International Symposium

“Cystic fibrosis in Asia from basics to clinics”

Date: **September 29-30, 2014**

Venue: **Noyori Conference Hall, Nagoya University, Japan**

Overseas Invited Speakers:

Adam Jaffe (Sydney)
Tzyh-Chang Hwang (Columbia)
Min Goo Lee (Seoul)
Shmuel Muallem (Bethesda)
Felix Ratjen (Toronto)
Julie Matel (Palo Alto)
Margarida Amaral (Lisbon)
Muxin Wei (Nanjing)
Jeong-Ho Kim (Seoul)
Noel G McElvaney (Dublin)
John Riordan (Chapel Hill)
Hsiao Chang Chan (Hong Kong)
Ann Harris (Chicago)
Claire Wainwright (Herston)
Garry Cutting (Baltimore)
Steven Rowe (Birmingham)
Brenda Button (Prahran)
Allan Powe (San Diego)

Topic 1: Epidemiology of cystic fibrosis in Asia
Topic 2: Assessment of nutritional status and management in cystic fibrosis
Topic 3: Asian-type CFTR mutations and their characteristics
Topic 4: Regulation of CFTR expression in cystic fibrosis
Topic 5: CFTR-related diseases in Asia

Hosted by Japan Intractable Diseases Research Foundation
Supported by Ministry of Health, Labour and Welfare, Japan
Co-hosted by Research Center of Health, Physical Fitness and Sports, Nagoya University

President: Tooru Shimosegawa
Tohoku University Graduate School of Medicine

http://www.htc.nagoya-u.ac.jp/~ishiguro/lhn/symposium.html

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Topics

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Assessment of nutritional status and management in cystic fibrosis
Asian-type CFTR mutations and their characteristics
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CFTR-related diseases in Asia

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President: Tooru Shimosegawa

(Professor of Gastroenterology, Tohoku University Graduate School of Medicine)

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http://www.htc.nagoya-u.ac.jp/~ishiguro/lhn/symposium.html

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Yoshifumi Takeyama (Osaka)

Hiroyoshi Endo (Tokyo)

Local Acting Members:

Yuka Usami, Itsuka Taniguchi, Makoto Yamaguchi, Yuka Mochimaru

Preface

On behalf of the organizing committee, we are pleased to welcome you to Nagoya and International Symposium "Cystic fibrosis in Asia from basics to clinics". This symposium is hosted by Japan Intractable Diseases Research Foundation and supported by Ministry of Health, Labour and Welfare, Japan.

Cystic fibrosis is rare in Asians while it is the most common genetic disease in Europeans. There was little information how to diagnose and treat patients with this intractable disease until recently in Japan. The Research Committee of Intractable Pancreatic Diseases has conducted nationwide surveys, characterized Asian-specific CFTR mutations, and established the registry system to share new information among physicians, health-care professionals, researchers, and pharmaceutical companies. Pancrelipase, Dornase Alfa, and tobramycin inhalation solution have recently become available in Japan owing to the extensive efforts by the family association. We are sure that this is the best time to hold an international symposium.

Opinion leaders in biology of CFTR, genetics, new therapeutic strategies, nutritional management, airway clearance physiotherapy, multidisciplinary team care, and personalized medicine in cystic fibrosis from 7 countries kindly accepted our invitation to this symposium. We have 20 poster presentations including 13 posters presenting case reports of cystic fibrosis in Japan and basic researches. This symposium provides the first opportunity for Japanese physicians and overseas opinion leaders meet face to face and to discuss the direction of clinical and basic researches on cystic fibrosis in Asia. We hope that many people involved in the medical practice of cystic fibrosis in Japan will attend this symposium.

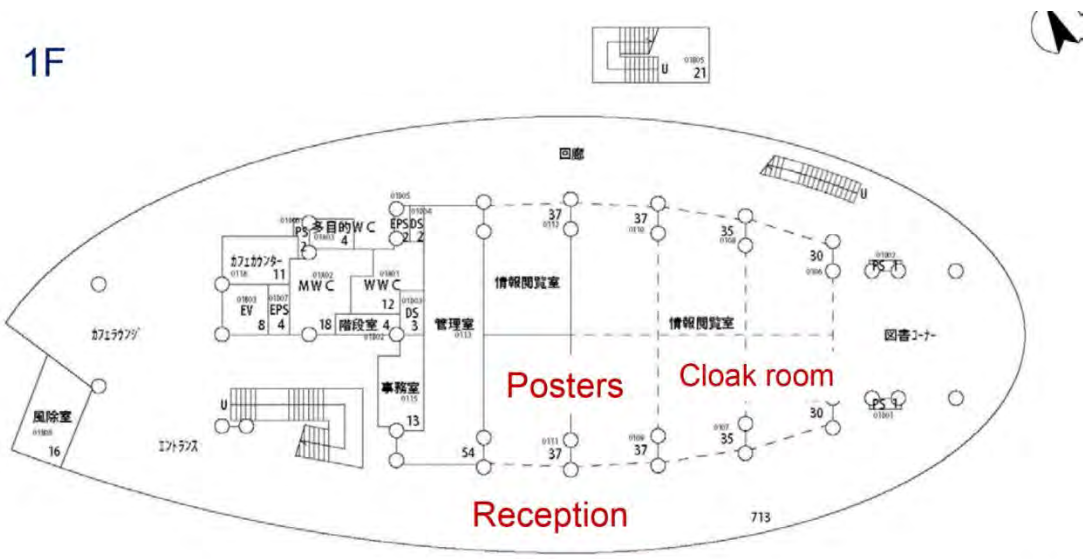
Tooru Shimosegawa
Yoshifumi Takeyama
Satoru Naruse
Hiroshi Ishiguro

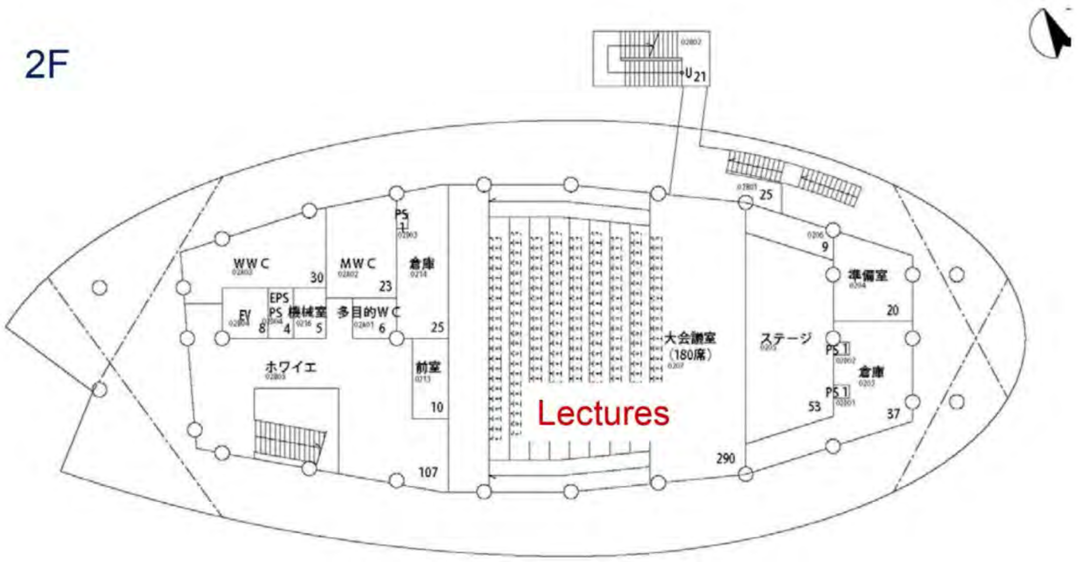
September 2014

Venue:

The scientific program is held at Noyori Conference Hall in the Higashiyama campus of Nagoya University.

(Furo-cho, Chikusa-ku, Nagoya, Aichi, 464-8602 JAPAN)





[Programs of oral presentations]

September 29 (Monday)

8:50~9:10

Opening remarks-1 Tooru Shimosegawa (Sendai)

Opening remarks-2 Hiroyoshi Endo (Japan Intractable Diseases Research Foundation)

9:10~10:30

Biology of CFTR Chair: Yoshiro Sohma (Tokyo)

1. CF, from the patient to discovery of the basic defect and back
   John R Riordan (Chapel Hill) 20 min talk + 10 min discussion
2. Molecular physiology of the CFTR chloride channel
   Tzyh-Chang Hwang (Columbia) 20 min talk + 10 min discussion
3. Structure and fluctuation of single CFTR molecules observed by high-speed atomic force microscopy
   Yoshiro Sohma (Tokyo) 15 min talk + 5 min discussion

(Coffee break 10 min)

10:40~12:30

Cystic fibrosis in Europeans and Asians Chair: Claire Wainwright (Herston)

1. Genotype-phenotype correlation in cystic fibrosis
   Garry Cutting (Baltimore) 20 min talk + 10 min discussion
2. Cystic fibrosis cases in Korea
   Jeong-Ho Kim (Seoul) 20 min talk + 10 min discussion
3. Overview and registry of cystic fibrosis in Japan
   Hiroshi Ishiguro (Nagoya) 15 min talk + 5 min discussion
4. CFTR gene mutations and pulmonary manifestations in Japanese patients with cystic fibrosis
   Kunihiko Yoshimura (Tokyo) 20 min talk + 10 min discussion

12:30~14:00

Lunch and poster discussion

14:00~15:40

CFTR and HCO_3^- secretion by pancreatic duct Chair: Hiroshi Ishiguro (Nagoya)

1. Regulation and synergism in epithelial fluid and HCO_3^- secretion

   Shmuel Muallem (Bethesda) 20 min talk + 10 min discussion

2. HCO_3^- transport by cystic fibrosis pancreatic duct

   Akiko Yamamoto (Nagoya) 15 min talk + 5 min discussion

3. Regulation of HCO_3^-/Cl^- permeability of CFTR

   Min Goo Lee (Seoul) 20 min talk + 10 min discussion

4. ARHGAP9, a GTPase-activating protein, for CDC42/RAC1/RAC2, inhibits CFTR chloride channel activity through the STAS domain of SLC26 transporters

   Shigeru Ko (Tokyo) 15 min talk + 5 min discussion

(Coffee break 10 min)

15:50~17:20

Pathophysiology of CFTR Chair: Steven Rowe (Birmingham)

1. CFTR in reproduction and embryo development

   Hsiao Chang Chan (Hong Kong) 20 min talk + 10 min discussion

2. Low-mortality airway-specific β-epithelial sodium channel (βENaC) transgenic mice as a model of cystic fibrosis lung disease

   Tsuyoshi Shuto (Kumamoto) 20 min talk + 10 min discussion

3. CFTR quality control checkpoints as drug target

   Tsukasa Okiyoneda (Sanda) 20 min talk + 10 min discussion

September 30 (Tuesday)

8:50~9:50

New therapeutic strategies of cystic fibrosis-1 Chair: Naoto Keicho (Tokyo)

1. Early detection of lung inflammation & infection in cystic fibrosis
   Adam Jaffe (Sydney) 20 min talk + 10 min discussion
2. Treatment of early pseudomonas aeruginosa infection in cystic fibrosis
   Felix Ratjen (Toronto) 20 min talk + 10 min discussion

(Coffee break 10 min)

10:00~11:30

New therapeutic strategies of cystic fibrosis-2 Chair: Kunihiko Yoshimura (Tokyo)

