Three Mutations in the Bilateral Frontoparietal Polymicrogyria Gene *GPR56* in Pakistani Intellectual Disability Families

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Abstract

Bilateral frontoparietal polymicrogyria (BFPP, MIM 606854) is a heterogeneous autosomal recessive disorder of abnormal cortical lamination, leading to moderate-to-severe intellectual disability (ID), seizure disorder, and motor difficulties, and caused by mutations in the G protein–coupled receptor 56 (*GPR56*) gene. Twenty-eight mutations in 40 different families have been reported in the literature. The clinical and neuroimaging phenotype is consistent in these cases. The BFPP cortex consists of numerous small gyral cells, with scalloping of the cortical–white matter junction. There are also associated white matter, brain stem, and cerebellar changes. *GPR56* is a member of an adhesion G protein–coupled receptor family with a very long N-terminal stalk and seven transmembrane domains. In this study, we identified three families from Pakistan, ascertained primarily for ID, with overlapping approximately 1 Mb region (chr16:56,973,335–57,942,866) of homozygosity by descent, including 24 RefSeq genes. We found three *GPR56* homozygous mutations, using next-generation sequencing. These mutations include a substitutional variant, c.1460T > C; p.L487P, (chr16:57693480 T > C), a 13-bp insertion causing the frameshift and truncating mutation, p.Leu269Hisfs*21 (NM_005682.6:c.803_804insCCATGGAGGTGCT; Chr16:57689345_57689346insCCATGGAGGTGCT), and a truncating mutation c.1426C > T; p.Arg476* (Chr16:57693446C > T). These mutations fully segregated with ID in these

Keywords

- ▶ G protein-coupled receptor 56
- ▶ adhesion G protein-coupled receptor G1
- ▶ bilateral frontoparietal polymicrogyria
- ▶ intellectual disability
- ▶ next-generation sequencing

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families and were absent in the Exome Aggregation Consortium database that has approximately 8,000 control samples of South Asian origin. Two of these mutations have been reported in ClinVar database, and the third one has not been reported before. Three families from Pakistan with *GPR56* mutations have been reported before. With the addition of our findings, the total number of mutations reported in Pakistani patients now is six. These results increase our knowledge regarding the mutational spectrum of the *GPR56* gene causing BFPP/ID.

Introduction

Bilateral frontoparietal polymicrogyria (BFPP; MIM-606854) is an autosomal recessive cortical disorder associated with abnormalities of neuronal lamination, which includes replacement of normal gyri by multiple (poly) and small (micro) gyri, alterations in white matter, and mild brain stem and cerebellar abnormalities.^{1,2} The neuroimaging characteristically shows a “cobble-stone like” polymicrogyria that either involves frontoparietal lobe and if it is generalized, then it has anterior to a posterior gradient. The sulci are shallow, and the interface between gray and white matter is irregular.³ There are cerebellar changes, including dysplasia, cortical and subpial cysts, and the disorganization of vermis. In brain stem, a hypoplasia with flattening of the pons and the posterior concavity is observed. Clinically, patients have moderate-to-severe intellectual disability (ID), hypotonia, seizures, and ocular movement disorders.³

In BFPP, the architecture of the frontal lobe is more severely affected than parietal and occipital lobes.⁴ The gyri are bigger in number but smaller in size and the six-layer structure of the cortex is disrupted.² In a mouse model study, it was demonstrated that loss of function of the protein *GPR56* results in an overmigration of neurons during development through a breached pial basement membrane into arachnoid space.⁵

GPR56 (also known as adhesion G protein-coupled receptor G1 [ADRG1 ENSG00000205336; MIM 604110]) is located on chromosome 16q12.2–21 and has 15 exons. The clinical and radiological findings were first described in two siblings in 1990,⁶ but in 2004, *GPR56* mutations were identified in association with BFPP by Piao and colleagues.⁴ Twenty-eight different mutations in *GPR56* have been reported in 40 different families across the world, from many different countries, including Qatar, French Canada, Arab, Palestine, Pakistan, India, Afghanistan, Italy, Bedouin origin, Turkey, and Spain. Twenty-seven of these mutations are homozygous, and one is heterozygous. Most of the families reported are consanguineous, but 10 are not consanguineous.^{1,3,4,7–12} Three of these mutations have been reported in families of Pakistani origin, and one of these families was nonconsanguineous. The total number of affected individuals in these families was six.^{3,4}

