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PATHOGENIC COMPOUND HETEROZYGOUS MUTATIONS IN A MEXICAN MESTIZO PATIENT WITH NIEMANN-PICK DISEASE TYPE B

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Summary: *Pathogenic compound heterozygous mutations in a Mexican mestizo patient with Niemann-Pick disease type B:* Niemann-Pick disease (NPD) type B is a lysosomal storage disorder caused by a deficiency of acid sphingomyelinase (ASM). We report the clinical follow-up of a 19-year-old Mexican mestizo woman with a NPD type B phenotype who presented hepatosplenomegaly, persistently low high-density lipoprotein (HDL) cholesterol and thrombocytopenia, without central nervous system involvement. After of a dengue fever episode with severe anemia and pancytopenia, leading to a bone marrow study in which foamy histiocytes were noticed and diagnosis of Niemann Pick disease was suspected; and confirmed by biochemical and molecular tests. The missense c.1343A>G (p. Tyr448Cys, formerly Y446C) and c.1426C>T (p.Arg476Trp, formerly R474W) mutations in the *SMPD1* gene were identified. These mutations have never been reported in the Mexican population. Since the c.1343A>G (Y446C) mutation has been previously reported in a Japanese patient with NPD type A, we suggest an attenuator effect of c.1426C>T (R474W) allele (previously associated with the NPD type B phenotype). In conclusion, this is the first description of the concomitant occurrence of Y446C and R476W mutations in a Mexican patient with NPD type B, showing the importance of increased awareness and availability of specialized diagnostic tests in the diagnosis of rare inherited metabolic diseases.

Key-words: Compound heterozygous – Gene – Lysosomal storage disorder – Mexico – Niemann-Pick type B.

INTRODUCTION

Niemann-Pick disease (NPD) comprises a group of autosomal recessive inherited lipid metabolic disorders, yielding to the accumulation of sphingomyelin and cholesterol involving a wide variety of tissues and organs leading to their dysfunction, which in turns explains most of the clinical features and course (2). The different forms of NPD are classified on the basis of their clinical presentation as well as their biochemical and molecular findings. Type A (Online Mendelian Inheritance in Man, OMIM, accession number 257200) and type B (OMIM 607616) NPD are caused by mutations leading to a deficient activity of the lysosomal acid sphingomyelinase (ASM), which is required to catalyze the breakdown of sphingomyelin into ceramide and phosphorylcholine (2, 7). NPD type B, like Gaucher type, may present at any

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age (one-third of patients in adulthood - late adulthood) and compared with type A, is less severe and implies a protracted disease course, without central nervous system (CNS) involvement, commonly presenting hepatosplenomegaly, thrombocytopenia and bleeding diathesis, respiratory complications, altered liver function tests, bone involvement, bruising, headache, abdominal pain, diarrhea and abnormal plasma lipids profile (7). Also, an intermediate phenotype exists, which encompassing a cluster of clinical variants combining features of both type A and type B NPD (7); while NPD type C is a different genetically and clinically entity from acid sphingomyelinase deficiencies, which exhibit signs and symptoms very similar and usually become apparent in childhood, although signs and symptoms can develop at any time (10). Herein we describe a 19-year-old woman with the clinical phenotype of NPD type B, in whom the low ASM activity was demonstrated to be the consequence of mutations previously associated with both types A and B of the disease. To the best of our knowledge, this form of compound heterozygosity has never been reported.

CASE REPORT

Our patient was born to healthy non-consanguineous Mexican mestizo parents on September 30, 1995, in the Sinaloa state, located in the northwest of Mexico. The patient received pediatric hospital care at the age of 3 years for epistaxis. At that time during the physical exam an abdominal mass was detected and a significant hepatosplenomegaly was confirmed by ultrasonography. Two years later, with the evaluation of a respiratory tract infection, at age 5 years, a chest X-ray showed bilateral interstitial infiltrates of miliary appearance. The patient was positive to the coccidiodin skin test, and a liver biopsy yielded a histologic preparation that showed cells with vacuolated foamy cytoplasm, further suggesting a lysosomal storage disorder.

