Genetics and Molecular Biology, 31, 2, 423-426 (2008)

www.sbg.org.br

Short Communication

Prevalence of the serpin peptidase inhibitor (alpha-1-antitrypsin) *PI*S* and *PI*Z* alleles in Brazilian children with liver disease

Guilherme Baldo[1,2*], Ana Ayala[2,3*], Matias Melendez[2,3], Karina Nonnemacher[2], Luciane Lima[5], Sandra Leistner Segal[5], Carlos Kieling[4], Sandra Gonçalves Vieira[4], Cristina Targa Ferreira[4], Themis Reverbel da Silveira[4], Roberto Giugliani[1,3,5] and Ursula Matte[2,4]

[1]*Programa de Pós-Graduação em Ciências Biológicas: Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.*
[2]*Centro de Terapia Gênica, Centro de Pesquisas, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil.*
[3]*Programa de Pós Graduação em Genética e Biologia Molecular, Departamento de Genética, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.*
[4]*Laboratório de Hepatologia Experimental, Centro de Pesquisas, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil.*
[5]*Serviço de Genética Médica, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil.*

Abstract

Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1 (SERPINA1) deficiency is one of the main genetic causes related to liver disease in children. In SERPINA1 deficiency the most frequent *SERPINA1* alleles found are the *PI*S* and *PI*Z* alleles. We used the polymerase chain reaction and the amplification created restriction site (ACRS) technique to investigate the prevalence of the *PI*S* and *PI*Z* alleles in a group of Brazilian children (n = 200) with liver disease and established the general frequency of the *PI*S* allele in our population. We found a significant association of the *PI*Z* allele and liver disease, but no such relationship was found for the *PI*S* allele. Our results show that SERPINA1 deficiency due to the *PI*Z* allele, even when heterozygous, is a frequent cause of liver disease in our group of Brazilian children but that the *PI*S* allele does not confer an increased risk of hepatic disorders in our group of Brazilian children

Key words: alpha-1-antitrypsin deficiency, liver disease, pediatric patients, *SERPINA1*.

Received: July 16, 2007; Accepted: October 15, 2007.

Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1 (SERPINA1, formerly known as alpha-1-antitrypsin (A1AT) is a glycoprotein mainly produced by hepatocytes whose major function is to inhibit the action of neutrophilic elastase, a serine protease that hydrolyses elastin fibers in the lung (Francavilla *et al.*, 2000). The protein is encoded by the highly polymorphic *SERPINA1* gene located in the long arm of chromosome 14 (14q31-32.3) (Schroeder *et al.*, 1985). Mutations in *SERPINA1* lead to a reduction or loss of the inhibitory capacity. Although more than seventy alleles for the enzyme are known, designated according to their isoelectric point (De Tomasso *et al.*, 2001), the *PI*S* and *PI*Z* alleles are the two major forms predominant among the deficient variants. The *PI*S* allele results from a point mutation in exon III of the gene, which leads to the substitution of a glutamic acid residue for a valine at position 264 and formation of an unstable protein (Long *et al.*, 1984). This is associated with a reduction of about 40% in the normal range of SERPINA1 and increased intracellular degradation (Cox, 2004). Even lower levels of SERPINA1 are produced by the *PI*Z* allele, this variant resulting from the substitution of a glutamic acid residue for a lysine at position 342 in exon V (Brind *et al.*, 1990) and is the most frequent *SERPINA1* deficient variant (Alpha-1 Foundation, 2003). Classical studies demonstrated an association of the *PI*Z* allele with liver disease (Lieberman *et al.*, 1972). Hepatic involvement in SERPINA1 deficiency due to the *PI*Z* allele seems to be due to the accumulation of mutant proteins in hepatocytes (Perlmutter, 2003) that have been associated with the clini-

Send correspondence to Ursula Matte. Centro de Terapia Gênica, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos 2350, Largo Eduardo Z. Faraco, Rio Branco, 90035-903 Porto Alegre, RS, Brazil. E-mail: umatte@hcpa.ufrgs.br.
*Both authors contributed equally to this work.

