Novel GNB1 de novo mutation in a patient with neurodevelopmental disorder and cutaneous mastocytosis: Clinical report and literature review

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A R T I C L E   I N F O

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A B S T R A C T

De novo monoallelic mutations in the GNB1 gene, encoding a β subunit of heterotrimeric G proteins, cause a newly recognized disorder with the typical clinical picture of severe developmental delay/intellectual disability, hypotonia and extrapyramidal symptoms. We describe another case of the condition with manifestations of cutaneous mastocytosis associated with a novel de novo mutation GNB1 NM_001282539.1: c.230G > T; p. (Gly77Val). We also present the detailed clinical and etiopathogenetic discussion on previously diagnosed patients as well as suggestions for the link of the mutation with skin disease.

1. Introduction

Severe intellectual disability (ID) is a complex disorder that typically presents with behavioural abnormalities, seizures, severe motor dysfunction and other neurological symptoms (Carulla et al., 2011). It has a number of genetic and non-genetic causes. Gillissen et al. demonstrated that about a quarter of such patients are diagnosed with the single-nucleotide variant by Whole-Exome Sequencing (WES) strategy (Gillissen et al., 2014). The majority of genetic ID cases are presumably due to autosomal dominant de novo pathogenic variants (Yang et al., 2013).

GNB1-related neurodevelopmental disorder (OMIM: 616973) has been clinically characterized by global developmental delay/intellectual disability, seizures, hypotonia and ophthalmological symptoms (Petrovski et al., 2016; Lohmann et al., 2017). Great majority of the observed cases of the condition have been caused by pathogenic de novo GNB1 gene variants (8/13 (Petrovski et al, 2016) and 10/14 (Lohmann et al., 2017)) with notable exception of R92L dominant variant identified in three families of different ethnic origin (Lohmann et al., 2017). Reported GNB1 causative mutations result in both loss and gain of function (Yoda et al., 2015, Petrovski et al, 2016; Lohmann et al., 2017).

Mastocytosis (or Mast cell disease, MIM:154800) is characterized by an excessive number of apparently normal mast cells in the skin and, sometimes, in other organs. Its cutaneous form is not associated with a hematologic disorder and occurs typically as urticaria pigmentosa.

Herein, we present a clinical report of GNB1-related encephalopathy with a novel pathogenic variant. The proband presented with the full clinical picture of an early-onset neurodevelopmental disability accompanied by hypotonia and extrapyramidal symptoms as well as mastocytosis. Cutaneous mastocytosis has not been reported previously in the GNB1-related disorder. We sum up the previously reported cases of the condition and discuss the possible link between G proteins and inflammatory manifestations.

2. Clinical report

The female proband was born to a nonconsanguineous healthy 40-year-old mother and a healthy 50-year-old father. No family history of note or exposure to teratogens was reported. The proband has two healthy half-brothers. She was born by cesarean section at 39 weeks gestation. Apgar scores were 8 and 9 after 1 and 3 min respectively. Birthweight was 2520 g (5th centile), length 53 cm (95th centile) and gestation. Apgar scores were 8 and 9 after 1 and 3 min respectively. Birthweight was 2520 g (5th centile), length 53 cm (95th centile) and occipito-frontal circumference (OFC) 33 cm (15th centile). No congenital anomalies or dysmorphic features were noted at birth except genital anomalies or dysmorphic features were noted at birth except genital anomalies or dysmorphic features were noted at birth except microretrognathia. Standard intracranial US revealed slightly increased right ventricle.

Although there were no feeding difficulties or slowing of physical growth within the first year of life, the achievement of major milestones was severely delayed when assessed for both gross and fine motor,
cutaneous mastocytosis as a possible part of GNB1-related neurodevelopmental disorder. Transmission revealed muscular hypotonia and extrapyramidal symptoms of speech, and social skills development (Table 1). Early neurological assessment revealed muscular hypotonia and extrapyramidal symptoms (upper-limb choreoathetosis).

Features of cutaneous mastocytosis have been evident in the proband since the end of the first year of life (Fig. 1). The diagnosis of urticaria pigmentosa form was confirmed by skin biopsy.

