Severe Phenotype in a Patient With Homozygous 15q21.2 Microdeletion Involving BCL2L10, GNB5, and MYO5C Genes, Resembling Infantile Developmental Disorder With Cardiac Arrhythmias (IDDCA)

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Homozygous and compound heterozygous mutations in GNB5 gene have been associated with a wide spectrum of clinical presentations, ranging from neurodevelopmental issues with or without cardiac arrhythmia (LADCI) to severe developmental delay with epileptic encephalopathy, retinal dystrophy, and heart rhythm abnormalities (IDDCA). While missense or missense/non-sense mutations usually lead to milder form, the biallelic loss of function of GNB5 gene causes the severe multisystemic IDDCA phenotype. So far, only 27 patients have been described with GNB5-associated disease. We report the first case of a patient carrying a homozygous 15q21.2 microdeletion, encompassing GNB5 and the two contiguous genes BCL2L10 and MYO5C. The clinical features of the child are consistent with the severe IDDCA phenotype, thus confirming the GNB5 loss-of-function mechanism in determining such presentation of the disease.

Keywords: 15q21.2 microdeletion, infantile developmental disorder with cardiac arrhythmias (IDDCA), GNB5, BCL2L10, MYO5C, epileptic encephalopathy, neurodevelopmental diseases

INTRODUCTION

Chromosomal aberrations are a common cause of developmental delay/intellectual disability (DD/ID) and congenital malformations (Stankievic and Beaudet, 2007; Sagoo et al., 2009). We now know that as many as 30–50% of cases of ID with or without other pathological features are caused by genetic or chromosomal anomalies (Cooper et al., 2011; Schaaf et al., 2011; Srivastava et al., 2019). In the present report, we describe a male patient affected by profound development delay with absence of motor and language acquisition, early onset epilepsy, bradycardia, nystagmus, visual impairment, and severe gastroesophageal reflux. Array-CGH analysis demonstrated the presence of a homozygous deletion in 15q21.2, spanning about 193 kb and involving BCL2L10, GNB5, and MYO5C genes. Among these three genes, GNB5 has been already associated with neurodevelopmental impairment and variable multisystemic dysfunction in 27 cases (Table 1).
GNB5 encodes a β subunit of heterotrimeric GTP-binding proteins. Its transcript binds members of the R7 family of G-protein-signaling regulators (RGS), supporting their negative regulation of G protein-coupled receptor signaling. The R7 RGS family is widely expressed in the central nervous system, and GNB5 transcript is involved in multiple signaling pathways in the brain. It has a central role in parasympathetic control of heart rate, neuronal development, motor function, and vision (Lodder et al., 2016). Recently, homozygous or compound heterozygous pathogenic variants in GNB5 have been reported as the cause of an autosomal recessive multisystemic syndrome with a wide spectrum of clinical presentation, categorized under two distinct phenotypes. The final presentation depends on the severity of the G protein β3 impaired function determined by the mutation. Specifically, homozygous carriers of missense variants, the most common being c.242 C > T p.(Ser81Leu), present a mild form characterized by language delay, cognitive impairment, attention-deficit/hyperactivity disorder (ADHD), with or without cardiac arrhythmia (LADCI, #617182) (Lodder et al., 2016; Shamseldin et al., 2016). Otherwise, homozygous carriers of non-functional alleles are affected by a severe form characterized by developmental delay occurring in severe intellectual disability with poor or absent speech, early epileptic encephalopathy, hypotonia, retinal abnormalities and sick sinus syndrome with bradycardia, escape beats and other arrhythmias in the absence of structural heart abnormalities (IDDCA, #617173) (Lodder et al., 2016; Turkdogan et al., 2017; Malerba et al., 2018; Vernon et al., 2018; Poke et al., 2019; Shao et al., 2019).

As this is the first case of homozygous deletion involving the GNB5 gene previously associated to diseases with complex phenotype in patients with homozygous mutations, here, we describe our patient and discuss the implications for the diagnostic assessment.