1. The central role of the neutrophil in cystic fibrosis related lung disease
   Noel G McElvaney (Dublin) 20 min talk + 10 min discussion
2. Personalized medicine for the treatment of cystic fibrosis
   Allan Powe (San Diego) 20 min talk + 10 min discussion
3. Treatment of the basic CF defect by modulating CFTR: Individualized monitoring and therapeutics
   Steven Rowe (Birmingham) 20 min talk + 10 min discussion

(Coffee break 10 min)

11:40~12:40

Management of patients with cystic fibrosis-1 Chair: Toyoichiro Kudo (Tokyo)

1. Multidisciplinary care for cystic fibrosis-some of the challenges
   Claire Wainwright (Herston) 20 min talk + 10 min discussion
2. Airway clearance physiotherapy for cystic fibrosis
   Brenda Button (Prahran) 20 min talk + 10 min discussion

12:40~14:10

Lunch and poster discussion

14:10~15:00

Management of patients with cystic fibrosis-2 Chair: Toshiaki Shimizu (Tokyo)

1. Nutritional management of cystic fibrosis
   Julie Matel (Palo Alto) 20 min talk + 10 min discussion
2. Exocrine function and nutritional status of Japanese patients with cystic fibrosis
   Kotoyo Fujiki (Nisshin) 15 min talk + 5 min discussion

(Coffee break 10 min)

15:10~16:30

Expression of CFTR in cystic fibrosis Chair: Garry Cutting (Baltimore)

1. Transcriptional networks regulating CFTR gene expression
   Ann Harris (Chicago) 20 min talk + 10 min discussion
2. Analysis of CFTR transcripts from nasal swab of Japanese patients with cystic fibrosis
   Miyuki Nakakuki (Nagoya) 15 min talk + 5 min discussion
3. Rescue of CFTR mutations with different molecular and cellular defects
   Margarida Amaral (Lisbon) 20 min talk + 10 min discussion

(Coffee break 10 min)

16:40~17:40

CFTR-related disorders in Asia Chair: Satoru Naruse (Miyoshi)

1. Comparative analysis of CFTR gene polymorphisms between chronic bronchitis and healthy Chinese population
   Muxin Wei (Nanjing) 15 min talk + 5 min discussion
2. Genetics of pancreatitis in Japan
   Atsushi Masamune (Sendai) 15 min talk + 5 min discussion
3. CFTR variants in Japanese patients with chronic pancreatitis
   Satoru Naruse (Miyoshi) 15 min talk + 5 min discussion

17:40~18:00

Closing remarks-1 Yoshifumi Takeyama (Osaka)

Closing remarks-2 Tooru Shimosegawa (Sendai)

[Poster presentations]

1. A Japanese case of cystic fibrosis-associated liver disease
   Koichi Ito (Nagoya)
2. A case of cystic fibrosis diagnosed in adulthood
   Kouko Hidaka (Kitakyushu)
3. Infantile-onset cystic fibrosis presenting with liver failure
   Rie Kawakita (Osaka)
4. Two childhood cases of cystic fibrosis in Japan
   Kosuke Yanagimoto (Kagoshima)
5. The first case of living donor lung transplantation for cystic fibrosis in Japan; 12 year's follow-up with multiple complications
   Tomoko Toma (Kanazawa)
6. Effect of aerosolized dornase alfa and tobramycin treatment on lung disease and quality of life in a Japanese cystic fibrosis patient
   Yoshiaki Harada (Osaka)
7. A case of cystic fibrosis in a 9-year-old Japanese child
   Daiei Kojima (Nagoya)
8. A case of cystic fibrosis diagnosed 20 years after first diagnosis of DPB
   Nanao Terada (Kanazawa)
9. A case of cystic fibrosis in a 7-year-old girl
   Reiko Shibata (Nagoya)
10. Improvement of growth retardation in a child with cystic fibrosis treated with dornase alfa and tobramycin inhalation
    Akira Endo (Iwata)
11. A case of 37 years old female cystic fibrosis, 9 years follow-up
    Yuichi Fukuda (Sasebo)
12. A Japanese infantile case of cystic fibrosis presenting pseudo-Bartter syndrome caused by H1085R and Y563H compound heterozygosity
    Tetsuro Matsuhashi (Sendai)
13. Pulmonary hypertension in a Japanese patient with CFTR-related bronchiectasis: a case report with autopsy
    Jiro Usuki (Kawasaki)

14. Vitamin C deficiency exacerbates respiratory function and emphysema in epithelial Na^+ channel-overexpressing mice

    Haruka Fujikawa (Kumamoto)

15. Aberrant splicing of zinc transporter ZIP2 causes mucus hypersecretory phenotype in CF airway epithelial cells

    Shunsuke Kamei (Kumamoto)

16. GLP-1 receptor agonist extendin-4 exacerbates mucus hypersecretory phenotype in epithelial Na^+ channel-overexpressing cells and mice

    Hirofumi Nohara (Kumamoto)

17. Increased IL-17C production by the TLR3 ligand POLY(I:C) in primary cystic fibrosis airway epithelial cells

    Yukihiro Tasaki (Kumamoto)

18. A homology modeling of human CFTR

    Yasutomo Ito (Nagoya)

19. Optimization of a mathematical model of ion transport by pancreatic duct cell

    Makoto Yamaguchi (Nagoya)

20. Expression and function of CFTR mutants found in Japanese CF patients

    Yingchun Yu (Tokyo)

ABSTRACTS

CF, FROM THE PATIENT TO DISCOVERY OF THE BASIC DEFECT AND BACK

John R Riordan, Tim Jensen, Lying Cui, Luba Aleksandrov, Lihua He, Andrei Aleksnadrov

Dept of Biochemistry and Biophysics and CF Center, University of North Carolina

Although infants with CF-like symptoms were mentioned in historic accounts from Europe in earlier centuries, CF was described as a defined disease entity only 75 years ago. As a heterogeneous disease of multiple epithelial tissues, recognition of the root cause was mystifying. Mucovisidosis was the prominent feature. Detection of altered electrolytes in sweat and other exocrine secretions and later measurement of increased bioelectric potential across airway (Knowles *et al*, *New Eng J Med* 305: 1489-95,1981) and sweat duct (Quinton. *Nature* 301: 421-22, 1983) epithelia led to extensive investigations of ion conductances and confirmation of an anion permeability defect. However identification of the primary molecular defect remained extremely challenging.

The recognized autosomal recessive inheritance of CF enabled the general proposal of Botstein and colleagues (*Am J Hum Genet* 32: 314-31,1980) that restriction fragment length polymorphisms (RFLPs) to be used to locate Mendelian disease loci. Lap-Chee Tsui seized upon this approach to CF and during the 1980s his group succeeded in locating the CF gene by what since has come to be known as positional cloning. During the same period my laboratory decided to pursue the sweat gland in which Paul Quinton had demonstrated the chloride permeability defect. Glands were dissected from skin biopsies of individuals with and without CF and primary cell cultures established. While attempts to detect disease related differences at the protein level failed, RNA and cDNA from the cultures that retained native electrophysiological properties were tested for hybridization to genomic fragments in the neighborhood of the CF genomic locus. One hybridizing partial cDNA was used to reprobe cDNA libraries from normal and CF cells to identify additional overlapping sequences covering a nearly complete open reading frame. Comparison of sequences of CF and control origins revealed a single codon deletion (ΔF508) in the former. Sequence similarities with other members of a family of transporters identified the product as a membrane protein and the pattern of expression in tissues was consistent with those affected by the disease. Additional experiments in the Tsui lab identified the same sequence difference on ~ 2/3 of CF chromosomes.

The greatest initial impact of finding the gene was to provide a focus for research in what had been a very broad and diverse field. The mutation consortium initiated by Lap-Chee and his fax machine led to the eventual uncovering of nearly 2000 different mutations. The many detailed genotype/phenotype studies collated in 'CFTR2' in combination with the output of high throughput small molecule modulator screens have already led CF into the era of personalized medicine.

Understanding of CFTR function and dysfunction is still incomplete, partly because determination of high-resolution structures has been impeded by its low abundance and strong self-association. Thermodynamic instability currently limits both efforts to crystallize the protein for 3D structure determination and to overcome the further destabilizing affect of the ΔF508 mutation. However progress is being made on this front and it is likely that the required fine rebalancing of thermal stability will be achieved. Anion channel activity has been well characterized but the permeation pathway is not fully defined, nor is the coupling of the ATP-binding and phosphorylated cytoplasmic domains with gating. This coupling which appears to be primarily allosteric rather than energetic (Alekesandrov *et al*, *Pfluger's Archiv* 453: 693-702, 2007; Kirk and Wang, *J Bio Chem* 286: 12813-19, 2011) is important to understanding the actions of both correctors and potentiators of mutant CFTRs.

Supported by the NIH and CFF.

MOLECULAR PHYSIOLOGY OF THE CFTR CHLORIDE CHANNEL

Tzyh-Chang Hwang

Department of Medical Pharmacology and Physiology, Dalton Cardiovascular Research Center, University of Missouri, Columbia, Missouri, USA

CFTR, whose dysfunction constitutes the fundamental basis of cystic fibrosis, is a phosphorylation-activated, but ATP-gated chloride channel. As a member of the ABC Transporter Superfamily, CFTR inherits two transmembrane domains (TMDs) that form a gated chloride-conducting pore, and two nucleotide-binding domains (NBD1 and NBD2), which coalesce into a "head-to-tail" dimer upon ATP binding with two ATP molecules sandwiched at the dimer interface as seen in other members of this Superfamily. Previous studies have shown that opening of CFTR's gate is coupled not to ATP binding but to post-binding NBD dimerization, whereas ATP hydrolysis facilitates gate closure likely by promoting dissociation of the NBD dimer. Consistent with insights derived from sequence analyses, biochemical studies demonstrated that only one of CFTR's two ATP binding sites can hydrolyze ATP (i.e., *site 2* formed by the "head" subdomain of NBD2 and the "tail" subdomain of NBD1). In the present talk, I will present our electrophysiological data collected over the past 5 years in support of two novel ideas regarding the coupling mechanism between opening/closing of the gate in TMDs and association/dissociation of NBDs. First, by applying ATP analogs to the CFTR channels at different times and/or for different durations, we showed that one ATP molecule is kept bound in catalysis-incompetent *site 1* for tens of seconds when the channel has undergone many rounds of opening-and-closing cycles, indicating that closing of the gate does not require a complete separation of the NBD dimer. This conclusion is further supported by the crystal structure of an ABC Transporter showing a conformation with partially separated NBDs. Second, two independent approaches reveal a conformation of CFTR wherein the gate remains open whilst *site 2* has been vacated following a partial separation of the NBD dimer, hence allowing a new ATP molecule to bind to this site before gate closure. Thus, contrary to the conventional thought, open/closing of the gate in TMDs is not strictly coupled to the association and partial separation of the NBDs. These results lead to a gating model featuring a probabilistic relationship between gating conformational changes in TMDs and association/dissociation of NBDs. In other words, NBD dimerization makes gate opening more likely to happen and gate opening facilitates NBD dimerization, an idea in accordance with the classical Monod-Wyman-Changeux model for allosteric modulation. Pathophysiological and pharmacological implications of this energetic coupling model of CFTR gating will be discussed.