cal features of SERPINA1 deficiency in the liver (mainly neonatal cholestasis) that can progress to chronic liver disease and cirrhosis (Sharp *et al.*, 1969; Teckman *et al.*, 1996; Lomas and Parfrey, 2004). Deficiency in SERPINA1 occurs in 1 in 1600 to 1 in 1800 live births, but prospective natural history studies indicate that only 10% to 15% of the affected population develops clinically significant liver disease (Sveger, 1976). Interestingly, the follow up of a cohort of patients with SERPINA1 deficiency showed that only 3% of children who are homozygous for the *PI*Z* allele develop liver disease (Sveger and Eriksson, 1995). More recently, a possible role of *SERPINA1* as a modifier gene of other forms of pediatric liver disease has been suggested (Campbell *et al.*, 2007).

The objective of the study reported in this paper was to evaluate the frequency of the *PI*S* and *PI*Z* alleles in pediatric patients with liver disease from different regions of Brazil and to compare these data with a control group of anonymous blood donors to establish the general frequency of the *PI*S* allele in our population.

We investigated DNA samples from 200 children (52% male, aged one month to 12 years with a median age of one year) with liver disease for *SERPINA1* mutations E264V (*PI*S* allele) and E342K (*PI*Z* allele). The children were referred to the Genetic Therapy Center at the Clinical Hospital in Porto Alegre (Centro de Terapia Gênica, Centro de Pesquisas, Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, Rio Grande do Sul, Brazil) by nine hospitals from different regions of Brazil, mostly from the Southern (84.5%), Southeastern (9.5%) and Center-West (1.5%) region. The main reasons for suspicion of SERPINA1 deficiency were cholestatic jaundice (23.5%), cryptogenic cirrhosis (10%) and neonatal cholestasis (10%). Other reasons include elevated transaminases, steatosis, hepatosplenomegaly, hepatic failure and ascites. The diagnosis of cirrhosis was based on clinical, biochemical, ultrasonographic and endoscopic findings and/or histology. Cryptogenic cirrhosis was diagnosed based on the absence of inborn errors of metabolism, sclerosing cholangitis, viral hepatitis B and C, congenital infectious disease, use of hepatotoxic drugs and Wilson disease. Blood samples were taken from the children as part of normal clinical investigations between 1998 and 2006.

The presence of the *PI*S* allele was also investigated in blood samples from 150 unidentifiable voluntary donors who donated blood at the HCPA blood bank in 1998. These samples had already been tested for the *PI*Z* allele (Lima *et al.*, 2001) but this time were used as a control to estimate the frequency of the *PI*S* allele in the general population, the use of adults as the control population being justified by the fact that only a small percentage of individuals with the *PI*ZZ* allele develop liver disease (Sveger and Eriksson, 1995).

Although we were not able to ethnically classify the children or adults who participated in our study almost 85% of them were from the Brazilian state of Rio Grande do Sul which has an ethnically heterogeneous population with a predominance of individuals of Mediterranean and Central European descent and, compared to the rest of Brazil, a small contribution of genes of African and Amerindian origin (Carvalho-Silva *et al.*, 2001). Informed consent was obtained from the parents of the children and the blood donors prior to their entering the study and the study was approved by the ethics commission of HCPA.