GPR56 is a G protein-coupled receptor (GPCR) that belongs to the family of adhesion GPCRs.⁹ *GPR56* protein has an extended extracellular N-terminus, a proteolytic site, and a seven transmembrane (TM) α -helix C terminal.¹³ *GPR56* messenger RNA is selectively expressed in hematopoietic stem cells and neural progenitors, suggesting a role in multipotent cell identity and tissue development.⁸

Collagen III interacts with *GPR56* and hinders neuronal migration.⁸

The BFPP-related missense mutations that are on extracellular regions of *GPR56* may prevent interactions between *GPR56* and other proteins.¹³ GPCR-interacting proteins are believed to play an important role in embryonic development, growth of central nervous system (CNS), and are essential for the functioning of the immune system. This has been reviewed by Bockaert and colleagues¹⁴ and by Druey.¹⁵ BFPP-related *GPR56* mutations include splicing mutations, frameshift mutations, and several missense mutations.

In this study, we report three different mutations (chr16:57693480T > C [NM_005682.5:c.1460T > C; p.Leu487Pro], chr16: 57689345_57689346insCCATGGAGGTGCT [NM_005682.6:c.803_804insCCATGGAGGTGCT; p.Leu269-His fs*21], and chr16:57693446C > T [NM_005682.5:c.1426C > T; p.Arg476*]) in three Pakistani families with multiple affected individuals ascertained for ID and clinical presentation consistent with BFPP. These mutations add to the number of disease-causing variants identified for BFPP. We have reviewed the literature for previously reported mutations in families of Pakistani origin.

Materials and Methods

We recruited three consanguineous ID Pakistani families (A–C). Family A was from the Gujranwala district, Punjab province (Punjabi language group), Family B from Bhakkar district, Punjab province (Saraiki language group), and Family C from D. I. Khan district, Khyber Pakhtunkhwa province (Saraiki language group). Affected individuals in Family C had already been diagnosed with polymicrogyria with the help of a computed tomographic (CT) scan. Affected individuals in two other families were only identified as suffering from ID. Written informed consent was obtained from participants, and the study was approved by the Institutional Research Ethics Boards at the Centre for Addiction and Mental Health in Toronto, Canada and National University of Sciences and Technology, Islamabad, Pakistan. Venous blood was drawn from all available affected and unaffected siblings, as well as their parents. DNA was extracted using standard methods. Clinical features for affected individuals from families A–C are summarized in ►Table 1.

Genome-Wide Homozygosity Mapping

Genotyping using Affymetrix 500K Nspl (Family B) or Illumina CoreExome (Families A and C) microarrays at The Center for Applied Genomics (TCAG) was performed on DNA from

Table 1 Clinical features of families A to C

Family	Gender/age (y)	Head circumference	Cognitive delay	Motor delay	Dys-conjugate gaze	Oromotor dyspraxia	Pyramidal signs	Cerebellar Signs (ataxia)	Epilepsy	Age of onset	Type of seizure	Epileptic activity
A II-2	M/14	53 cm	Severe/profound	Yes	Yes	Yes	Yes	Yes	Yes	1 y	Tonic-clonic	Ongoing
A II-3	M/9	52 cm	Severe/profound	Yes	Yes	Yes	Yes	Yes	Yes	1 y	Tonic-clonic	Ongoing till death
B III-2	F/12	51 cm	Severe/profound	Yes	Yes	Yes	Yes	Yes	Yes	1 y	Tonic-clonic	Ongoing
B III-6	F/21	52 cm	Severe/profound	Yes	Yes	Yes	Yes	Yes	Yes	1 y	Tonic-clonic	Ongoing
C II-4	F/23	52 cm	Severe/profound	Yes	Yes	Yes	Yes	Yes	Yes	2 y		Ongoing
C II-5	M/21	57 cm	Severe/profound	Yes	Yes	Yes	Yes	Yes	Yes	2 y		Ongoing
C II-6	F/19	51.5 cm	Severe/profound	Yes	Yes	Yes	Yes	Yes	Yes	2 y		Ongoing

affected family members along with one unaffected family member. Genotypes were analyzed for homozygosity-by-descent (HBD) regions using HomozygosityMapper and FSuite.¹⁶