Currently, at 4 years, the proband's weight is 14.8 kg (25th-50th centile), height 105 cm (50th-75th centile) and OFC 48.5 cm (10th-25th centile). She is globally developmentally delayed, although no formal psychological assessment has been performed yet. She understands simple commands and can speak a few words which seems to be her biggest challenge. Eye contact is weak. The gait is unstable and wide-based and when walking she tends to lean forward as if her movements were deconstructed. Generalized hypotonia requires her to prop up when standing up. The upper and lower limb reflexes are normal. The hand stereotypes (frequent hand-to-mouth movements) that add to an already existing extrapyramidal upper-limb activity are present. She also has bruxism, hypermobile tongue and apparently is unable to chew solid foods. The proband has bilateral severe vesicoureteral reflux that has already required unilateral nephrectomy. Subclinical hypothyroidism has been diagnosed as well. No significant behavioral abnormalities have been recorded in the proband. Discrete dysmorphic features such as prominent eyebrows and eyelashes, bilateral fifth finger clinodactyly (present in the relatives on the maternal side) and unilateral partial skin syndactyly of second and third toes are present.

In EEG generalized bursts of sharp and slow wave complexes were present but these did not require antiepileptic medication. MRI of the head performed twice was normal as were metabolic studies (TANDEM Mass Spectrometry and Gas Chromatography).

DNA extracted from the peripheral blood of the proband was analyzed with whole exome sequencing. Library was prepared with Nextera kit from Illumina; sequencing was performed on HiSeq 1500 to the mean depth of 50X; 82% of target bases were covered at a minimum of 20X whereas 95% had coverage of min. 10X, raw data were analyzed as previously described (Ploski et al., 2014) with Hg19 genomic build used for alignments. We found 563,432 variants passing a default quality filter. These variants were further filtered to include those affecting coding sequence (by changing aminoacid or splice site within 10bp from exon/intron boundary) and having < 1% minor allele frequency in all the following databases: ExAC (http://exac.broadinstitute.org), 1000Genomes (http://www.1000genomes.org/), ESP6500 (http://evs.gs.washington.edu/ESV), and an in-house database of ~500 Polish exomes. The final 705 variants were screened against known mutations listed in HGMD database (http://www.hgmd.cf.ac.uk) as pathogenic. In parallel, the 705 variants were searched for biallelic mutations consistent with autosomal recessive inheritance as well as for monoallelic variants potentially causative of an autosomal dominant or sex-linked condition (here we considered variants not affecting coding sequence). These variants were further filtered to include those reflecting a splice site variant.

Considering the phenotype and the characteristics of the variants, we prioritized a single variant in GNB1 exon 6 NM_001282539.1: c.230G > T; p.(Gly77Val) which has not been previously reported as causative of GNB1-related neurodevelopmental disorder. The novel GNB1 NM_001282539.1: c.230G > T; p.(Gly77Val) variant was submitted to NCBI GenBank database (accession number: MG210950).

Identified variant was has been predicted to be pathogenic by Polyphen2HVAR - D (damaging), Polyphen2HDIV - D (damaging), MutationAssessor - H (high), SIFT - D (damaging), FATHMM – D (damaging), MutationTaster – D (damaging), MetaSVM - D (damaging), and MetaLR - D (damaging). Using Sanger sequencing the mutation was confirmed in proband’s DNA and excluded in the parental samples (Fig. 2).

3. Discussion

The role of de novo dominant mutations has been widely discussed in human genetic disease (Veltman and Brunner, 2012). One hypothesis for their significant burden in certain or particular regions of these genes is that they exert strong negative fitness effects thereby reducing the role of inherited dominant variants. Of all the GNB1 gene mutations identified in 31 individuals close to a half are located in a small stretch of exons which is likely responsible for interaction...
between the proteins in the G protein complex (Petrovski et al., 2016; Lohmann et al., 2017; Brett et al., 2017; Steinrucke et al., 2016).

**GNB1** encodes a beta subunit of a heterotrimer known as the G protein complex. The complex interacts with G-protein-coupled receptors (GPCRs). GPCRs represent the largest family of seven transmembrane domain receptors that regulate vital cellular functions including neuronal signal transmission and cell proliferation, development as well as survival. There has been evidence for a critical role of **GNB1**-mutated cells in the regulation of central molecular pathways including cascades leading to tumor development (Yoda et al., 2015). Recently, a link has been established between GPCRs expressed in mast cells and host defense regulation in inflammatory disorders (Subramanian et al., 2016).

