Journal of the Neurological Sciences 371 (2016) 121–125

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

A novel mutation in *PGAP2* gene causes developmental delay, intellectual disability, epilepsy and microcephaly in consanguineous Saudi family

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ARTICLE INFO

Article history:
Received 14 June 2016
Received in revised form 28 September 2016
Accepted 17 October 2016
Available online 18 October 2016

Keywords:
PGAP2
Intellectual disability
Developmental delay
Epilepsy
Microcephaly

ABSTRACT

PGAP2 (Post-GPI Attachment to Proteins 2) gene is involved in lipid remodeling steps of Glycosylphosphatidylinositol (GPI)-anchor maturation. At the surface of the cell this gene is required for proper expression of GPI-anchored proteins. Hyperphosphatasia with mental retardation syndrome-3 is an autosomal recessive disorder usually characterized by severe mental retardation. Mutations in the *PGAP2* gene cause hyperphosphatasia mental retardation syndrome-3. We have identified a large consanguineous family from Saudi origin segregating developmental delay, intellectual disability, epilepsy and microcephaly. Whole exome sequencing with 100× coverage was performed on two affected siblings of the family. Data analysis in the patient revealed a novel missense mutation c.191C>T in *PGAP2* gene resulting in Alanine to Valine substitution (Ala64Val). The mutation was reconfirmed and validated by subsequent Sanger sequencing method. The mutation was ruled out in 100 unrelated healthy controls. We suggest that this pathogenic mutation disrupts the proper function of the gene proteins resulting in the disease state.

1. Introduction

The *PGAP2* gene was identified recently in 2013 as the source of "hyperphosphatasia, mental retardation syndrome-3" (HPMRS3; OMIM 614080) [1]. At chromosomal position 11p15.4 *PGAP2* is gene is localized involved in the synthesis of Golgi/ER-resident membrane protein that play a role in the final step of lyso-phosphatidylinositol (lyso-PI) remodeling to phosphatidylinositol (PI) containing saturated fatty acids. Absence of this protein leads to transport to surface of the cell of lyso-GPI-APs and resultantly are more prone to cleavage by phospholipase D [2,3].

Glycosylphosphatidylinositol (GPI)-anchored proteins (GPIAPs) are glycolipids made up of phosphatidylinositol, glucosamine, mannose, and carbohydrate residues. More than 150 proteins with multi roles are GPI anchored, including membrane associated enzymes, adhesion molecules, activation antigens, immunologically important proteins, differentiation markers, complement regulatory proteins and various other glycoproteins.

The precise role of GPI-anchored proteins are still not clear. Nonetheless, they participate as hydrolytic enzymes, receptors, adhesion molecules, protease inhibitors, and regulatory proteins, they share analogous characteristics, shown by their common glycolipid membrane anchors, hence act as cell adhesion receptors and play role in differentiation [4,5]. Some recent research studies have shown the significance of GPI-anchor proteins for normal embryonic and neuronal development [6].

These GPI-APs deficiencies are recently emerged as a group of disorders within inherited glycosylation disorders. These diseases are caused by aberrations in two different classes of genes. The first are PIG (Phosphatidyl Inositol Glycan) involved in transfer and biosynthesis of GPI, and secondly the PGAP (Post GPI Attachment to Proteins) involved in the structural remodeling of GPI once it has attached to proteins.

There are about 26 PIG and PGAG genes participate in GPI-anchor biosynthesis and remodeling pathway [7]. So far, mutations in 13 PIG and PGAP genes have been identified and found associated with these diseases [8]. The mode of inheritance for all is autosomal recessive except PIGA which is X-linked recessive. The patients with these disorders show wide range of clinical symptoms, occasionally leading to difficulties in their phenotypic recognition.

Here we report a large consanguineous family from Saudi Arabia with hyperphosphatasia, mental retardation syndrome-3. The patients were suffering from developmental delay, intellectual disability (IDs), poor hearing, epilepsy and marked microcephaly. The whole exome sequencing revealed novel mutation in *PGAP2* gene.

