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N1303K and IVS8-5T, clinical presentation within a family with atypical cystic fibrosis

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Abstract

The CFTR genotype N1303K/IVS8-5T can cause very mild cystic fibrosis (CF) and congenital bilateral absence of the vas deferens (CBAVD). We report one family consisting of five affected patients in two generations, presenting minor symptoms of CF at different ages, segregating the CFTR mutations N1303K and IVS8-T5-TG13 *in trans*. Common features were chronic sinopulmonary symptoms and borderline or slightly elevated sweat chloride values. One patient had CBAVD.

Keywords: Cystic fibrosis; CFTR; N1303K; 5T; TG13; Borderline sweat chloride; CBAVD

1. Introduction

The length of the polythymidine tract in intron 8 (IVS8-Tn polymorphism) of the cystic fibrosis transmembrane regulator (CFTR) gene affects the splicing efficiency of intron 8. Three alleles have been identified in IVS8, i.e. 9T, 7T and 5T. The 9T allele is associated with the most efficient usage of the intron 8 splice acceptor site and allows normal reading of the gene [1]. The splicing efficiency decreases with shorter polythymidine tracts (7T and 5T), which results in a lower than normal level of full-length CFTR mRNA and presumably a decrease in mature, functional CFTR protein. IVS8-5T is considered equivalent to a "mild" CFTR mutation with incomplete penetrance and is associated with the highest level of non-functional CFTR protein. In the general population, this 5T variant has a frequency of 5.2% [2] and has been found with a higher frequency in patients with CBAVD [2], pancreatitis [3], neonatal hypertrypsinemia [4] and chronic sinopulmonary disease [5,6]. Patients have been reported with a CF mutation *in trans* with IVS8-5T [2,6,7].

The variation in phenotypic expression of IVS8-5T can be explained by other polymorphic CFTR loci, such as the TGm locus localized immediately upstream of the Tn locus [8,9]. This IVS8-TGm polymorphism (with alleles ranging from 9 to 13 repeats) can further modulate exon 9 skipping but only when activated by the T5 allele: *in cis* with T5 the splicing efficiency of intron 8 decreases with longer TG tracts. The alleles T5-TG12 and T5-TG13 are found more frequently in affected patients than in controls [10].

We describe a patient with atypical CF and 4 family members with the same genotype, N1303K *in trans* with the IVS8-T5-TG13 allele (Fig. 1). The propositus was detected when she developed pneumonia after a history of chronic cough and recurrent respiratory infections. Genetic counselling identified four additional relatives. Apparently the mother and sister also suffered from chronic sinopulmonary symptoms, and borderline or slightly elevated sweat chloride concentration. One aunt showed a history of recurrent

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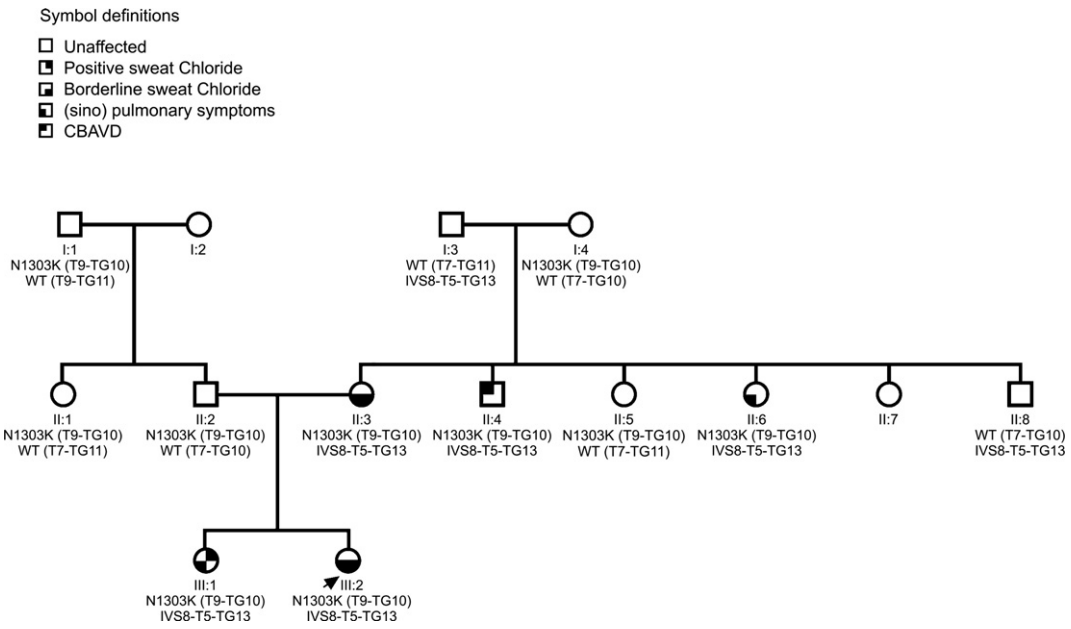

Fig. 1. Dummy.

bronchitis and sinusitis, one uncle was known to have azoospermia and CBAVD.

