
Article:
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Cover Letter for European Journal of Medical Genetics Revision Submission

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

Title of paper: *MAN1B1* causing a congenital disorder of glycosylation with a distinct phenotype

Date submitted: 31.5.2018

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Dear Prof. Verloes,

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

Thank you.

Yours sincerely,

Meena Balasubramanian

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

Professor Verloes

Editor-in-Chief

European Journal of Medical Genetics

Dear Professor Verloes,

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

Your's sincerely,

Meena Balasubramanian

Response to comments from the editors and reviewers:

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

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

Response: Apologies, this has now been added.

-Response to Reviewer 1

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

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

The following are corrections, and suggestions for improvement.

TITLE: should tell what is new; therefore I propose the following: "MAN1B1-CDG: three novel variants"

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

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

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

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

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

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

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

L 121-130: to be dropped.

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

L 136-147: to be dropped.

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

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

CLINICAL REPORT: L 186: aged 8 years, first

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

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

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

L 218: Brain MRI at 3.5 years

L 227-229: At 8 years of age, he was .....support, and was

L 231: He was able to speak in

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

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

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

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

L 291: by the family

L 298: but able to walk

L 302: (Fig. 2c). Weight was

L 307: showed a type 2 pattern.

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

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

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

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

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

L 407-409: reported before. In silico

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

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

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

FIGURE LEGENDS: L 804, 811 and 818: patient

L 806 and 813: Brain MRI shows

Response: Thank you, I have incorporated all the above changes in keeping with the style of written text.

-Response to Reviewer 2

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

1. The authors should show the results of transferrin glycoforms by IEF.

2. The authors should show the family trees.

3. All reported patients with MAN1B1-CDG should be summarized in a Table.

4. Screening for CDG should include O-linked type abnormalities.

Minor points

In page 5, golgi should be Golgi.

Line 519, then should be there.

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

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

Yours sincerely,

Meena Balasubramanian

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

Running Title: *MAN1B1* 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.

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

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

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

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

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

INTRODUCTION

Congenital disorders of glycosylation (CDG) are a rapidly growing group of inborn errors of metabolism with abnormal glycosylation of proteins and lipids. There are over 100 known CDG. CDG due to an N-glycosylation defect are divided into CDG-I (glycan assembly defects in cytosol and endoplasmic reticulum (ER)) and CDG-II (glycan remodelling defects in Golgi). CDG-type I rarely present with isolated ID; in contrast, CDG-type II has a highly

heterogeneous clinical presentation with lack of specific clinical clues to suggest an underlying diagnosis. This is especially true for MAN1B1-CDG[1].

MAN1B1-CDG is an autosomal recessive disorder characterized by variable intellectual/developmental disability, a characteristic facial dysmorphism, truncal obesity and hypotonia. The facial dysmorphism comprises prominent eyebrows with lateral thinning, downward-slanting palpebral fissures, bulbous tip of the nose, large ears and thin upper lip. Behavioural problems including overeating, verbal and physical aggression have also been reported in some cases. Serum transferrin IEF shows a type 2 pattern [2].

CLINICAL REPORT

Patient 1: Patient 1 is the older sibling in Family 1 aged 8-years, first child of healthy, non-consanguineous White European parents with no significant family history (Figure 1a). He was born following a normal pregnancy at term with a birthweight of 3.37 kilograms (25th centile). At the age of 6 months, he was noted to be hypotonic and delayed with his development. He sat at 14-months, crawled at 19-months and started walking at over 2 years but remained unsteady on his feet with a wide-based gait. He had no speech and communicated using Makaton. He was reported to have occasional night tremors but no overt seizures. He was reviewed in the Genetics clinic from 2-years and noted to be dysmorphic with anterior hair whorl, frontal bossing, hypertelorism, downward-slanting palpebral fissures and a mild pectus carinatum (Figure 1b). Weight was consistently above the 91st centile, height between the 50th-75th centiles with head circumference on 50th centile. Investigations at the time included normal 60K arrayCGH, chromosome breakage studies and metabolic work-up (including plasma and urinary amino acids and organic acids, CK, serum lactate, renal and liver function tests and bone profile). MRI-brain at 3.5-years was structurally

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

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

Patient 2: Patient 2 is the younger sibling of Patient 1 (Family 1) aged 6-years. He was born following a normal pregnancy at term with a birth weight of 4.22 kilograms (91^{st} centile). He was well immediately after birth but again was noted to be delayed with his development with cruising around furniture at 14-months, walking at 3-years but had no speech and unlike his brother, was not communicating by sign language. He was also noted to be hypotonic and wears glasses for hypermetropia. He was noted to be aggressive on occasions and had a quieter personality than his older sibling. He was initially reviewed in the Genetics clinic at 2.5-years of age and noted to be dysmorphic with similar facial appearance to his brother (Figure 2a). He also had mild 2-3 toe syndactyly, frontal bossing; weight~13.9 kilograms (50^{th}-75^{th} centile); height~89 cms (25^{th}-50^{th} centile) and head circumference~54 cms (98^{th} centile) at 2.5-years of age. MRI-brain at 2-years of age identified bilateral periventricular heterotopia with overlying cortical dysplasia which was thought to account for his more severe developmental impairment (Figure 2b).

At recent review aged 6-years, he was in a special needs school, he still remained in nappies. His sleep and appetite were reported to be normal. He wore glasses and a back brace for correction of scoliosis. He had no speech and was noted by the family to have occasional aggressive outbursts. He was in a wheelchair for long distances, but able to walk independently for short distances. There was less evidence of truncal obesity. On examination, he was noted to have similar facial dysmorphism as his brother (Figure 2c); weight~28.5 kilograms (98th centile); height~119 cms (75th centile) and head circumference of 57 cms (98th centile). Transferrin IEF, following identification of *MAN1B1* variants, showed a type 2 pattern.

