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Severe Congenital Neutropenia Type 4: A Rare Disease Harboring a *G6PC3* Gene Pathogenic Variant Particular to the Mexican Population

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ABSTRACT

Background: Severe congenital neutropenia type 4 (SCN4) is a rare autosomal recessive granulopoiesis disorder caused by *G6PC3* gene pathogenic variants. The estimated prevalence is 1/10,000,000 people. Over 90% of patients present a syndromic form with variable multisystemic involvement, including congenital heart defects, increased visibility of superficial veins (IVSV), inflammatory bowel disease, and congenital urogenital defects as prominent symptoms. **Objectives:** The objective of the study was to study non-hematological phenotypic findings that suggest a clinical diagnosis of SCN4. **Methods:** We examined medical records of patients diagnosed with neutropenia from January 2000 to December 2020, selecting cases with non-hematologic manifestations for phenotypic description and *G6PC3* gene sequencing. **Results:** We found 11 cases with non-hematologic features: congenital heart defects in 8, IVSV in 6, inflammatory bowel disease in 4, urogenital defects in 4, and similar facial appearance. In addition, Sanger sequencing confirmed 3 homozygous cases for the c.210delC variant, a compound heterozygous harboring this variant, and a c.199_218+1 deletion. **Conclusions:** Our findings of the c.210delC variant in very close geographical settings, to date, have only been reported among Mexicans, and a mutual uncommon surname in two families strongly supports a founder effect for the variant in the studied population. Furthermore, the described non-hematologic symptoms in patients with severe primary neutropenia should be explored, confirming SCN4 by investigating *G6PC3* gene mutations. (REV INVEST CLIN. 2022;74(6):328-39)

Keywords: Neutropenia. Severe congenital neutropenia. Severe congenital neutropenia type 4. *G6PC3* mutations.

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INTRODUCTION

Neutropenia is a wide-range hematopoiesis disorder characterized by granulopoiesis arrest at the promyelocyte maturation stage with peripheral blood absolute neutrophil count below 1500/µL or below 500/µL in severe neutropenia, for a normal range of 2500-6000/µL. Severe congenital neutropenia (SCN) includes a heterogeneous group of disorders of hematopoiesis deficient mature neutrophils[1]. SCN type 4 (SCN4) is a rare disease characterized by the presence of SCN, associated with non-hematologic multisystemic traits, that was simultaneously described by Dursun et al. (2009) and Boztug et al. (2009), identifying both groups of authors the underlying genetic cause[2,3]. The characteristic non-hematological manifestations include increased visibility of superficial veins (IVSV), congenital heart defects, and urogenital anomalies. In addition, less frequent findings are thrombocytopenia, failure to thrive, inner ear hearing loss, and endocrine disorders such as growth hormone deficiency, delayed puberty, and cutis laxa[4,5]. As a group, SCN has an estimated prevalence of 6/1,000,000 individuals; in contrast, SCN4 has a prevalence of 1/10,000,000 people[6]. The gene G6PC3, located in 17q21, encodes the ubiquitously expressed glucose 6-phosphatase-β enzyme (G6Pase-β or G6PC3)[7,8].

G6PC3 catalyzes the final step of glycogenolysis, hydrolyzing the glucose-6-phosphate to glucose and inorganic phosphate in the endoplasmic reticulum (ER) (4). The biallelic *G6PC3* gene variants trigger energy homeostasis impairment, failure in 1,5-anhydroglucitol-6-phosphate elimination, ER stress, and elevated apoptosis rate, causing dysfunctional neutrophils and SCN4[9,10].

Although SCN comprises a heterogeneous group of rare genetic diseases, SCN4 occurs mainly as a syndromic entity, including severe neutropenia and variable multisystemic non-hematologic features. In this respect, only seven cases of nonsyndromic neutropenia related to G6PC3 deficiency have been reported, all younger than 18 years when diagnosed[4,11-13]. Hence, the rarity of the disease, around one in 10 million people, and the identification of 11 patients suggestive of SCN4 in an adult tertiary medical center, prompted us to define the phenotypic, molecular, and geographical distribution characteristics of this apparent cluster in the Mexican population.

METHODS

Patient population

We conducted an observational retrospective study by reviewing the electronic medical records of patients with neutropenia (ICD-10 code D.70) seen from January 2000 to December 2020 at the National Institute of Medical Sciences and Nutrition "Salvador Zubiran" (Mexico City, Mexico). Syndromic neutropenia cases included at least one associated non-hematological disorder, such as IVSV, congenital heart defects, urogenital anomalies, and inflammatory bowel disease. Cases of secondary neutropenia due to other hematological or non-hematological disorders were excluded from the study.

Informed consent for study participation, molecular analysis, manuscript, and photographs publication is available from all the patients. The Institutional Review Board approved the study on May 21, 2021, (GEN-3729-21-22-1).

Clinical-genetic inclusion procedure

All patients included in the study were selected through a two-steps process by an experienced geneticist and a resident in medical genetics: (1) scrutiny from medical records of all cases with a diagnosis of neutropenia; and (2) a genetic assessment interview with each patient, including clinical exam, laboratory results, sociodemographic characteristics, pedigree construction, pre-test genetic counseling, signed consent approval and, from the patient and family members that accepted, a whole blood sample for the molecular study. Patients with phenotypic characteristics of other genetic or unrelated disorders were excluded from the study.

Molecular analysis

The DNA samples from all patients underwent direct Sanger sequencing of the whole coding region of the *G6PC3* gene (RefSeq NM_138387.4). The primer sequences for *G6PC3* (exon 1-6) were taken from a previous report[2] and ordered from Integrated DNA Technologies (IDT, Coralville, Iowa, USA). We used HotStarTaq Master Mix Kit for fragment amplification under manufacturer standard conditions (QIAGEN, Hilden, Germany). The primer sequences and

annealing temperatures are exhibited in Table S1. The PCR products were sequenced using a BigDye Terminator v3.1 Cycle Sequencing kit on an ABI Prism 3500 Genetic Analyzer under the procedure indicated by the manufacturer (Applied Biosystems, ThermoFisher Scientific, CA, USA). Sequence analysis was performed using the Unipro UGENE (version 39.0)[14].