2. Case report

The propositus (III.2), a 17-month-old girl, presented with a history of chronic cough and recurrent respiratory infections which were treated with oral antibiotics. The neonatal period was uneventful. Weight and height were on the 50th percentile. At the age of 17 months she developed high fever and chest X-ray showed a consolidation in the right middle lobe. She was hospitalized and the pneumonia was treated with IV antibiotics. A sweat test was performed because of the history of chronic respiratory symptoms. Sweat chloride values ranged from 48 to 64 mEq/l on 4 occasions. Pancreas elastase was normal, i.e. >250 μg/g faeces (normal values are >200 μg/g faeces). At follow up, 6 months after the event, chest X-ray showed residual infiltrate. Molecular analysis of the CFTR gene was performed by screening 35 frequent mutations in the Caucasian population (Inno Lipa™ CFTR19 and CFTR17+Tn, Innogenetics). This revealed one heterozygous mutation N1303K and an IVS8-Tn genotype T5/T9. Sequence analysis of the intron 8/exon 9 boundary (at the TGm locus) and segregation analysis performed in this family led us to conclude that for the propositus (and for the other affected relatives) the mutation N1303K is *in trans* with the IVS8-T5-TG13 allele, confirming a diagnosis of (atypical) CF. DGGE analysis of the full CFTR coding sequence including all 27 exons and the flanking splice sites was completed to exclude the presence of other mutations. Her chronic cough has resolved by treating her with nebulized mucolytics and physiotherapy. Current therapy recommendations include mucolytics and physiotherapy at time of respiratory symptoms.

As part of genetic counselling, DNA samples of family members were analyzed. The mother (II.3) and sister (III.1) of the propositus appeared to have the same mutations, i.e. N1303K *in trans* with T5-TG13. The father (II.2) seemed to be heterozygous for the mutation N1303K in trans with 7T. According to the parents of the propositus it is very likely that they would be related to each other, because mother and paternal great-grandmother of the propositus have the same family name and both families originate from the same region in Belgium.

The 3-year-old sister (III.1) suffered intermittently from chronic cough, which was treated with oral antibiotics. Height followed the 50th percentile and weight the 25th percentile. Sweat chloride value was 75 mEq/l, but pancreas elastase was >500 μg/g faeces, suggesting no pancreatic involvement. Chest X-ray revealed no abnormalities.

The mother (II.3) of the patients described above, mentioned chronic rhinitis and sinusitis. BMI was 24.5, i.e. normal. Sweat chloride concentration was 61 mEq/l and pancreas elastase was normal (496 μg/g faeces). She recently had an event of pleuritis, treated with antibiotics. Since this episode, she kept feeling tired and dyspnoeic and the coughing persisted. Her GP prescribed inhaled steroids and long acting beta-agonist. Forced expiratory volume in one second (FEV1) was 100% (with inhaled medication) and chest X-ray is normal. Recommendations for the mother and sister are the same as for the propositus.

Two additional family members with the same genotype were found after genetic counselling. The aunt of the propositus (II.6) also spoke of a history of recurrent bronchitis and sinusitis. Further investigations showed that she had a BMI of 19.1, a FEV1 of 94%, and chest X-ray was normal. She did have colonization of the sputum with Pseudomonas, which resolved with oral anti-pseudomonal

antibiotic treatment. Pancreatic function was normal, with pancreas elastase showing a value of 243 μg/g faeces. Recommendations are also mucolytics and physiotherapy when symptoms appear to reoccur.

The uncle's DNA (II.4) had already been analyzed a few years earlier due to sterility. He had been diagnosed with azoospermia and congenital bilateral absence of the vas deferens (CBAVD). No information is currently available regarding pulmonary symptoms.

3. Discussion

The 5T variant in the intron 8 polythymidine tract (IVS8-5T) is a common allele in the general population with a frequency of about 5% [2], but has been found with an increased frequency among patients with monosymptomatic forms of cystic fibrosis, i.e. in male patients with CBAVD [2], in patients with pancreatitis [3], in newborns with hypertrypsinemia [4], and in patients with atypical sinopulmonary disease [5,6].

Because T5 *in trans* with a severe CFTR mutation is not found exclusively in 'CF' patients but also in healthy subjects, IVS8-5T is considered as a disease mutation with partial penetrance. Genetic studies have shown that the IVS8-5T variant causes alternative splicing of exon 9 resulting in a high proportion of CFTR transcripts that lack exon 9 whose translation products will not contribute to apical chloride channel activity [1]. Variability in the efficiency of the splicing mechanism exists among different individuals carrying this T5 allele and between different organs of the same individual [11]. This can explain why T5 only affects sensitive organs. Interactions of T5 with other polymorphisms in the CFTR gene can explain the variability of the associated phenotypes [8]; i.e. the IVS8-TGm polymorphism (localized immediately upstream of the T tract) *in cis* with T5 further modulates exon 9 skipping with increasing number of TG repeats increasing the penetrance of T5. This and influences of other factors such as the environment and the absence or presence of modulator genes make the clinical phenotype not predictable from the genotype alone.

In this family, all five members identified as being compound heterozygous for N1303K and IVS8-T5-TG13 presented with minor symptoms of CF at different ages. Chronic cough and recurrent respiratory infections including pneumonia were the most prominent symptoms. Sweat chloride values of the propositus (48–64 mEq/l) and her mother (61 mEq/l) were borderline. Only the sister had a sweat chloride level of more than 60 mEq/l. Additionally, the male patient presented with an azoospermia and congenital bilateral absence of the vas deferens, which is consistent with earlier observations in patients with the genotype N1303K/5T [2].

In conclusion, this report confirms that the clinical phenotype and age of onset of atypical CF is not predictable from the genotype alone and that once a patient with atypical CF has been identified genetic counselling of the family is very important. Family members (siblings and parents) should undergo molecular analysis to exclude other cases of atypical CF, which can be associated with very mild expression. Relatives with the same genotype should therefore be followed to allow appropriate early intervention in case other symptoms appear.

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