Patient 3: This patient is the older sibling in Family 2, aged 10-years and is the first child of healthy, non-consanguineous White European parents with no significant family history (Figure 3a). There were no concerns in the pregnancy and she was born at term+2 weeks gestation with a birth weight of 4.30 kilograms (98th centile) by forceps delivery. She was in a good condition immediately after birth and there were no feeding problems noted. She walked at 19-months of age and her first words were at 18-months of age. She was in mainstream school with additional 1:1 support. She was also noted to have occasional outburst of aggressive behaviour.

This patient was initially reviewed in the Genetics clinic aged 7-years following a referral by the Community paediatric team in view of her developmental impairment. On examination, she was noted to be dysmorphic with downward-slanting palpebral fissures, hypertelorism with epicanthic folds, full lips (Figure 3b) and a café-au-lait patch on her right lower back; weight~31.5 kilograms (91st-98th centiles); height~126 cms (75th-91st centiles) and head circumference~54.3 cms (75th centile). She was recruited to the DDD study and saliva samples obtained for trio WES (Decipher ID: 294436).

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

Patient 4: This patient in the younger sibling of Patient 3. Aged 6-years, she was referred to Genetics following identification of a MAN1B1-CDG in the older sibling. There were initial concerns in the first year of life regarding dairy intolerance but this settled. She was subsequently referred to Ophthalmology for hypermetropia needing glasses. She was noted to have non-specific mild global developmental delay and referred to the Genetics clinic following the diagnosis in her older sibling.

MATERIALS AND METHODS

Both families 1 and 2 were recruited to the Deciphering Developmental Disorders (DDD) study. Trio-based exome sequencing was performed on the affected individual and their parents, as previously described[3]. Each affected individual also had a high-resolution analysis for copy number abnormalities using array-based comparative genomic hybridization (aCGH). Putative *de novo* mutations were identified from exome data using DeNovoGear software[4] and were validated using targeted Sanger sequencing.

RESULTS

Genetic analysis:

Trio WES through DDD study identified in Patient 1 a homozygous c.1311del,p.Leu438fs likely pathogenic variant in *MAN1B1* (NM_016219.4- HGVS nomenclature) (Figure 1f).

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

In Family 2, trio WES in Patient 3 identified compound heterozygous *MAN1B1* variants: a likely pathogenic c.761_764del,p.Ile254Thrfs*20 frameshift variant and a missense c.1000C>T,p.Arg334Cys in *MAN1B1* (NM_016219.4- HGVS nomenclature) which was biparentally inherited (Figure 3d and e). *In silico* analysis supports its likely pathogenicity confirming the diagnosis of MAN1B1-CDG. Both the variants are publicly accessible via the Decipher website (https://decipher.sanger.ac.uk) using their Decipher ID numbers.

DISCUSSION

MAN1B1 (OMIM 604346) which is situated on chromosome 9q34.3, has 13 coding exons and encodes a Golgi mannosidase. These enzymes are involved in N-glycan remodelling. These enzymes also contribute to the timing and disposal of misfolded glycoproteins through the ER associated degradation (ERAD) pathway. ERManI cleaves the terminal mannose from the middle branch of Man9GlcNAc2, producing a Man8GlcNAc2 isomer B. This is believed to play a critical role in glycoprotein quality control by targeting terminally misfolded proteins in ERAD. MAN1B1 was initially predicted to act as an ER-resident protein[5] but recent studies have shown that MAN1B1 localises to the Golgi apparatus in mammalian cells[6], further reinforcing the fact that quality control is not confined to ER alone but extends through the secretory pathway[1]. This provides further evidence that MAN1B1 operates as a check-point within the Golgi apparatus recycling misfolded proteins that escaped ERAD back to ER by interacting with the COP-I machinery resulting in retrograde transport of these

proteins[7]. It is also said to act as a lectin retrieving these proteins back to the ER prior to degradation[1].

The likely explanation for MANB1 deficiency resulting in a multi-system disorder is due to the fact that there is defective quality control as a result of *MAN1B1* genetic defects, resulting in defective check-point unable to minimise the level and toxicity of misfolded proteins within the cell. Interestingly, compared to most CDG phenotypes, MAN1B1-CDG considering how important MAN1B1 activity is within the cell, only presents with a milder phenotype. This supports the hypothesis that there may be more check-points within the ER-Golgi machinery. Further work on the secretory pathway and its regulation in various body systems will provide further insight into the phenotypic contribution of specific forms of CDG.

Recessive variants in *MAN1B1* were first identified in non-syndromic mental retardation-15 (MRT15; OMIM 614202) in four consanguineous families by Rafiq et al., 2011[8]. By undertaking WES and homozygosity mapping in these families with several affected siblings in one generation, they were able to identify several candidate genes narrowing it down to variants in *MAN1B1* as being causal[8]. The authors characterised the phenotype as being consistent with non-syndromic AR ID except in one family (MR43) with a nonsense variant where clear dysmorphism was identified, no photographs of this family were however published. Description of the facial features is however very similar to the facial phenotype we describe in our families. The authors concluded that ERAD is a new disease associated pathway and disruption of other ERAD pathway candidates may result in a similar clinical phenotype.