RESULTS

Patients

Eleven patients with neutropenia and non-hematologic traits were included in the study. The sex ratio female-to-male was 4:1. The current median age was 38.5 years (21-68 years); the median age at diagnosis was 32 years, although with a wide range (1 month-58 years). Regarding family backgrounds, patients 5 and 6 had first-degree relatives with recurrent infections, a sister and daughter, respectively, and patient 11 had two sisters with leukopenia.

Hematological findings

A varied hematological diagnosis was present in the four cases that were subsequently confirmed to have *G6PC3* gene pathogenic variants (PV), showing neutropenia, cyclic neutropenia, pancytopenia, and medullary aplasia (Table 1 and Fig. 1). Of the remaining seven cases, two presented severe neutropenia and two cyclic neutropenia. Other diagnoses were mild neutropenia, cytopenia, and medullary aplasia, one each.

Non-hematological findings

The most frequent non-hematological findings in the patients were cardiovascular disorders in 8/11 cases (77.7%), atrial septal defects (ASD) in four patients, and a persistent cardiac murmur in the other 4. Furthermore, seven patients (63.6%) showed peripheral vascular system-associated symptoms, of which 6 had IVSV, and one showed peripheral venous insufficiency. In addition, four patients had anomalies of the urogenital tract (36.4%). A similar number of cases (36.4%) presented inflammatory bowel disease, Crohn's disease in 2, and ulcerative colitis in one. Figure 2 shows the combined non-hematological manifestations of each of the 11 patients.

Molecular findings

Sanger sequencing of the *G6PC3* gene confirmed the clinical diagnosis of SCN4 in 4 of the 11 patients analyzed (Fig. 3). Patient one was a compound heterozygous for the c.210delC variant (rs769441127; NC_000017.11:g.44071174) and a 21bp deletion c.199_218+1del (rs1597905369; NC_000017.11:g.44071159-44071179del), predicted to impact the canonical splice site region between exon and intron 1. The remaining three cases were homozygous for the frame shift pathogenic c.210delC variant creating a premature stop codon, just 46 amino acids after the deletion site, giving place to a truncated protein of 115 amino acid residues (NP_612396.1: p.(Phe71Serfs*46)) that presumed to suffer the nonsense-mediated decay of the transcript[9]. Segregation analysis was available for patients 1, 2, and 4. The 21bp deletion of compound heterozygous (patient 1) was inherited from the mother and the 1bp deletion from the father. Both parents of patients 2 and 4 were carriers of the c.210delC variant. Cascade testing in the family of patient 2 confirmed homozygosity for the c.210delC variant in 2 cousins of the index patient. One is a 24-year-old female with recurrent aphtha, cardiac murmur, and inguinal hernia, and her brother, 16 y. o., with recurrent upper airway infections and bilateral cryptorchidism. Although in the family of patient three, parents and siblings rejected genotyping, the mother and the father should be both heterozygous for the c.210delC variant because patient three is homozygous for it.

Clinical description of positive cases

Table 1 compares the clinical findings observed in patients with a confirmed molecular diagnosis. Figure 1 shows the heterogeneous behavior of the magnitude of neutropenia over time in these four patients with the G6PC3 gene variants described.

Additional clinical findings observed in the cases with confirmed SCN4

Patient 1

Female, 25 y.o., born to an at-term uneventful pregnancy from healthy parents denying consanguinity. No other family member was affected (Fig. 4A). Soon

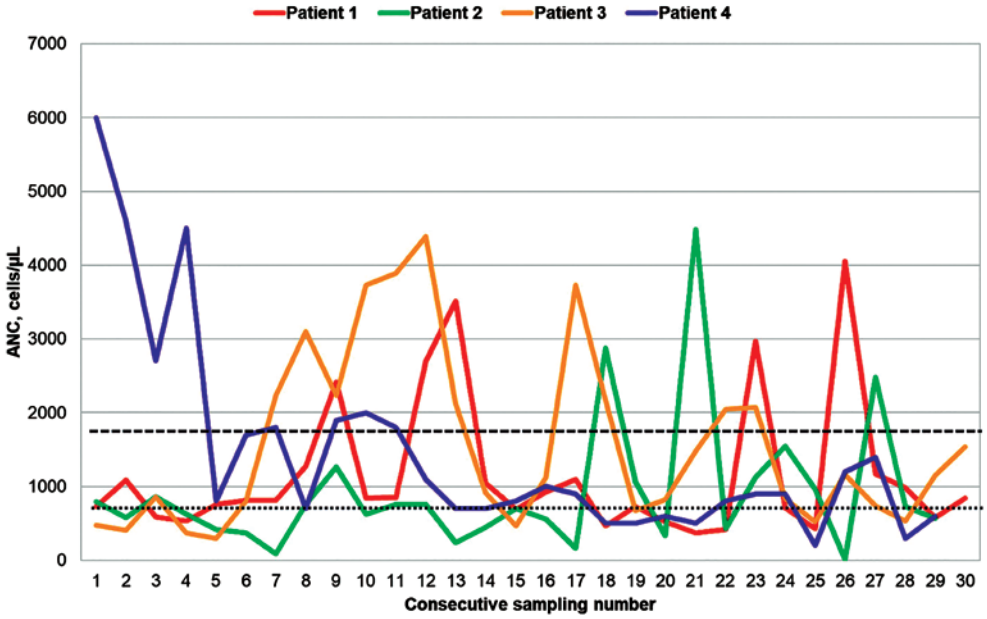
Table 1. Clinical description of patients with *G6PC3* gene pathogenic variants