**CASE REPORT**

Our patient is a male child, born from first cousin healthy parents of Egyptian ancestry; no remarkable issue was reported in family history, but the parental couple experienced five miscarriages before the patient was born. After an uneventful full-term pregnancy, he was born trough an emergency C-section, performed for fetopelvic disproportion and initial fetal distress. We were unable to access any documentation about neonatal parameters, but perinatal period was reported as physiologic, except for mild jaundice and the evidence of minor heart defects (patent foramen ovale and ductus arteriosus, both later spontaneously closed). At the age of 6 months he experienced the onset of infantile spasms epilepsy, for which he was initially treated with valproic acid (VPA), discontinued at 3 years of age after remission of the symptoms; VPA was reintroduced 1 year later, when the child started manifesting major critical episodes characterized by hypertonia and upward gaze deviation, multiple times a day. No episodes have been reported since the introduction of a combination treatment with VPA and levetiracetam (LVT). His current phenotype is that of a severe neurodevelopmental impairment (he managed to acquire partial head control by 1 year of age but no further developmental milestones; language is completely absent), cortical blindness with subcontinuous erratic eye movements, and generalized epilepsy. Auxometric parameters are within the normal range (8 years old: height 120 cm, −0.3 SD; weight 19 kg, −1.6 SD; head circumference 50 cm, −1.5 SD), and he does not present any facial peculiar characteristics or somatic malformation. Electroencephalographic registration revealed severe disorganization with recurrent generalized epileptic anomalies, predominant on the frontal lobes. Brain MRI showed moderate bilateral enlargement of the ventricular system and cerebral sulci, suggestive of global supra and subtentorial atrophy. His heart rate was reduced at heart auscultation, and ECG registration confirmed the presence of a marked sinus bradycardia, with heart rate of 39; heart ultra sounds demonstrated the absence of structural anomalies, while at the Holter monitoring, significant sinus arrhythmia, mostly nocturnal, was found. A subsequent cardiac evaluation ruled out the need, at that moment, of further interventions for the bradycardia. Nocturnal polysomnography was also performed, showing periodic breathing with several desaturations and episodic bradypnea, suggestive of autonomic nervous system impairment. Furthermore, marked alterations, displayed by electroretinogram, indicated the presence of retinal dystrophy. The patient also suffers from severe gastroesophageal reflux disease.

**Array-CGH Analysis**

DNA was extracted from peripheral blood using a specific kit (Gentra kit, Qiagen, Hilden, Germany). ACGH analyses were performed using the Cytosure Oligo ISCA180K platform, which comprises a research-validated collection of specific probes that enable reliable detection of CNVs with high resolution in regions associated to genetic disorders. Array design was performed by Oxford Gene Technology (OGT; Begbroke, Oxfordshire, United Kingdom) and manufactured by Agilent Technologies (Santa Clara, CA, United States). The DNA test was hybridized with sex-matched DNA from pooled controls (reference DNA; Promega, Madison, WI, United States), according to the manufacturer’s protocol. Hybridization was performed using MaiTai™ Hybridization System (SciGene). After 20 h, CytoChip Oligo array was washed and scanned using InnoScan 710 Microarray Scanner (Innopsys, Carbonne, FR). Amplifications or deletions are revealed by green (Cy3) or red (Cy5) signals, due to unbalanced ratio between the two fluorophores. Data were analyzed using Cytosure interpret software (Oxford Gene Technology). Clinical interpretation of Array-CGH results are based on published literature and public databases (ENSEMBL, USBC, Database for Genetic Variants, DECIPHER, the Italian database of Troina) following Cytogenetic European and International Guidelines (Kearney et al., 2011; Hastings et al., 2012). Genomic coordinates are based on the February 2009 Human Genome Build (GRCh37/hg19).
### Table 1

| Family A II.1 | 
| --- | 
| c.249G > A (p.Asp84Valfs*52) pat | Severe DD, epilepsy, nystagmus, hypotonia, CA, GER – Brain MRI: normal |
| c.994C > T (p.Arg332*) mat | Severe DD, epilepsy, nystagmus, retinopathy, hypotonia, hyporeflexia, CA, PFO, GER – Brain MRI: normal |

| Family A II.1 | 
| --- | 
| c.249G > A (p.Asp84Valfs*52) pat | Severe DD, epilepsy, nystagmus, hypotonia evolving into spasticity, CA – Brain MRI: cerebral atrophy |
| c.994C > T (p.Arg332*) mat | 

| Family B II.1 | 
| --- | 
| c.249 + 1G > T (p.Asp84Leufs*31) pat | Severe DD, epilepsy, nystagmus, hypotonia, CA, GER – Brain MRI: normal |
| c.249 + 1G > T (p.Asp84Leufs*31) mat | 

| Family C II.2 | 
| --- | 
| c.249 + 3A > G (p.Asp84Valfs*31) pat | Severe DD, nystagmus, hypotonia, CA, GER |
| c.249 + 3A > G (p.Asp84Valfs*31) mat | 

