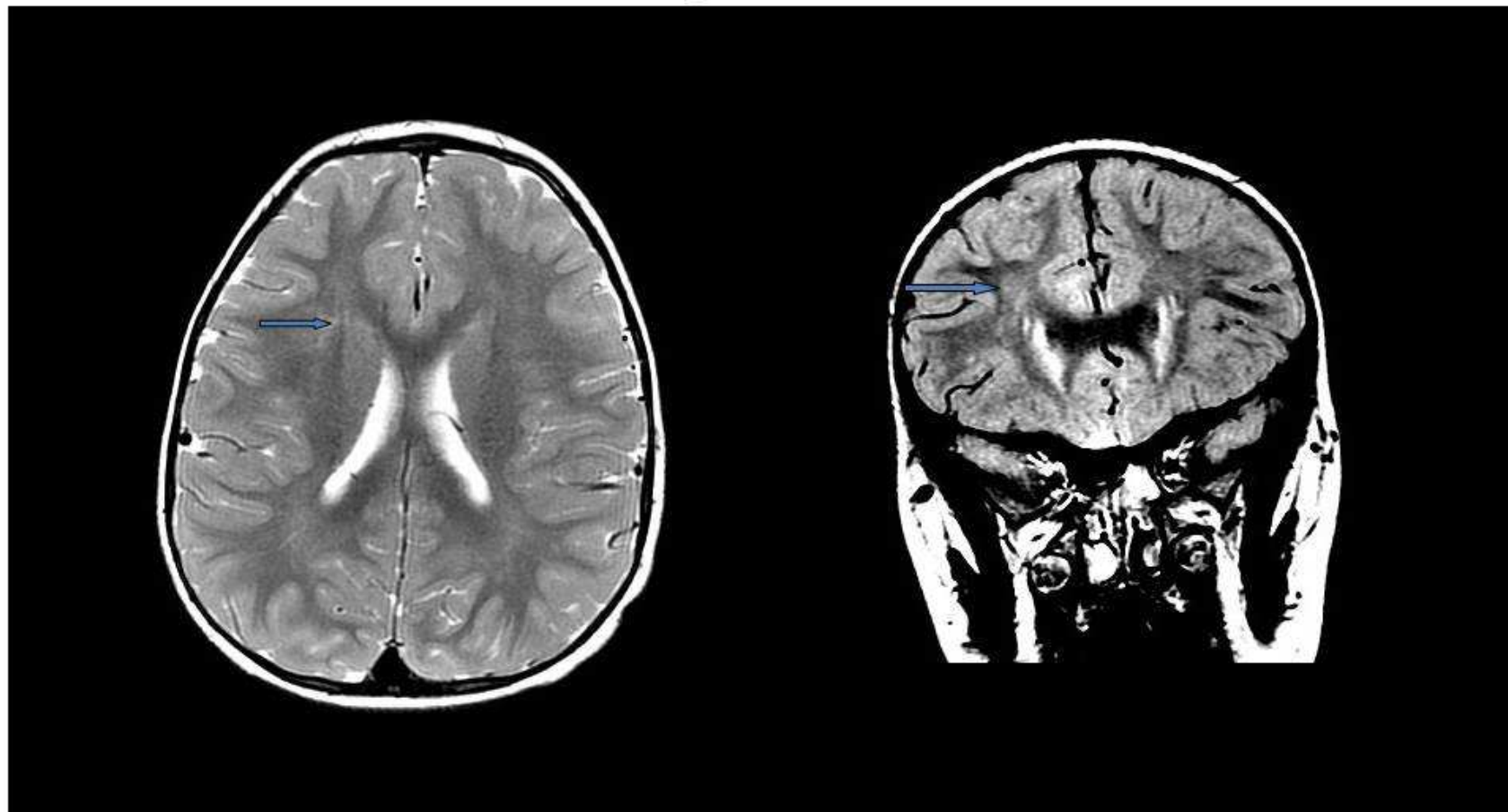
# Figure 1a



**Figure 1b**



Figure 1c



**Figure 1d**



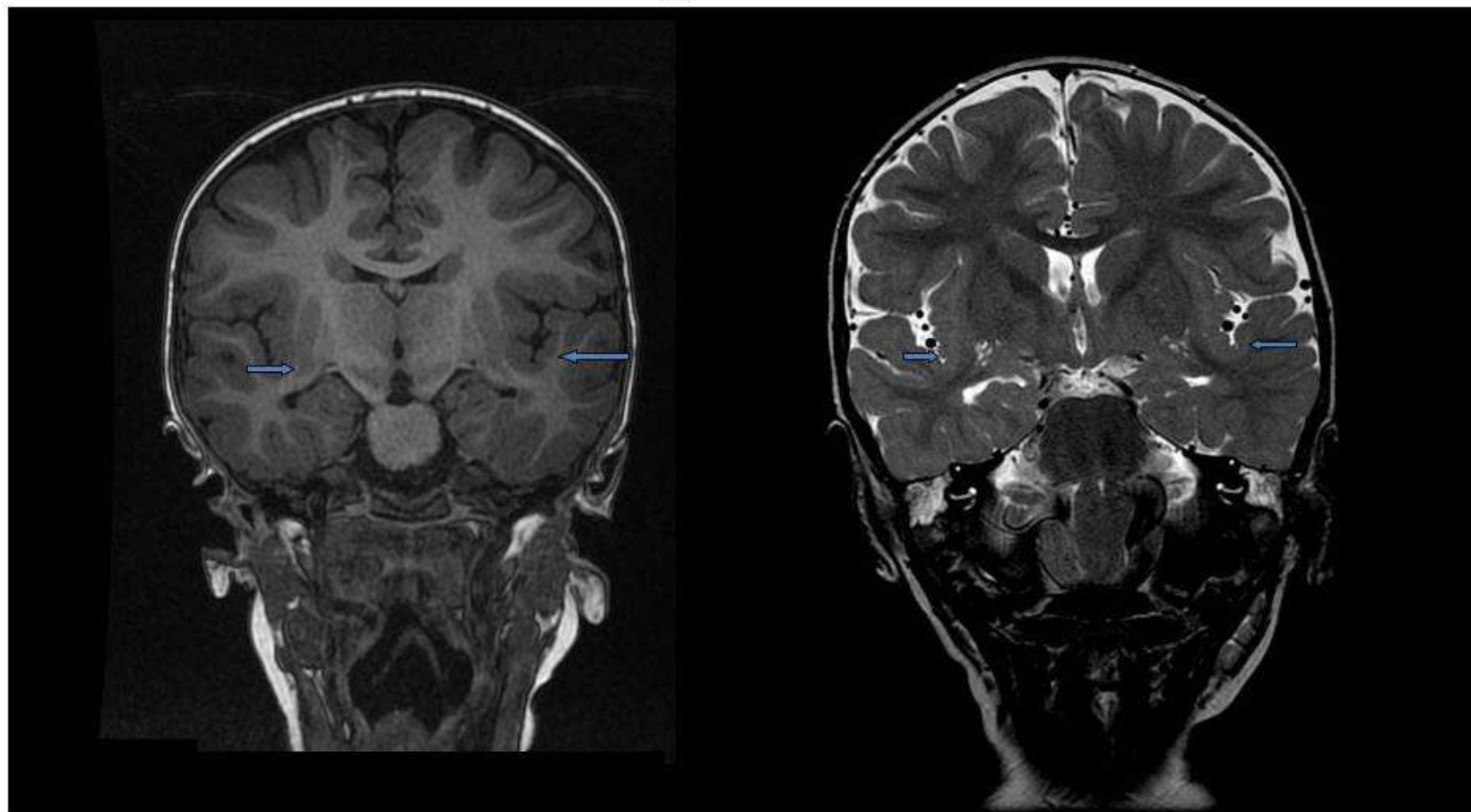