STRUCUTURE AND FLUCTUATION OF SINGLE CFTR MOLECULES OBSERVED BY HIGH-SPEED ATOMIC FORCE MICROSCOPY

Yoshiro Sohma and Hayato Yamashita

Department of Pharmacology, Keio University School of Medicine, Tokyo, Japan

Cystic Fibrosis Transmembrane conductance Regulator (CFTR), a unique member of ABC transporter superfamily, functions as an ATP-dependent anion channel after PKA-dependent phosphorylation of the regulatory (R) domain unique to CFTR. In CFTR, ATP-induced dimerization of its nucleotide binding domains (NBDs) and subsequent hydrolysis-triggered dimer separation are proposed to be coupled, respectively, to the opening and closing of the gate in its transmembrane domains.

Channel function of CFTR has been mainly studied by measuring ionic current going through the pore using the patch-clamp technique, which has given us many important findings about the mechanism of CFTR gating. On the structural aspects, recent advances in X-ray crystallography provide atomic-level structures for several bacterial and mammalian ABC transports whereas the crystal structure of whole CFTR molecule has not been solved. However, neither the electro-physiology nor the crystal structure can give us the information about the molecular dynamic processes of CFTR proteins.

Recently we have started up a project to image dynamic structural changes and interactions occurring in individual CFTR molecules by the high-speed atomic force microscopy (HS-AFM). In the preliminary study, HS-AFM visualized a dimeric formation of DMM-solubilized, purified WT-CFTR molecules attached on the HS-AFM stage over sideways. The HS-AFM image of CFTR molecule showed an ellipsoidal structure which was consistent with the one obtained from the single particle analysis[1)]. In addition, we found a small flap-like structure fluctuating at the bottom of putative cytoplasmic domain. HS-AFM showed an antibody against the R domain bound to the fluctuating flap structure, which suggested that the flap structure might be a part of the R domain.

Next we observed the solubilized CFTR molecules incorporated into the lipid bilayer expanded on the AFM stage. The CFTR molecules showed a fluctuation varied among them, which might be underlain by various pre-phosphorylation levels in the PKA-dependent regulatory domain.

1) Mio *et al*. *J Biol Chem* 283: 30300-10, 2008.

GENOTYPE-PHENOTYPE CORRELATION IN CYSTIC FIBROSIS

Garry R. Cutting, MD

McKusick-Nathans Institute of Genetic Medicine; Johns Hopkins University School of Medicine; Baltimore, MD USA

Cystic fibrosis (CF) is one of the most common lethal autosomal genetic disorders in the Caucasian population affecting approximately 70,000 individual worldwide. The classic form of the disease involves progressive obstructive pulmonary disease, exocrine pancreatic insufficiency and elevated concentration of chloride and sodium in sweat. Non-classic CF occurs in 10-15% of patients and includes pancreatic sufficient patients who do not have clinically evident pancreatic disease. Median survival for CF patients is currently 37 years and lung disease accounts for almost 90% of the mortality. Manifestations of CF are caused by abnormalities in electrolyte transport across epithelia leading to altered mucous viscosity and recurrent episodes of obstruction, inflammation and progressive destruction of affected organs. Cloning of the gene responsible for CF, the CF TRansmembrane conductance Regulator (*CFTR*) provided a major breakthrough in our understanding of the molecular basis of this disease. CFTR functions as a chloride channel and regulates the activity of separate channels, and possibly ion transporters. Analysis of the relationship between CFTR genotype and the CF phenotype can provide insight into the amount of CFTR function that needs to be achieved to effect clinical improvement. An association has been shown between the nature of the CFTR mutation and pancreatic status and between functional classes of CFTR mutations and sweat chloride concentration ([Cl^-]). Understanding the relationship between individual mutations and lung disease severity has been a highly sought goal but with only isolated success to date.

Treatment of CF took a major step forward in the successful deployment of Kalydeco (Ivacaftor, VX-770), a compound that potentiates the function of CFTR bearing the G551D mutation. Administration of Kalydeco resulted in substantial reductions in sweat chloride concentration [Cl^-] and improvement in lung function measurements. Unfortunately, we can’t predict how much improvement might be achieved with partial recovery of CFTR function. A powerful approach to this address this dilemma is to correlate the *in vitro* function of CFTR bearing different mutations with the clinical features of patients carrying these mutations. Prior studies utilizing genotype/phenotype correlation in patients and *in vitro* correction of CFTR function in cell-based systems have suggested that 10% function is required to avoid the life-limiting lung disease in CF. Alternatively, some studies have suggested that 5% function is sufficient while others propose that recovery of 20 to 25% of wild-type CFTR will be required to effectively treat CF.

To address this issue, we have utilized the clinical data collected from ~40,000 CF patients in the CFTR2 database (cftr2.org). We have discovered that CFTR chloride channel function appears to exhibit an exponential relationship with sweat [Cl^-] and likely with measures of lung function. Conversion of CFTR chloride current to an exponential is attractive as ion channels operate in a non-linear fashion. As passive dissipaters of ion gradients, channels like CFTR rapidly achieve maximal efficiency upon activation. An exponential relationship indicates that recovery of a small fraction of wildtype function would be expected to have a substantial impact on abnormal chloride gradients. Treatment of many CF patients with currently available compounds would be justified if we had reasonable confidence that lung function would be stabilized providing more years of life for patients as additional CFTR-targeted therapies are developed. With an unprecedented breadth of mutations and clinical data available from CFTR2, we are rigorously testing for correlations between CFTR function, lung function measures and sweat [Cl^-]. Quantification of these relationships will provide the basis for estimating the potential clinical benefit of a CFTR-target therapy and for deciding whether reduction in sweat [Cl^-] caused by molecular therapeutics predict improvement in lung function.

CYSTIC FIBROSIS CASES IN KOREA

Jeong-Ho Kim, Chang-Seok Ki, Won-Jung Koh, Min Goo Lee

Department of Laboratory Medicine, Yonsei University College of Medicine; Department of Laboratory Medicine and 3Division of Pulmonary Medicine, Department of Medicine, Samsung Medical Center and Sungkyunkwan University; Department of Pharmacology, Yonsei University College of Medicine

Cystic fibrosis (CF) is very rare in Korean like other Asian populations. A diagnosis of CF is based upon the presence of typical clinical features, history of CF in a sibling, positive sweat chloride test, identification of *CFTR* mutations in both alleles, and an abnormal nasal potential difference measurement. We describe a total of 16 disease-causing mutations of the CFTR gene were identified in the 9 Korean CF patients.

The respiratory symptoms were clinically documented in the CF patients as follows: chronic cough (N=7; 78%), sputum (N=4; 44%), sinusitis (N=4; 44%), recurrent or persistent pneumonia (N=7; 78%), and bronchiectasis (N=5; 56%). Infections related with CF were pulmonary tuberculosis (N=5; 56%), aspergillosis (N=1; 11%), and infections with Non-tuberculosis *Mycobacterium* (N=1; 11%), *Staphylococcus aureus* (N=5; 56%), *Pseudomonas aeruginosa* (N=4; 44%), and *Stenotrophomonas maltophilia* (N=1; 11%). Most of the patients had experienced infections of *S. aureus* and *P. aeruginosa*, which are well-known clinical infections of CF, during the course of their disease progression. Other symptoms observed in the CF patients were a history of failure to thrive (N=1; 11%), steatorrhea (N=3; 33%), clubbed fingers (N=1; 11%), fatty liver (N=2; 22%), pancreatic atrophy (N=1, 11%) and meconium ileus (N=2; 22%).

All patients were diagnosed with CF on the basis of classical clinical phenotypes and high sweat chloride concentration (>60 mEq/L). The median sweat chloride concentration of 7 patients was 93.2 mmol/L, and all patients tested showed high sweat chloride concentration. In 2 neonates, the sweat test could not be done because of their young age.

All identified mutations were detected by PCR and direct sequencing with the exception of a large deletion in exon 14a, which was detected by MLPA. No p.F508del mutations, known to be the most common mutation among Caucasians, were detected in the Korean patients so far. The identified mutations included 3 missense mutations (p.Q98R, p.Q1352H, and p.L441P), 3 nonsense mutations (p.Q220X, p.Q1291X, and p.L88X), 1 duplication with frameshift (c.3908dupA), 1 insertion with frameshift (c.2089-2090insA), 4 splice site mutations (c.1766+2T>C, c.3272-26A>G, c.579+5G>A, and IVS8-T5) and 2 deletion mutations (c.2052delA and c.2623-2751+?del). The p.Q98R mutation was the only recurrently observed mutation, with a frequency of 18.8% (3/16 alleles). Mutations on both alleles were identified in 7 out of 9 patients. All identified mutations of the CF patients examined were confirmed with targeted genetic tests in consenting family members. Eight out of 9 families were tested, and all parents of the patients, except in 1 family, were proven to be heterozygous carriers of the mutations with no phenotypic abnormalities.

The heterogenous mutational spectrum of the CFTR gene in the Korean population suggests that full sequencing, covering a broader range of the CFTR genome, and may require more efficient way to screening the patient with standardized sweat chloride or conductivity test or other method.

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OVERVIEW AND REGISTRY OF CYSTIC FIBROSIS IN JAPAN

Hiroshi Ishiguro, Akiko Yamamoto, Miyuki Nakakuki, Kotoyo Fujiki, Satoru Naruse, Kunihiko Yoshimura, Tooru Shimosegawa, Yoshifumi Takeyama, and the Research Committee of Intractable Pancreatic Diseases, the Ministry of Health, Labor, and Welfare of Japan

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Cystic fibrosis (CF) is rare in Asian populations including Japanese. Common disease-causing mutations of *CFTR* in Europeans such as F508del have rarely been identified in Japanese CF patients. To estimate the number of CF patients in Japan and to examine the clinical courses, nationwide surveys have been conducted every 5 years from 1994 by sending questionnaires to larger hospitals (>400 beds) with pediatrics department and children's hospitals. The number of patients treated for CF in Japan in the year 2009 was estimated to be 15 (95% CI: 12-18). The estimated prevalence rate was 1 per 1,500,000 population. The median survival time of 95 cases (males 47, females 48) registered from 1994 was ~ 20 years. Female patients have had significantly ($p<0.05$) higher mortality rate than male patients. Their clinical courses were similar to those of European CF patients and most of them died of respiratory failure. Now 25 patients (males 11, females 14, median age 12) are now registered and treated for CF in Japan. Among them, 80% of patients are pancreatic insufficient and 24% have liver disease. Fifteen consecutive patients since 2004 were analyzed for *CFTR* mutations in Department of Human Nutrition, Nagoya University Graduate School of Medicine. All exons, their boundaries, and promoter region of the *CFTR* gene were directly sequenced and genomic rearrangements were examined by multiplex ligation-dependent probe amplification (MLPA) using a commercial kit (SALSA P091-C1 CFTR, MRC Holland). European type mutations including F508del and R1066C were found in 8 alleles inherited from European ancestry. Among 22 alleles inherited from Japanese/Asian ancestry, a large genomic deletion spanning exons 16, 17a and 17b (*CFTR*dele16-17b) was detected in the 8 alleles by MLPA. Sequence of the junction fragment revealed a new mutation: c.2908+1085_3367+260del7201[1)] which has not been reported in Europeans. CF-causing mutations including an Asian-type mutation, p.Leu441Pro were found in 12 alleles. Since no CF-causing mutations were found in the other 2 alleles inherited from Japanese ancestry, we analyzed CFTR transcripts extracted from their nasal swabs. In one patient who carries *CFTR*dele16-17b in one allele, PCR analysis of the full-length cDNA, generated using the gene-specific primer on exon 24, revealed a deletion of exon 1 in the CFTR transcript with intact exons 16-17b[1)]. In summary, the nationwide survey for 20 years confirmed very low incidence and poor prognosis of CF in Japanese. Most patients carry Japanese/Asian-specific *CFTR* mutations and *CFTR*dele16-17b is the major CF-causing mutation in Japanese.