| Family C II.3 | 
| --- | 
| c.249 + 3A > G (p.Asp84Valfs*31) pat | DD, nystagmus, hypotonia, CA, GER |
| c.249 + 3A > G (p.Asp84Valfs*31) mat | 

| Family D II.2 | 
| --- | 
| c.906C > G (p.Tyr302*) pat | Severe DD, epilepsy, nystagmus, hypotonia, CA, GER – Brain MRI: normal |
| c.906C > G (p.Tyr302*) mat | 

| Family E II.1 | 
| --- | 
| c.242C > T (p.Ser81Leu) pat | Mild ID, language delay, CA |
| c.242C > T (p.Ser81Leu) mat | 

| Family E II.2 | 
| --- | 
| c.242C > T (p.Ser81Leu) pat | Mild ID, language delay, CA |
| c.242C > T (p.Ser81Leu) mat | 

| Family F II.1 | 
| --- | 
| c.242C > T (p.Ser81Leu) pat | Mild ID, CA |
| c.242C > T (p.Ser81Leu) mat | 

| Shamseldin et al. (2016) V:1 | 
| --- | 
| c.355delG (p.Ala119Profs*16) pat | Severe DD, epilepsy, nystagmus, hypotonia, autism, CA, microbrachycephaly |
| c.355delG (p.Ala119Profs*16) mat | Severe DD, epilepsy, nystagmus, hypotonia, CA, microbrachycephaly – Deceased 5m |

| V:2 | 
| --- | 
| c.355delG (p.Ala119Profs*16) pat | Severe DD, epilepsy, nystagmus, hypotonia, CA, microbrachycephaly – Deceased 7m |
| c.355delG (p.Ala119Profs*16) mat | 

| V:3 | 
| --- | 
| c.355delG (p.Ala119Profs*16) pat | Severe DD, epilepsy, nystagmus, hypotonia, CA, microbrachycephaly – Deceased 8m |
| c.355delG (p.Ala119Profs*16) mat | 

| V:6 | 
| --- | 
| c.355delG (p.Ala119Profs*16) pat | Severe DD, epilepsy, nystagmus, hypotonia, CA, microbrachycephaly – Brain MRI: normal |
| c.355delG (p.Ala119Profs*16) mat | 

| Turkoglu et al. (2017) V:1 | 
| --- | 
| c.355delG (p.Ala119Profs*16) pat | Severe DD, epilepsy, nystagmus, retinopathy, hypotonia/intermittent extremities hypertonia, upper limbs involuntary movements, CA, GER, left ear hearing loss, laryngomalacia – Brain MRI: thin corpus callosum |
| c.737G > A (p.Arg246Gln) pat | Mild ID, language delay, strabismus, CA, hypotonia |

| Malerba et al. (2018) | 
| --- | 
| c.222_226delTAAAGA (p.Asp74Glufs*52) mat | Mild ID, language delay, strabismus, CA, hypotonia |

| Shao et al. (2019) | 
| --- | 
| c.906C > A (p.Tyr302*) pat | Severe DD, epilepsy, nystagmus, hypotonia, hyporeflexia, CA, central sleep apnea – Brain MRI: long posterior corpus callosum |
| c.906C > A (p.Tyr302*) mat | 

| Poke et al. (2019) | 
| --- | 
| c.136delE (p.Glu46fs*8) pat | Severe DD, epilepsy, visual impairment, hypotonia, contractures, CA – Brain MRI: normal |
| c.136delE (p.Glu46fs*8) mat | 

| P3 | 
| --- | 
| c.242C > A (p.Ser81*) pat | Severe DD, epilepsy, nystagmus, hypotonia – Deceased 13y – Brain MRI: normal |
| c.242C > A (p.Ser81*) mat | 

| P4 | 
| --- | 
| c.242C > A (p.Ser81*) pat | Severe DD, epilepsy, nystagmus, hypotonia, CA – Brain MRI: mild ventricular asymmetry |
| c.242C > A (p.Ser81*) mat | 

| P8 | 
| --- | 
| c.906C > G (p.Tyr302*) pat | Severe DD, epilepsy, nystagmus, hypotonia, hyporeflexia, CA – Brain MRI: normal |
| c.906C > G (p.Tyr302*) mat | 

| Current case | 
| --- | 
| Ar[Hg19]15q21.2:52385564_52579282_x10 mat, pat | Severe DD, epilepsy, nystagmus, retinopathy, central hypotonia/intermittent extremity hypertonia, CA, GER, central sleep apnea – Brain MRI: cerebral and cerebellar cortical atrophy |