Characteristics	P1	P2	P3	P4
Sex	Female	Male	Female	Male
Age	25	37	28	19
Consanguinity	Denied	Denied	Denied	Denied
ANC (cells/mm3)	375–4051	84–20,942	297–4384	300–11,500
Lymphocytes (cells/mm^3)	175–825*	171–780*	257–884*	300*–5,814
Platelets (×10^9/L)	245-573	17†-257	91†-448	45†-499
Height (cm)	1.34	1.62	1.57	1.57
Dysmorphic features	Triangular face, midface hypoplasia, depressed nasal bridge, thick lips, and prognathism	Round face, midface hypoplasia, depressed nasal bridge, thick lips, and prognathism	Round face, midface hypoplasia, depressed nasal bridge, thick lips, and prognathism	Round face, midface hypoplasia, thick lips prognathism
Congenital heart defects	ASD	ASD	ASD	ASD
Skeletal findings	Scoliosis	None	None	Pectus carinatum and scoliosis
Increased visibility of superficial veins pattern	Upper and lower limbs	Upper and lower limbs	Chest, upper and lower limbs	Absent
Endocrine findings	Delayed bone age, central thyroid dysfunction	Hypergonado-tropic hypogonadism	None	None
Bone marrow findings	Not available	Hypercellular marrow with myeloid hyperplasia	Increased megakaryocytes	Hypocellular marrow with megaloblastic changes in myeloid cell line
Other findings	Delayed psychomotor development, juvenile rheumatoid arthritis (JRA), and Crohn's disease	Crohn's disease 46, XY	Chronic diarrhea	Delayed psychomotor development 46, XY
Genotype	Compound heterozygous [c.210delC] + [c.199_218+1del]	Homozygous [c.210delC]	Homozygous [c.210delC]	Homozygous [c.210delC]

*Values in the range of lymphocytopenia; †Values in the range of thrombocytopenia; ASD: atrial septal defect.

after birth, a urinary infection required hospitalization for 4 days, solved without complications. The heart murmur, diagnosed at birth as an ASD and later developing to pulmonary arterial hypertension, was surgically repaired at age 14. She showed failure to thrive, delayed psychomotor development, recurrent infections during childhood with fever, cyclic neutropenia, and later, symptoms of juvenile rheumatoid arthritis. When first seen by us, she showed short stature of 134 cm (< 3rd centile), a head circumference of 49 cm (< 3rd centile), with an upper/lower segment ratio of 0.90 and arm span/height ratio of 0.94. She had a triangular face, retrognathia, mild frontal bossing, hypotelorism, low nasal bridge, short philtrum, downturned mouth corners, midface hypoplasia, IVSV of the upper and lower limbs, scoliosis, bilateral clinodactyly of the fifth finger, amenorrhea, central thyroid dysfunction, and growth hormone release defect. She was diagnosed with cyclic neutropenia and nonregenerative hypochromic microcytic anemia secondary to iron deficiency. A colon biopsy performed due to chronic diarrhea showed a focal

Figure 1. Absolute Neutrophilic Count (ANC) in G6PC3 deficient patients. Long dashes indicate the threshold of 1500 cells/uL, below which ANC corresponds to neutropenia; short dashes indicate the threshold of 500 cells/uL, corresponding to severe neutropenia. Spikes are coincident with remission episodes.



zone of fibrosis, active inflammation in the terminal ileum, collapsed loops, and mesenteric adenomegaly diagnosed as Crohn's disease.

Patient 2

Male, 37 y.o., born to an at-term uneventful pregnancy of probably consanguineous healthy parents from a rural community (933 inhabitants); and a family history of a female second cousin who died at age 18 of severe neutropenia and Crohn's disease (Fig. 4B). At birth, he presented neutropenia, later diagnosed as cyclic neutropenia. He has bilateral cryptorchidism, surgically repaired at age 8. He has short stature (162 cm, $p < 5$), dysmorphic face features (Table 1), an IVSV pattern of the upper and lower limbs, acute kidney pain, kidney stones, and hypercalciuria. He presented intense-generalized abdominal pain and episodic diarrhea in late adolescence. A colon biopsy at age 19 showed acute severe ulcerative colitis and rectum with chronic proctitis diagnosed as Crohn's disease, treated surgically, and currently having an ileostomy. Incidentally, the patient was diagnosed with ASD at the same age, which was repaired. Sex hormone analysis confirmed hypogonadotropic hypogonadism, with a 46, XY normal karyotype. He is currently on and off granulocyte colony-stimulating factor treatment.

Patient 3

Female, 28 y.o., born to an at-term uneventful pregnancy of unknown consanguinity of healthy parents (Fig. 4C). She presented cytopenia, recurrent otitis media, and upper airway infections during childhood. At age 19, she started with episodic diarrhea and intense-generalized abdominal pain, mild neutropenia, thrombocytopenia, and lymphopenia. On genetic evaluation, she presented dysmorphic features (Table 1), with upper and lower limbs showing IVSV. Echocardiography at 28 years of age confirmed an ASD and mitral and aortic insufficiency.

Patient 4

Male 19 y.o., born to an at-term uneventful pregnancy and ignored consanguinity of healthy parents (Fig. 4D). He had recurrent upper-airway infections, hypotonia, and gastroesophageal reflux during the neonatal period. Recurrent upper airway infections and idiopathic neutropenia continue as current symptoms. At age 16, he underwent surgical repair of a previously diagnosed ASD. Imaging studies confirmed thoracic 2-4 vertebral fusion and dextroscoliosis. He also presented dysmorphic features and *Pectus carinatum* (Table 1).

Figure 2. Non-hematological manifestations observed in patients with syndromic neutropenia. †Height below the third percentile.