Genomic DNA was extracted from 5 mL of peripheral blood using the salting-out technique (Miller *et al.*, 1988) and DNA analysis performed using the polymerase chain reaction (PCR) and the amplification created restriction site (ACRS) technique (Andresen *et al.*, 1992). To detect the *PI*S* allele we amplified *SERPINA1* exon III in a final volume of 50 µL containing 30 pmol each of primers p7553 (5'-CGTTTAGGCATGAATAACTTCCAGCA-3') and p7702 (5'-GATGATATCGTGGGTGAGTTCATT TA-3'), 5 µL 10X buffer (200 mM Tris-HCl (pH 8.4) and 500 mM KCl), 1.5 mM of $MgCl_2$, 0.2 mM of each dNTP and 1 unit of Taq DNA polymerase. Amplification of the *PI*Z* allele *SERPINA1* exon VII used a final volume of 50 µL containing 40 pmol each of primer AT5f (5'-ATAA GGCTGTGCTGACCATCGTC-3') and AT5r (5'- GAAC TTGACCTCGAGGGGGATAGA-3'), 5 µL of buffer (75 mM Tris-HCl (pH 9), 50 mM KCl, 20 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$ and 0.001% (v/v) bovine serum albumin), 0.2 mM of each dNTP, 6% (v/v) DMSO and 1 unit of Taq DNA polymerase. Amplification was carried out in a Personal Thermocycler (Eppendorf, Germany) using an annealing temperature of 50 °C for both exons. Amplification length was 149 bp for *PI*S* and 97 bp for *PI*Z*. The PCR products were cleaved using the using the *Xmn*I endonuclease for the *PI*S* products and *Taq*I for the *PI*Z* products according to manufacturer's instruction (Invitrogen). Fragments were separated on 12% (w/v) polyacrylamide gel and stained with 0.004% (w/v) ethidium bromide. Expected fragment sizes were 133 bp and 16-bp for homozygous *PI*S* individuals and 111 bp, 22 bp and 16 bp for individuals without *PI*S*. For *PI*Z* homozygous individuals the fragments were 86 bp and 11 bp. When the mutation was not present, fragments were 64 bp, 22 bp and 11 bp. All reagents for PCR and digestion were purchased from Invitrogen (Carlsbad, USA).

Statistical analysis to compare the allele frequency in patients and controls was performed using the chi-square test with the Yates correction.

Our analysis showed that of the 200 children examined, 20 (10%) were *PI*Z* homozygous, 2 (1%) were *PI*S* homozygous, 2 (1%) were *PI*SZ* compound heterozygous, 13 (6.5%) were *PI*Z* heterozygous and 21 (10.5%) were *PI*S* heterozygous. The HCPA clinical findings for the children positive for the *PI*Z* and *PI*S* alleles are available as supplementary online material (Table S1). Cirrhosis was the final diagnosis in 2 children heterozygous for *PI*Z* and in 1 child heterozygous for *PI*S* who had associated biliary atresia. Eight *PI*ZZ* children developed cirrhosis and five

were given a liver transplant. The median time of follow-up for these patients was 7 years (range = 2 years to 19 years). One *PI*SZ* child had Overlap Syndrome and was listed for a liver transplant 6 months after diagnosis with severe decompensate cirrhosis. No liver disease was observed in children homozygous for the *PI*S* allele.

To obtain data about the general frequency of the *PI*S* and *PI*Z* alleles in our population we also investigated 150 blood donors, who acted as controls representing the frequency of these two alleles in the general population. In this group the *PI*S* allele frequency found was 6.66%. Eighteen (12%) individuals were heterozygous and only one (0.66%) was homozygous for *PI*S*. The *PI*Z* allele occurred in only 1 (0.66%) heterozygous individual.

Comparison of the calculated allele frequencies for the children and those for the general population as estimated from the control group revealed a significantly ($p < 0.001$) increased *PI*Z* allele (13.75%) frequency for the children with liver disease as compared to controls. Moreover, the association between liver disease and the *PI*Z* allele was significant ($p < 0.01$) even considering only heterozygous children. These children were confirmed to not have other changes in *SERPINA1* (data not shown). The frequency of the *PI*S* allele (6.75%) was not statistically different from that found in the general population for homozygous and heterozygous individuals (Table 1). Analysis of genotypic frequencies indicated that the *PI*Z* allele was in Hardy-Weinberg disequilibrium for the children with liver disease.