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CFTR GENE MUTATIONS AND PULMONARY MANIFESTATIONS IN JAPANESE PATIENTS WITH CYSTIC FIBROSIS

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Cystic fibrosis (CF) is the most common lethal autosomal recessive disease in Caucasians affecting 1 in 2,500 live births. In contrast, it has long been considered very rare in the Japanese population. By the efforts of our group and others, the characteristics of CF cases in Japan have been gradually documented.

First, we analyzed the gene coding for cystic fibrosis transmembrane conductance regulator (CFTR) in 27 consecutive Japanese individuals with highly suspected or confirmed CF who were referred to Jikei University, Toranomon Hospital or Omori Red Cross Hospital. The diagnosis of CF is based on the Guidelines of Cystic Fibrosis Foundation Consensus Report[1). The presence or absence of CFTR mutations was evaluated by direct sequencing of the whole 27 exons of the gene. Among 27 patients, 8 were confirmed as homozygotes, and 8 were compound heterozygotes of CFTR mutations. Other 11 individuals were heterozygotes of CFTR mutation despite the rigorous search. The detected mutations included 125C in 14 alleles, dele16-17b in 6, T1086I in 4, Q98R, M152R, L441P and 1540del10 in 3, E217G, R347H, H1085R and Q1352H in 2, and 460insAT, R75X, G85R, E267V, 5T, delF508, L548Q, I556V, L571S, T663P, 2848delA, D924N, V1381I, and R1453W in 1, respectively. Most of the mutations listed above were very rare or novel in reference with the Cystic Fibrosis Mutation Database[2).

Second, we evaluated the pulmonary manifestation of patients with CF accumulated to the Japanese CF Patient Registry. Among 18 registered patients with CF, thirteen individuals were analyzed for radiological characteristics by chest computed tomography (CT). Saccular and/or cylindrical bronchiectasis was observed in 11 patients (85%), infiltrative shadows in 8 (62%), centrilobular small nodular opacities in 7 (54%), atelectasis in 5 (39%), cystic changes in 3 (23%), respectively. The severity in radiological abnormalities was likely related to the age of patients and the presence of sustained airway infection with *Pseudomonas aeruginosa*. These findings detected by chest CT in Japanese individuals with CF were very consistent with those of Caucasian patients in the literature[3)4)].

In summary, the spectrum of CFTR mutations was distinctive from that of other populations including Caucasians. Pulmonary manifestations evaluated by CT scan were very similar with those of other ethnic populations documented in the literature. Further elucidation is needed for more thorough understanding of CF in the Japanese population, and introduction of the most updated therapeutic approaches for CF is urgently required for Japanese patients with this devastating disease.

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REGULATION AND SYNERGISM IN EPITHELIAL FLUID AND HCO_3^- SECRETION

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HCO_3^- secretion is a key function of secretory epithelia and involves HCO_3^- entry at the basolateral membrane and exit across the luminal membrane. In most epithelia the bulk of HCO_3^- entry is mediated by the Na^+-HCO_3^- co-transporter NBCe1-B and HCO_3^- exit is mediated by the combined and regulated action of CFTR and members of the SLC26 transporters. The function, regulation and interdependence of CFTR/SLA26 complexes are critical for the secretory process. In this presentation, the properties of and function of the HCO_3^- transporters by IRBIT and by intracellular Cl^- will be discussed in the context of synergism in epithelial fluid and HCO_3^- secretion and intracellular Cl^- as a key regulator of the transport process.

HCO_3^- TRANSPORT BY CYSTIC FIBROSIS PANCREATIC DUCT

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Cystic fibrosis transmembrane conductance regulator (*CFTR*) gene encodes a protein that functions as a cyclic AMP-activated anion channel in various epithelia. HCO_3^- secretion in the pancreas juice depends on CFTR. Defective CFTR leads to acidic and small-volume pancreatic juice containing dense mucus and the formation of protein plugs, which eventually results in the destruction of pancreatic parenchyma and cystic fibrosis (CF) of the pancreas[1)2)3)]. However, it is not very clear why defective CFTR results in acidic pancreatic juice. Among the SLC9A family of Na^+-H^+ exchangers (NHE), NHE1 is ubiquitously expressed in various organs and regulates intracellular pH, while NHE3 is localized in the apical membrane of epithelial cells and mediates $NaHCO_3$ absorption in kidney proximal tubule[4)] and H^+-coupled dipeptide absorption in the small intestine[5)]. In mice pancreatic duct, NHE1 was localized in the basolateral membrane while NHE3 and CFTR were co-localized and functionally coupled in the apical membrane[6)7)]. Our previous data suggested that the activity of apical NHE was greater in pancreatic duct cells from ΔF cystic fibrosis (ΔF/ΔF) mice and it was enhanced by forskolin stimulation. The high activity of apical NHE (not controlled by functional CFTR) may be involved in acidic and small-volume pancreatic juice in CF. In this study we have examined forskolin and acetylcholine (ACh)-stimulated fluid secretion/absorption in interlobular pancreatic duct segments (diameter ~100 μm) isolated from ΔF cystic fibrosis mice. Both ends of the isolated ducts sealed spontaneously during 24-hour culture. The sealed ducts were superfused at 37°C and the rate of fluid secretion into the closed luminal space was analyzed by video-microscopy from the increment in the luminal volume and expressed as secretory rate per unit area of epithelium (nl min^{-1} mm^{-2}). When isolated ducts from wild-type mice (wt/wt ducts) were superfused with the standard HCO_3^--CO_2-buffered solution, basal fluid secretion at the rate of 0.08 ± 0.09 nl min^{-1} mm^{-2} (n = 4, mean ± SD) was observed. Stimulation of wt/wt ducts with forskolin (1 μM) or ACh (10 μM) significantly ($p<0.05$) increased the fluid secretory rate to 0.41 ± 0.10 and 0.26 ± 0.13 nl min^{-1} mm^{-2}, respectively. The rate of basal fluid secretion in isolated ducts from ΔF cystic fibrosis mice (ΔF/ΔF ducts) was 0.06 ± 0.02 nl min^{-1} mm^{-2} (n = 4), which was not significantly different from that in wt/wt ducts. Upon stimulation with forskolin or ACh, ΔF/ΔF ducts started shrinking. The rates of fluid absorption were -0.30 ± 0.14 nl min^{-1} mm^{-2} under forskolin stimulation and -0.11 ± 0.01 nl min^{-1} mm^{-2} under ACh stimulation. Thus both cAMP- and Ca^{2+}-stimulated fluid secretion was abolished in ΔF/ΔF cystic fibrosis pancreatic ducts and the stimulated ΔF/ΔF ducts absorbed luminal fluid instead. The fluid absorption by cystic fibrosis pancreatic duct is consistent with the high activity of apical NHE and may be involved in acidic and small-volume pancreatic juice in CF.

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REGULATION OF HCO_3^-/Cl^- PERMEABILITY OF CFTR

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Human pancreas secretes pancreatic juice which contains as much as 140 mM bicarbonate (HCO_3^-). Recently, we have shown that $[Cl^-]_i$-sensitive activation of WNK1-OSR1/SPAK pathway plays a critical role in pancreatic HCO_3^- secretion by increasing the bicarbonate permeability (P_{HCO3}/P_{Cl}) of CFTR[1]. However, how $[Cl^-]_i$-sensitive kinases modulate P_{HCO3}/P_{Cl} of CFTR remains elusive. In the present study, we investigated molecular mechanisms that underlie the WNK1-OSR1/SPAK-induced regulation of CFTR anion selectivity. Overexpression and knockdown of each kinase in HEK 293 and epithelial cells revealed that WNK1 is the key molecule that governs overall effect of $[Cl^-]_i$-sensitive kinases on the CFTR bicarbonate permeability. Furthermore, experiments with truncated WNK1 indicated that N-terminal parts of WNK1 are required to regulate P_{HCO3}/P_{Cl} of CFTR. Interestingly, WNK1 affects permeability of other anions as well as bicarbonate in patch clamp recordings. Especially, the interval of relative permeabilities (P_x/P_{Cl}) between each anion was greatly narrowed by WNK1. Consequently, WNK1 increased the dielectric constant of the hypothetical selectivity filter of CFTR. These findings suggest that WNK1 increases the bicarbonate permeability of CFTR by modulating the polarizability of anion selectivity filter and provide insight into the fundamental question of how ion selectivity of anion channels can be regulated by cytosolic signaling at the molecular level.

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ARHGAP9, A GTPASE-ACTIVATING PROTEIN, FOR CDC42/RAC1/RAC2, INHIBITS CFTR CHLORIDE CHANNEL ACTIVITY THROUGH THE STAS DOMAIN OF SLC26 TRANSPORTERS

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[Aim] We have reported that SLC26 Cl^-/HCO_3^- transporters and a CFTR Cl^- channel, expressed in the luminal membrane of secretory epithelial cells, have physical interactions and are functionally coupled (Ko *et al*, *EMBO J* 2002). The intracellular domain located at the carboxyl terminus of SLC26 transporters, called Sulfate Transporter Anti-Sigma factor antagonist (STAS) domain, directly binds to the regulatory domain of the CFTR chloride channel and markedly activates Cl^- transport activity of the CFTR (Ko *et al*, *Nat Cell Biol* 2004). The aim of this study was to identify a functional molecule that binds to the SLC26 STAS domain and participates in the regulation of CFTR chloride channel activity by the STAS domain.

[Materials and Methods] A glutathione S-transferase (GST) tag was added at the N-terminus of the STAS domain (aa.515-754) of human SLC26A6 transporter. Soluble fraction of Capan-1 cells, one of the human pancreatic adenocarcinoma cell lines, was prepared and proteins that bind to the GST-fused SLC26A6 STAS domain were isolated using glutathione-sepharose 4B beads. The samples were analyzed by Liquid Chromatography-tandem Mass Spectrometry. Anion exchange activity of SLC26 transporters was measured with a pH-sensitive-dye BCECF using fluorescent microscopy. Chloride channel activity of CFTR was electro-physiologically measured by the patch clamp method.

[RESULTS] ARHGAP9, one of the GTPase-activating protein for CDC42/RAC1/RAC2, was identified as a protein bound to the SLC26A6 STAS domain. GAP domain of ARHGAP9 was responsible for the binding of ARHGAP9 to SLC26A6 STAS domain. GAP domain of

ARHGAP9 had no effect on the anion exchange activity of SLC26A3 and A6 transporters. Rho GTPase Rac 1, one of the target proteins of ARHGAP9, did bind to the GAP domain of ARHGAP9, but did not bind to the SLC26A6 STAS domain. The GAP domain of ARHGAP9 had no effect on the CFTR chloride channel activity, but almost completely inhibited the activation of CFTR chloride channel by the SLC26 STAS domain. The SLC26A6 STAS domain showed no measurable GTPase activity.

[CONCLUSION] We have identified a GTPase-activating protein ARHGAP9 as a protein binds to the STAS domain of SLC26 transporters and inhibited the activation of CFTR chloride channel activity by the STAS domain. SLC26A6 STAS domain interacts with ARHGAP9 in a GTPase-activity independent manner.

