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P01
Development of a bioinformatics analysis method for diagnosis of Primary Ciliary Dyskinesia
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Primary ciliary dyskinesia is a rare, autosomal recessive genetic based disease causing functional and/or structural defects of cilia. However, there are little information about the genetic basis of this disease. By this time, we know that, mutations on the DNA11 and DNASH genes are responsible for encoding of dynein proteins of cilia, causing this disorder. Discrimination of the PCD from other diseases like Cystic fibrosis is quite problematic. Discrimination and treatment will be easier if the genetic basis of the PCD is known. In the proposed work, we plan to carry out whole genome exome sequencing on 3 families with PCD siblings. Each these families have healthy parents and two sick children. As a result of exome sequencing, we will be able to identify common SNPs and/or affected common genes related with PCD. Then, the data obtained from exome sequencing will be used as the input to PANOGA to identify affected pathways. Thus, we will be able to study PCD aetiology via affected pathways. Eventually, we aim to provide a novel marker for diagnosis of PCD.

P02
Quality of life in patients with primary ciliary dyskinesia: a systematic review
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Background: As in most rare diseases, little is known on how primary ciliary dyskinesia (PCD) affects the patient’s daily functioning and mental wellbeing. We aimed to identify all studies that examined the psychosocial impact that PCD has on the lives of patients. Methods: We searched MEDLINE, EBSCO, Cumulative Index to Nursing and Allied Health Literature (CINAHL), PsycINFO and EMBASE for qualitative and quantitative studies. Results: We found 260 studies, of which 14 were included. Five of the included studies had qualitative approaches. Increasing age was associated with worsening of respiratory symptoms, physical, and mental domains of health-related quality of life, with a greater decline compared with reference populations. Patients felt physically impaired, e.g., less energy and difficulty keeping up with others. Social implications included embarrassment and a sense of isolation, with patients concealing symptoms and/or their diagnosis. Isolation intensified with lack of public and professional awareness of PCD. Patients were emotionally affected by PCD and expressed anxiety about getting sick or when thinking about their future health. Treatment burden was an issue for patients, whilst acknowledging treatments improved symptom. Conclusion: Health-related quality of life decreases with age in patients with PCD. For all age groups, PCD adversely affects physical, emotional, social functioning, and treatment burden. Interventions that ameliorate these issues need investigation.

P03
Estimation of cell number in a three-dimensional cell cluster comparison of different 3D nuclei calculating methods
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Efficient counting of cell numbers in three-dimensional structures (cell clusters, spheres or aggregates) often poses a challenge for the existing image analysis software. We present a novel approach to the problem, for the use on fluorescence-stained three-dimensional volumes (z-stacks) obtained using confocal microscope. The method combines the 2D segmentation of nuclei slices using grabcut algorithm, followed by three-dimensional region labelling and nuclei assembly. The software has been developed with the use of Python software, as well as the Bio-Formats, Open-CV and SciPy libraries. Time consumption, precision and scalability of the method have been compared with other available nuclei calculating methods (manual counting and available ImageJ plugins). We also explored the possibility to use of our algorithm on z-stacks obtained using regular fluorescent microscope. This research has received funding from the National Science grant number 2013/09/D/NZ4/01692.
P05
In vitro differentiation of respiratory epithelial cells in the sequential culture model
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In vitro culturing of the respiratory epithelial cells is a primary step in studies on the structure, functioning and differentiation of the respiratory epithelium (RE). It is also used during diagnostic process of primary ciliary dyskinesia, to obtain cilia without secondary changes caused by infection load. Two most commonly used methods of culturing airway epithelial cells are: the air-liquid interface (ALI) culture and the sequential culture (Jorissen) method. In the sequential culture cells are first cultured adhered to a layer of collagen gel, after reaching confluence, the cell sheets are released from the collagen layer and transferred into suspension. Although much is known about the expression of genes during differentiation of airway epithelial cells cultured at the ALI culture, not much information exists about the epithelium differentiation chronology in the sequential culture of RE cells. Here, using a panel of antibodies against proteins characteristic for different epithelial cell types, we characterized the different stages of epithelial differentiation. Basal cells of the epithelium were monitored using antibody against p63 protein, antibody against MUC5AC protein was used to detect secretory (goblet) cells and antibody against CC10 was used to detect club cells (previously known as Clara cells). Ciliated airway cells were detected using antibodies against cilia-specific proteins - acetylated α-tubulin or RSPH4A. Expression of proteins was analyzed at different time-points of the culture (at culture start, after proliferation period, at the beginning and the end of cell differentiation). In addition to the protein expression, the time-dependent changes in the gene expression level were monitored, using multiplex RT-PCR with primers against cDNA of several genes: DNAH5, CCDC40, ZMYND10, RSPH4A, CFTR, GAPDH.