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http://dx.doi.org/10.1016/j.jns.2016.10.027
0022-510X/

2. Materials and methods

2.1. Samples collection and ethical approval

The blood samples was taken from five members of the family (two affected and three normal) (Fig. 1), and 100 unrelated healthy controls of Saudi origin. Prior to the start of study, informed written consent was taken from all the participants according to the Helsinki declaration. The study was also approved the ethical committee of the Center of Excellence in Genomic Medicine Research, King Abdulaziz University, Jeddah. Examination of pedigree suggested autosomal recessive mode of inheritance and total 5 affected individuals were identified in the family, three of whom has died while two are still alive. Both the affected individuals underwent medical examination at King Abdulaziz University Hospital, Jeddah.

2.1.1. Patient 1

The proband (IV:4) is 25 months old boy with severely regression in developmental mile stones since age of 7 months. The patient also has poor hearing, intellectual disability, epilepsy and marked microcephaly. His head circumference was below the normal range i.e. 32 cm (−3.07 SD).

2.1.2. Patient 2

The patient (IV:3) is 5 years old girl and shows same features as patient 1 with developmental delay and growth retardation with poor hearing, epilepsy, intellectual disability and marked microcephaly. The patient was normal till age of 8 months. The patient is also not able to walk or speak and could not concentrate and see properly. Her head circumference was below the normal range i.e. 35 cm (−3.83 SD).

2.2. EEG testing

One hour long video-EEG recording was carried out for the child during wakefulness in patient (IV:4). Provocation: photic stimulation at 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 30 and 40 Hz during eye opening and eye closure. The background was moderately well stained and modulated 4–5 Hz theta posteriorly and centrally, 4 Hz theta anteriorly and diffuse low voltage beta.

2.3. Whole exome sequencing

The resulting VCF (variant call format) file contains 83,480 variants. These variants are filtered based on quality, genomic position, frequency, protein effect, pathogenicity and previous associations with the phenotype. Candidate variants were expected to follow an autosomal recessive inheritance pattern (homozygous or compound heterozygous state) given the reported positive family history. Homozygous variants were of primary interest based on the positive consanguinity. Looking for rare homozygous variants within the protein coding regions of all genes that have previously been associated with one of the symptoms provided yield one plausible candidate in the *PGAP2* gene. Mutations in *PGAP2* genes are associated with hyperphosphatasia with mental retardation syndrome-3, which is an autosomal recessive disorder leads to severe mental retardation, milder intellectual disability, hypotonia along with poor motor development, poor speech, increased serum alkaline phosphatase, sensorineural hearing loss and microcephaly. This autosomal recessive disorder could account for this patient's microcephaly, global developmental delay, intellectual disability, and epilepsy with hearing problems.

Thereafter, rare, potentially harmful variants present in those genes in (compound) heterozygous state (or possibly de novo) state were further considered. However, this approach did not yield a plausible candidate.

The analysis was then expanded to all genes, whereby we firstly focused on potentially harmful homozygous variants and then moved on to potential candidates in (compound) heterozygous state. However, none of these approaches yielded good candidate variants.

2.4. Sanger sequencing

To validate the exome sequencing data we performed Sanger sequencing (ABI 3730), in other affected patient (IV:3) along with 100 normal persons as controls as shown in Fig. 2.

2.5. PGAP2 docking

Swiss Dock output showed that the binding of Stearoyl Co-A with PGAP2 WT protein occur mostly within the alpha-helical structures (Fig. 3A). On the contrary, the stearoyl Co-A mostly binds with the coiled region outside the alpha-helical structures of PGAP2 MT (Fig. 3B). This strongly suggests that the mutation caused significant structural changes in PGAP2 and subsequently affects the proper binding of stearoyl CoA with the PGAP2 MT leading to a defect in the molecular function of PGAP2 in transferring the stearic acid to the GPI-APs.

3. Results

3.1. EEG report

No focal abnormalities or epileptiform discharges were seen interictally, however, very frequent spasms were recorded. Clinically