CFTR IN REPRODUCTION AND EMBRYO DEVELOPMENT

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Cystic fibrosis (CF) is characterized by a hallmark defect in electrolyte and fluid transport in almost all tissues with exocrine function, with a wide spectrum of clinical manifestations, including chronic lung disease, pancreas insufficiency and infertility[1)]. However, the possible role of CFTR in regulating different processes of human reproduction was not explored till recently. The first hint for broader impact of CFTR on human reproduction other than congenital bilateral absence of the vas deferens (CBVAD) in CF came from the screening study on 13 *CFTR* mutations showing increased mutation frequencies in a general population of men with reduced sperm quality[2)]. A possible role of CFTR in sperm function was further suggested by the demonstrated involvement of CFTR in mediating uterine HCO_3^- secretion and its effect on the fertilizing capacity of sperm[3)]. CFTR protein was later found in mouse and human sperm and demonstrated to be important for the activation of the HCO_3^- dependent soluble adenylyl cyclase (sAC) and downstream cAMP/PKA signaling known to be involved in both sperm motility and capacitation[4)]. Sperm from CF mice were shown to have reduced sperm motility and capacitation with reduced fertility rate *in vitro* and *in vivo*[4)], clearly indicating a role of CFTR in sperm functions. Interestingly, a recent study on aging Chinese males has also demonstrated that reduced sperm qualities, such as motility and fertilizing capacity, in aging sperm are associated with age-dependent down-regulation of CFTR and impairment of CFTR/HCO_3^- -dependent cAMP signaling[5)]. Recently, a study has also reported impaired CFTR-dependent regulation of spermatogenesis in CF mice as well as downregulation of CFTR with abnormal CREB phosphorylation observed in testicular samples from Chinese men with azoospermia[6)], supporting a role of CFTR in spermatogenesis other than CBAVD.

Women with CF are also known to exhibit symptoms such as anovulation, higher testosterone to estradiol ratio, which are similar to polycystic ovarian syndrome (PCOS), an endocrine disorder affecting 5~10% women of reproductive age. However, the pathophysiological basis for abnormal estrogen production in CF and PCOS remains obscure. A recent study has demonstrated a previously unsuspected role of CFTR in modulation of basal and FSH-stimulated ovarian estrogen biosynthsis in ovarian granulosa cells involving a HCO_3^- sensor, the soluble adenylyl cyclase (sAC)[7]. Reduced sAC-dependent CREB phosphorylation, aromatase expression as well as the FSH-stimulated estrogen production are observed with CFTR inhibition or in *cftr* knockout/deltaF508 mutant mouse ovaries or granulosa cells. Reduced ovarian CFTR expression is also found in polycystic ovarian syndrome (PCOS) mouse models and human patients, suggesting that defective CFTR-dependent regulation of aromatase expression may underline the ovarian disorders seen in both CF and PCOS. A recent study has also demonstrated the involvement of CFTR/sAC-dependent CREB phosphorylation in activation of mir-125b required for embryo development[8]. Taken together, these findings support an important role of CFTR in human reproduction and embryo development well beyond CF.

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LOW-MORTALITY AIRWAY-SPECIFIC β-EPITHELIAL SODIUM CHANNEL (βENAC) TRANSGENIC MICE AS A MODEL OF CYSTIC FIBROSIS LUNG DISEASE

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Airway mucus hypersecretion, overproduction and obstruction are pathophysiological characteristics of severe lung diseases including cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD). Despite the enormous impact of these disorders, there is only limited murine model that reproduces the mucous-related airway obstruction. Increasing evidence suggests that these pulmonary phenotypes are in part due to increased airway Na^+ absorption mediated by the amiloride-sensitive epithelial Na^+ channel (ENaC), which results in depletion of airway surface liquid (ASL). Interestingly, Mall *et al*. showed the mice, which overexpress β subunit of ENaC (βENaC) in the lower airway, as a possible murine model of CF lung disease (*Nat Med* 2004). However, these mice mostly die within 4 weeks after birth due to severe airway obstruction, which limits potential to further analyze the pulmonary phenotypes of these mice.

To circumvent this limitation, low-mortality βENaC-transgenic (Tg) mice line was established by crossing commercially available βENaC-Tg mice (Jackson Laboratories, Bar Harbor, ME) and C57/BL6 mice for 3 generations. Consistent with the previous finding, mucus hypersecretory, airway inflammatory and emphysema-like phenotypes were observed in our low-mortality βENaC-Tg mice line. Notably, DNA microarray analysis further confirmed the pulmonary phenotypes observed in our Tg mice line. Moreover, clinically acceptable respiratory parameters, such as elastance (E), compliance (C = 1/E), forced vital capacity (FVC), forced expiratory volume in 0.1 second (FEV0.1) and FEV0.1% (FEV0.1/FVC), were analyzed by invasive lung function measurements using the flexiVent (SCIREQ Inc. Montreal, Quebec, Canada), which is known to result in relatively precise and physiological variables. Importantly, airway elastance was decreased and compliance was increased in the Tg mice compared with their wild-type littermate mice, and FEV0.1/FVC, a marker of airflow

obstruction during expiration, was significantly decreased in this Tg line, suggesting the impaired pulmonary mechanics in our established βENaC-Tg mice.
Although these findings suggested that our established βENaC-Tg mice might serve as a useful animal model for the mucous obstructive pulmonary diseases, therapeutic and pathophysiological evaluations by drugs or in genetically and nutritionally modified mice are still ongoing. In this symposium, we will focus on the proteases- and the oxidative stress-dependent pathways, which are determined by DNA microarray analysis, as crucial pathways in the development of mucus obstructive pulmonary diseases in the mice.

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CFTR QUALITY CONTROL CHECKPOINTS AS DRUG TARGET

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Cystic fibrosis (CF), one of the most common inherited disease in the Caucasian population, is caused by mutations of the CF transmembrane conductance regulator (CFTR). CFTR, a cAMP-regulated anion channel, is confined to the apical plasma membrane (PM) and mediates transepithelial water and electrolyte transport. CFTR comprises of two membrane spanning domains (MSD1, MSD2) and three cytosolic domains; a regulatory (R) and two nucleotide-binding domains (NBD1, NBD2). Newly synthesized CFTR is co-translationally N-glycosylated and undergoes both cotranslational domain folding and posttranslational, coupled multi-domain assembly in the endoplasmic reticulum (ER), aided by a network of chaperones and co-chaperones. Folded CFTR undergoes complex-glycosylation upon traversing the Golgi complex and expresses at the PM. Deletion of F508 (ΔF508) in the NBD1, the most common CF mutation (~90%), causes global misfolding of the CFTR, resulting in marginal PM expression of the partially functional channel. Misfolded CFTR is recognized by the ER and cytosolic quality control (QC) mechanisms and degraded by ubiquitin-proteasome system, a process known as ER-associated degradation (ERAD). Although most of the ΔF508 CFTR molecules are eliminated by ERAD, a small amount could be detected at the PM in selected mouse and human tissues. This "residual" ΔF508 CFTR activity can be augmented by exposing to reduced temperature (e.g. 26°C), chemical chaperones or correctors (e.g. VX-809). However, ΔF508 CFTR is rapidly eliminated from PM by peripheral QC mechanism, hampering the therapeutic efforts[1)].

Compared to the ER QC mechanism, peripheral QC mechanism responsible for elimination of ΔF508 CFTR from the PM remained largely unknown. At the ER, misfolded ΔF508 CFTR is recognized by ER (e.g. calnexin, DNAJB12) and cytosolic (e.g. Hsp/Hsc70, DNAJA1, Hsp90, Aha1) chaperones and co-chaperones. The ER-retained ΔF508 CFTR is ubiquitinated by ubiquitin E3 ligase CHIP, Rma1 and Gp78, leading to ERAD pathway. Similarly, the PM-localized ΔF508 CFTR is eliminated by ubiquitin-dependent mechanism. Ubiquitinated ΔF508 CFTR is rapidly internalized and sorted to lysosome for degradation. Endosomal

Sorting Complex Required for Transport (ESCRT) complex including Hrs and TSG101 is responsible for efficient lysosomal sorting of ubiquitinated CFTR at endosomes. Our functional siRNA screen reveal that conformationally defective ΔF508 CFTR at the PM is recognized by Hsc70-Hsp90 chaperone system and ubiquitinated by chaperone-associated ubiquitin ligase CHIP with UbcH5, an ubiquitin conjugating E2 enzyme. Inhibiting the CHIP-mediated ubiquitination by ablating the peripheral QC machinery stabilizes PM-localized ΔF508 CFTR and increases the channel function, indicating that modulation of the peripheral QC mechanism could augment the therapeutic effort of maneuver improving ΔF508 CFTR trafficking to the PM[2].

Recent studies reveal effect of ΔF508 mutation on the CFTR misfolding at the ER. ΔF508 mutation not only renders NBD1 energetically unstable but also impairs its interdomain interactions, especially NBD1-MSD2 and coupled domain folding[3,4]. Efficient correction of both CFTR structural defects is necessary and sufficient to restore ΔF508 CFTR function to the wild-type level in most CF patients[3,4]. As a corollary, correction of one of the primary (NBD1 or the NBD1-MSD2 interface) or secondary (e.g. NBD2) structural defects could account for the limited ΔF508 CFTR rescue efficiency of correctors identified to date[5]. Our recent study reveals that NBD1-MSD2 interface and NBD2 are stabilized by class I (e.g. VX-809) and class II correctors (e.g. corrector 4a), respectively. None of correctors, but only chemical chaperones (e.g. glycerol), surrogates of class III correctors, stabilize human ΔF508-NBD1. Combined treatment of three classes of correctors robustly restores ΔF508 CFTR PM expression and function by improving the ER folding efficiency and PM stability[6]. Thus, circumventing the CFTR QC checkpoints at the ER and PM provides an effective therapeutic strategy in CF.

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EARLY DETECTION OF LUNG INFLAMMATION AND INFECTION IN CYSTIC FIBROSIS

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The lungs of children with cystic fibrosis (CF) are virtually normal at birth. However, it is known that inflammation is present as early as 4 weeks, even in the absence of detectable infection. Furthermore, abnormalities in lung function and lung structure may be abnormal as early as 3 months of age. As the principal of therapy is to prevent deterioration in lung function and structure, there is a need to commence appropriate treatment early in life. Infection with *Staphylococcus aureus*, *Haemophilus influenzae* and *Pseudomonas aeruginosa* are the commonest organisms infecting the lower airway but other organisms such as *Stenotrophomonas maltophilia* and more recently non tuberculous mycobacteria are emerging important organisms; left undetected and untreated, many of these organisms cause a decline in lung function. Hence early detection is important as infection can be detected in the lower airway in babies even in the absence of symptoms[1)]. Broncho-alveolar lavage (BAL) is the gold standard for the detection of infection in the lower airway in children under 5 years of age who cannot spontaneously expectorate sputum. However, it is invasive and often requires a general anaesthetic. While some centres undertake this yearly in children under 5 as part of the annual review, there is little evidence to support improved outcomes with this approach. Other less invasive techniques to detect infection in children with CF include sampling the upper airway and oropharynx by a deep throat swab, cough suction or cough plates, but there are conflicting data on the sensitivity and specificity of each of these techniques when compared to lower airway sampling. The Australian Cystic Fibrosis Bronchoalveolar Lavage study compared one group who underwent BAL as follows: routinely before 6 months; if hospitalized for an exacerbation; if *Pseudomonas aeruginosa* was detected on an oropharyngeal culture; and following *Pseudomonas aeruginosa* eradication, with a group who underwent orpharyngeal culture sampling only[2)]. At 5 years there was no difference between scores on chest computerized tomography (CT) or *Pseudomonas aeruginosa* infection. As a consequence, the Cystic Fibrosis Foundation does not recommend use of bronchoscopy to determine infection with *Pseudomonas aeruginosa*. Furthermore, although it is a safe procedure, there are increasing concerns that general anaesthesia in children may have long term effects on neuro-cognition. In older children unable to expectorate sputum, induced sputum using hypertonic saline is often effective although it is time consuming.

