

SHORT REPORT

Cationic trypsinogen and pancreatic secretory trypsin inhibitor gene mutations in neonatal hypertrypsinemia

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Neonatal hypertrypsinemia with normal sweat chloride detected during CF screening may be related to trypsin activation. We have looked for mutations of the cationic trypsinogen (PRSS1) and pancreatic secretory trypsin inhibitor (PSTI) genes in 50 hypertrypsinemic neonates with known CFTR genotypes and negative sweat test. No mutations were found in either gene. Two silent polymorphisms were detected in the PRSS1 gene. A polymorphism in the promoter region and an intronic polymorphism of the PSTI gene were found. No difference was observed in the frequency of PRSS1 or PSTI polymorphisms in neonates carrying or not carrying CF mutations. These results do not provide an indication for an increased frequency of mutations in the PRSS1 and PSTI genes in this group of neonates with transient hypertrypsinemia.

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Introduction

Immunoreactive trypsinogen concentration measurement (IRT) in dried blood spots is commonly performed as a first step in neonatal screening for cystic fibrosis (CF).¹ Infants with hypertrypsinemia are then tested for the most common CF gene mutations: neonates carrying two CF-causing mutations, and infants with one CF mutation and a positive sweat test are diagnosed as affected. Neonates with raised trypsin levels at birth, one CF mutation and normal sweat electrolyte concentrations are usually considered to be carriers. However high IRT levels are not specific to CF, and they can occur also in newborns not carrying any common CF mutations. Thus, other factors, possibly genetic, could predispose to neonatal hypertrypsinemia: genes associated with exocrine pancreatic function appear

to be likely candidates. The cationic trypsinogen gene (PRSS1) codes for the major form of trypsinogen.² The cleavage of the signal peptide leads to the activation of trypsinogen to trypsin, which subsequently converts all proteolytic proenzymes to their active form.³

Mutations in the PRSS1 gene have been associated with certain forms of hereditary pancreatitis (HP),⁴ a rare autosomal dominant disease with 80% penetrance and variable expressivity characterised by recurring episodes of severe abdominal pain often experienced by young patients.⁵ In particular, R122H, a missense mutation in exon 3 of the PRSS1 gene, abolishes the primary autolysis site of human trypsin, and it is clearly associated with HP.^{2,4} Also, mutation A16V in the PRSS1 gene, which interferes with the signal peptide cleavage, has been identified in chronic pancreatitis and in HP families with low penetrance.⁶ Other PRSS1 gene mutations have been reported to be associated with HP.^{7–9}

Ockenga *et al.*¹⁰ have recently shown that mutations of the CFTR gene but not PRSS1 are associated with recurrent or chronic idiopathic pancreatitis. It is not clear if there are

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relationships between CFTR and PRSS1 in the determining pancreatic phenotype.

Mutations in the pancreatic secretory trypsin inhibitor (PSTI, also known as serine protease inhibitor, Kazal type 1, SPINK1) gene which determine insufficient inhibition of trypsin have also been found in hereditary and in idiopathic chronic pancreatitis.^{11,12} In particular, PSTI gene N34S mutation has been detected in 18/96 unrelated children and adolescents with chronic pancreatitis,¹¹ in 5/55 familial pancreatitis, and in 23/57 idiopathic chronic pancreatitis cases.¹²

In this research we investigated whether mutations of the PRSS1 and PSTI genes are associated with neonatal hypertrypsinemia in newborns who had a normal sweat test.

Subjects and methods

The population under study consisted of 50 neonates from a CF neonatal screening programme cohort from North East Italy with IRT levels at birth above the 99.5th centile and normal sweat test as recently described for 47 of them.¹³

A panel of 15 common mutations, covering 85% of the CF alleles in our area, and denaturing gradient gel electrophoresis (DGGE) of all 27 exons and intron flanking regions of the CFTR gene had been previously performed as described for 18 of them,¹⁴ 33 out of 50 neonates had one CFTR mutation from the panel, and 15 of them also had other CFTR gene mutations; 17/50 had no mutation from the panel, and seven of them had other CF mutations, as shown in Table 1.^{13,14}

PRSS1 gene mutations R122H and A16V were analysed by enzymatic restriction with the appropriate endonuclease as described.^{4,6} PRSS1 gene exons 1–4 were analysed by DGGE as described,⁷ and exon 5 was analysed by direct DNA sequencing with Big Dye terminator kit (Perkin Elmer).

Mutation N34S in the PSTI gene was screened by restriction analysis of genomic DNA amplified by RG-PCR¹⁵ using the forward primer 5'-CAATCAGTTATCCCCAG-3' and the modified reverse primer 5'-TGGTGCATCCATTAAGTACA-3'. The reverse primer creates a restriction site for the endonuclease *SduI* when the mutation is present.

The PSTI gene was also analysed by DGGE of the promoter region, the four exons, and intron–exon boundaries in 49 neonates, as described.¹⁶ Fragments which showed an altered migration pattern by DGGE analysis were directly sequenced using the BigDye terminator Cycle Sequencing kit (Perkin Elmer) on a 377 ABI PRISM sequencer (Perkin Elmer).

