INTRODUCTION

✓ Mucopolysaccharidosis type VI (MPS VI) is caused by a deficiency in the activity of N-acetylglalactosamine 4-sulfatase (ARSB) enzyme. It is inherited as an autosomal recessive trait and presents a variable clinical spectrum, from severe to attenuated forms.

✓ Patients with MPS VI can be treated with enzyme replacement therapy, and cases treated early seem to present better treatment outcome.

✓ MPS VI is a very rare condition, which has a relatively high incidence in the county of Monte Santo, in Northeast Brazil (50,000 inhabitants, with 13 MPS VI cases identified so far). A common mutation (H178L) was identified in all cases.

✓ The objective of this project is to evaluate, initially in dried blood spot (DBS) samples, the possibility of adding to the standard newborn screening program established on this area (for PKU, congenital hypothyroidism - CH and hemoglobin disorders - HD) a program for MPS VI detection, using biochemical and molecular methods.

✓ The program includes the measurement of ARSB activity and the analysis of the mutation p.H178L (PCR followed by RFLP), which was the only one found in MPS VI patients of Monte Santo.

MATERIALS AND METHODS

✓ The DBS samples were collected in Monte Santo and sent to Salvador (where is located the state NBS lab) for the routine newborn screening; after performing screening for PKU, CH and HD, the same samples were sent to Porto Alegre (MPS reference center) for the screening of MPS VI.

✓ For the biochemical analysis, the activity of ARSB in DBS was assayed according to the method described by Civallero et al (Clin Chim Acta 372: 98-102, 2006), adapted to 96-well microplates.

✓ For the molecular analysis, DBS disks were used without prior treatment. PCR followed by RFLP was performed for the analysis of the p.H178L mutation according to Costa-Motta et al (Mol. Genet Metab 104: 603-7, 2011).

RESULTS

✓ The standardization of the techniques for enzyme assay, and mutation detection were already completed.

✓ Analysis of samples from neonates born from Jan 2011 to Jun 2014 was performed so far (2,000 babies, table1).

✓ From the 2,000 neonates evaluated, 88 have presented ARSB activity below the reference values and a follow-up investigation was established to rule out MPS VI.

✓ On the molecular analysis, 30 carriers for the p.H178L mutation were found. These subjects presented ARSB activity below lower reference limits.

DISCUSSION

✓ The relatively high proportion of neonates with enzyme activity below normal limit, specially suggests that the collection/transportation/storage process should be improved (this was not a problem on our case as the molecular analysis provided the necessary information for screening purposes).

✓ As the 30 carriers presented low ARSB activity, enzyme activity could also potentially be used to screen MPS VI carriers on this population, a finding which should be confirmed in a larger sample.

✓ The subjects with low ARSB activity should continue the investigation with the measurement of enzyme activity in leucocytes, as we cannot rule out their possibility of presenting MPS VI due to another mutation(s) on the ARSB gene.

✓ The frequency of heterozygotes found for the population of Monte Santo (30/2000, or 1.6%) was considered significant, taking into account the disease rarity.

✓ The program is in progress, as an increased sample size will allow us to more precisely estimate the frequency of the mutation and the expected number of heterozygotes and homozygotes in the region, which will be important for a population medical genetics approach to the community, including genetic counseling, prenatal diagnosis and early treatment.