Since then, there have been very few cases of MANB1-CDG reported so far and matchmaker repositories such as Genematcher, Phenome central and Decipher do not produce any

matches suggesting this remains a rare, potentially undiagnosed form of CDG. This is likely because of the varying presentation and what is initially thought to be a non-specific presentation as evidenced by our families. However, by including transferrin IEF in initial screening of global developmental delay, along with other routine investigations such as urinary organic acids, plasma amino acids will help pick this up early. Screening for CDG should also include O-linked type abnormalities. However, interestingly, the CDG-type II pattern seen may also indicate sample degradation requiring a repeat sample for confirmation which in this cohort may not always be possible. With further genomic advances (and resultant cost-benefit), use of first-line WES/ WGS in clinical practice for diagnostic work-up of children with developmental delay should hopefully address this issue. It is important, however, to ensure that WES/ targeted gene panels thus generated for developmental delay include genes associated with CDG.

Table 1 summarises all the patients reported so far with MAN1B1-CDG in comparison to our cohort. Rymen et al., 2013 reported seven patients with recessive variants in *MAN1B1* from their cohort of unsolved CDG-type II and were able to provide further functional evidence of MAN1B1 role in protein quality at the Golgi apparatus[1]. All patients had hypotonia, variable degree of ID, truncal obesity but with normal MRI-brain in all but one patient who also presented with epilepsy. Behavioural problems do not appear to be a major component of the phenotype unlike some other CDG identified so far.

Van Scherpenzeel et al., 2014 reported twelve patients with *MAN1B1* recessive variants from their cohort of molecularly undiagnosed CDG-type II patients[9]. Patients in this study showed a predominant neurological phenotype with moderate ID. They were also noted to have macrocephaly, truncal obesity, early hypotonia and characteristic facial dysmorphism including an oval face, bulbous nasal tip with thin upper lip. However, many of the patients did not have the classic CDG-type II features including inverted nipples, ataxia, abnormal fat

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[10,11]. 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[10]. 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*[11]. 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.

STATEMENTS:

A. Funding:

The DDD study presents independent research commissioned by the Health Innovation Challenge Fund [grant number HICF-1009-003], a parallel funding partnership between the Wellcome Trust and the Department of Health, and the Wellcome Trust Sanger Institute [grant number WT098051]. The views expressed in this publication are those of the author(s)

and not necessarily those of the Wellcome Trust or the Department of Health. The study has UK Research Ethics Committee approval (10/H0305/83, granted by the Cambridge South REC, and GEN/284/12 granted by the Republic of Ireland REC). The research team acknowledges the support of the National Institute for Health Research, through the Comprehensive Clinical Research Network.

B. Acknowledgements:

We would like to thank all these families for consenting to publication. This study makes use of DECIPHER (http://decipher.sanger.ac.uk), which is funded by the Wellcome.

C. Contributorship Statement:

All authors recruited their respective patients to the DDD study and provided data regarding their patients; DDD study provided trio exome sequencing data. MB planned the study; MB recruited Patient 1 to DDD; wrote manuscript; DSJ recruited Patient 3 to DDD; all authors reviewed and contributed to the manuscript.

D. Competing Interest: None to declare for all authors.

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FIGURE LEGENDS

Figure 1a-e: Family pedigree and photographs of patient 1 at 3 and 8 years of age demonstrating frontal bossing, oval face, down-slanted palpebral fissures, thin upper lip; MRI-brain demonstrating nonspecific right frontal high signal but otherwise essentially normal.

Figure 1f: Trio WES identified in Patient 1 a homozygous c.1311del,p.Leu438fs likely pathogenic variant in *MAN1B1* (NM_016219.4- HGVS nomenclature).

Figure 2a-c: Photographs of patient 1 at 3 and 6 years of age demonstrating similar facial dysmorphism to his older sibling; MRI-brain demonstrating bilateral temporal heterotopia.

Figure 3a-c: Pedigree and photographs of patient 3 at 7-years and 10-years of age demonstrating facial dysmorphism as previously described.

Figure 3d-e: Trio WES in Patient 3 identified compound heterozygous *MAN1B1* variants: a likely pathogenic c.761_764del,p.Ile254Thrfs*20 frameshift variant and a missense c.1000C>T,p.Arg334Cys in *MAN1B1* (NM_016219.4- HGVS nomenclature) which was biparentally inherited.

Table 1: Clinical features reported in patients with MAN1B1-CDG in comparison to our cohort

