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ARTICLE

ATB⁰/SLC1A5 gene. Fine localisation and exclusion of association with the intestinal phenotype of cystic fibrosis

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The Na^+-dependent amino acid transporter named ATB^0 was previously found to be located in 19q13.3 by fluorescence *in situ* hybridisation. Genetic heterogeneity in the 19q13.2-13.4 region, syntenic to the Cystic Fibrosis Modulator Locus 1 (CFM1) in mouse, seemed to be associated to the intestinal phenotypic variation of cystic fibrosis (CF). We performed fine chromosomal mapping of *ATB^0* on radiation hybrid (RH) panels G3 and TNG. Based on the most accurate location results from TNG-RH panel, mapping analysis evidenced that *ATB^0* is localised between STS SHGC-13875 (*D19S995*) and STS SHGC-6138 in 19q13.3, that corresponds with the immediately telomeric/distal segment of the strongest linkage region within the human CFM1 (hCFM1) syntenic region. Regarding to the genomic structure and exon organisation, our results show that the *ATB^0* gene is organised into eight exons. The knowledge of the genomic structure allowed us to perform an exhaustive mutational analysis of the gene. Evaluation of the possible implication of *ATB^0* in the intestinal phenotype of CF was performed on the basis of the functional characteristics of the encoded protein, its apparent relevance to meconium ileus (MI) and position in relation to the hCFM1 syntenic region. We have analysed this gene in samples from CF patients with and without MI. Several sequence variations in the *ATB^0* gene were identified, although none of them seemed to be related to the intestinal phenotype of CF. Even though no particular allele or haplotype in *ATB^0* appears to be associated to CF-MI disease, new SNPs identified should be useful in segregation and linkage disequilibrium analyses in families affected by other disorders caused by the impairment of neutral amino acid transport. *European Journal of Human Genetics* (2001) **9,** 860–866.

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Introduction

The transport of amino acids across the plasma membrane, essential for cellular metabolism, requires specialised transport proteins.[1] There are two amino acids transporter gene families based on ion dependence: Na^+-independent and Na^+-dependent. Na^+-dependent transporters actively transport amino acids driven by the electrochemical gradient of Na^+ and concentrate them inside the cell.[2] The brush border membrane of the absorptive cells of the small intestine and kidney expresses a Na^+-dependent, broad specificity, neutral amino acid transport system. This new member of this superfamily of transporters was cloned and named *ATB^0* (neutral amino acid transporter B^0)[3] which indicates broad substrate specificity and preference for zwitterionic amino acids. It has also been named *SLC1A5*.[3,4]

Human *ATB^0* (*$hATB^0$*) mRNA is detectable in placenta, lung, skeletal muscle, kidney and pancreas.[3] It was also reported to be expressed in rabbit jejunum and the human

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intestinal cell line Caco-2.[5] This transport system is likely to be important in the transport of neutral amino acids in the placenta, intestine and kidney.[3] ATB^0 has been recently demonstrated to act as a receptor for RD114/type D retrovirus.[6] Although the knowledge of the physiological function of the protein is increasing, little is known about the genomic structure and exon organisation of *ATB⁰* gene.

Some members of the Na^+-dependent amino acid transporter family are known to cotransport H^+ and Cl^- and countertransport K^+. Potassium dependence has not been reported for $hATB^0$.[3] No evidence of Cl^- transport has been reported to be associated to $hATB^0$ amino acid transport,[3] but the proper performance of the test has been questioned.[7]

Cystic fibrosis is caused by mutations that lead to the loss of function in CFTR (cystic fibrosis transmembrane conductance regulator),[8] a Cl^- channel in epithelial cell membranes. Nevertheless, the high heterogeneity of disease manifestations and severity observed among cystic fibrosis (CF) patients, even among the members of the same family, could not be explained totally by the presence of mutations in the *CFTR* gene. The molecular mechanisms are not yet completely understood and it has been suggested that other genetic as well as environmental factors could be implicated.

Besides the loss of Cl^- permeability, an increased Na^+ absorption across airway epithelia[9,10] and colon[11] in cystic fibrosis patients has been observed. Recent data from Wu *et al*[12] have demonstrated that CFTR senses the concentration of cytosolic electrolytes and requires high levels of them for full activity, indicating that cytosolic electrolytes are cofactors for CFTR gating. ATB^0 amino acid transport activity is known to be coupled to the cotransport of Na^+ inside the cell.[3] Due to these data, the altered transport of Na^+ could modulate CFTR activity and/or influence the phenotype causing milder or severer CF symptoms. We hypothesised that the increased absorption of Na^+ could be consequence of the altered expression of ATB^0.