Deficiency in SERPINA1 is a genetic disorder strongly related to hepatic disease and the need for liver transplantation. It affects all major racial subgroups, and there are about 120.5 million carriers and deficient individuals worldwide (De Serres *et al.*, 2003). The *PI*S* allele is a very common deficient variant, reaching incidences higher than 14% in countries such as Portugal and France (Roychoudhury and Nei, 1988). Luisetti and Seersholm (2004) published data on the worldwide *PI*S* and *PI*Z* allele frequencies, but presented no data for South America due to the lack of studies in this region. In our study, we analyzed the frequency of the *PI*S* allele in a sample of Brazilian children with liver disease and also in a group of blood donors representative of the general population. Comparing our data with that of previous investigations, the frequency of the *PI*S* allele in our population seems to be higher than in Holland (2.9%) and Denmark (2.2%) but lower than in Portugal (15%) and France (14.5%) (Roychoudhury and Nei, 1988; Luisetti and Seersholm, 2004). The intermediate frequency of the allele in our Brazilian population may be explained by the mixed origin of our population, which has a strong influence from Portuguese colonization but with a significant contribution from other Central European countries, as observed by mutation frequency data for other diseases (Castro *et al.*, 2007).

In our study, the *PI*Z* allele showed a high prevalence among the children, strengthening the hypothesis that the presence of the *PI*Z* allele contributes to hepatic disease and may have a role as a modifier gene of other forms of pediatric liver disease (Campbell *et al.*, 2007). However, it is important to point out the lack of correlation between hepatic disease and the presence of the *PI*S* allele seen in our investigation. Elliot *et al.* (1996) suggest that the *PI*S* variant has increased susceptibility to polymerization, although this increase is marginal when compared to the *PI*Z* allele. There has been some speculation regarding synergy between the variants which may lead to cirrhosis in *PI*SZ* adults (Mahadeva *et al.*, 1999). Hadzic *et al.* (2005) investigated *PI*SZ* patients and showed that although they had low SERPINA1 levels some of them may have had late hepatic disorders. This suggests that even though we found no correlation between the *PI*S* allele and hepatic disorders in children, individuals with the *PI*S* allele should be monitored in adulthood to evaluate late-onset hepatic problems. Furthermore, although testing for both alleles in children with liver disease may not be cost-effective further assessment is needed regarding the cost-effectiveness of testing for the *PI*S* allele in heterozygous adults with the *PI*Z* allele.

Summarizing, in this study of *SERPINA1* we found a significant association between liver disease in children and the *PI*Z* allele but no such relationship for the *PI*S* allele. These results indicate that while SERPINA1 deficiency due to the *PI*Z* allele is a frequent cause of liver

Table 1 - Serpin peptidase inhibitor (alpha-1-antitrypsin) *SERPINA1 PI*S* and *PI*Z* allele frequencies and homozygous and heterozygous genotype frequencies for Brazilian children (n = 200) with liver disease and adult blood donors (n = 150) with no liver disease compared using the chi-squared test with Yates correction.

	*PI*Z* allele			*PI*S* allele		
		Genotype frequency (%)			Genotype frequency (%)	
Group	Allele frequency (%)	Homozygous	Heterozygous	Allele frequency (%)	Homozygous	Heterozygous
Children	13.75	10.00	7.50	6.75	1.00	11.50
Blood donors	0.33	0.00	0.66	6.66	0.66	12.00
p value	< 0.001*	< 0.001*	< 0.01*	> 0.05^{ns}	> 0.05^{ns}	> 0.05^{ns}

*Within the same column differences between the values were significant at the stated probabilities (p).
^{ns}Within the same column differences between the values were not significant at the stated probabilities (p).

disease in children the *PI*S* allele does not confer an increased risk for pediatric hepatic disorders.

Acknowledgments

The authors are grateful for the support given by Fundo de Incentivo à Pesquisa e Eventos do Hospital de Clínicas de Porto Alegre (FIPE-HCPA), the Brazilian Higher Education Training Program (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES) and the Brazilian National Counsel for Technological and Scientific Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq).

References

Alpha-1 Foundation (2003) Genetic testing for alpha-1-antitrypsin deficiency. Am J Respir Crit Care Med 168:874-899.

Andresen BS, Knudsen I, Jensen PK, Rasmussen K and Gregersen N (1992) Two novel nonradioactive polymerase chain reaction-based assays of dried blood spots, genomic DNA, or whole cells for fast, reliable detection of Z and S mutations in the alpha 1-antitrypsin gene. Clin Chem 38:2100-2107.