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

Figure 1a

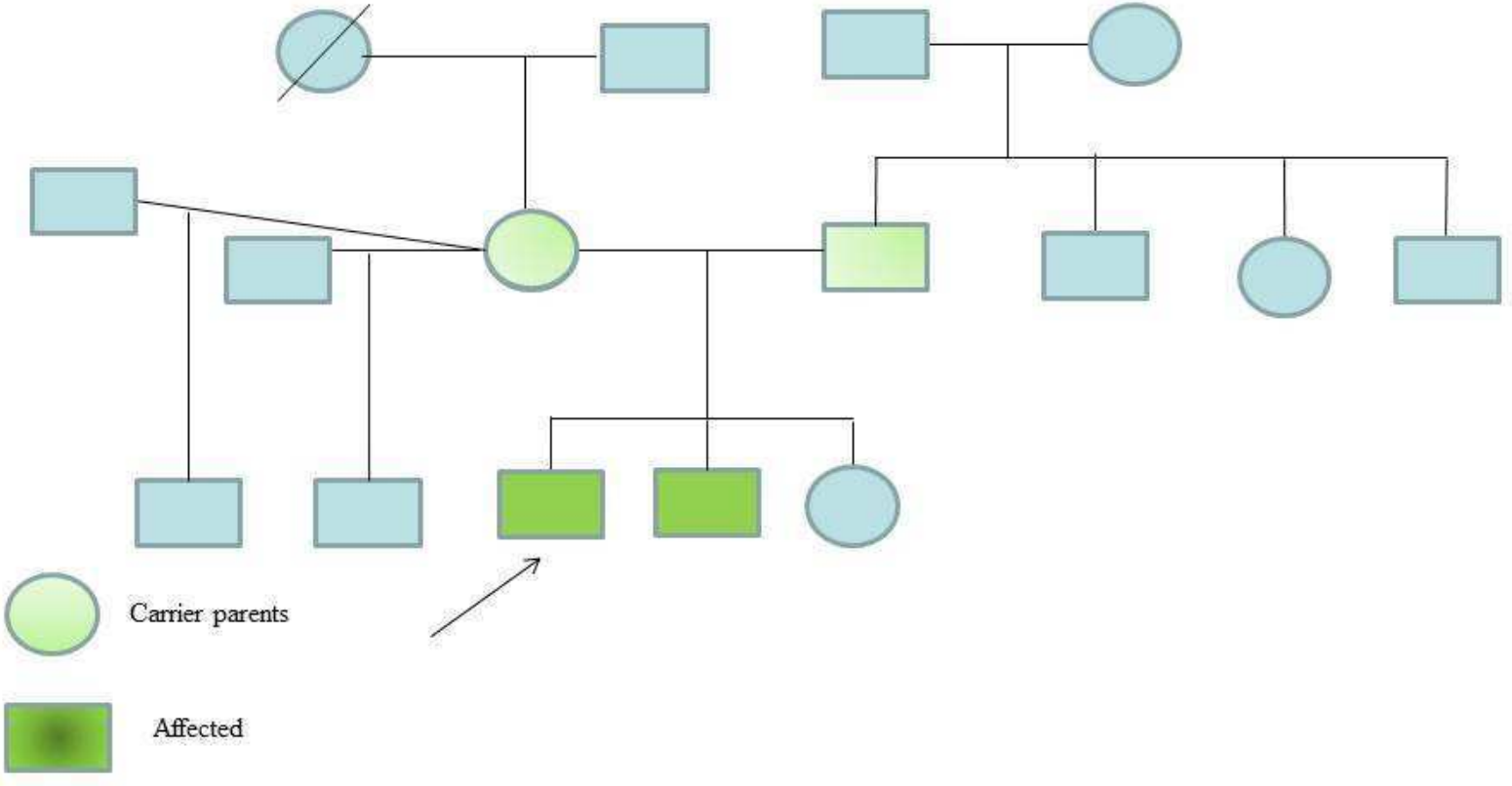

