primary transcript occurs preferentially if not completely at the aberrant splice site. The predicted protein encoded by the mutated transcript contains intact coding sequences for all double zinc finger domains except for the double zinc finger 4 in which the carboxy-terminal finger motif is interrupted. How this mutation might lead to the phenotype remains to be elucidated. It has previously been shown in the *Drosophila* transcription factor *Krüppel* that a missense mutation replacing one of the conserved cysteine residues within the second of five tandemly arranged finger motifs results in a null allele.[8] Therefore, the splice mutation reported here is likely to result in loss of biological function of the most carboxy-terminal double zinc finger domain. It remains unclear if this is sufficient to result in *SALL1* haploinsufficiency causing TBS. The predicted mutant protein is 116 amino acids shorter than the wild type protein and contains different carboxy-terminal amino acids because of the frameshift. An alternative explanation for the effect of the mutation could therefore be that the changed three dimensional structure of the mutated protein results in a non-functional SALL1 protein which is unstable or not able to bind to its target sequences.

The phenotype of the severely affected family members reported here is not significantly different from other TBS cases in which classical truncating mutations were found. Therefore, we assume that all mutations shown in this report will lead to haploinsufficiency for *SALL1*, as suggested to be the common result of all mutations previously reported.[4]

Data access: GenBank: http://www.ncbi.nlm.nih.gov/. Accession numbers: Y18264 (*SALL1* exon 1 and intron 1 genomic sequence (partial)), Y18265 (full *SALL1* coding sequence), X98833 (*SALL1* genomic sequence of intron 1 (partial), exons 2 and 3 and intron 2). Mutation accession numbers (Human Genetics Online Mutation Data Submission): H971415 (840delC), H971417 (IVS2-19T>A). Online Mendelian Inheritance in Man (OMIM): http://www.ncbi.nlm.nih.gov/OMIM (for Townes-Brocks syndrome, OMIM 107480).

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Genotype-phenotype correlation in three homozygotes and nine compound heterozygotes for the cystic fibrosis mutation 2183AA→G shows a severe phenotype

EDITOR—Cystic fibrosis (CF) is the most common lethal childhood disorder in white populations and occurs at a frequency of about 1/2500 with regional variations. Over 1000 mutations in the CF transmembrane conductance regulator (*CFTR*) gene accounting for the disease have been identified so far and the most common gene mutation is ΔF508.[1] The frameshift mutation 2183AA→G in exon 13 was first described in three Canadian CF patients[2] and later was shown to have a significant frequency in patients from mid and southern Europe. The frequency among CF patients is 9.3% in north east Italy,[3] 2.4% in the Tyrol,[4] 1-2.1% in Belgium,[3] 1.8% in Greece,[5] 1% in Bavaria, Bulgaria, and France,[3] and 0.4% in mid and northern Germany.[6] We identified three homozygotes among 120 Turkish patients (2.5%), two born to first cousin parents, three compound heterozygotes among 185 Bulgarian patients (0.8%), and seven compound heterozygotes among 650 Spanish patients (0.5%).[7] The mutation was detected by denaturing gradient gel electrophoresis or single strand conformational analysis followed by DNA sequence analysis.

We report here the genotype-phenotype correlation in 12 patients with CF with the mutation 2183AA→G (three homozygous and nine compound heterozygous for 2183AA→G and other mutations). The anamnestic, clinical, and laboratory data are summarised in table 1. Pancreatic insufficiency (PI) was assessed by the fat content of stools and requirement of pancreatic enzyme replacement therapy. Gastrointestinal symptoms (GI) are abdominal cramps and pain and frequent passage of foul and fatty faeces. The presence of pulmonary symptoms was defined as having at least one of the following clinical findings: increased rate of breathing, wheezing, dark coloured/profuse sputum, and recurrent attacks of coughing. Dehydration includes at least one of the following: decreased skin tonus and turgor, decreased output of urine, and sudden weight loss. The presence of bronchiectasis was evaluated by chest *x* rays and thin section computerised chest tomography.