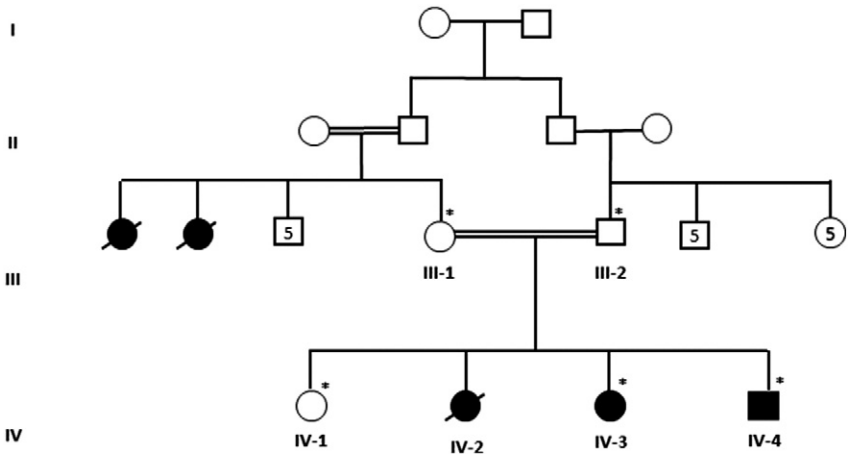

Fig. 1. A consanguineous family from Saudi Arabia showing the disease phenotype segregating in an autosomal recessive manner. The samples available for genetic testing are marked with asterisks.

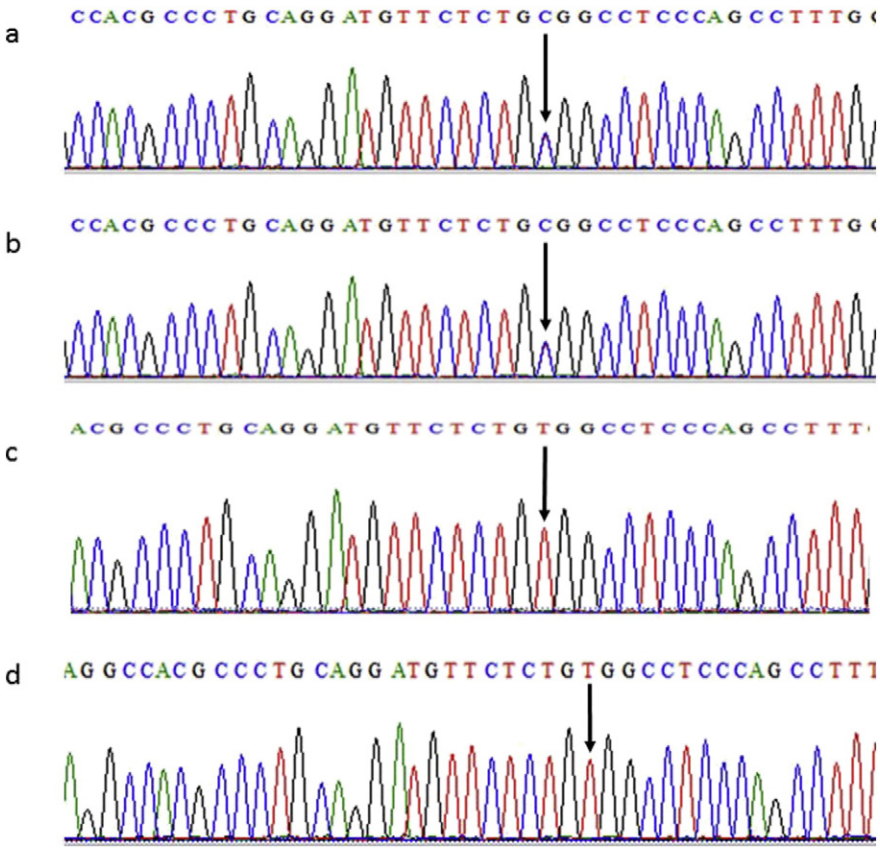

Fig. 2. Sanger sequence analysis: a and b (III-1 and III-2) are normal parents showing C and T in heterozygous state, while c and d (IV-3 and IV-4) are affected children showing only homozygous T in exon 3 of *PGAP2* gene.

