**Introduction**

Primary ciliary dyskinesia (PCD; MIM #242650) is a multisystem disorder characterized by respiratory tract symptoms (recurrent infections, sinusitis and bronchiectasis) and male subfertility. In about half of the patients, sinusitis and bronchiectasis are associated with situs inversus totalis, presenting a characteristic triad of symptoms (Kartagener syndrome, KS; MIM #244400). The disease phenotype is caused by the impaired motility of respiratory cilia and sperm tails, which in most cases is due to ultrastructural defects of these structures (Afzelius and Mossberg 1995). PCD/KS is usually inherited as an autosomal recessive trait, but genetics of the disease is far from being clear (for review see Geremek and Witt 2004). The broad variety of ciliary defects suggests genetic heterogeneity of PCD. Indeed, numerous linkage studies (Blouin et al. 2000; Meeks et al. 2000; Jeganathan et al. 2004) indicated several genomic regions potentially involved in PCD pathogenesis, but did not reveal any major PCD locus. Two human genes encoding dynein chains, important components of the ciliary ultrastructure, have been proved to date to cause the disease when mutated (Pennarun et al. 1999; Olbrich et al. 2002): DNAI1 on 9p13.3 and DNAH5 on 5p15–5p14. Mutations in these 2 genes account for ~24% of PCD cases (Guichard et al. 2001; El Zein et al. 2003; Hornef et al. 2006), indicating that other genes must be involved in the majority of PCD cases. Theoretically, one cannot exclude a scenario whereby the phenotypic expression of some small-effect mutations in putative PCD genes could be triggered by mutations in yet-unidentified modifier genes.

One of the possible candidates for the PCD modifier genes is CFTR, encoding the cystic fibrosis transmembrane conductance regulator, a cyclic AMP-mediated chloride channel. Mutations of CFTR cause cystic fibrosis (CF), the most frequent

---

**Carrier status for 3 most frequent CFTR mutations in Polish PCD/KS patients: lack of association with the primary ciliary dyskinesia phenotype**

Urszula Skrzypczak¹, Ewa Rutkiewicz¹, Andrzej Pogorzelski², Michal Witt¹,³, Ewa Ziżtkiewicz¹

¹Department of Molecular and Clinical Genetics, Institute of Human Genetics, Polish Academy of Sciences, Poznañ, Poland
²Institute of Tuberculosis and Lung Diseases, Pediatric Section, Rabka, Poland
³International Institute of Molecular and Cell Biology, Warszawa, Poland

**Abstract.** We screened a large group of primary ciliary dyskinesia/Kartagener syndrome (PCD/KS) patients and their siblings (148 patients from 126 unrelated families) for the presence of the CFTR mutations that are most frequently found in the Polish population: the severe F508del and 2,3del21kb, and the mild 3849+10kbC > T. No statistically significant increase in the frequency of these mutations was found in the studied group, as compared with the general population. This is consistent with an earlier observation in another population and indicates that the status of being a carrier of any of these CFTR mutations should not be considered as an important risk factor in PCD/KS pathogenesis.

**Keywords:** cilia, cystic fibrosis, primary ciliary dyskinesia.
autosomal recessive disease in Caucasians. Malfunction of CFTR causes aberrant transmembrane ion current, leading to abnormally viscous secretion, causing sino-pulmonary disease similar to PCD/KS, exocrine pancreatic insufficiency and male infertility. Over 1500 CFTR mutations have been identified to date (Consortium 2006). F508del is the most frequent (~70% CF alleles in Europeans) and only several others occur at frequencies > 1%, accounting for another ~13% of CF chromosomes (NIH CDP 1997). The Polish population differs slightly from the European average. For example, F508del in CF chromosomes is less frequent (~55% vs 70%) while the 3849 + 10kbC > T mutation is significantly more frequent (~4% vs 0.2%) (Witt et al. 1990; CFGA Consortium 1994; Walkowiak et al. 2001). The frequency of the East/Central-European-specific 2,3del21kb mutation in Polish patients (~3%, based on our recent unpublished data), although within the range observed in a number of Central and East European countries (16%), is much higher than in Western/Southern Europeans (only sporadic cases observed) (Dork et al. 2000). The phenotypic effects of CFTR mutations vary. F508del and 2,3del21kb are among those considered “severe”, resulting in the absence or dysfunction of the CFTR protein; 3849+10kbC > T is considered “mild” as it still produces a limited amount of normal mRNA by alternative splicing. Normally, the presence of 1 functional copy of the CFTR gene is sufficient for proper electrolyte levels, such that carriers of only 1 severe CFTR mutation are asymptomatic in terms of CF phenotype. On the other hand, heterozygous cystic fibrosis carriers seem to have a selective advantage over normal homozygotes by resistance to bacterial-toxin-mediated diarrhea (Romeo et al. 1989; Pier et al. 1998) and to cholera (Rodman and Zamudio 1991); heterozygous F508del CFTR have been shown to translocate fewer Salmonella typhi than wild-type CFTR protein (Pier et al. 1998). The above exemplifies “positive” phenotypic effects of the CFTR mutation carrier status, but also indicates that the association of heterozygous CFTR mutations with certain phenotypic effects different from CF is possible. It can be therefore hypothesized that the phenotypic presentation of PCD may be a result of the ciliary malfunction accompanied by a severe CFTR gene mutation resulting in only 50% of the normal level of the CFTR protein or by a mild mutation with a diminished quantity of protein formed.

The earlier study aiming to determine whether mutations in CFTR predispose to PCD/KS revealed no association between PCD and 12 CF mutations and several markers of the CFTR gene (Liechti-Gallati and Kraemer 1995). However, the study was based on 5 PCD families only, and a purported association, even if present, could have been missed. We took advantage of the large collection of blood samples from PCD/KS patients available in our laboratory and screened the samples for the presence of the CFTR mutations that are most frequently found in the Polish population: the severe F508del and 2,3del21kb, and the mild 3849 + 10kbC > T.

Materials and methods

Blood samples were collected from patients diagnosed with PCD or KS. All of them were Caucasians, from various regions of the country (Wielkopolska, Pomerania, Silesia, Malopolska). The clinical picture in patients from all 126 families included coexisting chronic bronchitis, sinusitis and otitis media (in some patients bronchiectasis and bronchial deformations were observed); 63 families were classified as Kartagener syndrome because in at least 1 affected family member pulmonary symptoms were accompanied by situs inversus. Clinical diagnosis was additionally confirmed by: negative results of a saccharine test (performed in adult and sub-adult patients); low levels of nasal NO; the presence of akinetic or dyskinetic bronchial cilia in light microscopic examination; and in ~50% of the families the clinical diagnosis was confirmed by transmission electron microscopic analysis of bronchial ciliary ultrastructure, which revealed defects of outer or outer/inner dynein arms. In 2 of the families, PCD symptoms overlapped with those of retinitis pigmentosa (RP).

DNA was isolated from peripheral blood by using a routine salting-out technique. Patients from 126 unrelated families were analyzed (63 with KS, 63 with PCD). In 10 KS and in 12 PCD families more than 1 affected child were available – in these cases all affected siblings were genotyped (a total of 148 PCD/KS patients). Wherever a mutation carrier was found, the available healthy siblings were analyzed. Genomic regions encompassing the 3 CFTR mutations were PCR-amplified and analyzed by using routine protocols (Witt et al. 1993; Augarten et al. 1993; Dork et al. 2000).