**Abbreviations:** DD, Developmental Delay; ID, Intellectual Disability; CA, Cardiac Arrhythmia; PFO, Patent Foramen Ovale; GER, GastroEsophageal Reflux; ADHD, Attention Deficit/Hyperactivity Disorder; MRI, Magnetic Resonance Imaging; m, months/y, years. § Reported in Poke et al. (2019).
RESULTS

We screened copy number variations by Array-CGH, and we detected a homozygous deletion in 15q21.2. The proximal breakpoint is between nucleotide 52,366,562 and 52,385,564, and the distal breakpoint is between 52,579,282 and 52,602,756, thus spanning about 193–236 kb. The deleted region involved BCL2L, GNB5, and MYO5C genes (Figure 1). Both parents carried a heterozygous deletion overlapping with that and showed no clinical sign of disease.

No cases with similar homozygous microdeletion are present in public databases (Decipher, ClinVar Long Variants, ClinGen CNVs), while in the literature, 27 patients carrying GNB5 homozygous or compound heterozygous mutations can be found (Lodder et al., 2016; Shamseldin et al., 2016; Turkdogan et al., 2017; Malerba et al., 2018; Vernon et al., 2018; Poke et al., 2019; Shao et al., 2019). MIM database indicates that mutations in GNB5 are associated with autosomal recessive disorders: infantile developmental disorder with cardiac arrhythmias (IDDCA, MIM# 617173) and language delay and ADHD/cognitive impairment with or without cardiac arrhythmia (LADCI, MIM# 617182). IDDCA may occur with a severe phenotype, when caused by loss-of-function mutations in both alleles, or with less severe clinical features when caused by compound heterozygous non-sense/missense mutations (Shamseldin et al., 2016; Malerba et al., 2018; Vernon et al., 2018); LADCI is a mild/moderate form of disease, caused by missense mutations in both alleles.

The clinical features of our patient were almost completely overlapping with severe phenotype of IDDCA, so we can infer

**FIGURE 1 |** Array-CGH genomic profile (screenshot from the Cytosure software analysis) focused on the 15q21.2 chromosome region of the proband (purple line), his mother (green line), and his father (blue line). The log2 ratio of microdeletion of the patient was about −3, indicating homozygous deletion, while log2 ratios of the microdeletion carried by both parents were −1, indicating heterozygous loss of the region. Also indicated in the software figure is the gene content of the region: the same region with its gene content is also enlarged in the lower image, captured in the UCSC genome browser.
that homozygous GNB5 deletions determine the syndrome, as well as homozygous loss-of-function mutations of the gene.

**DISCUSSION**

Infantile developmental disorder with cardiac arrhythmias (IDDCA) is an autosomal recessive multisystem disorder characterized by neurodevelopmental impairment, delayed motor development, seizures, hypotonia, retinal disease, nystagmus, sinus node dysfunction, and gastro-esophageal reflux. This disorder is severe and is caused by homozygous loss-of-function mutations in the GNB5 gene that encodes for one out of five variants of the beta subunit of the G protein (G protein β5) (Lodder et al., 2016). The IDDCA phenotype is very similar to that of our patient, so that GNB5 must be considered the main factor in determining the clinical features of the child, given its phenotypic overlap with patients carrying GNB5 null mutations.

G protein β5 is involved in regulation of a plethora of cellular activities (Simonds and Zhang, 2000). Human GNB5 is expressed in the brain, pancreas, kidney, heart (Jones et al., 1998) and retina (Watson et al., 1996). Within the brain, the highest expression is found in the cerebellum, cerebral cortex, occipital pole, frontal lobe, temporal lobe, and caudate putamen, while the lowest expression was in the corpus callosum and spinal cord (Jones et al., 1998). It is a part of the neurotransmitter signaling cascade of the G protein-coupled receptor and plays a crucial role in psychiatric functions (Catapano and Manji, 2007; Meye et al., 2014), heart rate regulation (Posokhova et al., 2010), motor functions (Zhang et al., 2011), and vision (Shao et al., 2019). The mouse Gnb5 knockout model shows high mortality rate (Chen et al., 2003; Zhang et al., 2011), somatic runty at birth, and then a small body size, significant developmental milestone delays, abnormal gate, balance and motor learning, hyperactivity, delayed Purkinje cell development, reduced dendritic arborization, abnormal hippocampal development, and changes in transcription levels of several genes (Zhang et al., 2011). Both the knockout mice and the patients with homozygous loss-of-function mutations show phenotypic overlap with our patient: such considerations strongly suggest that GNB5 deletion plays a predominant role in defining the clinical presentation of our child.