Sanger Sequencing of GPR56

Primers were designed for all 15 exons of *GPR56* (► **Supplementary Table 1**, online only) for Sanger sequencing (Family C) and for confirming mutations detected by whole exome sequencing (WES, Families A and B). Polymerase chain reaction (PCR) with standard methods was used to amplify each exon, and PCR products were Sanger sequenced by TCAG (www.tcag.ca).

Whole Exome Analysis

Whole exome sequencing was used to screen DNA from one affected family member (II-2) from Family A using the Illumina HiSeq 2500 platform (a description of the library preparation and variant analysis pipeline is given elsewhere,¹⁷ and from Family B (III-6) using the Ion Proton platform, with sequence capture using AmpliSeq PCR-based exome amplification, according to manufacturer's recommendations, and analysis using the Ion Reporter suite.

The Illumina sequencing was performed using the Rubicon Genomics ThruPLEX DNAseq library preparation kit with the SureSelectXT V5 Exome Capture system. After raw files Agilent were generated from the sequencer, the reads were aligned to the Human hg19 reference genome and variants were called using GATK V 3.7¹⁸ with duplicates marked using Picard (<http://picard.sourceforge.net>). Annotation was performed using ANNOVAR¹⁹ and Diagnostics and Eone-Diagnostics Genome Center (EDGC) Annotator (<http://www.edgc.com>), which is based on Variant Effect Predictor database. A-II-2 has a median length of 192 bp and a mean depth of 47.5X. The Ts/Tv ratio is 2.9 with 90.1% of the raw reads exceeding Q30.

B-III-6 sequencing was performed on the Ion Proton Sequencing system using the AmpliSeq Exome Capture and the Ion Chef Ion Proton Prep system. The raw reads were aligned using Torrent Mapping Alignment Program (TMAP, <https://github.com/iontorrent/TMAP>) to the hg19

reference genome. Variants were called using the Ion Proton Variant Caller and annotated using EDGC Annotator and the Ion Reporter Suite.

Variants were then filtered based on allele frequency from Exome Aggregation Consortium (ExAC) and the various pathogenicity scores from Sift, Polyphen2, MutationTaster, and the regions identified from Homozygosity Mapping.

Results

Family A

The parents were first cousins. There was no reported history of ID in the family. There are two male members affected in this family with a very similar clinical presentation. In both boys, the first cry at birth was delayed. Tonic-clonic seizures started in the second year of their life. Motor development was delayed, and they walked around 6 years of age. They spoke only a few (five–six) single words. They started speaking these words at the age of 7 years. They never spoke full sentences. They were not toilet trained. They experienced frequent seizures, which were tonic-clonic in nature. Medication used for seizures included phenobarbitone, sodium valproate, and clonazepam. They were dependent on others for eating, washing, and other personal care. They had ataxia. The level of ID was severe to profound.

The younger brother (II-3) died in October 2015 after a febrile illness caused by pneumonia. An MRI was performed on the older brother, who showed classic “BFPP” with a gradient (anterior more than posterior). The microgyri were in a somewhat simplified gyral pattern (cobblestone). In ► **Fig. 1**, we provide the MRI from II-2.

Family B

This family has two branches, and the two female patients are first cousins. The parents are first cousins. The clinical presentation in both the families was similar. In both the girls, the first cry at birth was delayed. Tonic-clonic seizures started before their second birthday. Motor development was delayed, and they walked around 5 years of age. They spoke only a few

Fig. 1 MRI from individual II:2, Family A. (A) MRI showing frontoparietal polymicrogyria transverse plane; (B) transverse plane showing cerebellum and cerebellar vermis; and (C) sagittal plane showing pons. MRI, magnetic resonance imaging.