A severe intestinal obstruction is detected in 15–20% of CF patients at birth that is called meconium ileus (MI). MI represents a functional disorder, mainly detected during the second trimester of gestation, due to an abnormal thickened meconium within the terminal ileus. Studies in CFTR knockout mice have evidenced a CF modifier locus (CFM1) mapping near the centromere of mouse chromosome 7, related to the modulation of the intestinal disorders that are deleterious in homozygous mutant mice.[13] A recent study[14] has reported its syntenic region on human chromosome 19. The region of 7Mb comprises from 19q13.2 to 19q13.4 (*D19S408*-telomere). *ATB⁰/SLC1A5* mapped in chromosome 19 in the 19q13.3 region by fluorescence *in situ* hybridisation[4] and was found to be located near the glycogen synthase gene (GYS: 19q13.3 *D19S412-D19S867*), indicating the presence of the *ATB⁰* gene inside the human CFM1 (hCFM1) corresponding region. This fact further supported our hypothesis of a potential role of ATB^0 as a regulator of CFTR function.

Here, we localise precisely the *ATB⁰* gene on chromosome 19 using G3 and TNG RH panels. We also report the genomic structure of *ATB⁰* that has facilitated an exhaustive mutational analysis of the open reading frame (ORF) of the gene and its immediate flanking regions in CF patients with or without an intestinal phenotype of MI.

Materials and methods

Human *ATB⁰* radiation hybrid mapping

The chromosomal localisation of the gene was determined by PCR screening of the Stanford G3 and TNG[15] radiation hybrid panels. The *ATB⁰* gene was detected by using primers HNAT-8 and HNAT-9,[4] to produce a predicted 163–173 bp product (depending on the length of the $(GT)_n$ sequence contained in the fragment amplified). Two-point linkage analysis was performed using the RHMAP-2.0 on the RH Server at the Stanford Human Genome Centre (http://www-shgc.standford.edu/RH/index.html).

Genomic structure

To determine the genomic structure, the Blast N program was used to align genomic sequences (finished as well as unfinished) with the *ATB⁰* cDNA sequence (GeneBank accession no. U53347 and NM 005628).

Analysis of *ATB⁰* nucleotide changes

DNA was isolated from peripheral blood lymphocytes as previously described.[16] After the deduction and identification of the different exons (Table 1), *ATB⁰* DNA was amplified in eight segments (Table 2) comprising the 5′- and 3′-untranslated regions (UTR), coding exons and the immediately flanking intronic sequences of the gene. The 5′-fragment that incorporates 606 bp 5′-UTR and translation initiation site was amplified using the GC-rich PCR system (Roche Molecular Biochemicals, Indianapolis, IN, USA) due to its high content of Gs and Cs. Primers are described in Table 2. Variations from wild type sequence were detected by SSCP/heteroduplex analysis. Direct automated sequencing for all abnormal bands was performed.

A simple sequence repeat $(GT)_n$ in the 3′-UTR of *ATB⁰* was also determined by PCR for each sample. PCR parameters and primers were as previously described.[4] The amplified products were run on an AbiPrism® 310 Genetic Analyser and the size was determined using Genotyper® software (v. 2.5) (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

The distribution of $(GT)_n$ alleles in the general population, CF-nonMI and CF-MI was examined by the Shapiro-Wilk test of normality and the Kruskal-Wallis non-parametric test. A binary logistic regression model was used to analyse the effect of $(GT)_n$ length on the phenotype.

Contingency two-by-two tables with significance calculation using Yates' correction of χ^2 test and the Fisher test to the

Table 1 Description of the eight *ATB⁰* exons

Exon	3′ Splice acceptor	5′ Splice donor	cDNA position No. U53347[a]	cDNA position No. NM005628[a]	Size (bp)
E1	cccatcATGGTG ...	... TGCGAG**gt**	620–1185	591–1156	566
E2	**ag**AAATAT ...	... CGCTCA**gt**	1186–1228	1157–1199	43
E3	**ag**TACTCT ...	... GTGAAG**gt**	1229–1276	1200–1247	48
E4	**ag**GTGCCC ...	... CATGTG**gt**	1277–1443	1248–1414	167
E5	**ag**GTACGC ...	... TTCCAG**gt**	1444–1677	1415–1648	234
E6	**ag**TTCCGC ...	... CATCCT**gt**	1678–1872	1649–1843	195
E7	**ag**GGTCAC ...	... GCTAGT**gt**	1873–2007	1844–1978	135
E8	**ag**CGACCG ...	... GTCATGtaa	2008–2242	1979–2213	235

The exon-intron structure of *ATB⁰* is detailed. Splice acceptor and donor sequences are shown in boldface type. Size of each exon is also included. [a]GeneBank accession no.