**Figure 1e**



**Figure 2a**



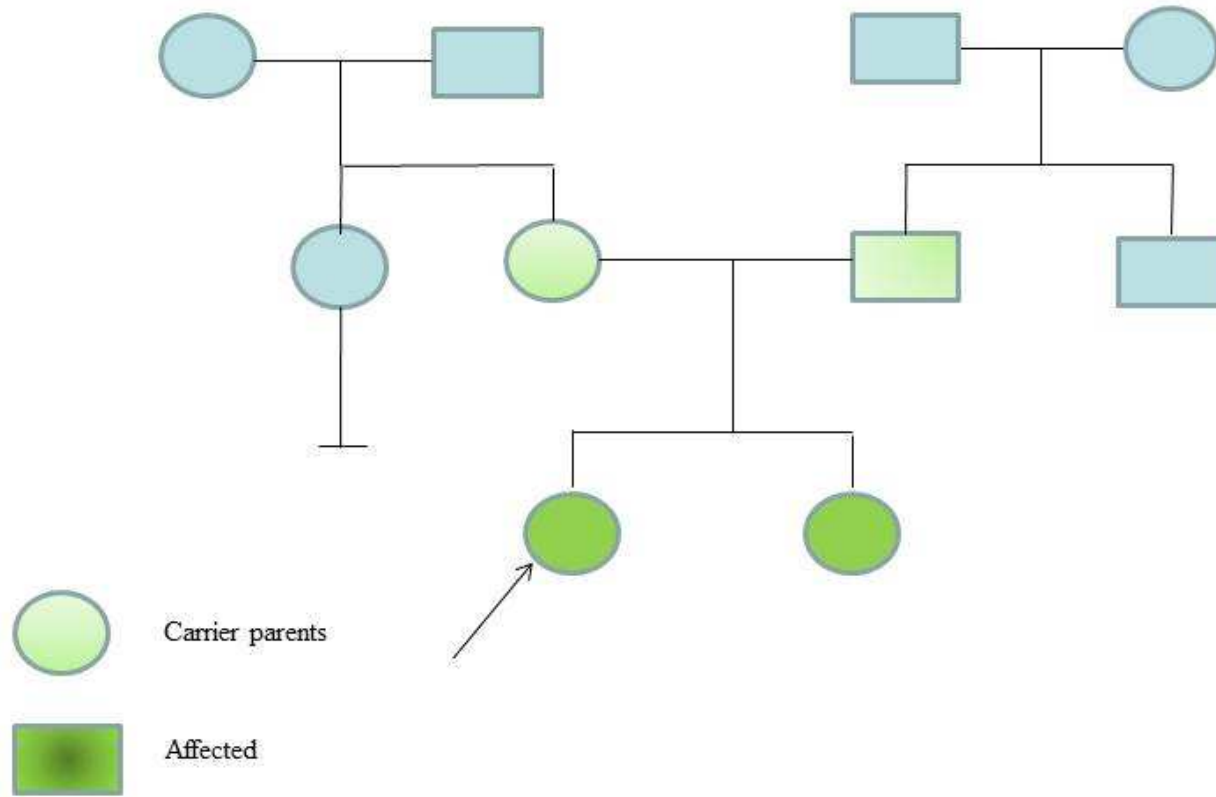
Figure 2b



**Figure 2c**



Figure 3a



**Figure 3b and c**