Figure 1b

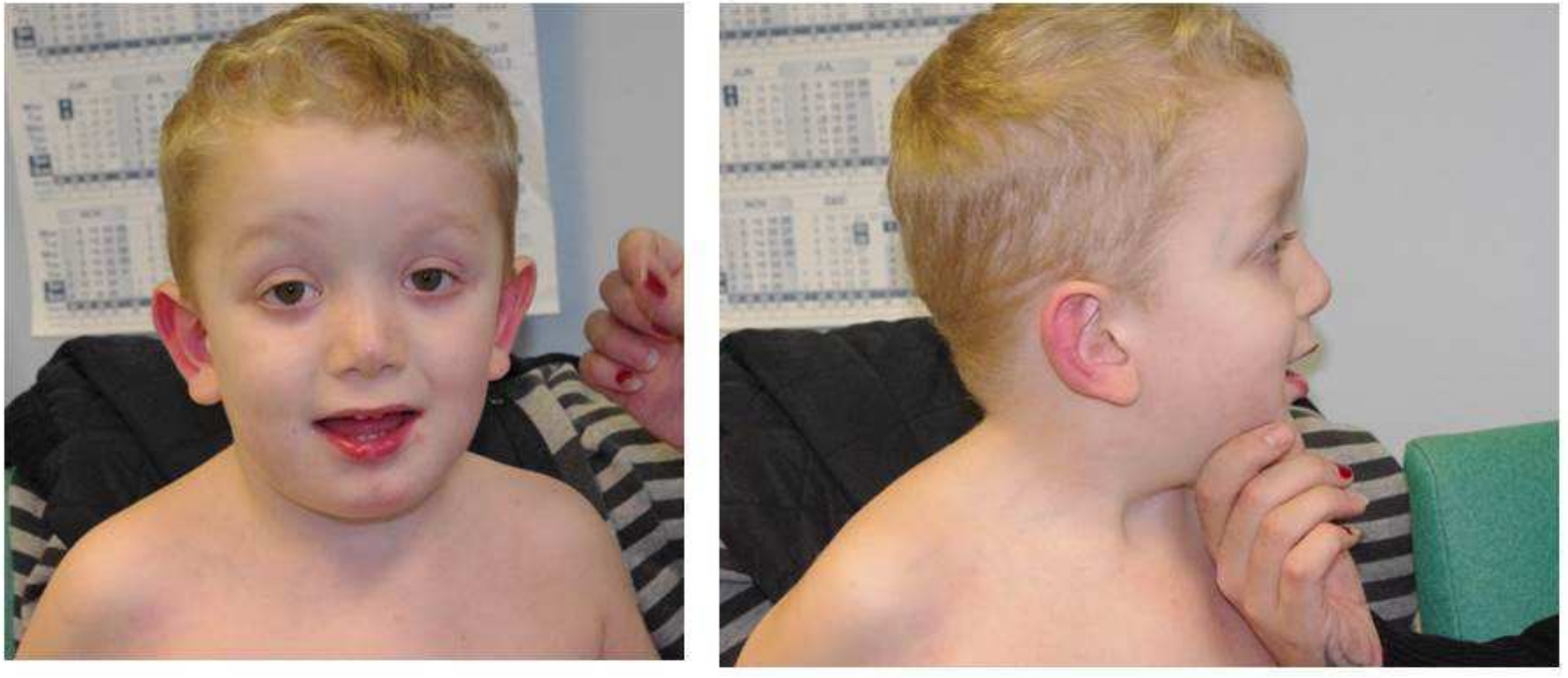

Figure 1c

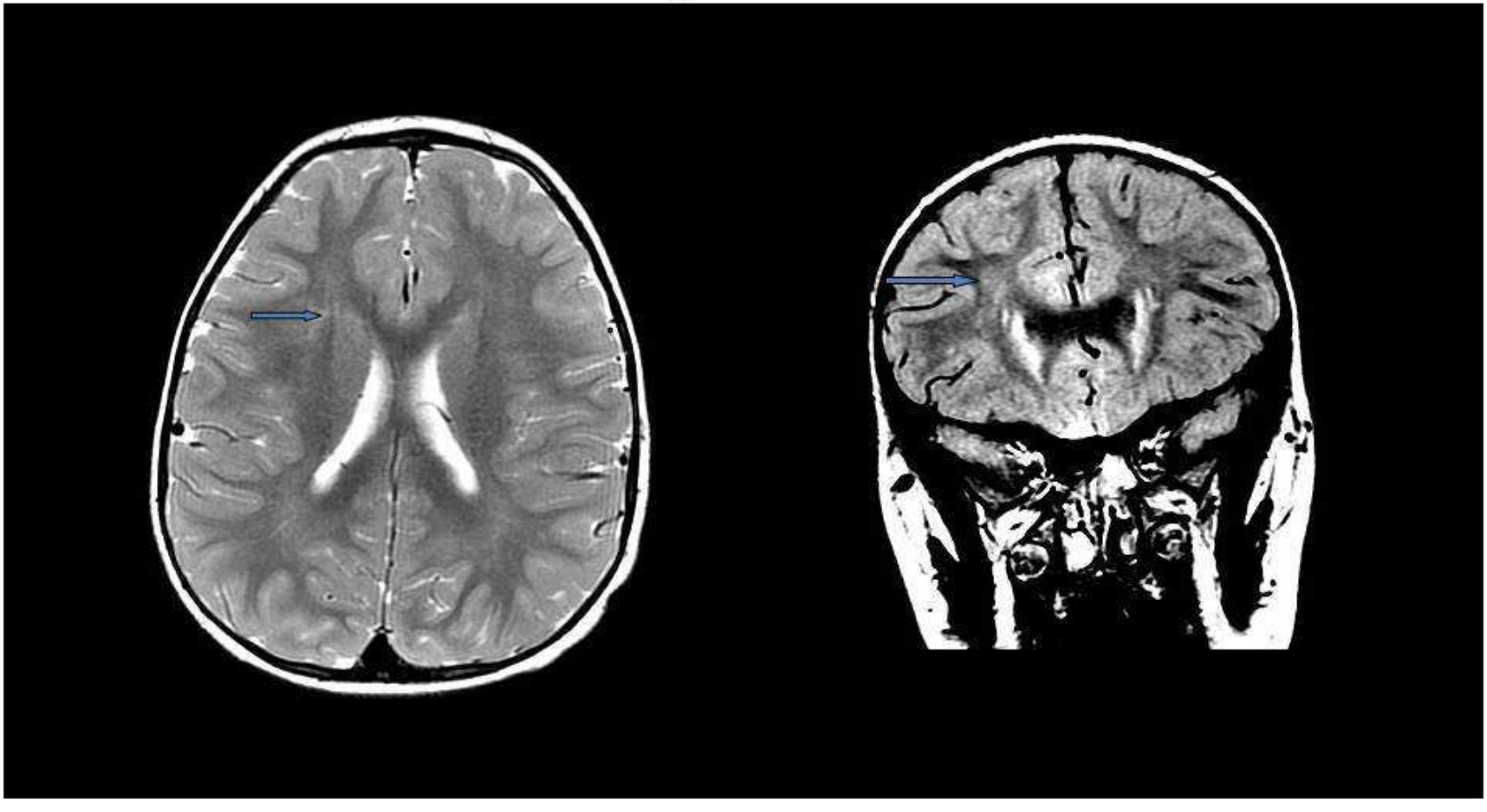