Table 2 Sequences of oligonucleotides used as PCR primers

ATB⁰ region	*5′ Primers*	*3′ Primers*	*Expected size*	*Annealing T[a]*
5′ UTR-Exon 1	D14: aggctttctctggctggtaac	R10: tgggggcattttcggcactg	1134	60°C
Exon 2	E2D: cctcaccccgaacccacaat	E2R: gtccacccccatcccctca	341	64°C
Exon 3	E3D-B: tttccacctctggaaaatgg	E3R-B: gggaaaagaggggacaaagt	398	62°C
Exon 4	E4D: gccatccctgacgactaata	E4R: ggtgccccatcccaaaca	437	62°C
Exon 5	E5D: cgtggaagaggcaacaagc	E5R-B: cctgaagtatggcccctgta	388	64°C
Exon 6	E6D: agggtggttgggtgacagg	E6R-B: atgatggccagagtgaggac	525	66°C
Exon 7	E7D-B: cctaacctcccaccttctcc	E7R-B: cttcccgaggtcacaacagt	451	68°C
Exon 8	E8D: ccctttccttccctctcacc	E8R: gctcatccatttatccattcc	381	57°C
3′-UTR-(GT)n	HNAT-9*: gggatgttacaacaccatgc	HNAT-8*: ctgttattgtggagggaatag	163–173	60°C

*Published by Jones *et al*.[4] Oligonucleotides and the size of the fragment amplified are described.

5% limit were employed to test for independence between physiological and genetic variables.

Subjects of study

CF patients were clinically diagnosed and selected for the presence or absence of meconium ileus (MI). Twenty-five unrelated CF patients presented MI and pancreatic insufficiency. No signs of MI and pancreatic sufficiency were found in 23 CF patients. All of CF patients but one (with a clear CF phenotype) presented two CF mutations in their genotype after complete screening of the *CFTR* gene (Table 3). Four MI patients had a sibpair with an identical CF genotype but presented discordance of intestinal phenotype (Table 3). Twenty-three non-CF subjects were also selected for the analysis of the distribution of the $(GT)_n$ allele and of the P17A and V512L mutations in Spanish general population.

Results

Chromosomal gene localisation

We have mapped the *ATB⁰* gene using primers that amplify a polymorphic $(GT)_n$ repeat in 3′-UTR[4] using the G3 and TNG radiation hybrid (RH) panels. These primers produce a single product when used to amplify human genomic DNA, but do not amplify sequences in hamster genomic DNA (data not shown).

RH mapping linked *hATB⁰* to 19q13.3 in both G3 and TNG RH mapping panels. G3 hybrid panel revealed that the gene was linked to STS SHGC-9630 (*D19S985*) and SHGC-112 with lod scores of 8.23 and 7.94 respectively, at an approximate distance of 19 and 29 cRs (399 and 546 kb).

The highest lod score results from the TNG radiation hybrid panel were 7.82 and 7.17 linked to markers STS SHGC-6138 and SHGC-13875 (*D19S995*) respectively, at an approximate distance of 27 and 33 cRs (108 and 132 kb).

Based on our RH mapping results and given the highest accuracy in the distance estimates obtained from the TNG RH panel, we conclude that the *ATB⁰* gene is located in the SHGC-13875 (*D19S995*) – SHGC-6138 interval (Figure 1).

Genomic structure of *ATB⁰*

Two BAC clones from chromosome 19 were found to contain the coding region of *ATB⁰* gene by using BlastN-htgs database (unfinished sequences): GeneBank accession nos. AC025983 (44 pieces) and AC008622 (27 pieces). The genomic structure of *ATB⁰* was determined by comparing the sequence of the BAC with *ATB⁰* cDNA sequence (accession no. U53347). The BAC no. AC025983 was selected due to the highest identity with the cDNA sequence. This allowed us to determine the exon/intron boundaries of the gene. Eight exons were identified (Table 1) and most of the intronic sequence. Each intron is flanked by the canonical GT-AG splice site dinucleotides. The length of the exons varies from 43 bp of exon 2 to 566 bp of exon 1 (Table 1). Intron sizes range from 101 bp (intron 6) to 3.5 kb (intron 4).

