Expanding the Phenotypic Spectrum of GPI Anchoring Deficiency Due to Biallelic Variants in GPAA1

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Abstract

Background and Objectives

To expand the clinical knowledge of GPAA1-related glycosylphosphatidylinositol (GPI) deficiency.

Methods

An international case series of 7 patients with biallelic GPAA1 variants were identified. Clinical, biochemical, and neuroimaging data were collected for comparison. Where possible, GPI-anchored proteins were assessed using flow cytometry.

Results

Ten novel variants were identified in 7 patients. Flow cytometry samples of 3 available patients confirmed deficiency of several GPI-anchored proteins on leukocytes. Extensive phenotypic information was available for each patient. The majority experienced developmental delay, seizures, and hypotonia. Neuroimaging revealed cerebellar anomalies in the majority of the patients. Alkaline phosphatase was within the normal range in 5 individuals and low in 1 individual, as has been noted in other transamidase defects. We notably describe individuals either less affected or older than the ones published previously.

Discussion

Clinical features of the cases reported broaden the spectrum of the known phenotype of GPAA1-related GPI deficiency, while outlining the importance of using functional studies such as flow cytometry to aid in variant classification.

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The glycosylphosphatidylinositol (GPI) anchor pathway plays an essential role in posttranslational modification of a variety of proteins integral to cell signaling and function. Proteins targeted for GPI modification are captured in the cytoplasm and translocated to the endoplasmic reticulum where they acquire the GPI moiety. They are then transferred to the Golgi apparatus for remodeling and maturation of the GPI-anchored protein (GPI-AP), before being transferred to the plasma membrane. These proteins localize to numerous cell types and play integral roles in a variety of cellular processes. Examples of GPI-APs include Fcγ receptors such as CD16, regulators of the complement pathway such as alkaline phosphatase (ALP). In the nervous system, GPI-APs play a vital role in axon growth, axon regeneration, synapse formation, and synaptic plasticity. Dysfunction of neural GPI-APs has been linked to a variety of neurologic diseases including autism spectrum disorder, schizophrenia, and MS.

Numerous neurodevelopmental disorders have been associated with variants in genes involved in the biosynthesis of GPs. Inherited GPI deficiency disorders, also referred to as glycosylphosphatidylinositol biosynthesis defects or GPIBDs, are a group of autosomal recessive or X-linked recessive syndromic intellectual disability disorders. In 2006, the first cases of inherited GPI deficiency were documented, with the descriptions of patients with thrombosis and seizures who were found to have biallelic pathogenic variants in PIGM. More recently, extensive phenotypic descriptions of patients with variations in several genes (e.g., PIGN, PIGT, PIGY, and PIGK) in the pathway have been published. GPIA1 encodes a subunit of the GPI-lipid anchor transamidase complex. Its role in human cancers has been described since 2006; however, its delineation as a cause of syndromic intellectual disability is more recent.

A cohort of 10 patients from 5 families are the only reported individuals affected by GPPAI-related GPI deficiency (OMIM 617810), also known as GPI biosynthesis defect 15. Patients with pathogenic variants in GPPAI have developmental delay, hypotonia, seizures, cerebellar atrophy, dysarthria, dysmetria, ataxia, osteopenia, and variable dysmorphic features. In this study, we describe and broaden the clinical and biochemical variability associated with GPPAI-related GPI deficiency in an additional 7 families.

Methods

Seven individuals from unrelated families with biallelic GPPAI variants (GenBank: NM_003801.3) are included in this case series. Individuals were identified from international centers through direct communication between clinicians and the authors of the original GPPAI case series. Whole-exome sequencing (WES) was performed on both research and clinical bases according to protocols laid out by the respective laboratories (Supplemental Data, links.lww.com/NXG/A479). Developmental assessments were performed by pediatricians, pediatric neurologists, and developmental pediatricians. Seizure and EEG classification were performed by neurologists or pediatric neurologists. Dystrophology examinations were performed by medical geneticists.

Standard Protocol Approvals, Registrations, and Patient Consents

Informed consent including consent for publication of photographs, imaging, and clinical information was obtained from each family in accordance with guidelines established by the institutional review boards at their primary site of care. CARE reporting guidelines were followed while preparing this article.