CF is largely a neutrophilic driven disease and while the presence of inflammation in early life in the lower airway, particularly free neutrophil elastase, predicts bronchiectasis on CT scans at 3 years of age[3)] its presence does not usually change management. To help address this, the COMBAT study is currently underway in Australia assessing the anti-infective and anti-inflammatory role of azithromycin in CF babies from 3 months of age until 3 years of age by BAL and CT scans at 1 year and 3 years.

There is a clear need to develop sensitive and specific non-invasive markers to assess early inflammation and infection in CF. Exhaled breath condensate remains a promising tool to detect inflammation, but needs further development[4)]. Similarly, the utility of the lung clearance index deserves further attention after one study found that an abnormal lung clearance index in children with CF under 5 years of age was suggestive of infection with *Pseudomonas aeruginosa* and increased lower airway inflammation[5)].

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TREATMENT OF EARLY PSEUDOMONAS AERUGINOSA INFECTION IN CYSTIC FIBROSIS

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P. aeruginosa is one of the major pathogens in cystic fibrosis (CF) lung disease. Its prevalence increases with age and *P. aeruginosa* becomes the main bacteria in adults with CF. The mechanisms favouring initial colonisation include breakdown of mucociliary clearance due to depletion of airway surface liquid (ASL), changes in ASL composition as well as structural differences of CF airway epithelial cells. The incidence of *P. aeruginosa* infection differs between centers and hygiene measures such as cohort isolation appear to be important for the prevention of *P. aeruginosa* acquisition. Initial colonisation may be transient, but is often followed by a period of persistence of bacteria in the lower airways. Even this stage of infection with non-mucoid strains of *P. aeruginosa* is generally not associated with a change in the patient's clinical status. If untreated, most patients will eventually develop chronic infection with mucoid strains of *P. aeruginosa*, which, in most cases, cannot be eradicated even with intensive antibiotic treatment. A major focus in CF care is therefore to treat patients in the early phase of acquisition to avoid the shift to chronic mucoid *P. aeruginosa* infection.

Initially, intravenous antibiotic therapy alone was used for early *P.aeruginosa* infection similar to the treatment approach for pulmonary exacerbations in patients with chronic infection. Long term success was limited, but no randomized study has been performed to date. The concept of using inhaled antibiotics is based on achieving high concentrations in the airways with limited systemic toxicity and multiple studies have shown success with inhaled tobramycin alone. Two larger comparative trials, ELITE and EPIC, have assessed the efficacy of different treatment regimens. In ELITE 28 days inhaled tobramycin inhalation solution (TIS) was compared to 56 days of TIS demonstrating a short term success rate of over 80% for both treatment regimens and no superiority of longer treatment duration. The majority of patients remained *P. aeruginosa* free during the 2 year follow-up in both treatment arms. EPIC did not show any superiority of adding ciprofloxacin to inhaled tobramycin alone and treatment only at times of positive culture was equally effective to regular 3 monthly treatment. Subsequent studies have compared 28 days of inhaled tobramycin alone to the combination of oral ciprofloxacin and inhaled colistin the latter being popular in Denmark and the UK; both regimens were found to be equally effective. A recent study reported similar success rates for inhaled azthreonam as well, but this study did not include a control arm. Currently, trials are underway to assess whether either intravenous antibiotic therapy as an eradication protocol or adding azithromycin to inhaled tobramycin increase the success rate of initial therapy. Overall, the focus is now shifting to optimizing treatment of patients failing initial therapy; a treatment pathway was developed at our institution that will be presented at the meeting.

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THE CENTRAL ROLE OF THE NEUTROPHIL IN CYSTIC FIBROSIS RELATED LUNG DISEASE

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Cystic fibrosis (CF) is one of the commonest genetically inherited lung diseases. It is characterized by recurrent respiratory tract infections eventually leading to respiratory failure. One of the hallmarks of this disease is a persistent and predominantly neutrophil driven inflammation. Normally, neutrophils provide the first line of lung defence by killing and digesting phagocytosed bacteria and fungi, yet despite advances in our understanding of the molecular and cellular basis of CF, there remains a paradox of why recruited CF neutrophils fail to eradicate bacterial or fungal infections in the lung. Not only do CF neutrophils fail to protect the lung by killing pathogens they also actively contribute to lung destruction in CF by release of proteases and oxidants. In this presentation we will evaluate the factors subtending the CF neutrophil's inability to effectively kill pathogens in the CF lung. We will discuss the decreased degranulation of secondary and tertiary granules seen in the CF neutrophil and the pathways by which this occurs. We will evaluate how the CFTR defect contributes directly to this. We will also evaluate the increased degranulation of primary granules in neutrophils from individuals with CF, leading to increased neutrophil elastase release as well as release of other proteases and oxidants. We will discuss how these proteases and oxidants damage the lungs and lung defences and how their effects may be controlled. Finally we will evaluate the mechanisms controlling the excessive neutrophil migration into the CF lung and determine how much is due to dysregulation caused by inflammation and infection and how much is due to the intrinsic CFTR defect. This presentation will describe mechanisms involved in microbial killing, neutrophil migration and apoptosis leading to inflammatory resolution. We will discuss dysregulated neutrophil activity and consider genetic versus inflammatory neutrophil reprogramming in CF and ultimately pharmacological modulation of the CF neutrophil for therapeutic intervention.

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PERSONALIZED MEDICINE FOR THE TREATMENT OF CYSTIC FIBROSIS

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The underlying cause of cystic fibrosis (CF) is the loss of epithelial chloride transport due to mutations in the CF transmembrane conductance regulator gene (*CFTR*) that encodes the CFTR protein. The CFTR protein is a chloride channel that is normally present at the cell surface of epithelial cells, where it is opened and closed (channel gating) by ATP binding and hydrolysis when activated by protein kinase A. CFTR normally transports chloride to regulate salt, fluid, and pH balance in multiple organs. In patients people with CF, the loss of chloride transport due to defects in the CFTR protein results in the accumulation of thick, sticky mucus in the bronchi of the lungs, loss of exocrine pancreatic function, impaired intestinal absorption, reproductive dysfunction and elevated sweat chloride concentration. More than 1900 CFTR mutations have been identified. Many of these mutations result in the loss in chloride transport and presentation of the disease phenotype, with individual mutations varying widely in their severity. Evaluation of the molecular defect in the CFTR protein caused by CFTR mutations has shown that the loss of chloride transport can be due to a reduction in the quantity and/or function of CFTR channels at the cell surface. A potential therapeutic strategy to treat CF is to enhance chloride transport using small molecules known as CFTR modulators. Two complimentary CFTR modulators include CFTR correctors and CFTR potentiators. CFTR correctors increase the amount of functional CFTR at the cell surface to enhance chloride transport. CFTR correctors include lumacaftor (also known as VX-809) and VX-661. CFTR potentiators potentiate the channel gating activity (open probability) of the CFTR channels at the cell surface to enhance chloride transport. Ivacaftor (also known as KALYDECO^{TM}, VX-770), is a CFTR potentiator. Ivacaftor can potentiate the channel gating activity of CFTR channels delivered to the cell surface by lumacaftor or VX-661 to enhance chloride transport more than either agent alone. To test which mutant CFTR forms respond to CFTR potentiators and CFTR correctors *in vitro*, a panel of cell lines expressing different mutant CFTR forms and primary human airway cells derived from people with CF were used to monitor CFTR processing, trafficking, and chloride transport. The results of these *in vitro* studies may help select people with CF for clinical studies to investigate the potential clinical benefit of CFTR potentiators and correctors.

TREATMENT OF THE BASIC CF DEFECT BY MODULATING CFTR: INDIVIDUALIZED MONITORING AND THERAPEUTICS

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Based on the functional consequences of various CFTR mutations, specific therapeutic strategies to restore deficient or defective protein function are being developed by altering CFTR expression or function. Based on ambitious high-throughput screening efforts[1)2)3)4)5)], the benefits of these new CFTR modulators have begun to come to fruition for CF patients. Results in the clinic demonstrate that the rescue of the CFTR protein by the archetype CFTR modulator ivacaftor is associated with marked improvements in the clinical outcome that compare favorably to previous therapies widely used by CF patients[6)7)8)]. The CFTR potentiator ivacaftor (formerly VX-770) was the first to successfully advance as an approved CF treatment among patients with the G551D gating mutation, an allele represented in ~4% of CF patients. Ivacaftor and other CFTR potentiators function to activate surface-localized CFTR channels by potentiating cAMP-mediated channel gating via decoupling with ATP hydrolysis[4)9)10)] and may be more broadly useful as it demonstrates activity in vitro against a greater variety of CFTR missense alleles, including other Class III gating mutations and other CFTR forms that exhibit residual activity at the cell surface (i.e. conductance and mild processing mutants)[11)12)]. The highly efficacious treatment benefit observed with ivacaftor therapy has engendered considerable interest towards recapitulating its effects among other more common CFTR alleles[13)]. This includes correctors of F508del CFTR misfolding, termed correctors, that attempt to restore normal CFTR processing to the most common CFTR mutation. Recently, the investigational corrector lumacaftor (formerly VX-809) in combination with ivacaftor was shown to modestly improve lung function as well as the risk of pulmonary exacerbations in a large Phase 3 clinical trial program. Other agents that induce readthrough (or suppression) of premature termination codons (PTCs) to induce expression of full-length CFTR to an otherwise foreshortened protein are also under development and have shown promise in proof-of-concept clinical trials[14)15)16)17)]. An archetype small molecule investigational agent ataluren is presently in Phase 3 testing. Other approaches beyond these small molecule CFTR modulators are also being explored. For example, gene replacement by viral and non-viral gene therapy remains an approach under active investigation[18], as well as newer strategies that attempt to express CFTR through transduction of mRNA alone[19][20]. In total, this class of agents that target the basic CF defect serve as a prime example of the potential for new genetic-based approaches in CF, and serve as a seminal example for other genetic diseases. Since many patients (~40%) are complex heterozygotes for more than one CFTR mutation[21], combination therapeutics addressing more than one CFTR allele, or use of multi-drug therapy seem likely in the future, and will dictate a need for individualized therapeutics optimized for particular patients based on their underlying disease and other genetic covariates[13]. New technologies to monitor the effects of CFTR therapeutics, including in vivo and ex vivo biomarkers such as optical coherence tomography imaging[22] or intestinal organoid swelling[23], are rapidly emerging to address these needs. Effective modulation of CFTR function has also provided a new opportunity to determine the mechanisms of disease pathogenesis, including heretofore unanticipated effects of CFTR. For example, ivacaftor augments mucociliary clearance as observed by clearance of inhaled Tc99 radiolabeled particles[24]. Infection rates of *P. aeruginosa* also improves within 6 months, suggesting the possibility that innate defense is augmented simply by enhancement of CFTR function[24]. The relatively large magnitude of weight gain observed with ivacaftor in CF patients with G551D-CFTR has recently been attributed to its beneficial effect on intestinal pH via bicarbonate secretion, raising the possibility that mucosal integrity might also be improved throughout the gut[24]. Other potential avenues for exploration include the effect of CFTR modulation on other manifestations of CF, such as glucose metabolism, innate immunity and leukocyte function, osteopenia, pancreatic insufficiency and gastrointestinal absorption, some of which may be directly tied to CFTR activity.