Table 3 *CFTR* mutations of the CF patients under study with and without meconium ileus (MI)

CF-non MI CFTR *mutations*	n	*CF-MI* CFTR *mutations*	n
F508del/R117H	2	F508del/F508del	7
F508del/R334W	3	F508del/L365P	1
F508del/R347P	1	F508del/G542X	1
F508del/621+1G>T[a]	1	F508del/621+IG>T[a]	1
F508del/M1101K	1	F508del/R1066C	1
F508del/1609delCA[a]	1	F508del/W1089X	1
F508del/2789+5G>A[a]	3	F508del/R1162X	1
F508del/3849+10kbC>T	1	F508del/1609delCA[a]	1
G542X/G85E	1	F508del/Q1281X	1
G542X/V232D	1	F508del/1811+1.6kbA>G	1
G542X/1811+1.6kb A>G[a]	1	F508del/2789+5G>A[a]	1
G542X/2789+5G>A	1	F508del/2869insG	1
Q890X/L206W	1	F508del/unknown	1
1811+1.6kbA>G/P205S	1	I507del/I507del	1
R1162X/3272-26A>G	1	G542X/1078delT	1
N1303K/R347H	1	G542X/1811+1.6kbA>G[a]	1
N1303K/A1006E+5T	1	S549R/CFTR50kbdel	1
2789+5G>A/405+1G>A	1	R1066C/R1066C	1
		W1282X/712-1G>T	1

[a]CF patient with a sibling presenting identical *CFTR* genotype and discordance of intestinal phenotype.

3′ *ATB⁰* polymorphism, single nucleotide polymorphism (SNP) and mutational analysis

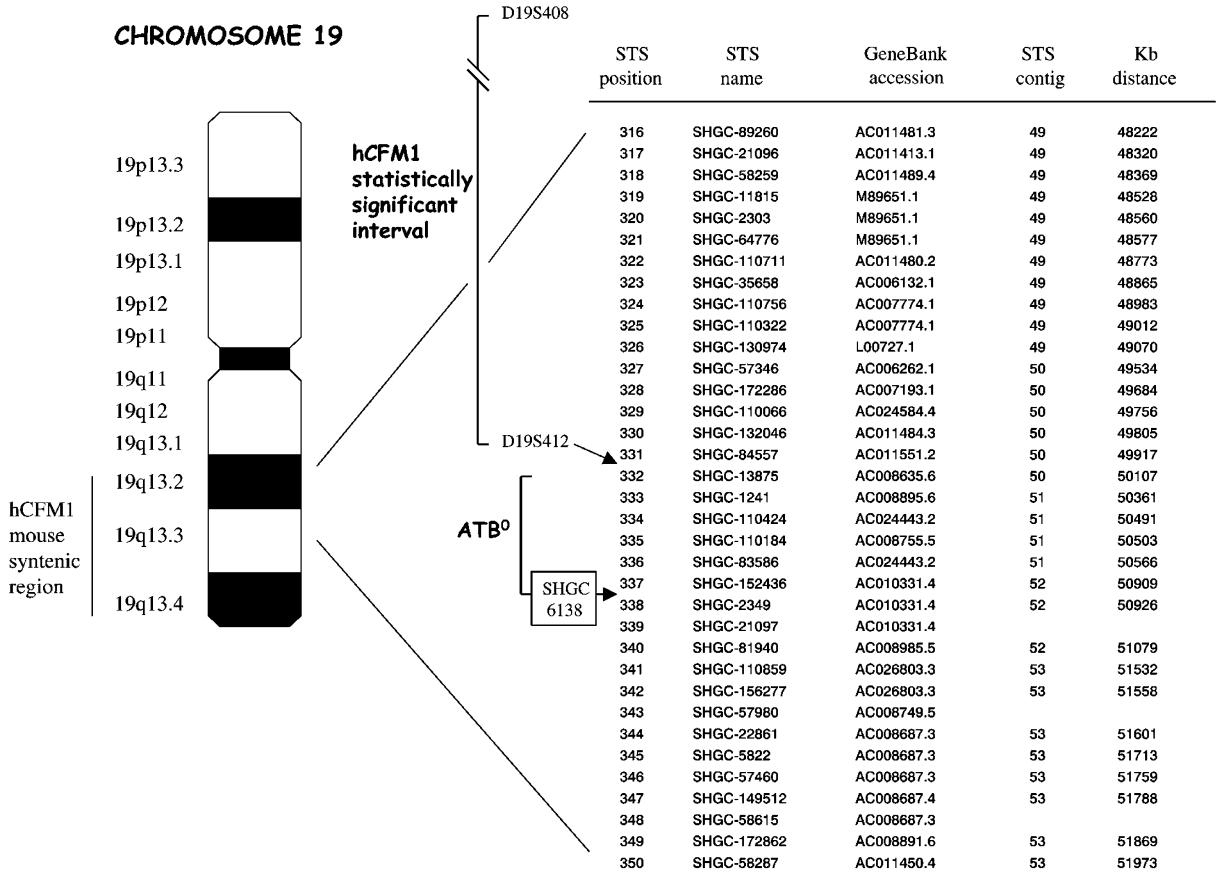
The polymorphic $(GT)_n$ sequence in the *ATB⁰* 3′-UTR was analysed as previously described[4] in 23 CF-nonMI and 25 CF-MI patients. This microsatellite was also analysed in 23 individuals of the general population in order to determine the $(GT)_n$ allelic distribution in Spanish population. The results are shown in Table 4. The GT repeat lengths in each group were compared with those in the others showing similar results: the mean of the $(GT)_n$ allele was 167.1 in general population, 167.5 in CF-nonMI and 166.7 in CF-MI. The frequency of each allele was also compared *vs* the frequency of the rest of the alleles among the different groups. No statistical difference was detected, except for allele 167, which was significantly more frequent (χ^2= 0.2; P=0.02) when comparing general population and CF patients, but not between CF subgroups (χ^2=0.01; $P>0.99$). Although the frequency of allele 169 was increased in the CF-MI subgroup (Table 4), there was no statistical significance when compared with the general population (χ^2=0.13, P=0.14) nor with CF-nonMI (χ^2= 0.3; P=0.35). Analysis of the distribution of the $(GT)_n$ alleles in each group of individuals showed that they were not normally distributed (P=0.010). The results of applying the Kruskal-Wallis test showed no significant difference among the three groups (P=0.604). When a binary logistic regression model was fitted to the data, with the phenotype as the response and the GT repeat length as the predictor, no significant effect of the GT repeat length was observed (CF-MI/CF-nonMI P=0.25, estimated odds ratio=93 and 95% CI=0.82–1.05; general population/CF P=0.93, estimated odds ratio=0.99 and 95% CI=0.89–1.11; general population/CF-MI P=0.5, estimated odds ratio=0.95 and 95% CI=0.83–1.09).