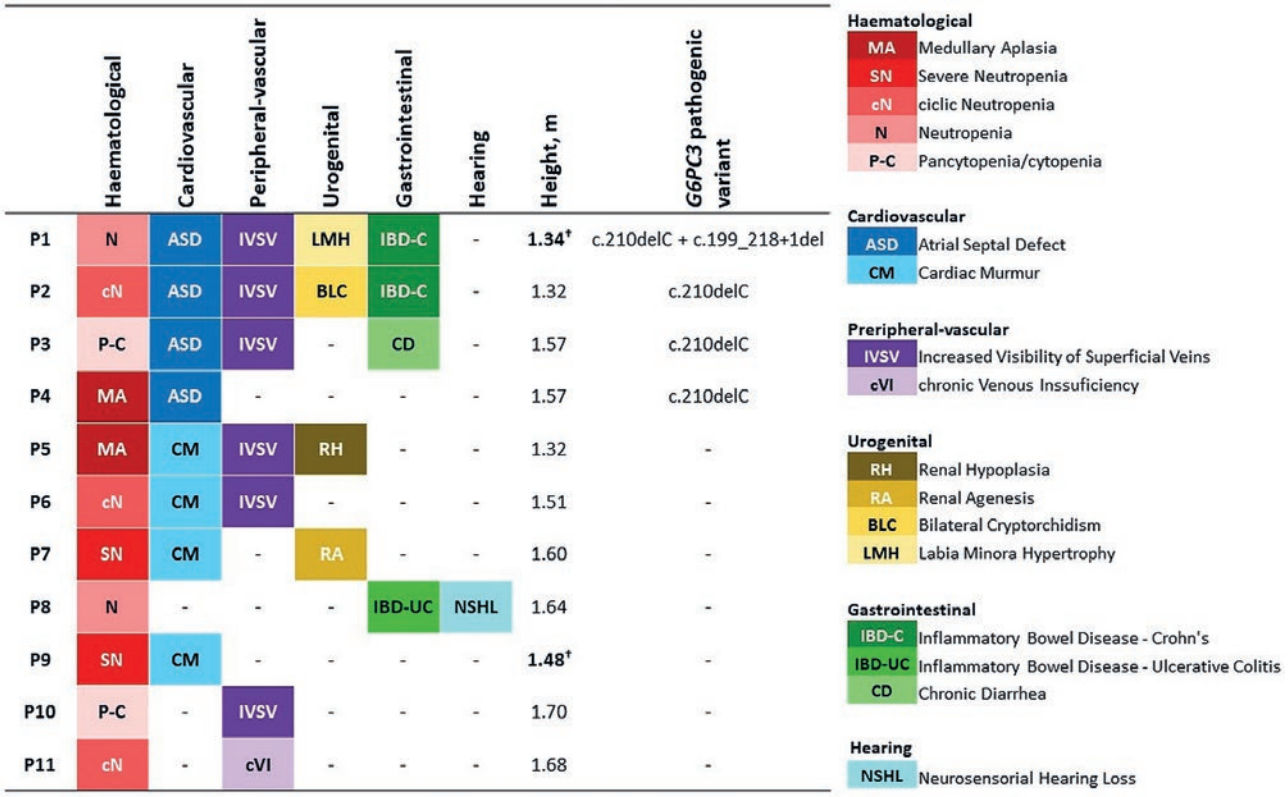


Figure 3. Electropherograms of the *G6PC3* reference sequence and the two identified pathogenic variants. Homozygous c.210delC in patients 2, 3, and 4, and compound heterozygous c.210delC c.199_218+1del variants in patient 1. The first variant was inherited from the father and the second from the mother.