Fluorescence-Activated Cell Sorting Analysis

Blood samples collected from 3 of the 7 patients (II, III, and VII), 3 different healthy controls, and the father of an affected individual were subjected to flow cytometry to assess the effect of GPPAI variants on the cell surface GPI-APs on granulocytes. Blood samples were stained for an hour on ice with phycoerythrin-conjugated anti-human CD16 (BioLegend), fluorescein-labeled proaerolysin (FLAER)-Alexa 448 (Cedarlane), and fluorescein isothiocyanate-conjugated mouse anti-human CD55 or CD59 (BD PharMingen). Samples were treated with fluorescence-activated cell sorting Lysing Solution (BD Biosciences) before flow cytometry analysis (BD FACS Canto II system (BD Biosciences). FlowJo software (v9.5.3, Tomcy Digital) was used to analyze the data.

Data Availability

Original data can be made available on request; genetic data or clinical data sharing may be subject to privacy restrictions.

Results

Clinical presentations of the 7 patients are outlined in Table 1. Patients were 43% male (3/7). The age of the patients was variable, ranging from 6 weeks to 3 years at the time of diagnosis. Symptom onset was generally between birth and 4 months, when hypotonia was noted. All patients presented with developmental delays or intellectual disability of varying severity. Epilepsy was seen in 6/7 individuals, with the first seizure occurring between 8 months to 3 years. Numerous seizure types were observed, with 4 patients experiencing...
febrile seizures, 2 patients with absence seizures, 3 patients with bilateral tonic-clonic seizures, 2 patients with myoclonic jerks, and 1 patient experiencing epileptic spasms. Only 1 patient, aged 22 months, had not experienced a seizure. EEG data were available for 5 patients and showed variable findings, including normal, nonspecific changes, spike/polyspike discharges, multifocal spike and wave, and slow wave epileptiform discharges; no common EEG pattern was seen. Patients were maintained on a variety of antiepileptic medications (Supplemental Data, links.lww.com/NXG/A479).

Six of 7 patients were described as hypotonic and 2 demonstrated spasticity. In all patients with hypotonia, it was either present at birth or noted before 4 months of age. Cerebellar atrophy was seen in 3/6 patients (Figure 1 and eFigure 2, links.lww.com/NXG/A479). It was progressive in 1 patient (eFigure 1), with imaging at 7 months of age demonstrating benign enlargement of the subarachnoid space of infancy with a normal cerebellum. Imaging was repeated at age 2 years and demonstrated mild diffuse and symmetrical brain atrophy with progression in the cerebellum. The second patient did not have neuroimaging until age 21 years; this demonstrated cerebellar atrophy, which has been stable on repeat imaging at ages 25 and 29 years. The third patient with cerebellar atrophy has not been assessed with serial MRIs. In 2 of these individuals, additional abnormalities were noted, including slightly delayed myelination and mild thinning or hypoplasia of the corpus callosum.