Figure 1d

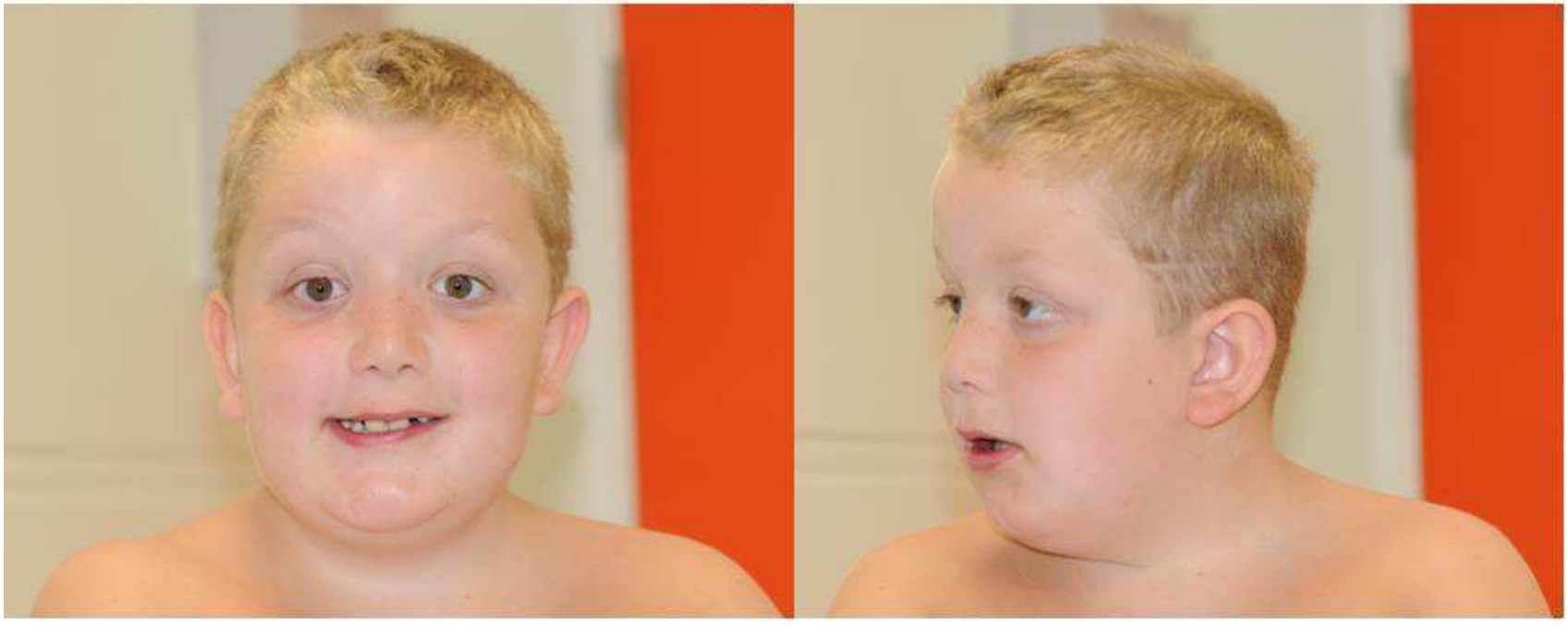

Figure 1e

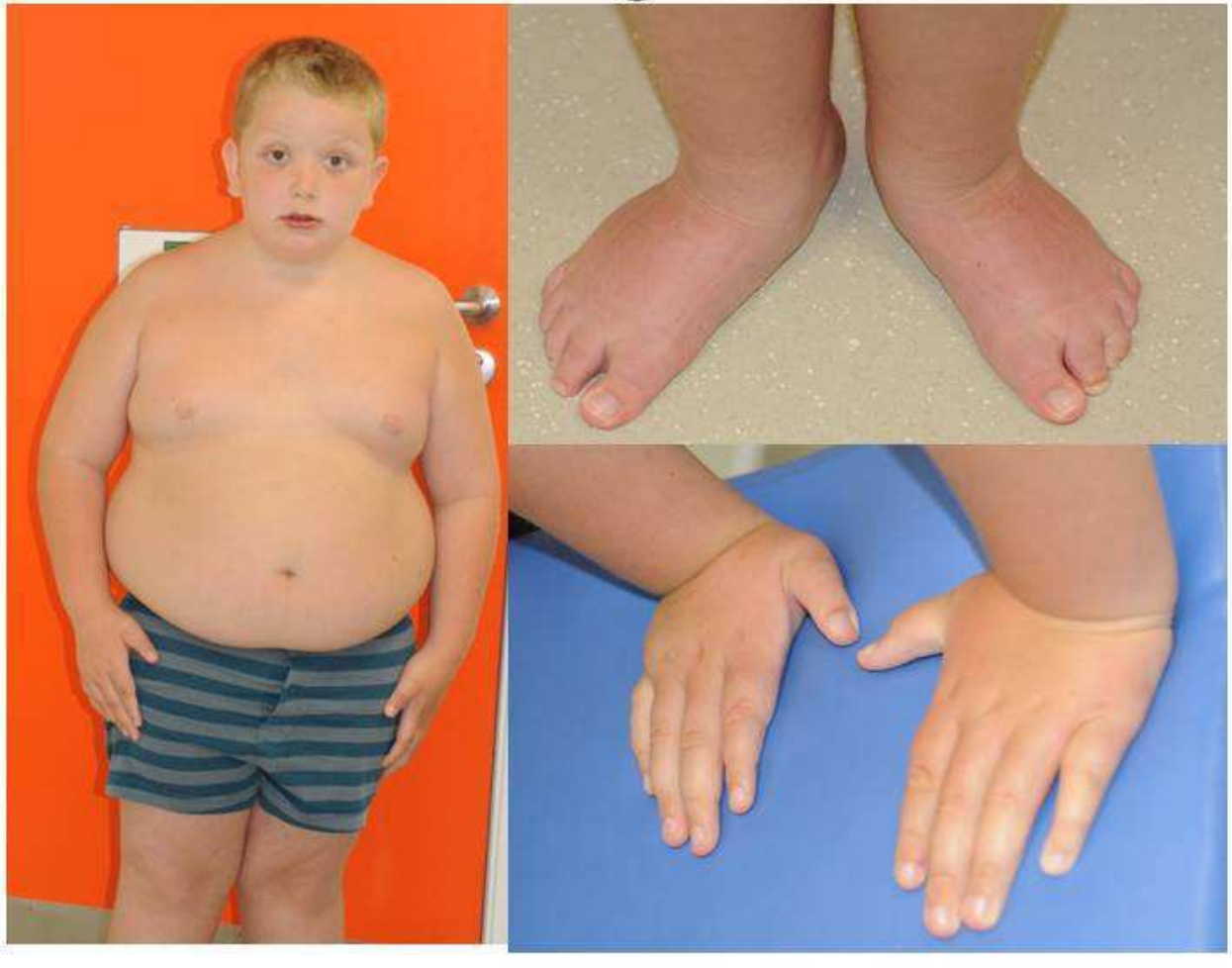