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MULTIDISCIPLINARY CARE FOR CYSTIC FIBROSIS -SOME OF THE CHALLENGES

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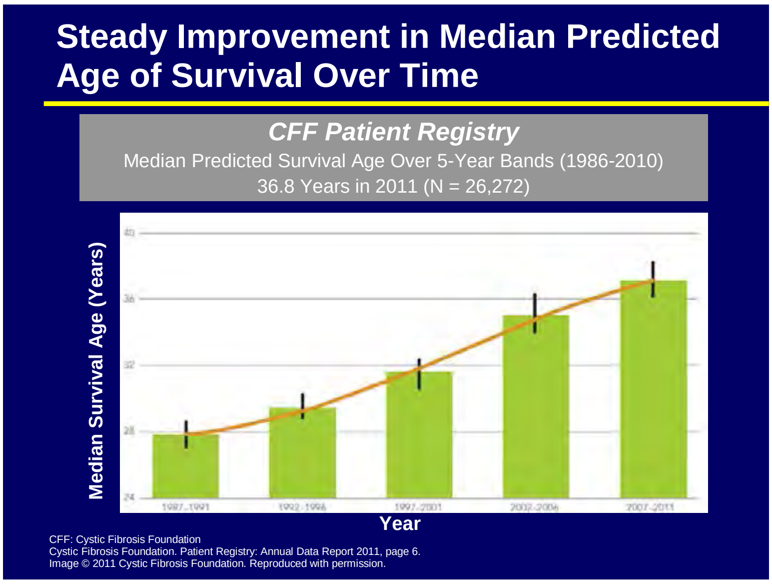
Cystic fibrosis (CF) is a complex multisystem genetic disease and the clinical outcomes for people with CF have changed dramatically over the last 20-30 years with a steady improvement in median survival (Figure 1). There are likely many factors responsible for this improvement but the establishment of specialist multidisciplinary care as the cornerstone of management has been regarded as one of the key factors responsible for this improvement.

Figure 1.



There have not been any randomised controlled trials of use of specialist multidisciplinary care for people with CF however two good quality observational studies have been reported. One study reported a step-wise improvement in nutritional outcomes (body mass index) and associated with improved forced expiratory volume in one second (FEV_1) and chest XR scores in adults related to the amount of care received in a CF specialist centre with multidisciplinary care. There was more infection seen with *Pseudomonas aeruginosa* associated with specialist multidisciplinary CF centre care in this study which was conducted prior to *P. aeruginosa* eradication and infection control measures were instituted in clinics to reduce the potential risk of cross infection and reduce chronic infection[1)]. A study in children managed in 3 types of care in Wales also showed a step-wise improvement in FEV_1 according to amount of CF specialist centre care they received[2]. One observational study showed no difference between groups according to the amount of CF specialist centre care however this was a small study[3)]. A consistent effect was seen in two high quality observational studies across both adult and paediatric patients and in both studies a "dose response" effect of CF specialist centre care was observed suggesting that specialist multidisciplinary care provides significant advantage.

In many countries Standards of care for CF have been established and present a consensus view of what constitutes an acceptable multidisciplinary team and the facilities that should be available for managing patients with CF.

It is now well recognised nationally and internationally that there is huge variation in health care practice and outcomes for chronic diseases. The National Health Service (NHS) in the United Kingdom (UK) has compiled an Atlas of Variation in Healthcare (www.rightcare.nhs.uk). This Atlas highlights the enormous variation across the UK for use of screening tools, medication, admissions to hospital, complications of diabetes etc. Understanding and exploring variation provides opportunities to improve health care outcomes, improve efficiency and reduce risks for poorer outcomes using quality improvement strategies but also highlights the many factors that are important in ensuring optimal outcomes for patients. Similarly, despite overall improvements in health outcomes over the last few decades, it is well recognised that there is a large variation between clinical outcomes for different CF specialist centres nationally and internationally[4)5)]. Although social, economic and clinical factors contribute to this variation, quality improvements within clinics significantly improve outcomes, suggesting that not only is a specialist multidisciplinary team important in improving health outcomes but that this alone is not enough and the function and behaviour of the team in managing patient outcomes is also hugely important in determining outcomes for patients [6)]. The quality improvement (QI) model established by the CF Foundation in the US using clinical measures to drive quality improvement programs has led to significant improvements in health outcomes[7)]. To optimise health outcomes using QI measures requires data collection and benchmarking of clinical outcomes and data registries established in many countries provide the opportunity to do this.

The conclusion is that CF specialist Centres with multidisciplinary teams associated with measurement and benchmarking of clinical outcomes along with quality improvement processes provide the optimal model for managing people with CF.

There remain many challenges and unknowns in establishing CF specialist centers with specialized multidisciplinary teams. What is the optimal size of a viable, effective CF specialist center and what is the smallest and largest size for such a center to remain optimal for clinical outcomes? The optimal staff to patient ratios for paediatric and adult care remains elusive and outcomes may be more related to the values, approaches and leadership of multidisciplinary teams rather than specific staff /patient ratios further complicating the establishment and optimization of CF multidisciplinary care. Specialist teams are usually established in large tertiary health care settings which may be at some considerable distance from where patients live making access more challenging. The costs of healthcare continue to increase in almost all settings and achieving the best value for money invested in health care is a growing concern to governments, health care providers and taxpayers alike. Are there risks in keeping the same model over time and how can the CF specialist model adapt to maintain optimal health outcomes but provide care closer to home that might be less disruptive of family and working life for patients and how can costs of care be contained? How should health outcomes be measured for people with CF in countries without CF data registries? How can this be done in countries with limited resources such as in many African countries, in India, Pakistan, Lebanon etc. Can regional registries be established or can established registries offer sharing of use of their registries across international borders? The European CF data registry has shown that a registry can work effectively across multiple different countries with different languages and availability of newborn screening etc. How could we fund and establish data warehousing to compare outcomes across all registries at a global level? Could established CF specialist centers with excellent health outcomes partner with emerging clinics or clinics with poorer outcomes in other countries to enable sharing and learning between groups?

The CF research and clinical community has led the way in improving health outcomes for a condition that is complex and was in the past considered hopeless and must continue to adapt and meet the many future challenges ahead as well as reach out on a global scale to improve the lives of people with CF everywhere.

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AIRWAY CLEARANCE PHYSIOTHERAPY FOR CYSTIC FIBROSIS

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Evidence based airway clearance techniques endorsed by the International Physiotherapy Group for CF are presented. Full descriptions of the techniques can be accessed online via the ECFC website. No one technique or device has been found to be superior. Rather, selections are made based on each patient's individual problems, preferences and lifestyles.

Modern airway clearance therapy (ACT) has developed over the past thirty years. The mainstay treatment of the last century was postural drainage in head down tilted positions. While assisting in sputum clearance, the cost was poor adherence and many side effects including precipitation of gastro-oesophageal reflux(GOR), nausea, regurgitation and vomiting. GOR may worsen lung disease by reflex bronchospasm or inhalation of noxious gastric gases or aspiration. Further common side effects were headaches, sinus pain and desaturation.

From 1980 onwards a number of modern evidence based airway clearance techniques were developed. The first of these was the Active Cycle of Breathing (ACBT) first developed in NZ, and researched and modified in the UK. It is a technique requiring no equipment, can be used independently and is flexible to meet the needs of each individual. The cycles consist of thoracic expansion exercises (deep breathing) with optional inspiratory pauses (breath holds), breathing control (relaxed breathing) followed by a series of forced expirations (FET also known as huffing. Coughing is carried out when the secretions have reached the upper airways. The technique is used in upright sitting or horizontal positions. The technique employs collateral ventilation, interdependence and movement of the equal pressure point together with the use of shear forces to move secretions from the small airways towards the mouth. The technique can be started from 2 years of age and gradually being able to be used independently from early childhood and throughout life. It is easy to learn and teach.

Concurrently a different breathing technique was being developed in Belgium called

Autogenic Drainage (AD). This technique aims to generate the highest airflow in all generations of bronchi without causing dynamic collapse. It involves the patient breathing at different lung volumes starting in the more distal airways with small tidal volume breaths and inspiratory pauses followed by expirations with increased flow velocity. As patients hear secretions towards the end of expiration they increase the inspiratory volume and expiratory airflow and gradually work up the range of respiration from expiratory reserve volume towards inspiratory reserve volume. The technique aims to generate shearing forces to erode the mucus from the airway wall and gradually move secretions towards the upper airways for FET/huffing, coughing and expectoration. The technique is gentle, can be used in upright sitting or horizontal positions. Expirations need to be carried out through an open glottis for optimal efficacy. Cough needs to be controlled until secretions are moved up to the upper airways. The technique requires no equipment, but takes longer to learn and teach.

At the same time in Denmark a technique able to be carried out independently, positive expiratory pressure (PEP) therapy was developed. This is a flow operated technique through a sealed mask developing a positive expiratory pressure of 10-20 cmH_2O via a resistor during exhalation in the tidal volume range for 10-15 consecutive breaths. This is followed by a series of FET/ huffing to assist in movement of secretions to the upper airways for coughing and expectoration when ready. This technique employs collateral ventilation and a temporary increase in functional residual capacity (FRC) during the series of breathing against the resistance of the mask and selected resistor. A manometer can be attached to the circuit to measure the amount of positive pressure developed with individual resistors in order to make a selection of the optimal resistor for each individual. The technique can be carried out in upright sitting or horizontal positions. It can be used from diagnosis in infancy throughout life.

Another version of PEP was developed in Austria in the 1980s using the same equipment but employing higher pressures called HiPEP. In this technique pressures of 30-100 cmH_2O are used including forced expirations and coughing against the pressure of the resistor in the mask set-up. This technique employs movement of the equal pressure point and splinting of the airways during its expirations, huffing and coughing.

Oscillating positive expiratory pressure (OscPEP) developed in Europe in the 1990s and was first available using the Flutter® device. This flow operated technique consists of a mouthpiece, cone, stainless steel ball which rests in the cone and a cap to hold the device together. As air is exhaled via the device the ball moves up and down in the cone developing an oscillating positive pressure throughout expiration. As with all techniques cycles of OscPEP are interspersed with FET/huffing and coughing as individually required. This device is gravity dependent and needs to be held in the optimal position Patients need to be sitting leaning their elbows on a table and holding the mouthpiece at an angle that results in an oscillatory frequency in the mid range of around 15-16 Hz.

A further development of OscPEP occurred in the USA with the manufacture of the flow operated Acapella® device. This device employs a mouthpiece and chamber with a cone and resistor operated by two magnets which allows variation in the amount of PEP incorporated in the OscPEP cycles. A control at the back of the device allows more or less PEP to be dialed by moving the magnets closer together or further apart changing the strength of the magnetic field. The device is not gravity dependent and can be used in upright sitting or horizontal lying.

OscPEP therapy utilises vibration of the airways to loosen secretions, and alters the rheological properties of the sputum to be less viscous through vibration of the mucus. The airways are splinted by the PEP generated and secretions are expelled with FET/huffing. Physical exercise such as walking, running, swimming, cycling, jumping, rowing and team sports incorporating whole body exercise can be used as airway clearance therapy utilizing deep breathing, position changes and combined with FET/huffing, coughing and expectoration when secretions reach the upper airway.