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P06
Preliminary results of whole exome sequencing in Turkish primary ciliary dyskinesia patients - Hacettepe University Experience
“Three candidate genes, five novel and two known mutations”
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Background: Primary ciliary dyskinesia (PCD) is a rare, genetic disease characterized by cilia dysfunction. Recurrent lower respiratory tract infection and bronchiectasis play an important role in morbidity. The most frequently isolated microorganisms in the respiratory tract are Haemophilus influenzae, Streptococcus pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa and non-tuberculosis mycobacteria. Frequent respiratory tract infections often lead to the use of antibiotics in patients. Aim: Our aim in this study is to investigate the relationship between antibiotic use and resistance in PCD patients with H. influenzae in respiratory tract specimens during the last year. Methods: The sputum and bronchoalveolar lavage cultures of patients diagnosed with PCD in the Department of Paediatric Chest Diseases of Hacettepe University were examined for antibiotic susceptibility of H. influenzae in 2016. Antibiotic susceptibilities of isolates were determined by disk diffusion method. Antibiotic use of these cases was evaluated retrospectively in the last year. Results: H. influenzae were detected in 74 cases (33 M/ 41 F and the age range
The role of Rabconnectin3a in cilia length regulation

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Using the zebrafish mutant for the deltaD gene (dlld<sup>−/−</sup>), we showed that Notch signaling was involved in the control of cilia length in the cells of the fish lateral organ, the Kupffer’s Vesicle (KV). A microarray analysis of the list of genes differentially expressed in KV cells from dlld<sup>−/−</sup> mutants and wild-type embryos, identified a gene 16 fold down-regulated. This gene – rabconnectin3a or rbca3a codes for a large, ~325 kDa protein, highly conserved in multicellular organisms, which associates with its obligate binding partner, Rbc3b and sub-units of the v-ATPase complex. Rbc3a was implicated in the regulation of the activity of the V-ATPase. Its absence has been shown to decrease the acidification of intracellular vesicles, which can affect not only the cellular vesicular trafficking, but also the cytosolic Ca<sub>2+</sub> concentration. This gene has been associated with Notch signalling in Drosophila and mammalian cells through the regulation of the V-ATPase activity. Rbc3a has also been associated with vesicular acidification in zebrafish hair cells and with vesicular endocytosis and maturation in zebrafish neural crest migration. Furthermore, it was found that V-ATPase H+ pump, together with SNX10, regulated ciliogenesis both in vitro and in vivo. Using a Morpholino against rbca3a (Rbc3a MO), we caused a decrease in the cilia length of the KV and consequent randomization of organ situs. We propose that the decrease in ciliary length of dld<sup>−/−</sup> mutant KV cells is partially caused by the de-regulation of the V-ATPase pump. Using the vital dye LysoTracker Red we showed the decrease of acidic vesicles both in rbc3a morphant and dld<sup>−/−</sup> mutant embryos. We co-localized the V-ATPase to the apical membrane and to the basal body and used concanamycin, a specific V-ATPase inhibitor, to assess its effect on cilia length and we registered a similar expected reduction. We show that the reduction in rbca3a transcription levels negatively regulates the V-ATPase activity, resulting in a generalized decrease of the late endocytic vesicular trafficking, and in a shorter ciliary length.