At age 11 years, the laboratory test results with clinical significance were: elevated liver enzymes activity, prolonged times on clotting tests; hemoglobin concentration 9.9 g/dl (reference range: 12-14 g/dl) and blood lymphocytes count 900 (reference range: 1.0-4.2 per $10^3/\mu l$). A computed tomography scan confirmed the persistent hepatomegaly and an enlarged spleen with heterogeneous density.

At age 17 years, when she was on her 16th week of pregnancy, her laboratory tests results showed a normochromic anemia with hemoglobin concentration of 10.3 g/dl, thrombocytopenia (90,000 platelets/mm3), hypertriglyceridemia and low high-density lipoprotein (HDL)

of 22 mg/dL (normal range: 40-60 mg/dL). Nonetheless, the blood total leucocytes, neutrophils and lymphocytes count, as well as the liver function assays, tested normal. A daily supplement of folic acid and ferrous fumarate were instituted. Fortunately, a term baby girl was born by cesarean section without medical complications.

On October 2014, at age 19 years, the patient was admitted to the Hospital General de Culiacán with a 2-days history of fever, myalgia, arthralgia and thrombocytopenia (73,000 platelets/mm^3). Dengue fever infection was confirmed by means of a positive NS1 antigen test. On the fourth day of hospital stay the patient presented gingivorrhagia (bleeding gums), moderate vaginal bleeding, leukopenia (0.37 per 10^3/µl), neutropenia and anemia (hemoglobin 10 g/dL, hematocrit 30.5%, erythrocyte count 3.49 per 10^6/uL). The plasma lipid profile showed a low concentration of both HDL (9 mg/dL) and total cholesterol (127.33 mg/dL: as mean value of three estimates along ten days), as well as hypertriglyceridemia. The low platelet count worsened (18,000 platelets/mm^3), which motivated the transfusion with 10 platelet concentrates and a platelet aphaeresis. The pancytopenia was the reason for a bone marrow biopsy in which foam cells were demonstrated which prompted NDP diagnosis. A brain MRI was performed without important findings and symptoms that could indicate neuromuscular disease were not mentioned during the interview.

On March 2015 a tandem mass spectrometry analysis showed significantly low ASM activity in dried blood spots (0.05µmol/L/h; reference value: >2.17µmol/L/h). Gaucher disease was also discarded and used as an internal control (β-glucocerebrosidase activity of 6.67 µmol/L/h; reference range: >3.61µmol/L/h). The molecular analysis by means or Sanger direct gene sequencing revealed a compound heterozygous genotype (Fig. 1). The pathogenic missense alleles c.1343A>G (p.Tyr448Cys, formerly Y446C) and c.1426C>T (p.Arg476Trp, formerly R474W) in the *SMPD1* gene were found and deposited in the ClinVar with accession number SCV00257365.

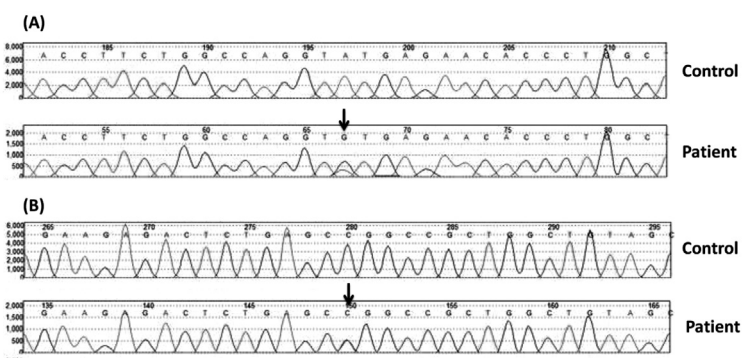

Figure 1:
Sequence chromatogram showing the normal sequence (control) and mutated (patient with Niemann Pick type B) of the SMPD1 gene. c.1343A>G, p.Tyr448Cys (A) and c.1426C>T, p.Arg476Trp (B) mutations. Sites of mutations are denoted by black arrows.

DISCUSSION

The clinical hallmarks of NPD type B tend to worsen over time, which include among other features childhood- or adolescence-onset hepatosplenomegaly, pulmonary disease and dyslipidemia, thrombocytopenia and leucopenia, without CNS involvement.