We have also analysed 606 bp of the 5′-UTR, the complete sequence of human *ATB⁰* coding exons and their respective intronic flanking regions for the presence of mutations in CF-MI and CF-nonMI individuals. The sequence differs from the published sequence of the human neutral amino acid transporter *hATB⁰* (GeneBank accession no. U53347). We confirmed the existence of four errors in the original sequence, as published previously[6] (accession no. AF105230), and detected two additional differences: the insertion of G in the position 129 (5′-UTR) and the presence of a point mutation (T>A), leucine changes to glutamine, in position 750. To further investigate these differences, we sequenced 25 additional cDNAs from lymphocytes from individuals of the general population (data not shown) confirming that they were errors in the original sequence of *hATB⁰*. A new nucleotide sequence (NM005628) is now available and is in agreement with our results. An additional comparison between U53347 and NM005628 sequences (using Blast N program) showed the presence of a C in position 410 of sequence U53347, that was absent in NM005628. We determined the presence of this nucleotide indicating that there is an error in NM005628 in the corresponding position (380insC).

On the other hand, several different SSCP migrating patterns were identified among 25 CF patients with MI. Sequencing of abnormal bands showed the presence of 14 nucleotide changes along the *ATB⁰* gene (Table 5). Four of these changes were found in the 5′-UTR (three nucleotide changes and one small deletion), six in the non-coding (introns 2, 3, 4, 6) and four in the coding regions (exons 1, 5, 8). Two of these amino acid changes resulted in amino acid substitutions: proline in position 17 to alanine, P17A (668C>G) in exon 1, and valine in position 512 to leucine, V512L (2153G>C) in exon 8. Two additional nucleotide changes C>T in position 1447 (exon 5) and T>C in position 2074 (exon 8) did not result in an amino acid change, thus they are polymorphic substitutions. Frequencies are detailed in Table 5. Nucleotide substitutions 272C>T, 337del6 and 349C>T in the 5′-UTR were found to be associated with the P17A mutation, while 1229-4A>G is associated to 1229-106A>G in unrelated patients both in CF-MI and CF-nonMI.

In order to evaluate a potential involvement of these nucleotide changes in the intestinal phenotypic variation of the CF disease, we analysed the presence of these SNPs in 23 CF-nonMI subjects. We found similar frequencies as in CF-MI patients and no statistically significant differences for these changes (Table 5).