Brind AM, McIntosh I, Brock DJ, James OF and Bassendine MF (1990) Polymerase chain reaction for detection of the alpha-1-antitrypsin Z allele in chronic liver disease. J Hepatol 10:240-242.

Campbell KM, Arya G, Ryckman FC, Alonso M, Tiao G, Balistreri WF and Bezerra JA (2007) High prevalence of alpha-1-antitrypsin heterozygosity in children with chronic liver disease. J Pediatr Gastroenterol Nutr 44:99-103.

Carvalho-Silva DR, Santos FR, Rocha J and Pena SD (2001) The phylogeography of Brazilian Y-chromosome lineages. Am J Hum Genet 68:281-287.

Castro SM, Weber R, Matte U and Giugliani R (2007) Molecular characterization of glucose-6-phosphate dehydrogenase deficiency in patients from the southern Brazilian city of Porto Alegre, RS. Genet Mol Biol 30:10-13.

Cox DW (2004) Prenatal diagnosis for alpha1-antitrypsin deficiency. Prenat Diagn 24:468-470.

De Serres FJ, Blanco I and Fernandez-Bustillo E (2003) Genetic epidemiology of alpha-1 antitrypsin deficiency in North America and Australia/New Zealand: Australia, Canada, New Zealand and the United States of America. Clin Genet 64:382-397.

De Tommaso AM, Rossi CL, Escanhoela CA, Serra HG, Bertuzzo CS and Hessel G (2001) Diagnosis of alpha-1-antitrypsin deficiency by DNA analysis of children with liver disease. Arq Gastroenterol 38:63-71.

Elliot PR, Stein PE, Bilton D, Carrell RW and Lomas DA (1996) Structural explanation for the disfunction of S alpha-1-antytripsin. Nat Struct Biol 3:910-911.

Francavilla R, Castellaneta SP, Hadzic N, Chambers SM, Portmann B, Tung J, Cheeseman P, Rela M, Heaton ND and Mieli-Vergani G (2000) Prognosis of alfa-1 antitrypsin deficiency-related liver disease in the era of paediatric liver transplantation. J Hepatol 32:986-992.

Hadzic N, Francavilla R, Chambers SM, Castellaneta S, Portmann B and Mieli-Vergani G (2005) Outcome of PiSS and PiSZ alpha-1-antitrypsin deficiency presenting with liver involvement. Eur J Pediatr 164:250-252.

Lieberman J, Mittman C and Gordon HW (1972) Alpha 1 antitrypsin in the livers of patients with emphysema. Science 175:63-67.

Lima LC, Matte U, Leistner S, Boop AR, Scholl VC, Giugliani R and da Silveira TR (2001) Molecular analysis of the *PI*Z* allele in patients with liver disease. Am J Med Genet 104:287-290.

Lomas DA and Parfrey H (2004) Alpha-1-antitrypsin deficiency. 4: Molecular pathophysiology. Thorax 59:529-535.

Long GL, Chandra T, Woo SL, Davie EW and Kurachi K (1984) Complete sequence of the cDNA for human alpha 1-antitrypsin and the gene for the S variant. Biochemistry 23:4828-4837.

Luisetti M and Seersholm N (2004) Alpha-1-antitrypsin deficiency: Epidemiology of alpha-1-antitrypsin deficiency. Thorax 59:164-169.

Mahadeva R, Chang WS, Dafforn TR, Oakley DJ, Foreman RC, Calvin J, Wight DG and Lomas DA (1999) Heteropolymerization of S, I, and Z alpha1-antitrypsin and liver cirrhosis. J Clin Invest 103:999-1006.

Miller SA, Dykes DD and Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215.

Perlmutter DH (2003) Alpha1-antitrypsin deficiency: Liver disease associated with retention of a mutant secretory glycoprotein in the endoplasmic reticulum. Methods Mol Biol 232:39-56.