Figure 2a

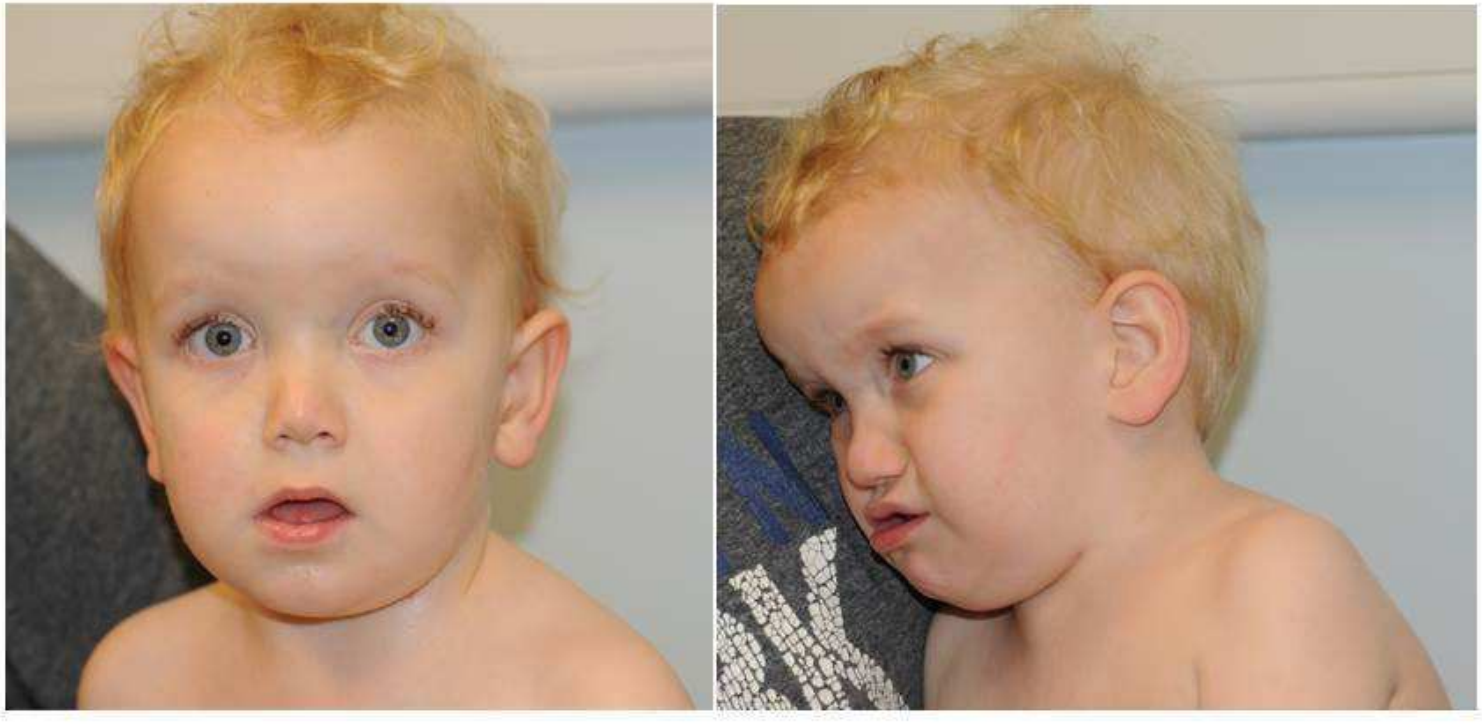

Figure 2b