Frequencies for P17A and V512L in the general population were also determined. Statistical analysis showed that the higher incidence for P17A and the lower incidence for V512L observed in the general population



STS position	STS name	GeneBank accession	STS contig	Kb distance
316	SHGC-89260	AC011481.3	49	48222
317	SHGC-21096	AC011413.1	49	48320
318	SHGC-58259	AC011489.4	49	48369
319	SHGC-11815	M89651.1	49	48528
320	SHGC-2303	M89651.1	49	48560
321	SHGC-64776	M89651.1	49	48577
322	SHGC-110711	AC011480.2	49	48773
323	SHGC-35658	AC006132.1	49	48865
324	SHGC-110756	AC007774.1	49	48983
325	SHGC-110322	AC007774.1	49	49012
326	SHGC-130974	L00727.1	49	49070
327	SHGC-57346	AC006262.1	50	49534
328	SHGC-172286	AC007193.1	50	49684
329	SHGC-110066	AC024584.4	50	49756
330	SHGC-132046	AC011484.3	50	49805
331	SHGC-84557	AC011551.2	50	49917
332	SHGC-13875	AC008635.6	50	50107
333	SHGC-1241	AC008895.6	51	50361
334	SHGC-110424	AC024443.2	51	50491
335	SHGC-110184	AC008755.5	51	50503
336	SHGC-83586	AC024443.2	51	50566
337	SHGC-152436	AC010331.4	52	50909
338	SHGC-2349	AC010331.4	52	50926
339	SHGC-21097	AC010331.4		
340	SHGC-81940	AC008985.5	52	51079
341	SHGC-110859	AC026803.3	53	51532
342	SHGC-156277	AC026803.3	53	51558
343	SHGC-57980	AC008749.5		
344	SHGC-22861	AC008687.3	53	51601
345	SHGC-5822	AC008687.3	53	51713
346	SHGC-57460	AC008687.3	53	51759
347	SHGC-149512	AC008687.4	53	51788
348	SHGC-58615	AC008687.3		
349	SHGC-172862	AC008891.6	53	51869
350	SHGC-58267	AC011450.4	53	51973

Figure 1 Schematic representation of human chromosome 19, indicating the region harbouring the *ATB^0* gene. hCFM1 mouse syntenic region comprises from 19q13.2 to 19q13.4 (D19S408-telomere).[14] hCFM1 statistically significant interval was defined as a region of 3.75 Mb (D19S408 – D19S412) that presented statistically significant association to the modulation of the intestinal phenotype of CF.[14] STS data were extracted from the Science human genome supplementary information[17] (http://www. sciencemag.org). *D19S412* marker is located 5′ immediately from marker SHGC-13875 (GeneMap 99 information http://www.ncbi.nlm.nih.gov/genemap99/). SHGC-6138 (associated to *ATB^0* on TNG RH panel) is the marker located immediately 5′ to SHGC-2349 (GeneMap 99 information http:// www.ncbi.nlm.nih.gov/genemap99/).

Table 4 Distribution of GT repeat alleles within the *ATB^0* gene of CF patients with and without intestinal phenotype and of general population individuals

(GT)n Allele Size	*General population* n	*Frequency (%)*	*CF-nonMI* n	*Frequency (%)*	*CF-MI* n	*Frequency (%)*
163	9	19.5	11	23.9	12	24
165	7	15.2	7	15.2	11	22
167	15	32.6	7	15.2	8	16
169	7	15.2	9	19.5	14	28
171	3	6.5	5	10.8	1	2
173	5	10.8	7	15.2	4	8

were not significantly different when compared with the frequency in CF-MI subgroup (Table 5).

The distribution of individuals with two mutations (P17A/P17A, P17A/V512L) in the CF-MI and CF-nonMI groups indicated no association between these double heterozygous genotype and the presence or absence of MI phenotype (Table 5).

Discussion

Despite the *ATB^0* gene mapping to 19q13.3 by Jones *et al*,[4] fine localisation of *ATB^0* had not been shown. This lack of information has resulted in the gene being absent from the Gene Map database (http://www.ncbi.nlm.nih.gov/genemap99/) even 5 years after its original description. Our RH panel mapping results in fine chromosomal localisation and

Table 5 Nucleotide and amino acid changes in *ATB⁰* in cystic fibrosis (CF) patients with and without meconium ileus (MI)