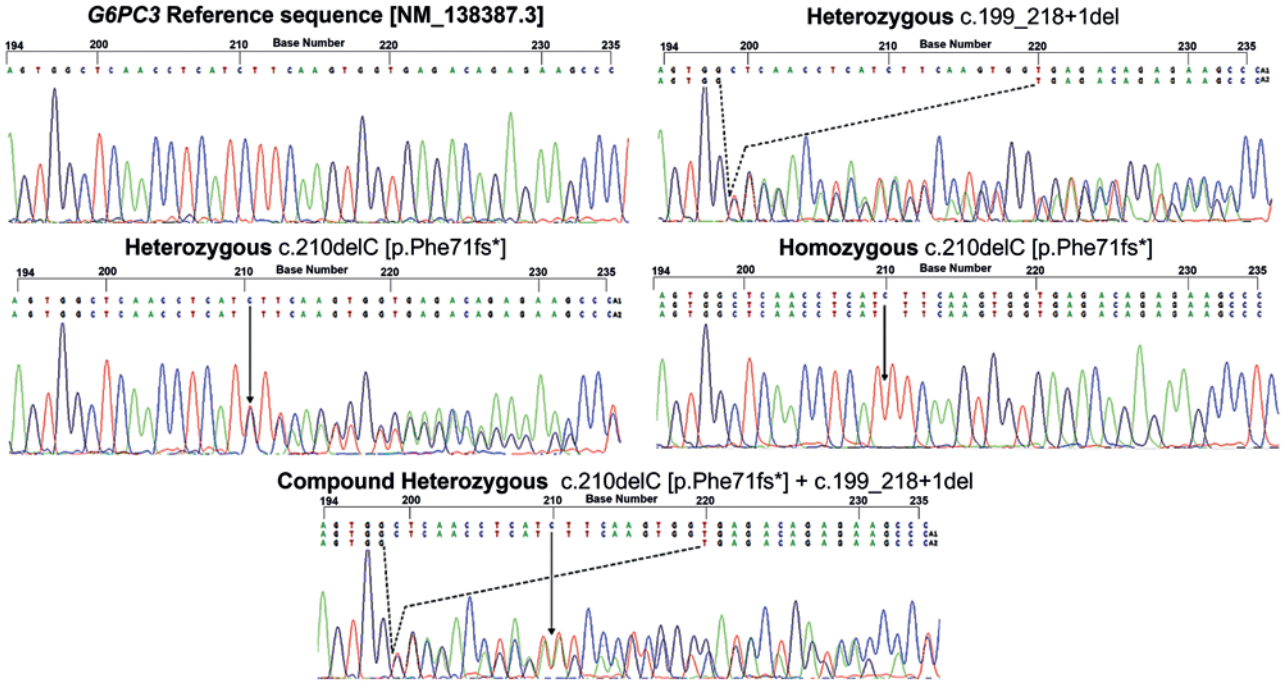
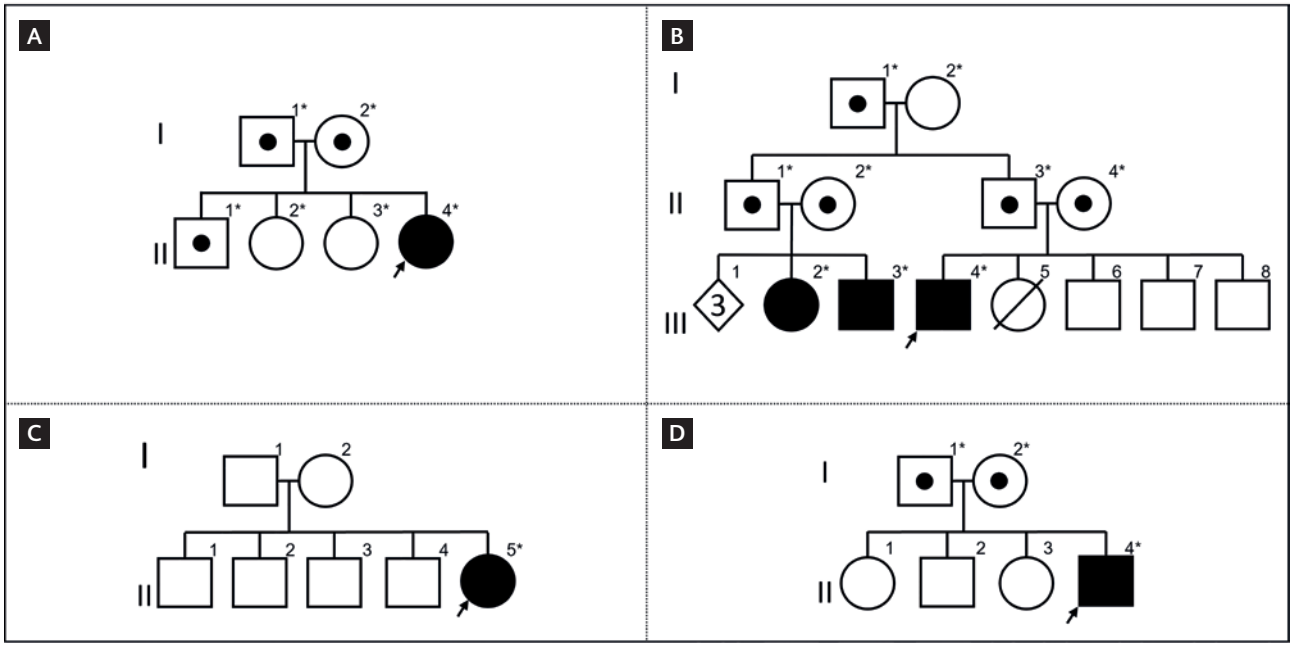


Figure 4. Sequencing analysis results and genealogies of *G6PC3* mutation positive. Family pedigrees illustrate the four cases in which a pathogenic variant (PV) in the *G6PC3* gene was present. In addition, segregation analysis and cascade testing were done to determine the parental origin of variants and carriers in three families. Circles represent females; squares indicate males; arrows refer to probands; fully shaded figures represent SCN4 patients, symbols with a black dot inside represent PV carriers. *Family members who underwent genetic testing. Arabic numerals-individual identifiers; Roman numerals-generations.



Interestingly, even though patients denied or ignored kinship and consanguinity, the information on geographic family origin from the father of patient 1, both parents of patient 2, and the father of patient 3 confirmed that they are all from neighboring towns of the same state (patient 1, Ixtlahuaca; patient 2, Jiquipilco; patient 3, Toluca; patient 4, Chalco). Furthermore, the distances between locations show geographical proximity: Ixtlahuaca-Jiquipilco 14 km, Ixtlahuaca-Toluca 34 km, Jiquipilco-Toluca 31 km, and Chalco-Toluca-Ixtlahuaca-Jiquipilco, 77–97 km. This strongly suggests a founder effect for the *G6PC3* gene c.210delC variant. Moreover, sharing an uncommon surname by two of the families further supports the possibility of a common ancestor, probably for all families.

DISCUSSION

We conducted a phenotypic, biochemical, and genetic analysis in 11 patients with severe cyclic congenital neutropenia. Sanger sequencing identified two previously rare reported *G6PC3* gene PVs in 4 of the 11 patients with non-hematologic traits (36.4%). Patient 1 had a rare splice donor site variant (c.199_218+1del), which has only once been submitted to the *ClinVar* database (ID: 691994), in a patient with pulmonary arterial hypertension, leukopenia, and an ASD, probably having SCN4, although not specified in the report[15]. The c.210delC variant has been well characterized as pathogenic and has only been described in patients of Hispanic descent[5,16]. Interestingly, 31 heterozygous carriers for the c.210delC variant are reported in the *gnomeAD* database, all in Latino/Admixed Americans[17]; in addition, 395 alleles were identified in 92,041 individuals (184,082 alleles) of Native Mexican ancestry, with a prevalence of 0.00215 in the Mexico City Prospective Study *(MCPS)* database[18].

Furthermore, in the same database, this variant has the highest frequency of all PV of the *G6PC3* gene, with a prevalence of 0.00143 in 138,200 DNA-sequenced samples. Interestingly, the c.210delC variant was only found in individuals confirmed to be of Native Mexican ancestry but not in Mexicans with an admixture of African, Asian or European ancestry[18]. Although our patients denied inter-family relationships, the family residences of patients 1, 2, and 3 are

within a 35 km distance, which makes it possible that these families share a common ancestor, also supported by the fact that two of the families share an uncommon surname. In another study of Mexican patients, Velez-Tirado et al. reported 5 patients carrying this variant; 3 of them were homozygotes, and although they denied consanguinity, the authors mentioned that they might come from geographically close communities[16]. Our findings and the above reports strongly support that the c.210delC variant is particular to the Mexican population and sheds light on a possible founder effect[5,16-18].