Late diagnosis of the PCD is still a great risk

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We are presenting a typical case of a PCD patient with late diagnosis. Nine-year-old girl with dextrocardia and polypsilaenia and with recurrent respiratory infections was referred to our department for a suspicion of PCD. She was born full-term with short ICU admission and she has had rhinorhea since birth. She has got a chronic wet cough all year round. During physical activity, she has suffered from dyspnoea and worsening of cough. She has complained of hearing problems and persistent ear secretions. She had pneumonia twice before the age of eight. The PICADAR score of our patient was 12. HRT examination revealed chronic infiltrations, collapse of the right lower lobe, bronchiectasia and mild hilar and mediastinal lymphadenopathy. Lung function examination at the time of diagnosis detected FVC 1.48 L (77% predicted) and FEV1 0.95 L (58% predicted) with only mild response to bronchodilator. Chronic bronchitis with purulent mucus production and tracheomalacia was found during flexible bronchoscopy. Cultures from bronchoalveolar lavage revealed Haemophilus influenzae. ENT examination found a mild conductive hearing disorder and chronic secretory otitis. Impressive history suggested PCD. CF was ruled out; laboratory findings did not show any immunopathology. Nasal NO was 10.2 nl/min, HSVA demonstrated akietic cilia. Ultrastructural examination showed absent outer dynein arms (ODA) in ciliary axonema, inner dynein arms were present. Recently introduced immunofluorescence examination revealed DNASH deficit, which supported our diagnosis of PCD with ODA defect. We have initiated the complex therapy consisting of targeted antibiotic therapy, inhaled mucolytic therapy, inhalation of ICS/LABA and chest physiotherapy including flutter. Thanks to this intensive therapy the patient’s condition has significantly improved despite the delayed diagnosis. This case indicates that despite typical symptoms the diagnosis of PCD may still be unnecessarily delayed. Increased awareness of the disease among both medical professionals and lay public is highly desirable.
Ten years’ experience of primary ciliary dyskinesia diagnostic testing
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Background: The Southampton primary ciliary dyskinesia (PCD) Diagnostic Centre has been commissioned by NHS England since 2006. The centre contains state of the art diagnostic facilities and specialised multidisciplinary team dedicated to PCD diagnosis and management. We aimed to describe the characteristics of patients diagnosed at the centre.

Methods: We conducted a cross-sectional study using data from local PCD registry, electronic health records and medical notes for all patients diagnosed with PCD in Southampton from 2006 to 2016. Diagnosis was always based on at least one diagnostic test. The number of tests has increased in the last 5 years particularly in genotyping (65% vs 56% before 2011). Nasal nitric oxide was measured in 82% of patients over 5 years of age. Testing was done on samples collected from patients attending clinic (77%) and on samples sent by courier (23%). Overall, 84% patients had high-speed video analysis, of which 70% showed predominantly static cilia and only 1.7% were deemed normal, 97% had transmission electron microscopy, of which 70% showed ‘hallmark’ defects, and 59% were genotyped. Median age at initial assessment was 10 years (IQR 3 to 23.5 years), younger for the 43% with situs abnormalities (7 vs 12 years, p=0.062). 11% had congenital heart disease, considerably higher than reported elsewhere (3-6%). 72% experienced neonatal respiratory symptoms, of which 58% needed respiratory support. Mean FEV1% was low in both children and adults (79% vs 71% respectively, p=0.26) at the time of diagnosis.

Conclusion: We found a high prevalence of congenital heart disease and neonatal respiratory symptoms. Lung function was impaired in both children and adults. These findings highlight the need for early diagnosis and care for PCD in specialised centres.

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