### Table 1 Key Clinical Features of Previously Reported and New Patients

| Feature            | New patients | Total          | Nguyen et al.² |
|--------------------|--------------|----------------|---------------|
| Sex                | M F F F M M  | 3/7 male       | 5/10 male     |
| Diagnosis          | 15 mo 10 w 3 y 3 mo 6 w 8 mo 22 mo 12 mo — — | — — | — — |
| Last assessment    | 38 y 22 mo 3 y 9 mo 5 y 3 mo 3 y 9 mo 5 y 5 mo 3 y — 4-30 yo | — — | — — |
| Height (cm)        | 170 (17%) 75 (<1%) 88 (<1%) 111 (33%) 90 (1%) 105 (7%) 97 (59%) — — | — — | (−1−59%) |
| Weight (kg)        | 78 (72%) 10.1 (23%) 12.1 (3%) 26 (96%) 11 (<1%) 17.7 (31%) 15.3 (72%) — — | — — | (−16−58%) |
| OFC (cm)           | 59.5 (93%) 45.3 (13%) 46 (1%) 45 (<1%) 46 (2%) 51.5 (69%) 49 (34%) — — | — — | (−1−87%) |
| DD/ID              | ++ + +++ +++ +++ Present +++ 100% (7/7) 100% (10/10) | 100% (10/10) | 100% (10/10) |
| Hypotonia          | Yes No Yes Yes Yes Yes Yes 86% (6/7) 100% (10/10) | 100% (10/10) | 100% (10/10) |
| Seizures           | Febrile, absence, and BTC Febrile, epileptic spasms, and myoclonic jerks Febrile Febrile, startle, myoclonic, absence, BTC, and status epilepticus Yes BTC 86% (6/7) 70% (7/10) | 70% (7/10) | 70% (7/10) |
| Cerebellar atrophy | Yes No Yes No Yes NA No 50% (3/6) 90% (9/10) | 90% (9/10) | 90% (9/10) |
| Neurologic features| At, DA, and DM None Sp At and Ny Ny and Sp None At — — | — — | — — |
| Osteopenia         | Yes (Z = −2.3) NA NA NA NA NA No 50% (1/2) 100% (8/8) | 100% (8/8) | 100% (8/8) |
| Dysmorphic features| Prominent forehead, hypertelorism Prominent forehead, deep set eyes, downslanting PFs, small chin, and tented upper lip Broad forehead, upslanting PFs, epicanthus, arched narrow palate, tented upper lip, and protruding ear lobes Epicantal folds, upslanting palpebral fissures, tented upper lip, and flat nasolabial folds Single palmar crease No 57% (4/7) 80% (8/10) | 80% (8/10) | 80% (8/10) |
| Plasma ALP         | Normal Normal Low Normal Normal Normal NA Normal 83% (5/6) 100% (10/10) | 100% (10/10) | 100% (10/10) |

Abbreviations: + = mild; ++ = moderate; +++ = severe; At = ataxia; BTC = bilateral tonic-clonic; DA = dysarthria; DD = developmental delay; DM = dysmetria; ID = intellectual disability; Ny = nystagmus; OFC = occipitofrontal circumference; PF = palpebral fissures; Sp = spasticity.
Associated features included nystagmus in 2 patients, dysarthria in 1 patient (not assessed in 4 patients given their age or language capacity), dysmetria in 1 patient, and ataxia in 3 patients. Osteopenia was present in 1 patient with a bone density Z score of $-2.3$; however, it was not assessed in 5 patients in the cohort. Plasma ALP levels were within the normal range in 5 patients and low in 1 patient. Variable dysmorphic features were described, with common descriptions including prominent forehead and a tented upper lip. Three patients were not dysmorphic.

We identified novel variants in GPAA1 in all cases (Table 2, Figure 2). Variants were identified on clinical WES in 5 patients and research WES in the remaining 2 patients. Indications for testing included developmental delay, hypotonia, seizures, and ataxia (Supplemental Data, links.lww.com/NXG/A479). In total, there were 10 new variants identified in the 7 patients, as 3 patients were homozygous for GPAA1 variants, and 2 unrelated patients were observed to carry the same variant. Seven variants were missense: c.164T>C (p.Met55Thr), c.1049T>G (p.Leu350Arg), c.917A>G (p.His306Arg), c.1559T>G (p.Leu520Arg), c.947C>T (p.Ala316Val), c.1831T>C (p.Trp611Arg), and c.149T>A (p.Met50Lys). Three variants were deletions resulting in frame-shifts: c.1233_1239del (p.Pro412Tyrfs*19), c.1477_1478del (p.Arg493Glyfs*152), and c.619delA (p.Met207Cysfs*21). No nonsense variants were identified in this cohort of patients (eTable 1).

Figure 1: Sagittal T1 Images From Patients With GPAA1-Related Glycosylphosphatidylinositol Deficiency

(A) Neuroimaging from patient I, taken at age 21 years. Note the cerebellar atrophy with prominent folia. (B) Sagittal T1 MRIs from patient II, in whom biallelic GPAA1 variants were found incidentally on exome sequencing. The cerebellum is normal in this patient. (C) Neuroimaging from patient III from 2018. Although her initial MRI did not report cerebellar hypoplasia/atrophy, subsequent cerebellar atrophy was noted on this follow-up imaging (shown) performed at age 2 years. Slightly delayed myelination and mild thinning of the corpus callosum was also identified. (D) Sagittal T1 MRI images from patient V, showing severe vermian hypoplasia/atrophy and thinning of the superior cerebellar peduncle and bilateral cerebellar hemisphere atrophy. The corpus callosum was also noted to be hypoplastic.