ATB⁰ *region*	*Nucleotide change**	*Amino acid change*	*CF-MI (%)*	*CF-nonMI (%)*	P *value (CF-MI/CF-nonMI)*	*GP (%)*	P *value (GP/CF-MI)*
5′-UTR	281 T>G	–	12/25 (48)	10/23 (43.5)	0.78		
5′-UTR	272 C>T	–					
5′-UTR	337del6#	–					
5′-UTR	349 C>T	–					
E1	668 C>G	P17A	11/25 (44)	9/23 (39)	0.77	16/25 (64)	0.17
I1	1186–123 G>A	–	23/24 (95.8)	21/23 (91.3)	0.61		
I2	1229–4 A>G	–	2/24 (8.3)	1/23 (4.3)	0.49		
I2	1229–106 A>G	–					
I4	1443+51 G>T	–	20/21 (95)	22/23 (95.5)	>0.99		
E5	1447 C>T	silent	7/23 (30)	10/23 (43.5)	0.54		
I5	1678–27 C>G	–	2/20 (10)	3/22 (13.6)	>0.99		
I5	1678–127 A>G	–	3/20 (15)	1/22 (4.5)	0.33		
E8	2074 T>C	silent	3/23 (13)	5/23 (21.7)	0.47		
E8	2153 G>C	V512L	12/23 (52)	10/23 (43.5)	0.76	6/23 (26.1)	0.13
		P17A/V512L	5/25 (20)	1/23 (4.3)	0.19	2/23 (8.7)	0.42
		P17A/P17A	1/25 (4)	2/23 (8.7)	0.60	4/25 (16)	0.34

*Positions of nucleotide change are named taking GeneBank accession no. U53347 as reference. #Deletion of TAGGGC from 337. GP: general population.

supports the 19q13.3 location of *ATB⁰*.[4] We determined the fine chromosomal location of *ATB⁰* in 19q13.3 between markers SHGC-13875 (*D19S995*) – SHGC-6138. At the time of the elaboration of this article, a more accurate RH map was published.[17] This fact has allowed us to map *ATB⁰* more accurately, between 50107 and 50926 kb of chromosome 19 draft sequence (Figure 1). This location is within the hCFM1 syntenic region that comprises the locus that modulates the intestinal phenotype of CF patients localised in chromosome 19q13.2–13.4.[14] Our RH panel results led us to place *ATB⁰* immediately telomeric to the 3.75 Mb region (*D19S408*–*D19S412*) inside the hCFM1 syntenic region (Figure 1), a region demonstrated to present the strongest linkage with MI.[14]

ATB⁰ was evaluated as a potential modulator of CFTR activity on the basis of the position in relation to the hCFM1 syntenic region, functional characteristics of the encoded protein and their apparent relevance to MI phenotype.

In order to perform an exhaustive mutational analysis of the gene, we determined the genomic structure of *ATB⁰* that consists of eight exons whose sizes vary from 43 bp (exon 2) to 566 bp (exon 1).

Due to the potential relationship of *ATB⁰* gene to the intestinal phenotypical variation of CF, we analysed a simple sequence repeat DNA polymorphism in *ATB⁰* 3′-UTR $(GT)_n$ and examined the *ATB⁰* gene for sequence variations that could be related with the presence or absence of meconium ileus in CF patients. The estimation of the association of a specific allele of the 3′-UTR polymorphism with intestinal phenotype of CF showed no statistical differences in the CF-MI group as compared to CF-nonMI, nor in the general population (Table 4). Only allele 167 was found in a statistically different frequency in the general population when compared with CF, suggesting that this gene may be modifying CF in general and not only the intestinal phenotype of CF. As regards sequence variations, 14 nucleotide changes were identified (Table 5), 10 of them were found in the non-coding regions and four in the coding regions of the gene. Two of these nucleotide changes were translated into amino acid substitutions, P17A in exon 1, and V512L in exon 8. The first change implies the presence of a smaller and hydrophobic amino acid in position 17 and the second, in position 512, varies exclusively the size of the amino acid and not the charge. Moreover, these changes affect neither the conserved regions in the family of amino acid transporters (ex. ASCT1 present K instead of P in position 17 and E instead of V in position 512), nor the transmembrane domains of the protein,[7,18] so that they are not predicted to greatly affect the properties of the protein. Although the groups of patients are fairly small (only 15–20% of patients present MI), statistical analysis suggested that none of the changes identified is associated to the intestinal phenotype of CF, as their frequencies were found to be similar in both CF-MI and CF-nonMI subjects. The studies in CFTR knockout mice[13] showed that there was an increased number of mice homozygous for the syntenic region associated to the modulation of the intestinal problem. In our study, no association was determined between the presence of two mutations and a specific intestinal phenotype either. Our results suggest that there is no evidence to support that these *ATB⁰* variants alone contribute to the intestinal phenotypical variation of CF. Zielenski *et al*[14] found no particular allele or haplotype associated with the MI phenotype from the preliminary analysis of a potential transmission disequilibrium of hCFMI, suggesting the potential involvement of multiple variants or mutations on multiple loci at the modifier locus site. Our results may help to define precise

haplotypes that could have a synergetic effect on other modifier loci in the same region.