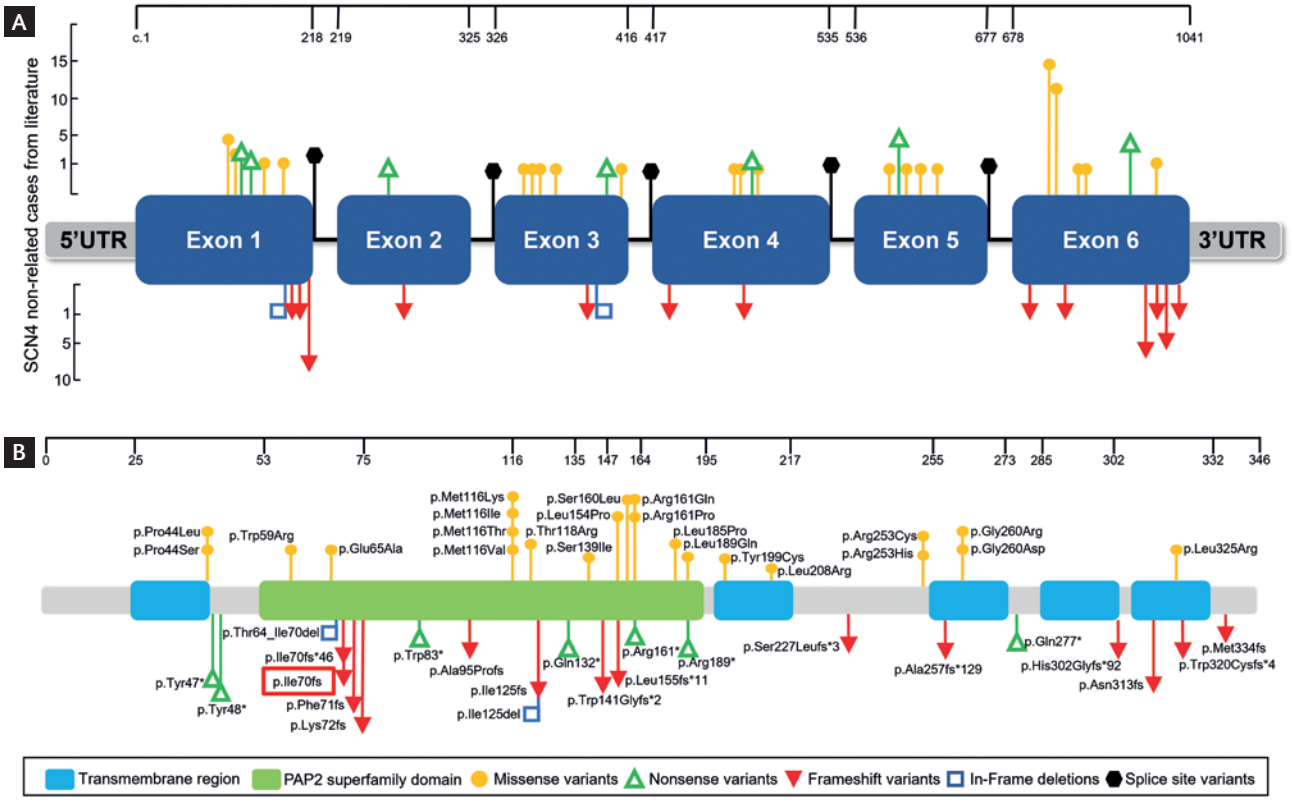
We describe four patients with sequencing diagnoses of *G6PC3* gene PV, 3 of which are homozygous for the c.210delC nonsense variant. Although the disease is known as SCN4, the blood analysis findings in these patients show that the hematological phenotype is much broader. Furthermore, our patients (Table 1) fulfilled the criteria for SCN and cyclic neutropenia, as all showed variable degrees of severe neutropenia associated with periods of remission and affected platelet and lymphocytes cell lines. Regarding non-hematological abnormalities, congenital heart defects were the most frequent, as others have noticed[16,19]. We found that in all positive cases presented with ASD, these defects usually occurred as soft murmurs or were asymptomatic for a long time, making them a common undiagnosed abnormality[20]. In our group of patients, IVSV was a relatively common finding. Although patient four did not show this anomaly, veins may probably become more prominent with age[5], being this finding present in two-thirds of patients[16].

Regarding patients without *G6PC3* PV, despite the unspecific diagnosis of a heart murmur, none of these patients had an ASD, as observed in the four patients with a *G6PC3* PV, which has been frequently associated with mutations in this gene[16]. Although 3/7 had IVSV, in our sample of cases, it seems not to be a defining phenotypic marker of the syndromic SCN4. In addition, except for one patient with ulcerative colitis, no other patients showed gastrointestinal symptomatology. The common aspect in both groups was the heterogeneous character of hematologic manifestations. It is of note that SCNs are a group of heterogeneous diseases with different inheritance and multigene etiology[1]. Therefore, the syndromic form is probably not always exclusively due to mutations in the *G6PC3* gene.