Results

PRSS1 gene

Cationic trypsinogen gene mutations R122H and A16V were not found, nor any other mutation reported to be associated with hereditary or idiopathic pancreatitis. Two polymorphisms in the PRSS1 gene were detected by DGGE analysis and DNA sequencing. Silent polymorphism 133807 C/T (numbering referred to the human beta TCR

locus¹⁷) in exon 4, in 21 neonates, and silent polymorphism 134359 C/T in exon 5, in 22 neonates, were detected. The genotypes of the 50 neonates relative to the two polymorphisms are shown in Table 1.

PSTI gene

Restriction analysis demonstrated that the N34S PSTI gene mutation was not present in any neonate. DGGE analysis of the PSTI gene in 49 neonates and subsequent sequencing demonstrated the presence of two polymorphisms: –253T/C in the promoter region of the PSTI gene in 16 neonates, and IVS2-23A/T in intron 2 in two neonates. The genotypes of the 50 neonates relative to the two PSTI gene polymorphisms are shown in Table 1. The PSTI polymorphisms found in this group of hypertrypsinemic neonates were found with the same frequency in a sample of 50 normal control individuals from the Italian population (16/50).

PRSS1, PSTI, and CFTR genes

In 33/50 neonates having 'bona fide' CF mutations (CFGAC site: www.genet.sickkids.on.ca/cftr/,¹⁸) 19 carried PRSS1 polymorphisms (133807 C/T and/or 134359 C/T) and 13 carried PSTI polymorphisms (IVS2-23 A/T and/or –253 T/C), while in 17/50 neonates not having 'bona fide' CF mutations 11 carried PRSS1 polymorphisms and four carried PSTI polymorphisms. The difference is not significant.

Discussion

This study was implemented following the speculation that newborns with neonatal hypertrypsinemia could carry particular PRSS1 and PSTI gene mutations depending on the presence or the absence of CF-causing mutations. The implication of CFTR gene mutations in the development of transient hypertrypsinemia was already described.^{13,14}

This study detected the presence of polymorphisms in the PRSS1 and in the PSTI genes in hypertrypsinemic neonates. These polymorphisms do not seem to have a functional significance in gene expression or disease modification and have been previously reported not to be related to hereditary pancreatitis or sporadic chronic pancreatitis.^{8,11,12}

The results of this study do not show evidence for PRSS1 or PSTI gene mutations in the group of neonates with hypertrypsinemia here described. We observed an homogeneous distribution of PRSS1 or PSTI polymorphisms in neonates carrying or not carrying CFTR gene mutations.

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Table 1 Hypertrypsinaemic neonate genotypes at the CFTR, PRSS1, and PSTI loci

	CFTR mutations	PRSS1 polymorphisms	PSTI polymorphisms
1	*R1162X, 3041-71G/C, 4002A/G	–	–
2	DELTA508	133807 C/T, 134359 C/T	IVS2–23A/T
3	*R1162X/R117H	–	n.s.
4	*DELTA508/E527G	–	–
5	DELTA508	133807 C/T, 134359 C/T	–
6	*N1303K, 2622+14G/A	133807 C/T, 134359 C/T	–
7	DELTA508	–	–253T/C
8	*DELTA508/1716G/A	133807 C/T, 134359 C/T	–253T/C
9	DELTA508	133807 C/T	–
10	DELTA508	133807 C/T, 134359 C/T	–253T/C
11	R1162X	133807 C/T, 134359 C/T	–253T/C
12	N1303K	–	–
13	*G542X, 1716G/A	–	–
14	DELTA508	133807 C/T	–
15	DELTA508	133807 C/T, 134359 C/T	–253T/C
16	*N1303K, 2622+14G/A	133807 C/T, 134359 C/T	–
17	*DELTA508/Y301C	–	–
18	2183AA/G	–	–
19	DELTA508	–	–253T/C
20	*1717-1G/A/ D1152H	133807 C/T	–
21	DELTA508	134359 C/T	–253T/C
22	*G542X, L997F	–	–
23	DELTA508	–	–
24	*1717-1 G/A/1716 G/A	134359 C/T	–
25	*DELTA508, L997F	–	–253T/C
26	R1162X	134359 C/T	–253T/C
27	*DELTA508/ M1137V	133807 C/T, 134359 C/T	–
28	DELTA508	133807 C/T, 134359 C/T	–
29	2183 AA/G	133807 C/T	–253T/C
30	*R553X/L997F	–	–
31	R553X	134359 C/T	–
32	*DELTA508	–	–253T/C
33	R553X/ D1152H	134359 C/T/ 134359 C/T	–253T/C
34	–	134359 C/T	–253T/C
35	*_	–	–
36	–	–	–
37	–	–	–
38	*R117H	–	–
39	–	133807 C/T	–
40	*2377 C/T	133807 C/T	–253T/C
41	*R75Q, 2082C/T	–	–
42	*1716G/A	133807 C/T	–
43	*L997F, F1052V	133807 C/T	–
44	*F1052V	134359 C/T	–253T/C, IVS2-23A/T
45	–	134359 C/T/ 134359 C/T	–253 T/C
46	–	134359 C/T/ 134359 C/T	–
47	–	133807 C/T/ 133807 C/T, 134359 C/T	–
48	–	133807 C/T, 134359 C/T	–
49	–	133807 C/T, 134359 C/T	–
50	*4404 C/T	–	–

*Data from Castellani *et al.* 2001. n.s.: N345 mutation was tested but the gene was not screened by DGGE analysis. –: no polymorphism was found.

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