(five–six) single words. They started speaking these words at the age of 7 years. They never spoke full sentences. They were not toilet trained. The frequency of seizures was more than one seizure every week. They received carbamazepine and sodium valproate that were only partially effective. They were dependent on others for eating, washing, and other personal care. They had ataxia. The level of ID was severe to profound.

Family C

The parents are cousins. Three children are affected; two females and one male. The disease was noted in the second year of life. Diagnoses included profound ID. By their teenage years, the patients still did not have any language at all. Their gait was unsteady; they had nystagmus and muscle spasticity. The seizures started within 4 to 5 years of life; they were tonic–clonic in nature. The frequency was 2 to 3 per week, and response to carbamazepine and sodium valproate was poor. CT scan showed polymicrogyria.

In Family A (►Fig. 2, Family A-i), a 5.5-Mb homozygosity by descent (HBD) region was identified flanked by single nucleotide

polymorphisms (SNPs) rs3095600 and rs437947 (Chr16: 52,431,954–57,942,866), shared by affected individuals. Exome sequencing identified a homozygous missense mutation (chr16:57693480T > C; NM_005682.5:c.1460T > C; p.Leu487 Pro). Both affected individuals were homozygotes, and one normal female sibling and both parents heterozygous (►Fig. 2, Family A-ii and A-iii). This variant is listed in the ClinVar database (variation ID 158619; rs587783653, ExAC minor allele frequency = 0)²⁰ in association with BFPP in a single case. In Family B (►Fig. 2, Family B-i), many HBD regions were identified, including a 1.9-Mb homozygous stretch on 16q21 was identified for individual III-6 from SNPs rs2306237 to rs2133783 (Chr16: 56,973,335–58,881,897). Exome sequencing of III-6 identified a homozygous nonsense mutation Chr16:576 93446C > T; NM_005682.5:c.1426C > T; p.Arg476* that was also confirmed in III-2 (►Fig. 2, Family B-ii). This variant is listed in the ClinVar database (variation ID 158618; rs587783652, ExAC minor allele frequency = 0)²⁰ in association with BFPP with multiple submitters and also reported in a patient from India whose parents were related.¹²

Fig. 2 A–C (i) Pedigree drawings for Families A to C, respectively. Dark symbols indicate individuals affected by ID. Electropherograms showing the homozygous mutations identified in *GPR56* in affected individuals (ii), heterozygous carriers (iii), and wild-type sequence (iv) (Families A and B only). For Family C, electropherograms from the reverse sequence are shown. ID, intellectual disability.

In Family C (►Fig. 2, Family C-i), HBD mapping identified a 4.2-Mb region on 16q21. Because of the diagnosis of BFPP, and the presence of *GPR56* within this locus, Sanger sequencing was used, and affected individuals were shown to be homozygous for a novel 13-bp insertion mutation (Chr16: 57689345_57689346 insCCATGGAGGTGCT; NM_005682.6:c.803_804insCCATGGAGGTGCT; p.Leu269Hisfs* 21) in exon 7 of *GPR56*. Three unaffected siblings and both parents were heterozygous for this mutation (►Fig. 2, Family C-iii). This variant was not reported previously in the ClinVar database.

All three mutations segregated with disease status in the respective families, and all three mutations were absent in the ExAC database of 65,000 controls (including > 8,000 South Asians).

Discussion

The *GPR56*-associated mutations are a major cause of BFPP globally. We identified two previously reported and one novel mutation using homozygosity mapping, whole exome

sequencing analysis followed by Sanger sequencing validation. In two of these families we had neuroimaging data confirming the diagnosis of BFPP. In family B, it was not

Species	487
Human	SAIFLHFSLITCLSWMGLEGYN
Rhesus	SAIFLHFSLIACLSWMGLEGYN
Armadillo	SAIFLHFSLIACLSWMGLEGYN
Mouse	SAMFLHFSLIACLSWMGLEGYN
Opossum	SAIFLHFSLIACLTWMGIEGYN
Platypus	SAIFLHFSLIACLTWMGLEGYN
Chicken	GGLFLHFSLISCLTWMGIEGYN
Xenopus	SGMLLHFTQLCMFTWMGIEGFN
Zebrafish	VAVFLHFFLITSFTWMGLESIH
Fugu	VAVLLHYFLITSFTWMGLESIH

Fig. 3 Multiple sequence alignment (UCSC: Vertebrate Multiz alignment and conservation track) for *GPR56* across the Leu487Pro missense mutation indicates conservation at this residue spanning the vertebrate lineage.