We describe a female patient in whom a novel **GNB1** exon 6 NM_001282539.1: c.230G>T; p.(Gly77Val) *de novo* mutation was identified. This particular residue was not listed by Yoda et al. as a site of recurrent somatic **GNB1** mutations in tumor samples, however the mutation at this locus, as well as other close variants in exon 6, putatively confers cytokine-independent growth and activates canonical G protein signaling via the disruption of the interaction between α, β, and γ subunits of G proteins (Yoda et al., 2015). Individual 9 in Petrovski et al. study had a different variant at the same p.Gly77 residue which suggests that this may be another recurrent site for **GNB1** mutations (Petrovski et al., 2016).

The neurodevelopmental phenotype of the girl is consistent with the previous reports on **GNB1**-related disorder. Global developmental delay, hypotonia, and seizures are observed in most patients with **GNB1** mutation. In Table 2 we have summarized major clinical features identified in already published series of **GNB1**-mutation positive individuals. Bilateral vesicoureteral reflux and hypothyroidism, respectively, have been observed in single cases (Petrovski et al., 2016).

An interesting feature noted exclusively in our patient was urticaria pigmentosa, a cutaneous form of Mast cell disease (MIM: 154800). The excessive presence of mast cells within the skin tissue was confirmed pathologically. No **KIT** gene variants were identified on exome sequencing, however somatic mutations are found in most genetic cases of mastocytosis so, since whole blood was used for analyses, we currently cannot exclude the presence of somatic **KIT** variant in our patient. Yoda et al. showed that downstream signaling of the mutated beta subunit of G protein complex results in leads activation of cell growth (Yoda et al., 2015). This may accordingly lead to the development of tumors, possibly including myeloid neoplasms. Importantly, the co-occurrence of **GNB1** mutations with acute lymphoblastic leukemia was recently shown by Brett et al. (Brett et al., 2017). Potentially, targeting the abnormally activated G protein cascade may thus inhibit cancerous cell growth. Another important role of G protein subunits was shown by Block et al. who linked GPCR-mediated downstream signaling to chemokine-induced leukocyte recruitment (Block et al., 2016). G proteins may thus be involved in inflammatory reactions. Recent data has allowed to demonstrate the presence of a novel MAS-related G-protein coupled receptor X2 (MRGPRX2) which is selectively expressed in mast cells (Subramanian et al., 2016). These receptors are also present in mast cells residing in the skin which might explain chronic urticaria phenotypes (Fujisawa et al., 2014). Thus mastocytosis phenotype in our case may be linked to abnormalities in GPCR downstream targeting due to G protein beta subunit activation. Alternatively, mastocytosis may be merely an incidental finding in our patient.

In conclusion, our report confirms neurodevelopmental phenotype of **GNB1**-related diseases and is the first one to suggest mastocytosis as a possible part of the clinical picture of these disorders. Understanding the role of G protein beta subunit in neurodevelopmental and skin disorders may help to develop novel therapeutic approaches.

### References


### Table 2

<table>
<thead>
<tr>
<th>Clinical feature</th>
<th>Previous reports (n = 31)</th>
<th>Our case (n = 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intellectual disability/developmental delay</td>
<td>30 (+)</td>
<td>+</td>
</tr>
<tr>
<td>Hypotonia</td>
<td>20 (+)</td>
<td>+</td>
</tr>
<tr>
<td>Seizures/Abnormal EEG</td>
<td>20 (−)</td>
<td>−</td>
</tr>
<tr>
<td>Ophthalmological symptoms</td>
<td>20 (−)</td>
<td>−</td>
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<tr>
<td>Brain MRI abnormality</td>
<td>14 (−)</td>
<td>−</td>
</tr>
<tr>
<td>Extrapyramidal features including hypertension</td>
<td>12 (+)</td>
<td>+</td>
</tr>
<tr>
<td>Growth delay</td>
<td>11 (−)</td>
<td>−</td>
</tr>
<tr>
<td>Dysmorphism</td>
<td>some (+)</td>
<td>+</td>
</tr>
<tr>
<td>Vesicoureteral reflux</td>
<td>1 (+)</td>
<td>+</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>1 (+)</td>
<td>+</td>
</tr>
<tr>
<td>Cutaneous mastocytosis</td>
<td>0 (−)</td>
<td>−</td>
</tr>
</tbody>
</table>

Fig. 2. Sequencing results: A) Pedigree of the studied family, proband is marked with black arrow. B) Presentation of NGS results of variant p.Gly77Val in **GNB1** gene in proband using Integrative Genomic Viewer (IGV). C) Presentation of Sanger sequencing results in **GNB1** gene in proband and healthy parents using Variant Reporter 1.1.