Patient 1 was homozygous for 2183AA→G. She was admitted to hospital at 2 months of age and died within a week. Clinical findings were clearly of CF with pancreatic insufficiency. The second homozygous patient (patient 2) was examined for CF because all his four sibs had died of the disease before the age of 1 year. He had pulmonary insufficiency at 15 days. He had fatty and foul stools, bronchial hyperactivity, and early *Pseudomonas* colonisation. As a result of medical treatment, he no longer has steatorrhoea

Table 1 Anamnestic, clinical, and laboratory data for CF patients carrying the mutation 2183AA→G

Patient No	1	2	3	4	5	6	7	8	9	10	11	12
Nationality	Turkish	Turkish	Turkish	Bulgarian	Spanish	Spanish	Spanish	Spanish	Spanish	Spanish	Bulgarian	Bulgarian
Second CF mutation	2183AA→G	2183AA→G	2183AA→G	ΔF508	ΔF508	ΔF508	ΔF508	ΔF508	G542X	G542X	G1244E	2789+5G→A
Sex	F	M	M	F	M	F	M	M	F	F	F	F
Onset of symptoms	25 d	15 d	2 mth	At birth	3 mth	1 mth	At birth	ND	ND	ND	3 mth	2 mth
Meconium ileus	No	No	No	Yes	No	No	Yes	No	No	No	No	No
Disease progression	Died 2 mth	Severe	Severe	Died 1 mth	Moderate	Moderate	Severe	Moderate	Moderate	Moderate	Died 12 y	Severe
No of affected sibs	0	4	1	0	0	1 (11 y)	0	1 cousin	0	0	0	0
Disease progression for CF sibs	—	All died <1 y	Died 10 mth	—	—	Moderate	—	ND	—	—	—	—
Data at diagnosis												
Age at diagnosis	2 mth	15 d	4 mth	At birth	3 mth	1 mth	2 mth	3 mth	4 y	1 y	3 mth	2 mth
Sweat Cl- (mEq/l)	ND	99	80	ND	100	100	100	90	93	110	132	130
Pancreatic insufficiency	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Failure to thrive	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No
Gastrointestinal symp	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No
Pulmonary symp	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	No	Yes	Yes
Dehydration	Yes	No	No	No	No	Yes	Yes	No	No	No	No	No
Data at latest visit												
Age	2 mth	7.5 y	7 y	—	2 y 6 mth	6 y	8 y	14 y	14 y	3 y 6 mth	12 y	7 y
Weight centile	3	25–50	25	—	ND	<3	ND	10–25	ND	ND	10–25	ND
Lung colonisation with bacterial pathogens	—	Pa*	Pa	—	No	No	Pa	Pa	No	No	Pa, Sa	No

*Before medication. ND = not determined. Pa = *Pseudomonas aeruginosa*. Sa = *Staphylococcus aureus*.

or *Pseudomonas* infection. The third homozygous patient (patient 3) was diagnosed early because his brother died of similar clinical findings at the age of 10 months. The clinical symptoms were gastrointestinal and pulmonary; in addition, vitamin deficiency, malnutrition, and severe anaemia (probably resulting from severe vitamin A deficiency) were observed. At present, he has early *Pseudomonas* colonisation, steatorrhoea, recurrent lung problems, and malnutrition.

The remaining nine patients are all compound heterozygotes. Patients 4-8 carry ΔF508 as the other CF allele. Patients 4 and 7 were diagnosed with meconium ileus. Other clinical data available for patient 4 are malnutrition, chronic respiratory insufficiency, and steatorrhoea. Patient 7 also has β thalassaemia. Patient 8 has bronchiectasis. Patients 9 and 10 carry the nonsense mutation G542X[8] on the other CFTR chromosome. Patient 9 has hepatomegaly, probably resulting from nutritional deficiency. Patient 10 was first diagnosed as having coeliac disease, then CF as well, and also has anorexia. Patient 11 carried the missense mutation G1244E[9] on the other CFTR chromosome. Pancreatic insufficiency was confirmed by a fat load test, which showed a poor rise in the plasma triglyceride level and an excretion of 60 mmol/day (normal is 20 mmol/day). An ultrasound examination of the liver showed a normal sized liver with markedly increased echogenicity, suggesting hepatic involvement. The enlarged portal vein had a diameter of 12 mm and the spleen and kidneys were normal. A chest radiograph showed widespread peribronchial thickening with interstitial markings, but there were no areas of atelectasis, and in general the changes were not severe. Two years later the chest radiograph showed a quite marked deterioration with much more widespread bronchiectatic changes. Patient 12 has 2789+5G→A, a splice site mutation,[10] on the other CFTR chromosome. She has recurrent respiratory infections.