FLAER staining is used as a marker of total GPI-APs, and specific GPI-APs such as CD16 can be quantified via flow cytometry. To assess the effect of the GPAA1 variants on the biosynthesis of GPI-APs, flow cytometry analyses were performed on blood samples from 3 patients. On the granulocytes of patients II, III, and VII, there was a 47%, 37%, and 12% decrease in the CD16 level compared with healthy controls, respectively (Figure 3). CD55, CD59, and total GPI-AP levels in these individuals showed slightly decreased or no change in the levels compared with healthy controls. The father of an affected parent, who carries a frameshift variant in GPAA1, did not have abnormalities on flow cytometry (eFigure 3, links.lww.com/NXG/A479).

Discussion

The present report of 7 new cases of GPAA1-related GPI deficiency both affirms and expands on the phenotype previously described. The most common features seen in the series are seizures, developmental delay, and hypotonia.

Variability in this cohort was marked. Patient II had a considerably milder clinical phenotype compared with the other patients in both this and the previously reported cohort. At age 22 months, the patient had developmental delay, most notably in motor milestone acquisition and adaptive development. Her problem solving, receptive language, and
socioemotional function was slightly below expected for her age. She has no seizures, hypotonia, or cerebellar atrophy (Figure 1). In all other individuals, hypotonia presented within the first 4 months of life. Seizures are more variable in onset; however, most patients in this case series had onset of epilepsy before 22 months (Supplemental Data, links.lww.com/NXG/A479). Her main medical concern was early onset inflammatory bowel disease; GPAA1 variants were considered incidental findings on exome sequencing. However, flow cytometry assays support the pathogenicity of the variants by demonstrating reduction in CD16 in granulocytes. Similar to other patients in this series, there was no decrease in other markers. Taken together, her clinical and biochemical findings could suggest a milder variant, and she will continue to be monitored for disease manifestations.

Patient I is, to date, the oldest individual reported with GPAA1 deficiency. His clinical presentation includes many cardinal features of the disorder, including neonatal hypotonia, generalized epilepsy with multiple seizure types, stable global cerebellar atrophy (Figure 2, links.lww.com/NXG/A479), osteopenia, and normal ALP. He exhibits several cerebellar symptoms including ataxia, dysarthria, and dysmetria. He has impairments in fine motor skills and is unable to stand, read, or write, however he does eat and undress independently.

Clinical features reported in these patients reflect what was described in the first cohort of patients with GPAA1-related GPI deficiency. Developmental delay of variable severity was universal in both cohorts. Two patients are nonverbal; all others had delayed language acquisition and ongoing impaired communication. All patients had delayed motor development: 1 patient cannot sit independently; others achieved this milestone between 11 months and 4 years. Ambulation is present in 1 patient, achieved at 6 years; however, the majority of patients in the cohort remain below age 6 years and may ambulate in the future (Supplemental Data, links.lww.com/NXG/A479). Hypotonia and seizures were also seen in comparable frequencies (Table 1). No common seizure type or EEG pattern was identified. Cerebellar atrophy was seen less frequently, with only 50% of patients demonstrating this finding on neuroimaging. Other intracranial abnormalities are seen in some individuals, including delayed myelination, mild thinning of the corpus callosum, and hypoplasia of the corpus callosum. Similar anomalies have been described in patients with other GPIBDs, reflective of the importance of GPI-APs in neural development. Flow cytometry identified decreased levels of only CD16 compared with previously reported patients with decreased CD16, CD59, and total GPI-AP. A characteristic flow cytometry profile in granulocytes has been described for individuals with PIGU and PIGT variants. GPAA1 deficiency does not demonstrate a characteristic pattern; however, measuring GPI-APs through flow cytometry remains a mechanism by which functional consequences of GPAA1 variants can be investigated.