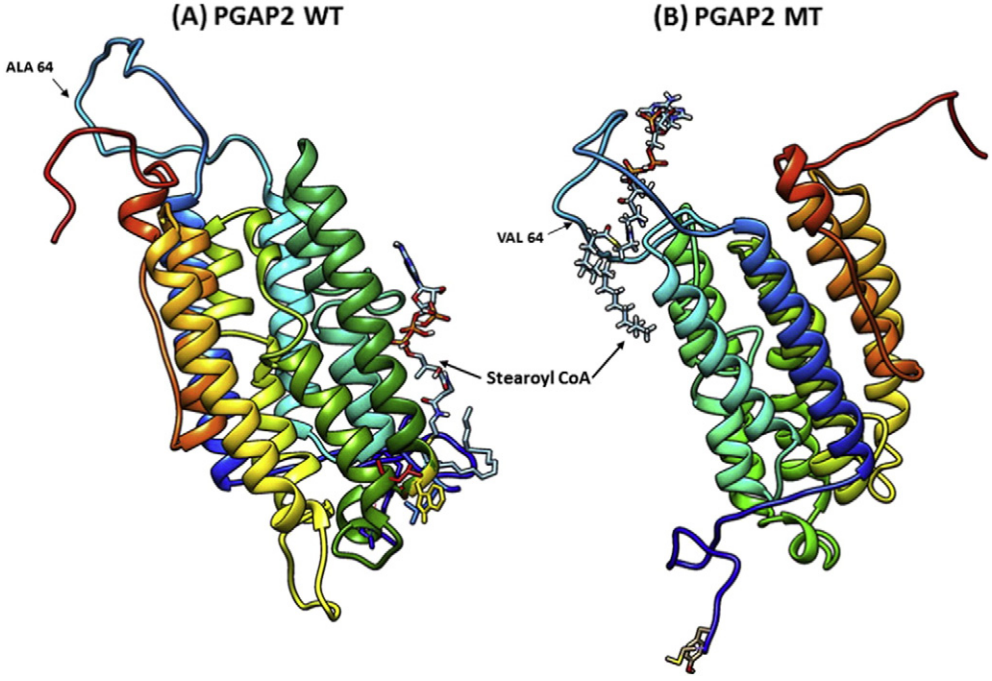

Fig. 3. Homology models for PGAP2 WT and PGAP2 MT were generated using the RaptorX web server to preform molecular docking studies with stearoyl CoA. (A) The Swiss Dock output clusters indicated that the binding of stearoyl CoA with PGAP2 WT protein occurs mostly within the alpha-helical structures. (B) Conversely, the stearoyl Co-A binds primarily with the coiled region outside the alpha-helical structures of PGAP2 MT. The mutation of Ala 64 to Val 64 in PGAP2 causes significant structural changes that hampers the binding of stearoyl CoA and, thus, leading to a defect in the molecular function of PGAP2.

they were seen as head flexion and subtle extension-flexion of the limbs and trunk. On EEG this was accompanied by high voltage biphasic central discharges or electrodecrements at times with superimposed fast activity. Single lead ECG shows a regular cardiac rhythm.

Abnormal video-EEG record showing frequent epileptic spasms on a somewhat preserved background that is only mildly slow for age but with poor reactivity. The findings are still consistent with epileptic encephalopathy and would support the diagnosis of infantile spasms without hypsarrhythmia; likely reflecting medication effects on West syndrome.

3.2. Whole exome sequencing

Whole exome sequencing revealed pathogenic mutation in *PGAP2* gene where C at position 191 replaced by T resultantly amino acid Alanine at position 64 is converted to valine showed a novel missense mutation c.191C>T in affected members of the family as shown in Table 1.

3.3. Sanger sequencing

This mutation c.191C>T was confirmed in other available affected individual with Sanger sequencing (ABI 3730) as shown in (Fig. 2). This mutation further validated in 100 unrelated healthy persons, but no one has this sequence variation.

3.4. PGAP2 docking with stearoyl Co-A

PGAP2 is involved in one of the lipid remodeling steps of GPI-Anchor Protein (GPI-AP) maturation. It adds a stearic acid from stearoyl co-A substrate to the GPI-AP which is critical for the stable expression of GPI-anchored proteins at the cell surface [5]. Since the crystal structure of PGAP2 is not yet available, we have generated the homology models, by imputing the amino acid sequences for PGAP2 wild type (PGAP2 WT) and PGAP2 mutant (PGAP2 MT) in FASTA format, using the Raptor X web server [9–12]. The docking studies were performed using appropriate homology models with Stearoyl Co-A by Swiss Dock web server [13]. SwissParam [14] is automatically used by SwissDock to perform the ligand set-up before docking Stearoyl Co-A with the homology models generated for PGAP2 WT and PGAP2 MT respectively (Fig. 3A, B). The view dock plugin of UCSF Chimera has been used to explore the predicted binding modes [15].