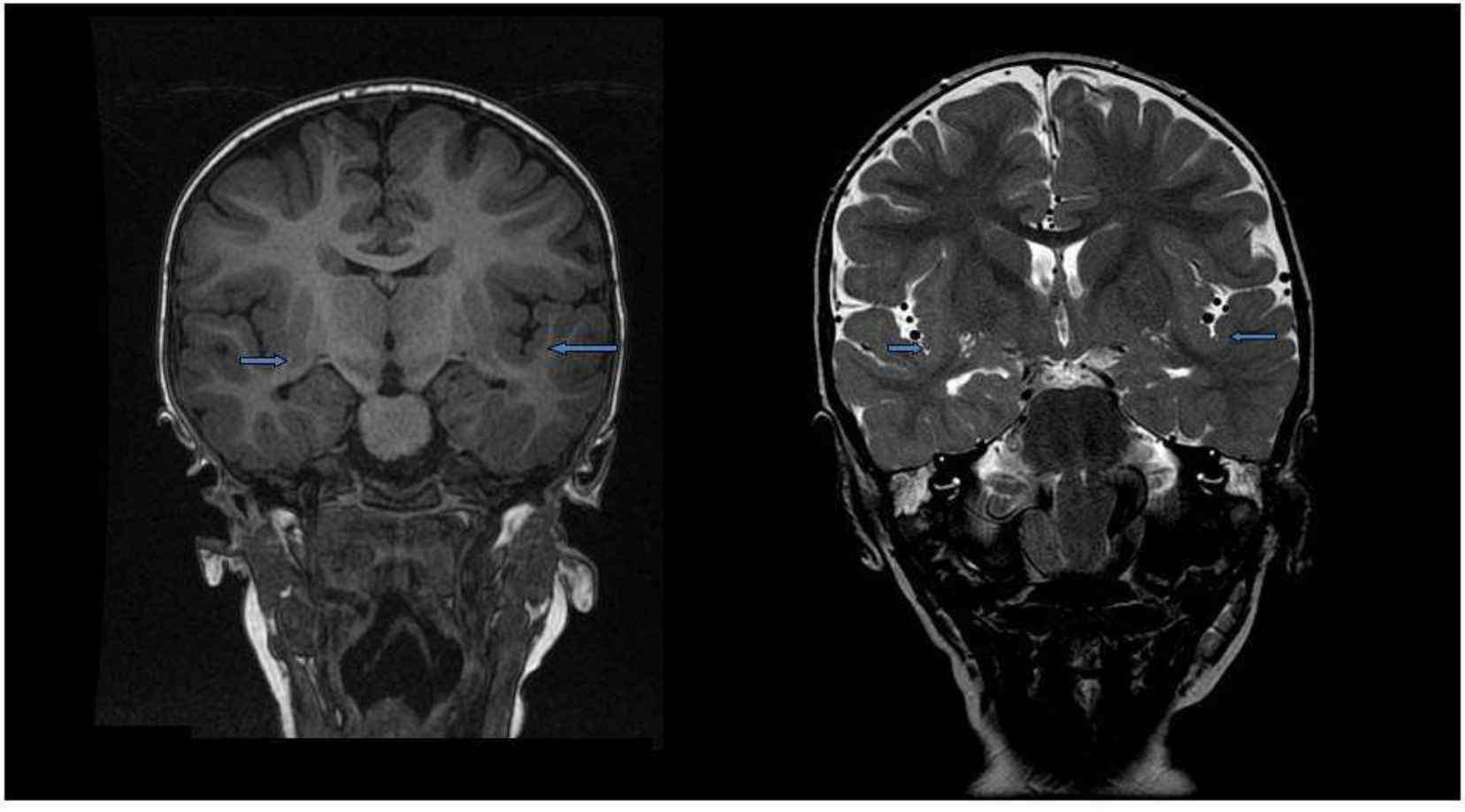

Figure 2c

Figure 3a

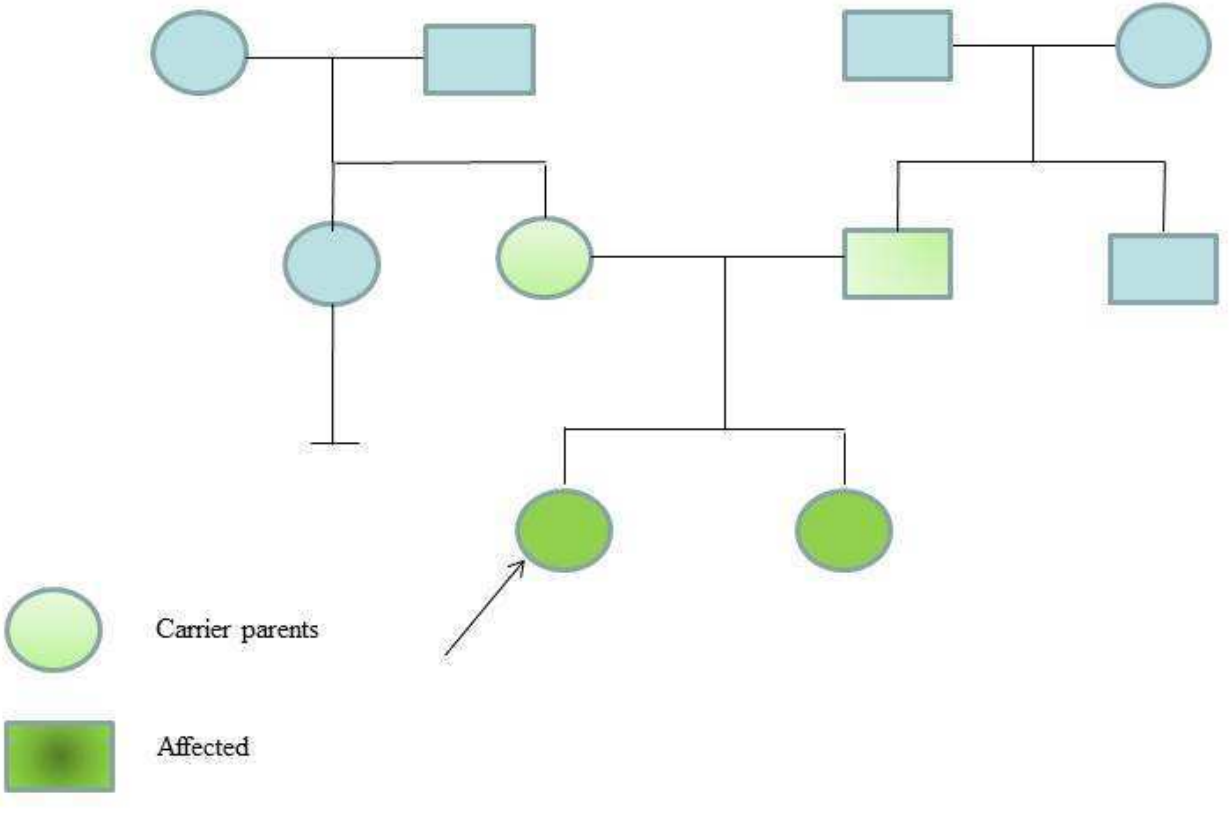

Figure 3b and c