**Table 2 Genetic Description of GPAA1 Variants**

| Patient | Genomic variant | cDNA variant | Protein variant | Zygosity | Inheritance | Pathogenicity score (CADD) |
|---------|-----------------|-------------|----------------|----------|-------------|---------------------------|
| I       | chr8: g.145138116T>C | c.164T>C | p.Met55Thr | Homozygous | Maternal + paternal | 24 |
| II      | chr8: g.145139663T>G | c.1049T>G | p.Leu350Arg | Homozygous | Maternal + paternal | 27 |
| III     | chr8: g.145139419A>G | c.917A>G | p.His306Arg | Compound heterozygous | Maternal | 23.3 |
|         | chr8: g.145140583T>G | c.1559T>G | p.Leu520Arg | Compound heterozygous | Paternal | 27.3 |
| IV      | chr8: g.145139449C>T | c.947C>T | p.Ala316Val | Homozygous | Maternal + paternal | 28.3 |
| V       | chr8: g.145139449C>T | c.947C>T | p.Ala316Val | Compound heterozygous | Paternal | 28.3 |
|         | chr8: g.145140014-145140020del | c.1233_1239del | p.Pro412Tyrfs*19 | Compound heterozygous | Maternal | 28.3 |
| VI      | chr8: g.145140501-145140502del | c.1477_1478del | p.Arg493Glyfs*152 | Compound heterozygous | Maternal | 32 |
|         | chr8: g.145140993T>C | c.1831T>C | p.Trp611Arg | Compound heterozygous | Paternal | 28.3 |
| VII     | chr8: g.145139039del | c.619delA | p.Met207Cysfs*21 | Compound heterozygous | Paternal | 24.6 |
|         | chr8: g.145138101T>A | c.149T>A | p.Met50Lys | Compound heterozygous | Maternal | 24.6 |

*a UCSC Genome Browser hg19.
*b GenBank: NM_003801.3.
*c uWashington Combined Annotation Dependent Depletion (CADD) Score Calculator (GRCh37-v1.4)—cadd.gs.washington.edu/.
Figure 2 Variant Localization and Residue Conservation

(A) Schematic representation of missense and frameshift $GPAA1$ variants, with previously reported variants indicated in blue, and novel variants indicated in red. (B) Amino acid conservation in vertebrates. (C) Three-dimensional modeling of the luminal domain of $GPAA1$, which spans residues 66–348. Shown in red are 2 missense variants within this domain.
conserved in all eukaryotes. All variants were novel; however, a recurrent variant was identified in 2 unrelated individuals. The p.Ala316Val substitution was found in an individual of Kurdish Turk descent in a homozygous state and in an unrelated Israeli individual with compound heterozygous variants. Both patients have severe global developmental delay; however, physical anomalies vary between the 2, including the presence of cerebellar atrophy in only the patient with compound heterozygous variants. That patient also has a more severe epileptic disorder, with poor seizure control despite multiple medications (Supplemental Data, links.lww.com/NXG/A479).

Osteopenia was a universal finding in the first cohort of patients identified by Nguyen et al. in 2017. Only 2 patients in this series have been assessed with dual-energy X-ray absorptiometry scans, with osteopenia present in 1. With ongoing surveillance of individuals with GPI-related disorders and osteopenia, it will hopefully be determined if its presence leads to adverse health outcomes. Given the independent association of osteopenia and osteoporosis with epilepsy, antiepileptic use, and decreased mobility, one may anticipate association of osteopenia and osteoporosis with epilepsy, leading to adverse health outcomes. Given the independent and osteopenia, it will hopefully be determined if its presence leads to adverse health outcomes. At least 1 individual with PIGT-related GPI deficiency has had fractures due to decreased bone mineral density.29

Osteopenia and osteoporosis are hypothesized to be secondary to decreased alkaline phosphatase (ALP) expression on the surface of osteoblasts, impairing their function and leading to decreased bone formation and therefore bone mineral density. Hyperphosphatasia is a consequence of deficient ALP membrane attachment. ALP tends to be elevated when proteins in later steps of the GPI pathway are dysfunctional, as the GPI anchor cannot be appropriately attached to the protein. The cell is left with soluble ALP that is then released into the serum.3 The GPI-transamidase complex, which includes proteins encoded by GPAA1, PIGK, PIGS, PIGT, and PIGU, mediates the attachment of the GPI anchor to targeted proteins.20 None of the patients described in the literature with pathogenic variants in GPI-transamidase complex genes have elevated ALP.2,14,21,22 In fact, several patients with PIGT and PIGK pathogenic variants have decreased alkaline phosphatase.7,9 Our cohort of patients is consistent with this pattern, as none had elevated ALP or hyperphosphatasia, but 1 patient had a decreased ALP.