The mutation 2183AA→G causes premature termination of translation 38 codons downstream on exon 13. The clinical data presented for three patients homozygous for the mutation and eight compound heterozygous patients who carry a severe mutation (ΔF508, G542X, and G1244E) on the other CFTR chromosome indicate that the mutation causes a severe CF phenotype. Severe pancreatic insufficiency is the most common clinical feature, being exhibited by all these 11 patients. Pancreatic insufficiency had also been reported for all three Canadian compound heterozygous patients.[2] Current moderate progression of the disease in some of these patients is probably the result of treatment with pancreatic enzyme supplements and antibiotics. The disease phenotype is also severe in the compound heterozygote with the mutation 2789+5G→A. It has been shown that this mutation has a mild phenotype which allows synthesis of some normal mRNA.[10]

The phenotype of the mutation 2183AA→G was assessed to be severe with pancreatic involvement, failure to thrive, and variable lung involvement (9/12 patients). In 5/10, colonisation with bacterial pathogens was observed. Two patients died too young (1-2 months) for bacterial colonisation to be assessed. Two of the ΔF508/2183AA→G patients had meconium ileus. The mutation may cause various other complications, with two patients exhibiting hepatic involvement and two bronchiectasis. All patients studied were diagnosed very early. Grouping the patients and their sibs together, six homozygotes died within the first year of life and two compound heterozygotes died at the ages of 1 month and 12 years.

In most of our heterozygous patients, the *CFTR* gene was only partially screened for mutations using either DGGE or SSCP. Thus, it is possible but unlikely that some

of these patients carry a third CF mutation. Spanish and Turkish patients were analysed for the IVS8-6(T) alleles[11] and the mutation 2183AA→G was found to be associated with the allele 7T, except in patients 1 and 3, who were homozygous for the allele 9T. Spanish and Turkish patients were also studied for the microsatellite loci IVS8CA, IVS17bTA, and IVS17bCA.[12–14] While all six Spanish patients shared the same haplotype (16-30-13) for the mutation 2183AA→G, Turkish patients were homozygous for two other haplotypes, 16-31-13 (patients 1 and 3) and 16-32-13 (patient 2). These three haplotypes are among the most common on normal chromosomes[15] and each can be derived from any of the other two. Alternatively, the mutation may have arisen independently in the two populations or even within the Turkish population. Despite the possible heterogeneous genetic background observed, in particular between the homozygous patients, the severity of the disease is similar.

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Alkaptonuria in Italy: polymorphic haplotype background, mutational profile, and description of four novel mutations in the homogentisate 1,2-dioxygenase gene

EDITOR—Alkaptonuria (AKU, OMIM 203500) is a rare disorder caused by the deficiency of homogentisate 1,2 dioxygenase (HGO, EC 1.13.11.5).[1] HGO catalyses the conversion of homogentisate (HGA) to maleylacetoacetate in the phenylalanine/tyrosine catabolic pathway.[2] As a consequence, affected subjects excrete HGA in their urine, which becomes dark upon exposure to air. The medical interest in this condition stems from its association with ochronosis, or the deposition of a brownish pigment in connective tissues including cartilage, where its accumulation can produce a debilitating degenerative joint disease.[3]

AKU occupies a unique place in the history of human genetics because it was the first disorder to be described as a Mendelian recessive trait.[4–6] Recent advances in the understanding of the molecular basis of AKU[7–9] have verified that loss of function mutations in the *HGO* gene are responsible for the disease. A few mutations have been repeatedly detected in patients from different European countries. Since these mutations segregated with specific haplotypes, they should be considered to be old mutations that have spread throughout western Europe with migration. However, allelic heterogeneity is the main feature emerging from the above and other studies.[7 9–12] Most *HGO* mutations were found in just one family and did not involve CpG dinucleotides. Rather, a preferential occurrence in the CCC sequence motif and its inverted complement GGG has recently been reported.[12] Furthermore, some AKU chromosomes escaped mutation detection within the *HGO* coding region, suggesting the existence of *HGO* alleles whose defect might be related to gene expression.

To determine the extent of allelic heterogeneity in Italian patients, we started a systematic search of AKU families through announcements at relevant National Congresses. We present here the results leading to the identification of four novel mutations. Our data should facilitate future mutation screening in Italian AKU patients and carrier identification by DNA typing.

Ten affected subjects from five unrelated Italian AKU pedigrees were included in the study. In three families it turned out that the patients' parents were consanguineous. Three patients were diagnosed at birth as having AKU through analysis of homogentisic aciduria. The remaining