G6PC3 deficiency due to pathogenic and probably PV of the *G6PC3* gene is considered within the group of rare diseases. The 127 reported cases of SCN4 worldwide present 53 different *G6PC3* PV. Of these, 22 are missense (41.5%), 22 nonsense or frame shift (41.5%), 6 splice site variants (11.3%), and 3 in-frame deletions (5.7%); gene location and detailed characteristics of each one is shown in figure 5 and table 2. Most described cases are in the pediatric age group, and in most, a diagnosis has been reached via WES, meaning that clinical diagnosis is rarely suspected; therefore, it is possible that SCN4 may be underdiagnosed. The 4 patients reported herein reached adulthood without a diagnosis, until now. An early confirmed diagnosis can diminish complications such as Crohn's disease, believed to be caused by intrinsic defects in hematopoietic cells, with inadequate control of gut microbiota, secondary to reduced survival and function of neutrophils, leading to a dysregulated inflammatory response; this association is not specific to G6PC3 deficiency[21]. There are more than 240 risk genes for IBD, including Crohn's disease; however, the pathogenic mechanism of contribution is not well understood. IBD is an immune-mediated disorder, and several identified genes participate in regulating and maintaining the local immune response to viruses and bacteria. The particular genetic background may predispose to altered immunoglobulin production, enhancement of specific cytokine expression, and subsequent tissue inflammation[22]. Crohn's disease or another IBD may not be influenced directly by *G6PC3* mutations but is aggravated by neutropenia. In addition, well-timed allogeneic hematopoietic stem cell transplantation has been proven beneficial in the remission of inflammatory bowel disease symptomatology[23].

It is known that SCN is a heterogeneous disease with multigene involvement and diverse inheritance patterns, as well as variable phenotypic manifestations, being *ELANE* (neutrophil elastase gene) PV responsible in most cases with the disease. However, such a form of SCN occurs without specific non-hematological manifestations[1]. Moreover, only two Mexican patients were reported with PV in *ELANE* and were sporadic[24]. Meanwhile, five identified SCN patients were found to have genetic alterations on *G6PC3*[16]. Therefore, the characterization of non-hematologic SCN-associated multisystemic external and internal

Figure 5. *G6PC3* reported pathogenic variants (PV) in patients with SCN4. **(A)** the absolute frequency of unrelated patients (one per family) of known PV in confirmed SCN4 cases reported in the literature consulted in *Scholar Google*. **(B)** illustration of known *G6PC3* PV presented on the protein with structural domains indicated. The red rectangle encloses a pathogenic variant found only in the Mexican population. All variant details and references can be found in Table II and Appendix S1, respectively.



abnormalities in the patients in this study shed light on defining the SCN4 syndromic form of the disease.

The findings in the four patients, mainly those present in the three patients homozygous for the c.210delC variant, do not allow to propose a definitive genotype-phenotype correlation, even though patients share the same congenital heart defect and other phenotypic features, such as IVSV, gastrointestinal, and characteristic facial appearance. Regarding the rest of the systems involved, findings were heterogeneous, which may represent, to some extent, a variable expression of this pathogenic variant, although other yet unknown modifying factors may affect the occurrence of non-hematological manifestations.

Finally, the present uniqueness of the c.210delC variant in Hispanics of Mexican descent, the regional proximity of the families' residence, and the sharing of an uncommon surname between the members of two of the families, strongly support the possibility of ignored consanguinity, an ancient common ancestor, and a founder effect for this *G6PC3* gene variant.

Our study showed that searching for non-hematological features in patients with severe neutropenia could identify cases with *G6PC3* PV. Therefore, it is highly recommended that patients with primary neutropenia be examined mainly for congenital heart defects, peripheral vascular system anomalies, inflammatory bowel disease, urogenital defects, and facial appearance, to guide genetic testing for decision-making. Furthermore, our findings suggest this disorder may have a higher prevalence in Mexicans than has been described, most probably due to a founder effect of the c.210delC pathogenic variant, particular to the Mexican population.

Table 2. Compilation of G6PC3 mutations reported in cases with SCN4

Exon	NM_138387.4	NP_612396.1	Short	MC	GRCh38 chr17	dbSNP	ClinVar	Interpr.	MT	CADD	Ref.
1	c.[130C>T]	p.[Pro44Ser]	P44S	MS	44071095	rs775224457	189781	P	DC	28	1-5
1	c.[131C>T]	p.[Pro44Leu]	P44L	MS	44071096	rs762019955	na	na	DC	29	6-8
1	c.[141C>G]	p.[Tyr47Ter]	Y47*	N	44071106	rs118203970	1039	P	DC	37	6,8,9
1	c.[144C>A]	p.[Tyr48Ter]	Y48*	N	44071109	rs1194477276	632283	likely-P	DC	34	10,11
1	c.[175T>C]	p.[Trp59Arg]	W59R	MS	44071140	rs752966267	853860	US	DC	32	8,12
1	c.[190_210]delACCGAGTG-GCTCAACCTCATC	p.[Thr64_Ile70del]	T64_I70del	IFD	44071155-44071175	na	na	na	DC	na	2,3
1	c.[194A>C]	p.[Glu65Ala]	E65A	MS	44071159	rs745318917	na	na	DC	31	7,13
1	c.[207delC]	p.[Ile70fsTer46]	I70fs*	FS	44071172	na	na	na	DC	na	14
1	c.[207dup]	p.[Ile70fsTer17]	I70fs*	FS	44071171-44071172	rs1191239079	691992	P	DC	33	6
1	c.[210delC]	p.[Phe71fsTer46]	F71fs	FS	44071175	rs769441127	189782	P	DC	35	6,15 This publication
1	c.[214delA]	p.[Lys72fsTer45]	K72fs*	FS	44071179	rs1177939839	na	na	DC	na	16
1	c.[218+1G>A]	p.?	p.?	SDL	44071184	na	1430600	P	DC	35	6,17
1	c.199_218+1delCTCAACCTC ATCTTCAAGTGG	p.?	p.?	SD	44071159-44071179	rs1597905369	691994	P	DC	na	This publication
2	c.[249G>A]	p.Trp83Ter	W83*	N	44074190	na	na	na	DC	39	18
2	c.[257delA]	p.[Glu86Glyfs*31]	E86Gfs*	FS	44074198	na	na	na	DC	na	19
2	c.[282delA]	p.[Ala95fsTer22]	A95fs*	FS	44074223	na	na	na	DC	na	5
2	c.[295C>T]	p.[Gln199Ter]	Q99*	N	44074236	na	na	na	DC	38	20
3	c.[326-1G>C]	p.?	p.?	SAL	44074679	na	na	na	DC	33	7
3	c.[346A>G]	p.[Met116Val]	M116V	MS	44074700	rs267606834	1042	P	DC	25	21
3	c.[347T>C]	p.[Met116Thr]	M116T	MS	44074701	na	na	na	DC	26	1,5
3	c.[347T>A]	p.[Met116Lys]	M116K	MS	44074701	na	na	na	DC	28	22
3	c.[348G>A]	p.[Met116Ile]	M116I	MS	44074702	rs1373865222	na	na	DC	30	6
3	c.[353C>G]	p.[Thr118Arg]	T118R	MS	44074707	rs766706036	na	na	DC	25	14
3	c.[372delC]	p.[Ile125*]	I125*	N	44074726	na	na	na	DC	na	23
3	c.[373_375delATA]	p.[Ile125del]	I125del	IFD	44074727-44074729	na	na	na	DC	na	11
3	c.[394C>T]	p.[Gln132Ter]	Q132*	N	44074748	na	na	na	DC	36	7,24
3	c.[416G>T]	p.[Ser139Ile]	S139I	MS	44074770	na	na	na	DC	39	6,25
4	c.[417-1G>A]	p.?	p.?	SAL	44074968	rs763408993	na	na	DC	34	26
4	c.[421delT]	p.[Trp141fsTer2]	W141fs*	FS	44074973	na	na	na	DC	na	15