Roychoudhury AK and Nei M (1988) Human Polymorphic Genes: World Distribution. Oxford University Press, Oxford, 393 pp.

Sharp HL, Bridges RA, Krivit W and Freier EF (1969) Cirrhosis associated with alpha-1-antitrypsin deficiency: A previously unrecognized inherited disorder. J Lab Clin Med 73:934-942.

Schroeder WT, Miller MF, Woo SL and Saunders GF (1985) Chromosomal localization of the human alpha 1-antitrypsin gene (PI) to 14q31-32. Am J Hum Genet 37:868-872.

Sveger T (1976) Liver disease in alpha-1-antitrypsin deficiency detected by screening of 200,000 infants. N Engl J Med 294:1216-1221.

Sveger T and Eriksson S (1995) The liver in adolescents with alpha 1-antitrypsin deficiency. Hepatology 22:514-520.

Teckman JH, Qu D and Perlmutter DH (1996) Molecular pathogenesis of liver disease in alpha1-antitrypsin deficiency. Hepatology 24:1504-1516.

Internet Resources

Alpha-1 Foundation http://www.alphaone.org/.

Supplementary Material

The following online material is available for this article:

- Table S1: Clinical findings in *PI*S* and *PI*Z* patients followed at Hospital de Clínicas de Porto Alegre.

This material is available as part of the online article from http://www.scielo.br/gmb.

Associate Editor: Paulo A. Otto

Table S1: Clinical findings in Pi*S and Pi*Z patients followed at Hospital de Clínicas de Porto Alegre

Patient	Gender	Clinical Feature	Age	Genotype	Clinical Diagnosis	Follow-up	Outcome
1	M	Cirrhosis	24 months	Z/N	Sclerosing cholangites	10 years	Liver transplantation (alive)
2	M	Cholestasis	1 month	Z/Z	Cirrhosis	19 years	Liver transplantation (alive)
3	F	Cholestasis	3 months	Z/Z	Cirrhosis	18 years	Liver transplantation (died)
4	M	Ascites	8 months	Z/N	Budd-Chiari Syndrome	4 years	Well
5	M	Cholestasis	2 months	Z/N	Idiophatic neonatal cholestasis	7 years	Lost
6	F	Hepatosplenomegaly	2 months	S/S	Portal Vein Thrombosis	10 years	Well
7	M	Cholestasis	2 months	Z/Z	Cirrhosis	15 years	Liver transplantation (alive)
8	M	Cirrhosis	108 months	Z/Z	Cirrhosis	4 years	Liver transplantation (alive)
9	M	Abnormal transaminases	2 months	S/N	Carbohydrate -deficient glycoprotein syndrome 1b	3 years	Abnormal transaminases
10	M	Cholestasis	1 month	Z/Z	Alpha-1-antitrypsin deficieny	2 years	Compensated cirrhosis
11	F	Cholestasis	2 months	Z/N	Cirrhosis	Lost	Lost
12	M	Abnormal transaminases	24 months	S/N	Drug hepatotoxicity	3 years	Well
13	M	Hepatomegaly	60 months	Z/N	Steatosis	Lost	Lost
14	M	Cholestasis/ Hepatosplenomegaly	36 months	S/Z	Overlap Syndrome	2 years	Liver transplantation (alive)
15	F	Cholestasis/abnormal transaminases	72 months	S/N	Autoimmune hepatitis	2 years	Compensated chronic hepatitis
16	M	Cholestasis	2 months	Z/Z	Alpha-1-antitrypsin deficiency	Lost	Lost
17	F	Cholestasis	1 month	S/N	Biliary atresia	3 years	Liver transplantation (alive)

18	M	Abnormal transaminases/ Hepatosplenomegaly	144 months	S/N	Congenital Disceratosis	3 years	Severe portal hypertension
19	M	Cholestasis	1 month	Z/Z	Alpha-1-antitrypsin deficiency	7 years	Compensated cirrhosis
20	M	Cholestasis	1 month	Z/Z	Alpha-1-antitrypsin deficiency	6 years	Liver transplantation (alive)