Here we discuss on a case of compound heterozygosity with missense mutations associated with both NPD type A (c.1343A>G) and type B (c.1426C>T), explaining an NPD type B phenotype and an ASM activity <1% the lower normal reference value.

The phenotypic variability observed for NPD type B may be due to wide spectrum of mutations, and their nature, in the *SMPD1* gene, which can possess higher frequencies in certain populations due to local founder effects. For example, p.deltaR608 mutation is considered "neuroprotective" due to it only occurs in non-neurological ASM-deficient NPD patients, and has been found in patients from Western Europe, North America and North Africa, mainly (11), whereas the H421Y and K576N mutations, specific to Saudi Arabia, are associated with early-onset and more severe phenotype; and R474W mutation known for its link with less severe phenotype, as our patient, also for its higher prevalence on Portuguese/Brazilian patients (8). More recently, Acuña *et al.* described the clinical expression on 13 Chilean patients, homozygous for the p.(Ala359Asp) variant, and all of them had moderate to severe NPD type B disease and further they suggest an Amerindian origin for this variant (1).

Unfortunately, for Y446C (c.1343A>G) mutation don't exist reports about its clinical consequences and distribution, except a patient described by Takahashi, *et al.* in a Japanese infant with hepatosplenomegaly, failure to thrive and neurologic deterioration, diagnosed as NPD type A (9). Nevertheless, this mutation has been found by the Dr. Schuchman´s group in several Mexican mestizo patients (personal communication).

To our knowledge, no previous reports in scientific literature exist about the combination of mutations identified in our patient, who fulfills a phenotype pattern of NPD type B. For Mexican population only three patients with NPD type B have been reported in the literature so far, without molecular characterization (13). The knowledge of reported mutations would help to establish the variable genotype-phenotype correlations, geographic distribution, as well as the genetic and phenotypic heterogeneity in Mexican patients.

We propose that in our case a certain "phenotype attenuator" role could exist for the c.1426C>T (p.Arg476Trp) mutation, at least when found

together with Y446C, a NPD type A pathogenic mutation. An opposed phenomenon to R474W mutation of "attenuated phenotype" can occur for the G→T transversion of coding nt 1487, known also as R496L, a mutation among NPD patients of Ashkenazi Jewish ancestry, displaying a type B disease in heterozygosis, and type A in homozygous patients (4).

An additional rarity in our patient was the comorbidity with Dengue fever, which could cause a more profound decline of the blood platelet count, further compromising life. Nonetheless, our patient survived. The concomitant development of these conditions has not been reported in the literature so far.

Monitoring of liver function is critical for the evaluation and management of patients with NPD. For that, liver enzymes activity can be useful laboratory evaluation of disease severity in NPD type B. Abdominal imaging by means of ultrasound, computed tomography and MRI scans are also important resources for the progression of disease with respect to structural visceral implications. Furthermore, low ASM activity increases the risk of atherothrombotic disease in adult patients with NPD type B, possibly through the adverse lipid profile prone to atheroma plaques formation (3). Not of minor importance is the genetic counseling and testing to the offspring of adult individuals affected with NPD type B. As with most enzyme deficiencies determined genetically, NPD is an autosomal recessive disorder in which all members of the offspring are obligatory carriers of one of the responsible mutations, provided that only one parent contribute with a diseased allele.

So far no public data exist regarding a definitive treatment or an effective enzyme replacement therapy. Bone marrow and liver transplantation have been successful in prolonging life span, but this strategy can reach only a limited number of patients (5, 6). Recently, a phase 1B clinical trial was sponsored, designed and conducted by Sanofi Company in five adults with nonneuronopathic ASM disease. The recombinant human acid sphingomyelinase (olipudase alfa) demonstrated tolerability and safety of a recombinant human ASM (12), which prompted to an ongoing phase 2, currently recruiting, will include more centers and there will be both an adult and a children trials.

CONCLUSION

In conclusion, this is the first description about a composed heterozygous Mexican patient with NPD type B caused by the concomitant occurrence of previously described pathogenic mutations associated

with both NPD type A and B. The possible attenuation effect of the missense c.1426C>T mutation (previously associated with the NPD type B phenotype) over the c.1343A>G pathogenic allele (previously associated with the NPD type A phenotype) merits further study.