4. Discussion

A rare, homozygous missense variant was identified in the *PGAP2* gene of this affected female. This gene play a role in maturation of the GPI anchor on GPI-anchored proteins. Recessive mutations in *PGAP2* are known to cause hyperphosphatasia with mental retardation syndrome-3 (HPMRS3), which is an autosomal recessive disorder normally showed severe mental retardation, hypotonia delay or no speech, and increased serum alkaline phosphatase [1]. Mostly individuals affected by this disease develop recurrent seizures (epilepsy) in early childhood. Seizures are usually the generalized tonic, tonic-clonic type, muscle twitching along with uncontrolled jerking of the body which involve muscle rigidity, convulsions, and loss of consciousness. Furthermore, the severity of the disorder also vary from severe to milder ID [16].

GPI-anchored proteins involved in many biological processes and play important roles, while any mutations affecting proteins involved in the synthesis of the GPI anchor are reported to cause an extensive spectrum of intellectual disabilities (IDs). *PGAP2* mutations have quite recently been discovered as a cause of ID.

Table 2
Mutation spectrum in PGAP2 gene.

S. no	Mutation	Consequence	State	Origin	Reference
1	c.296A>G	p.Tyr99Cys	Homozygous	Syrian	Hansen et al. [1]
2	c.530G>C	p.Arg177Pro	Homozygous	Pakistani	Hansen et al. [1]
3	c.46C>T	p.Arg16Trp	Homozygous	Finnish	Krawitz et al. [16]
4	c.479C>T	p.Thr160Ile	Homozygous	Finnish	Krawitz et al. [16]
5	c.380T>C	p.Leu127Ser	Homozygous	Turkish	Krawitz et al. [16]
6	c.2T>G c.221G>A	p.? p.Arg74His	Heterozygous	Poland	Jazela-Stanek et al. [8]
7	c.191C>T	p. Ala64Val	Homozygous	Saudi Arabia	Present study

Krawitz et al. [16], reported two unrelated patients with variable severity of HPMRS3. One was a Turkish boy, born of consanguineous parents, with severely delayed psychomotor development, lack of ability to walk, sensorineural hearing loss, seizures, atrial septal defect, Hirschsprung disease, hypotonia, cleft palate, and microcephaly. The other was a 28-year-old Finnish woman who had mild intellectual disability and childhood epilepsy. Hansen et al. [1] also identified two homozygous missense mutations in the *PGAP2* gene in patients with severely delayed development, pronounced muscular weakness and hypotonia, but no seizures.

Only a few studies about *PGAP2* mutations are available till date, future studies will add additional information, but all symptoms reported for this patient are associated with *PGAP2* mutations, hence this variant seems to be a good candidate to explain the patient's phenotype. To date only six mutations in the *PGAP2* gene has been reported so far (Table 2). In our study, we have identified 7th mutation in our family from Saudi Arabia origin. The associated clinical features of patients include poor hearing, developmental delay, intellectual disability, epilepsy and microcephaly. The variant detected in the patients results in an alanine to valine amino acid substitution at position 64 in exon 3. Its predicted deleterious effect according to several in silico tools indicate the possible relevance of this variant. However, its exact effect remains to be elucidated and it was not yet reported as pathogenic in literature before. Hence, further validation was carried out in all affected patients of family and 100 unrelated health persons as controls. This mutation was not present in any healthy individual, which confirmed it to be pathogenic.

Conflict of interest

All authors declare that there is no conflict of interest.

Author's contribution

M.I.N. conceived and designed the project. M.I.N. and M.R. performed experiments and confirmed these results. M.I.N., M.R., and A.G.C. analyzed and interpreted the whole-exome data. M.I.N., A.M.A., M.M.J., provided and interpreted phenotypic details for the patients. P.N.P. prepared the protein modeling structure. M.H.A., advised on the study design and writing of the manuscript. M.I.N. and M.R. wrote the manuscript.

Table 1
Primary findings.

Category	Gene	Disease	Inheritance	Variant	cDNA	Zygosity
Likely pathogenic	PGAP2	Hyperphosphatasia with mental retardation	Autosomal recessive	Ala64Val	191C>T	Homozygous

Acknowledgement

This project was funded by the King Abdulaziz City for Science and Technology (KACST), Riyadh, Kingdom of Saudi Arabia, under grant number APR-34-13. The authors acknowledge, with thanks, KACST, Science and technology unit King Abdulaziz University for technical and financial support.

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