SNPs are stable inherited, biallelic, single base pair differences which are present in the human genome at a density of 1 to 10 per 1000 nucleotides. It is thought that these SNPs contribute to the functional heterogeneity in gene expression and protein activity among individuals. Although no particular allele or haplotype in the *ATB^0* gene appears to be associated to CF-MI disease, new SNPs identified should be useful in segregation and linkage analysis in families affected by the impairment of neutral amino acid transport such as Hartnup disease, believed to be caused by a genetic defect in a specific system responsible for neutral amino acid transport across the brush-border membrane of renal and intestinal epithelium. Knowledge of DNA sequence variation may help us understand how genetic variability leads to functional variability, and will contribute to the development of new therapies in order to re-establish the normal phenotype.

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References

1 Christensen HN: Role of amino acid transport and countertransport in nutrition and metabolism. *Physiol Rev* 1990; **70**: 43 – 77.
2 Stevens BR: Vertebrate intestine apical membrane mechanisms of organic nutrient transport. *Am J Physiol* 1992; **263**: R458 – R463.
3 Kekuda R, Prasad PD, Fei Y-J, *et al*: Cloning of the sodium-dependent, broad-scope, neutral amino acid transporter B^0 form a human placental choriocarcinoma cell line. *J Biol Chem* 1996; **271**: 18657 – 18661.
4 Jones EMC, Menzel S, Espinosa R, Le Beau MM, Bell GI, Takeda J: Localisation of the gene encoding a neutral amino acid transporter-like protein to human chromosome band 19q13.3 and characterisation of a simple sequence repeat DNA polymorphism. *Genomics* 1994; **23**: 490 – 491.
5 Kekuda R, Torres-Zamorano V, Fei YJ, *et al*: Molecular and functional characterization of intestinal Na(+)-dependent neutral amino acid transporter B0. *Am J Physiol* 1997; **272**(6 Pt 1): G1463 – 1472.
6 Rasko JE, Battini JL, Gottschalk RJ, Mazo I, Miller AD: The RD114/simian type D retrovirus receptor is a neutral amino acid transporter. *Proc Natl Acad Sci USA* 1999; **96**: 2129 – 2134.
7 Palacín M, Estevez R, Bertrán J, Zorzano A: Mammalian plasma membrane amino acid transporters. *Physiol Rev* 1998; **78**: 969 – 1054.
8 Kerem B, Rommens JM, Buchanan JA, *et al*: Identification of the cystic fibrosis gene: genetic analysis. *Science* 1989; **245**: 1073 – 1080.
9 Boucher RC, Stutts MJ, Knowles MR, Cantley L, Gatzy JT: Na+ transport in cystic fibrosis respiratory epithelia. Abnormal basal rate and response to adenylate cyclase activation. *J Clin Invest* 1986; **78**: 1245 – 1252.
10 Schwiebert EM, Benos DJ, Egan ME, Stutts MJ, Guggino WB: CFTR is a conductance regulator as well as a chloride channel. *Physiol Rev* 1999; **79**: S145 – S166.
11 Mall M, Bleich M, Kuehr J, Brandis M, Greger R, Kunzelmann K: CFTR-mediated inhibition of epithelial Na+ conductance in human colon in defective cystic fibrosis. *Am J Physiol* 1999; **277**: G709 – G716.
12 Wu JV, Joo NS Krouse M, Wine JJ: CFTR gating requires cytosolic electrolytes. *J Biol Chem* 2001; **276**: 6473 – 6478.
13 Rozmahel R, Wilschanski M, Matin A, *et al*: Modulation of disease severity in cystic fibrosis transmembrane conductance regulator deficient mice by a secondary genetic factor. *Nat Genet* 1996; **12**: 280 – 287.
14 Zielenski J, Corey M, Rozmahel R, *et al*: Detection of a cystic fibrosis modifier locus for meconium ileus on human chromosome 19q13. *Nat Genet* 1999; **22**: 128 – 129.
15 Stewart EA, McKusick KB, Aggarwal A, *et al*: An STS-based radiation hybrid map of the human genome. *Genome Res* 1997; **7**: 422 – 433.
16 Kunkel LM, Smith KD, Boyer SH, *et al*: Analysis of human Y-chromosome specific reiterated DNA in chromosome variants. *Proc Natl Acad Sci USA* 1977; **74**: 1245 – 1249.
17 Olivier M, Aggarwal A, Allen J *et al*: A High-Resolution Radiation Hybrid Map of the Human Genome Draft Sequence. *Science* 2001; **291**: 1298 – 1302.
18 Arriza JL, Eliasof S, Kavanaugh MP, Amara SG: Excitatory amino acid transporter 5, a retinal glutamate transporter coupled to a chloride conductance. *Proc Natl Acad Sci USA* 1997; **94**: 4155 – 4160.