Despite the seriousness of the phenotype, neuroradiological study is normal in most patients reported in literature, or it sometimes shows minor dysmorphic anomalies (Lodder et al., 2016; Vernon et al., 2018; Poke et al., 2019). In our patient, a diffuse mild cerebral and cerebellar atrophy was found; such sign has been developed only by one patient, reported in both Lodder et al. (2016) and Poke et al. (2019) papers. We also underline the detection in our patient of nocturnal periodic breathing with numerous desaturations and episodic bradypnea. Sleep apneas have only been reported once by Shao et al. (2019): we can confirm the possible presence of such comorbidity in IDCCA that, along with bradycardia and arrhythmias, points out an autonomic nervous system impairment resulting from GNB5 loss of function.

**DATA AVAILABILITY STATEMENT**

The patient’s dataset is available in the public database DECIPHER (case number 411168).

**ETHICS STATEMENT**

Written informed consent was obtained from the minor(s)’ legal guardian/next of kin for the publication of any potentially identifiable images or data included in this manuscript.

**AUTHOR CONTRIBUTIONS**

FS interpreted the Array-CGH analysis, conceived and co-wrote the study. CC and SD’A performed the clinical assessment and...
follow-up of the patient and co-wrote the manuscript. CS helped in studying the case and drafting the manuscript. FF and FG performed the laboratory tests. CP reviewed the manuscript for intellectual content and supervised the work.

REFERENCES

Bultema, J. J., Boyle, J. A., Malenke, P. B., Martin, F. E., Dell’Angelica, E. C., Cheney, R. E., et al. (2014). Myosin Vc interacts with Rab32 and Rab38 proteins and works in the biogenesis and secretion of melanosomes. J. Biol. Chem. 289, 33513–33528. doi: 10.1074/jbc.M114.578948

Catapano, L. A., and Manji, H. K. (2007). G protein-coupled receptors in major psychiatric disorders. Biochem. Biophys. Acta 1768, 976–993. doi: 10.1016/j.bbadis.2006.09.025

Chen, G. K., Eversole-Cire, P., Zhang, H., Mancino, V., Chen, Y. J., He, W., et al. (2003). Instability of GDI domain-containing RGS proteins in mice lacking the G protein beta-subunit Gbeta5. Proc. Natl. Acad. Sci. U.S.A. 100, 6604–6609. doi: 10.1073/pnas.0631825100

Cooper, G. M., Coe, B. P., Santhosh Girirajan, S., Rosenfeld, J. A., Vu, T. H., Carl Baker, C., et al. (2011). A copy number variation morbidty map of developmental delay. Nat. Genet. 43, 838–846. doi: 10.1038/ng.909

Hammer, J. A. III, and Sellers, J. R. (2012). Walking to work: roles for class V myosins as cargo transporters. Nat. Rev. Mol. Cell. Biol. 13, 10–26. doi: 10.1038/nrm3248

Hastings, R., Howell, R., Dagna Bricarelli, F., Kristoffersson, U., and Cavani, S. (2016). The epileptology of GNB5 encephalopathy. Front. Genet. 7:626. doi: 10.3389/fgene.2016.00625

Rodriguez, O. C., and Cheney, R. E. (2002). Human myosin-Vc is a novel class V myosin expressed in epithelial cells. J. Cell. Sci. 115, 991–1004.

Shamseldin, H. E., Masuho, L., Alenizi, A., Alyamani, S., Patil, D. N., Ibrahim, N., et al. (2016). GNB5 mutation causes a novel neuropsychiatric disorder featuring attention deficit hyperactivity disorder, severely impaired language development and normal cognition. Genome Biol. 17:195. doi: 10.1186/s13059-016-1061-6

Simonds, W. F., and Zhang, J. H. (2000). New dimensions in G protein signaling: G beta 5 and the RGS proteins. Pharm. Acta Helv. 74, 333–336. doi: 10.1016/s0167-4889(98)00017-2

Vernon, H., Cohen, J., De Nittis, P., Fatemi, A., McClellan, R., Goldstein, A., et al. (2011). Knockout of G Protein β5 causes a neurodevelopmental syndrome with autism and cognitive disability. Annu. Rev. Genet. 45, 171–192. doi: 10.1146/annurev-genet-010610-112428

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