About half of patients with GPI-related disorders have abnormalities on neuroimaging including hypoplasia of the corpus callosum and white matter loss.14 Cerebellar hypoplasia and atrophy is noted in 10 different disorders (PIGA, PIGL, PIGN, PIGO, PIGG, PIGK, PIGS, PIGT, GPAA1, and PGAP1).2,6,9,14,23-28 In the original cohort of patients with GPAA1 deficiency, 9 of 10 patients had cerebellar anomalies; the 1 patient without cerebellar atrophy was only 1 year of age and perhaps too young to have developed the finding. Acquired cerebellar atrophy was noted in patient III of this cohort. Neuroimaging at 7 months showed a normal cerebellum; however, a repeat MRI at 2 years confirmed diffuse brain atrophy with the most striking progression in the cerebellum (see eFigure 1, links.lww.com/NXG/A479). Acquired, rather than congenital, cerebellar abnormalities may prove to be a common feature of the disorder, not yet fully appreciated as most children have MRI scans after onset of clinical manifestations caused by the presence of cerebellar atrophy. However, symptoms of nystagmus, dysarthria, and dysmetria were not congruent with the presence of cerebellar anomalies, suggesting that neuroimaging should be considered even in the absence of these signs and symptoms.

No patients had vascular anomalies including venous or capillary malformations. It was recently suggested that heterozygous loss-of-function variants in GPAA1 are associated with these anomalies.29 Data from patient cohorts published to date suggest GPAA1-related disorders are inherited in a recessive manner, and parents who carry loss-of-function variants in GPAA1 have not been found to have any health effects including vascular anomalies. As more patients are
identified with biallelic GPAA1 variants, family studies to assess the dermatologic and vascular phenotypes in heterozygotes may provide clarity regarding this relationship. Of interest, that same publication showed that GPAA1 knock-out models of zebrafish embryos develop numerous differences, including shortened length, back hyperextension, and smaller eyes, suggesting that GPAA1 plays a critical role in embryonic development. More studies will be needed to extrapolate the relevance of these findings to affected patients.

At present, treatments for GPI-related disorders are limited and primarily focused on seizure control. There is a case report of an individual with GPI deficiency due to variants in PIGM whose severe seizures were treated successfully with the histone deacetylase inhibitor sodium butyrate. However, this is likely not a feasible treatment for all GPI-related disorders, as the patient’s specific pathogenic variant was in the PIGM promoter and led to decreased acetylation and therefore decreased gene expression. Two brothers with early infantile epileptic encephalopathy due to PIGA pathogenic variants had resolution of their seizures after initiation of the ketogenic diet, but the mechanism for this has not been fully elucidated. Two patients in this cohort are currently or were previously managed on the ketogenic diet. In at least one of the patients, a partial but significant reduction in seizure frequency was observed. Previous case reports have discussed the efficacy of pyridoxine (B6) in seizure management in hyperphosphatasia with intellectual disability syndromes (caused by variants in PIGV, PIGY, PGAP2, PIGW, and PGAP3). ALP allows pyridoxal phosphate to cross the blood-brain barrier; thus, deficiency on cellular surfaces due to impaired GPI protein anchoring may lead to cerebral B6 deficiency, which can be addressed with B6 supplementation. Three of 7 patients in this report are using B6 as a part of their treatment regimen for seizure control. In 2 of these patients, control is suboptimal with ongoing breakthrough seizures; the third patient has well-controlled epilepsy. B6 is not felt to have contributed significantly to seizure control.

This cohort of patients supports and expands on the previously described phenotype, as it includes individuals who are either older or more mildly affected than those previously described. This will allow clinicians to begin to appreciate what long-term outcomes may look like for their affected patients. Functional assays such as flow cytometry aid in identifying true pathogenic variants causing decreased GPI-anchored protein expression vs variants of no functional significance, helping establish a library of causative variants. The quantitative nature of these assays in conjunction with access to clear clinical information may assist scientists in establishing a clear genotype-phenotype correlation. As is often the case in the era of next-generation sequencing, the full spectrum of GPAA1-related disorders will continue to evolve as additional patients are identified and characterized.

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**Appendix (continued)**

| Name                      | Location                                                                 | Contribution                                                                 |
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