(Continues)

Table 2. Compilation of G6PC3 mutations reported in cases with SCN4 *(continued)*

Exon	NM_138387.4	NP_612396.1	Short	MC	GRCh38 chr17	dbSNP	ClinVar	Interpr.	MT	CADD	Ref.
4	c.[461T>C]	p.[Leu154Pro]	L154P	MS	44075013	na	na	na	DC	28	27
4	c.[481C>T]	p.[Arg161Ter]	R161*	N	44075033	rs1056739194	na	na	DC	35	15,18
4	c.[482G>A]	p.[Arg161Gln]	R161Q	MS	44075034	rs1485073209	na	na	DC	27	6
4	c.[482G>C]	p.[Arg161Pro]	R161P	MS	44075034	na	1679807	US	DC	26	28
4	c.[535+1G>A]	p.?	p.?	SS	44075088	na	na	na	DC	34	7,24
5	c.[554T>C]	p.[Leu185Pro]	L185P	MS	44075328	rs118203969	1038	P	DC	24	15
5	c.[565C>T]	p.[Arg189Ter]	R189*	N	44075339	rs745582203	653016	P	DC	36	6,8,18
5	c.[566G>A]	p.[Arg189Gln]	R189Q	MS	44075340	rs140294222	262367	US	DC	18	22
5	c.[596A>G]	p.[Tyr199Cys]	Y199C	MS	44075370	rs1597910284	na	na	DC	27	20
5	c.[623T>G]	p.[Leu208Arg]	L208R	MS	44075397	na	na	na		26	3
5	c.[677+1G>A]	p.?	p.?	SS	44075452	rs778208850	1066709	likely-P	DC	34	10
6	c.[1000_1001delAT]	p.[Met334fs]	M334fs	FS	44076002-44076003	na	na	na	DC	na	1
6	c.[680_684delinsT]	p.[Ser227Leufs*3]	S227Lfs*	FS	44075682-44075685	na	na	na	DC	na	11
6	c.[757C>T]	p.[Arg253Cys]	R253C	MS	44075759	rs765927570	960968	likely-P/US	DC	31	29
6	c.[758G>A]	p.[Arg253His]	R253H	MS	44075760	rs118203968	1037	P	DC	31	10,18,20,22,30
6	c.[935dupT]	p.[Asn313fs]	N313fs+39aa	FS	44075933-44075934	rs797044567	189784	P	DC	na	6,7,10,26,27
+38 AA	FS										
	44075767	rs748931188	1342134	P	DC	33	7,33-36				
6	c.[766_768delGGG]	p.[Gly256del]	G256del	IFD	44075768-44075770	na	na	na	DC	na	20
6	c.[778G>C]	p.[Gly260Arg]	G260R	MS	44075780	rs200478425	30874	P/US	DC	28	6,10,14,18,22,26,33,34
6	c.[779G>A]	p.[Gly260Asp]	G260D	MS	44075781	na	na	na	DC	27	6
6	c.[829C>T]	p.[Gln277Ter]	Q277*	N	44075831	rs148559256	189783	P	DC	33	6,10,35,36
6	c.[882_903dup]	p.[His302GlyfsTer92]	H302Gfs*	FS	44075884-44075905	na	na	na	DC	na	5
6	c.[765_766delAG]	p.[Ala257CysfsTer129]	A257Cfs*+38aa	FS	44075767	rs748931188	1342134	P	DC	33	7,33-36
+39 aa	FS	44075933-44075934	rs797044567	189784	P	DC	na	6,7,10,26,27			
6	c.[960delG]	p.[Trp320CysfsTer4]	W320Cfs*	FS	44075961	na	na	na	DC	na	32
6	c.[974T>G]	p.[Leu325Arg]	L325R	MS	44075976	na	na	na	DC	25	37

MC: molecular consequence; MT: *mutation Tester* prediction; MS: missense; N: nonsense; FS: frameshift; IFD-in-frame deletion; SDL: splicing donor lost; SD: splicing donor; SAL: splicing acceptor lost; SS: splicing site; P: pathogenic; na: not available; US: uncertain significance; DC: disease causing; CADD: *Combined Annotation Dependent Depletion* score. †References mentioned in this table are placed in the Suplementary Data.

SUPPLEMENTARY DATA

Supplementary data are available at *Revista de Investigación* Clínica online (10.24875/RIC.22000234). These data are provided by the corresponding author and published online for the benefit of the reader. The contents of supplementary data are the sole responsibility of the authors.

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