Table 2 Mutations reported in Pakistani families and additional reports in other families

Mutation first reported in Pakistani families	Reported in additional families
E5 1G > C ⁴	None
739–746 del CAGGACC ⁴	One Afghan and one Indian family ⁴ and two further families from India ¹²
c 1254 C > G ³	None
chr16:57693480T > C; NM_005682.5:c.1460T > C; p.Leu487Pro (this study)	ClinVar
Chr16:57693446C > T; NM_005682.5:c.1426C > T; p.Arg476 (this study)	ClinVar and one Indian family ¹²
(Chr16: 57689345_57689346insCCATGGAGGTGCT; NM_005682.6:c.803_804insCCATGGAGGTGCT; p.Leu269Hisfs*21) (this study)	Novel

possible to get either a CT or a MRI scan. The mutation identified in Family B has been reported in an Indian patient with the diagnosis of BFPP. Prior evidence of association of this mutation with BFPP and a clinical picture consistent with BFPP makes it likely that patients in Family B suffered from BFPP. The frameshift mutation in Family C has not been reported before. The phenotype in all the three families is severe and includes the features described in other cases. When we recruited these families, only one of them had been diagnosed with BFPP based on the CT findings. Access to appropriate diagnostic and treatment facilities was possibly a factor in this. In many countries, there are competing demands on limited health resources, and clinicians face a dilemma to prioritize the expensive investigation procedures such as neuroimaging and genetic work-up. However, an early diagnosis for these families would have avoided some of the distress that parents experienced. With the availability of exome sequencing, after the birth of the first child if a mutation is identified, then families are empowered with a choice to make an informed decision about further pregnancies. In countries where consanguinity is high, the impact of genetic testing facilities could be significant, and if we take into account the economic and social cost of providing care to people with such severe disability, it would be cost-effective in the long term.

In this study, the missense mutation found in Family A (p.L487P) is at a highly conserved residue in vertebrates (► Fig. 3). It is located within a putative transmembrane (TM) domain (the third out of seven predicted TM domains), two residues distal to a previously reported BFPP mutation, S485P.³

The mutation found in Family B (p.Arg476*) would truncate the protein, removing much of the C-terminal portion of the protein, including seventh TM domain. The mutation in Family C (p.Leu269Hisfs*21) would truncate the protein and remove the C-terminal portion of the protein including the fifth TM domain. Mutations, including 13 missense, reported in the literature span across different exons and affect many different domains including the luminal region, the GPS (proteolytic site) domain and TM

domains (reviewed by Santos-Silva and colleagues).¹¹ ClinVar lists four additional missense mutations as either pathogenic or likely pathogenic: His89Tyr, Glu256Asp, Leu503Pro, and Trp623Ser.²⁰

Regarding clinical presentation, ID usually severe in degree, epilepsy, delay in motor development, cerebellar ataxia, and eye movement abnormalities are frequently reported in BFPP, and the three families reported here appeared fairly typical. BFPP caused by *GPR56* mutations has previously been reported in three Pakistani families. A frameshift mutation in exon 5 (739–746 del CAGGACC) is shared by one Afghani and three Indian families.^{4,12} A splicing mutation in exon 5 (E5 1G > C) was reported in a nonconsanguineous Pakistani family.⁴ In ► Table 2, we provide a summary of the mutations reported in Pakistani families.

Here in another study, a missense mutation in exon 10 (c 1254 C > G) was reported in a Pakistani family.³ None of the mutations we describe have been reported before in any Pakistani patients. It appears that spectrum of mutations in this gene in Pakistani patients is broad. A more systematic effort to study *GPR56* mutations in Pakistani patients recruited from neurology clinics may help discover further mutations in this gene. The mutation in Family C (p.Leu269Hisfs*21) is in exon 7. This is the first mutation reported in this exon.

Conflict of Interest

The authors report no conflict of interest.

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