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REFERENCES

1. *ACUÑA M., MARTÍNEZ P., MORAGA C., HE X., MORAGA M., HUNTER B., NUERNBERG P., GUTIÉRREZ R.A., GONZÁLEZ M., SCHUCHMAN E.H., LUIS SANTOS J., MIQUEL JF., MABE P., ZANLUNGO S.:* Epidemiological, clinical and biochemical characterization of the p.(Ala359Asp) SMPD1 variant causing Niemann-Pick disease type B. *Eur. J. Hum. Genet.*, 2016 *24*, 208-213.
2. *GOÑI F.M., ALONSO A.:* Sphingomyelinases: enzymology and membrane activity. *FEBS Lett.*, 2002; *531*, 38-46.
3. *LEE C.Y., KRIMBOU L., VINCENT J., BERNARD C., LARRAMÉE P., GENEST J., JR., MARCIL M.:* Compound heterozygosity at the sphingomyelin phosphodiesterase-1 (SMPD1) gene is associated with low HDL cholesterol. *Hum. Genet.*, 2003, *112*, 552-562.
4. *LEVRAN O., DESNICK R.J., SCHUCHMAN E.H.:* Niemann-Pick disease: a frequent missense mutation in the acid sphingomyelinase gene of Ashkenazi Jewish type A and B patients. *Proc. Natl. Acad. Sci.*, 1991, *88*, 3748-3752.
5. *MENDES M.S., PORTELA F.X., REIS R.C., CASTRO J.D., GARCIA J.H., HOLANDA M.A.:* Liver transplantation in a patient with Niemann-Pick disease and pulmonary involvement. *J. Bras. Pneumol.*, 2012, *38*, 269-271.
6. *SCHNEIDERMAN J., THORMANN K., CHARROW J., KLETZEL M.:* Correction of enzyme levels with allogeneic hematopoietic progenitor cell transplantation in Niemann-Pick type B. *Pediatr. Blood Cancer*, 2007, *49*, 987-989.
7. *SCHUCHMAN E.H., WASSERSTEIN M.P.:* Types A and B Niemann-Pick disease. *Best Pract. Res. Clin. Endocrinol. Metab.*, 2015, *29*, 237-247.
8. *SIMONARO C.M., DESNICK R.J., MCGOVERN M.M., WASSERSTEIN M.P., SCHUCHMAN E.H.:* The demographics and distribution of type B Niemann-Pick disease: novel mutations lead to new genotype/phenotype correlations. *Am. J. Hum. Genet.*, 2002, *71*, 1413-1419.
9. *TAKAHASHI T., SUCHI M., SATO W., TEN S.B., SAKURAGAWA N., DESNICK R.J., SCHUCHMAN E.H., TAKADA G.:* Identification and expression of a missense mutation (Y446C) in the acid sphingomyelinase gene from a Japanese patient with type A Niemann-Pick disease. *Tohoku J. Exp. Med.*, 1995, *177*, 117-123.
10. *VANIER M.T.:* Niemann-Pick disease type C. *Orphanet J. Rare Dis.*, 2010, *5*, 16.

11. *WASSERSTEIN M.P., DESNICK R.J., SCHUCHMAN E.H.:* Types A and B Niemann-Pick Disease. In: *Lysosomal Storage Disorders. A Practical Guide*. Mehta A.B and Winchester B. (eds) Chichester, UK, John Wiley & Sons, Ltd, 2012, 81.

12. *WASSERSTEIN M.P., JONES S.A., SORAN H., DIAZ G.A., LIPPA N., THURBERG B.L., CULM-MERDEK K., SHAMIYEH E., INGUILIZIAN H., COX G.F., PUGA A.C.:* Successful within-patient dose escalation of olipudase alfa in acid sphingomyelinase deficiency. *Mol. Genet. Metab.*, 2015, *116*, 88-97.

13. *ZARCO-ROMÁN J., GARCÍA-PÉREZ A.N., ANDRADE-ALDAMA P., CARBAJAL-RODRÍGUEZ L., RODRÍGUEZ-HERRERA R.:* Enfermedad de Niemann-Pick tipo B. Estudio de tres casos y revisión de la literatura. *Acta Pediatr. Mex.*, 2014, *35*, 30-37.

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