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NUTRITIONAL MANAGEMENT OF CYSTIC FIBROSIS

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Cystic Fibrosis (CF) is an autosomal recessive disorder, which affects approximately 30,000 adults and children in the United States and 70,000 individuals worldwide[1]. CF occurs as a result of a mutation in the CF transmembrane conductance regulator (CFTR), a sodium chloride channel, and presents in many systems, including the pulmonary, gastrointestinal, and reproductive organs[2][3]. Individuals eventually succumb to the pulmonary manifestations of the disease, however, maintaining an optimal nutritional status, especially early in life may have a significant impact on the trajectory of CF.

Numerous studies have shown that nutritional status is related to pulmonary function and health outcomes in people with CF. In the 1980's, a classic study comparing outcomes at two individual CF centers, Boston versus Toronto, illustrates this point. Despite similar medical management, there was a distinct survival advantage seen at the CF center in Toronto. The distinguishing feature was the high fat, less restrictive diets and more aggressive approach to nutrition at the Toronto site[4].

Although a connection between lung function and nutrition has been demonstrated, the question remains, which comes first? Is it that improved nutrition results in improved pulmonary outcomes or vice versa? A study by Konstan et al points to the former. In this study investigators found that children with a weight-for age percentile of < 5% at age 3 had the lowest FEV1% predicted values, which were < 90% at age 6. In addition, children with a weight-for-age percentile <10% at age 3 who improved to > 10% at age 6, had higher FEV1 values than those who did not improve[5].

From the body of literature that exists, it appears that not only does nutrition affects outcomes in CF, but that better nutritional status early in childhood and its positive effect on growth parameters is particularly important. A recent study by Yen and colleagues determined that greater weight at age 4 years is associated with greater linear growth, fewer complications and improved survival through 18 years of age[6].

Given the importance of nutrition in CF, what aspects of CF impose a risk for declining nutritional status in individuals with this disease? There are several factors that influence nutrition in individuals with CF, including the presence of malabsorption, present in 85-90% of CF patients, maldigestion, increased metabolic needs, related to increased work of breathing associated with frequent infections, decreased intake, and altered sense of taste and smell. Despite these challenges, the goal is to provide adequate nutrition in the form of a high calorie, high fat diet, along with optimal enzyme therapy to address pancreatic insufficiency. These strategies are implemented in order to achieve normal growth and development in children, along with the preservation of lean body mass and weight restoration in adults.

While high calorie high fat diets have been standard of care in CF for several decades, recently, this approach has met with some concerns[7]. As this patient population lives longer and as obesity rates increase, what is the impact of consuming a high fat diet, particularly trans fat and saturated fat, on inflammation and cardiovascular risk factors. These questions will undoubtedly become topics for future research.

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EXOCRINE FUNCTION AND NUTRITIONAL STATUS OF JAPANESE PATIENTS WITH CYSTIC FIBROSIS

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Background: Pancreatic insufficiency is the most common pathology of cystic fibrosis (CF). Insufficient pancreatic enzymes cause malabsorption of fat, protein, and several micronutrients. Better growth and improved survival are achieved by nutritional intervention. Although several guidelines for CF nutritional care are published in Europe and United States, there is no consensus in Asia.

Purpose: To analyze the nutritional status of Japanese patients with cystic fibrosis.

Methods: We performed anthropometric survey and biochemical analysis in 17 patients (2 to 37 years old) with cystic fibrosis in Japan.

Results: Fourteen patients (82.4%) had pancreatic insufficiency, 6 patients (35.3%) had anemia, and 2 patients (11.8%) had hypoalbuminemia. For all CF children (under 11 years old, n=10), the body weight was less than 25 percentile and the height was less than 10 percentile. The body mass index in adult CF patients (n=7) was 16.3 ± 3.4 kg/m^2.

Conclusion: Japanese patients with cystic fibrosis have growth impairment and malnutrition.

TRANSCRIPTIONAL NETWORKS REGULATING CFTR GENE EXPRESSION

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Normal expression of the cystic fibrosis transmembrane conductance regulator (CFTR) gene is controlled by many different regulatory mechanisms. Moreover, though the basal promoter is required for gene expression, tissue-specificity is conferred by cis-regulatory elements located outside the promoter and recruitment of a distinct set of elements occurs in different cell types. A higher order chromatin structure associated with the active CFTR locus is established and maintained by chromatin architectural proteins, CCCTC-binding factor (CTCF) and Cohesin. Within this 3D structure distal cis-regulatory elements such as enhancers bind cell-type-selective transcription factors and loop into close association with the gene promoter to activate CFTR expression. Critical enhancer elements that cooperate to drive CFTR expression in the intestinal and genital duct epithelium are located within DNase I hypersensitive sites (DHS) in introns 1 and 11. Elements located -44kb, -35kb and -3.4 kb upstream of the promoter work together to enhance expression in the airway. We identified factors that bind to each of these elements and contribute to their function as enhancers.
The enhancer in intron 11 (DHS11), which is located about 100kb away from the promoter, associates with enhancer signature proteins, such as p300, in addition to tissue-specific transcription factors (TFs). These factors include forkhead box A1/A2 (FOXA1/A2), hepatocyte nuclear factor 1 (HNF1), and caudal type homeobox 2 (CDX2). Mutation of the binding sites for each of these factors in the intron 11 core compromised its enhancer activity when measured by reporter gene assay. Moreover, siRNA-mediated knockdown of FOXA1/FOXA2 together and of CDX2 caused a significant reduction in endogenous CFTR transcription in intestinal cells, suggesting that these factors are critical for maintaining high CFTR expression levels in these cells. Chromatin immunoprecipitation (ChIP) analysis also showed that these TFs interact with multiple cis-regulatory elements across the CFTR locus implicating a more global role in intestinal expression of the gene. The transcriptional network that includes FOXA1/A2, HNF1 and CDX2 has other important roles in regulating epithelial ion channel and transporter gene expression. In contrast, the -35 kb enhancer (DHS-35kb) is evident in both primary human tracheal epithelial cells and many lung cell

lines. We showed that elements within DHS-35 kb bind interferon regulatory factor-1 (IRF1) or IRF2 and also nuclear factor Y (NF-Y). SiRNA-mediated depletion of IRF1 or overexpression of IRF2, an antagonist of IRF1, reduces CFTR expression in 16HBE14o- cells. NF-Y is critical for maintenance of H3K4me1 enrichment at DHS-35kb since depletion of NF-YA, a subunit of NF-Y, reduces H3K4me1 enrichment at this site. Moreover, depletion of SETD7, an H3K4 monomethyl transferase, reduces both H3K4me1 and NF-Y occupancy suggesting a requirement of H3K4me1 for NF-Y binding. NF-Y depletion also represses Sin3A and reduces its occupancy across the CFTR locus, which is accompanied by an increase in p300 enrichment at multiple sites and an associated elevation in CFTR expression. Our data suggest that airway-specific regulation of CFTR expression is mediated by sequences within DHS-44kb and DHS-35kb and that this depends on molecular mechanisms that involve both transcription factor binding and epigenetic modification of histones. These two processes are of fundamental importance at many sites across the locus though their relative contributions at individual regulatory elements may vary. These data illustrate the complexity of the cell-type-specific transcriptional networks that coordinate CFTR expression in different cell lineages and throw some light on the mechanisms that underlie the widely divergent abundance of CFTR in epithelial cells within the lung and the digestive system. Understanding these mechanisms may facilitate novel therapeutic approaches to modulate CFTR expression levels in vivo. This would be particularly relevant to CF patients with CFTR mutations that reduce the amounts of functional CFTR mRNA below the critical threshold.

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ANALYSIS OF CFTR TRANSCRIPTS FROM NASAL SWAB OF JAPANESE PATIENTS WITH CYSTIC FIBROSIS

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Cystic fibrosis (CF) is rare in Japan. Since 2007, we have analyzed mutations in the *CFTR* gene in 18 consecutive patients suspected of CF. CF-causing mutations were detected in 22 alleles by sequence analysis of all exons, their boundaries, and promoter region. Two novel types of large genomic deletion were detected by multiplex ligation-dependent probe amplification (MLPA) analysis, dele2-3 [c.54-1760_c.274-10222del18185] in one allele, and dele16-17b [c.2908+1085_c.3367+260del7201] in 8 alleles. Since no CF-causing mutations were found in the other 5 alleles of 4 patients, we analyzed CFTR transcripts extracted from their nasal swabs.

In patient 1 (1-year-old boy) who carries dele16-17b in one allele, PCR analysis of the full-length cDNA, generated using the gene-specific primer on exon 24, revealed a deletion of exon 1 in the CFTR transcript with intact exons 16-17b[1]. He was pancreatic insufficient (fecal elastase: 18 μg/g) and his sweat Cl^- level was 122 mM.

Patient 2 was a 7-year-old girl who carries CFTR p.Leu441Pro in one allele. She was pancreatic sufficient (fecal elastase: 777 μg/g) and her sweat Cl^- level was 114mM. RT-PCR of the full-length CFTR transcript from nasal swab was performed using the primers on 5'-untranslated region (-786) and exon 24 (4649). The size of the amplified fragment was ~4630 bp and the purified product lacked exon 1. An alternative 5' upstream exon of CFTR (exon -1a)[2] was directly spliced to exon 2. However, the splicing variant was also detected in nasal swab from a control subject.

The other 2 patients were suspected of CF because of recurrent pneumonia with mucoid strains of *Pesudomonas aeruginosa* and borderline sweat Cl^- levels. Fecal elastase levels were normal in both patients. Patient 3 was a 23-year-old man who carries p.Glu217Gly in one allele and has a genotype of 12/12 TG repeats, 7/7 poly T, and M/V at p.M470V. Patient 4 was a 38-year-old woman who carries p.Ile556Val in one allele and has a rare genotype of 12/12 TG repeats, 7/7 poly T, and V/V at p.M470V. We several times failed to extract full-length CFTR transcripts from nasal swab of both patients. Thus we next tried to compare the amount of the CFTR transcripts between these 2 patients and a control subject (11/12 TG repeats, 7/7 poly T, M/V at p.M470V, and I/V at p.Ile556Val). RT-PCR of CFTR exons 1-5 and exons 7-10 was performed. AQP5 was used as an intrinsic indicator since CFTR and AQP5 are both localized to submucosal gland acinar cells of respiratory epithelia. The amount of CFTR exons 1-5 transcripts in these 2 patients were reduced to 10-15% compared to the control subject. The ratio of exon 9 skipping was ~45% in these 2 patients. The data suggest that the amount of full-length CFTR transcripts in nasal epithelial cells in these 2 patients is reduced to less than 10% compared to the control subject.

Analysis of CFTR transcripts from nasal swab is recommended, when conventional sequence analysis proves to be negative in Japanese CF.

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RESCUE OF CFTR MUTATIONS WITH DIFFERENT MOLECULAR AND CELLULAR DEFECTS

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Cystic fibrosis (CF) is a major life-shortening genetic disease leading to severe respiratory symptoms caused by mutations in CF transmembrane conductance regulator (CFTR), a chloride/bicarbonate channel expressed at the apical membrane of epithelial cells. Absence of functional CFTR from the surface of respiratory cells reduces mucociliary clearance, promoting airways obstruction, chronic infection and ultimately lung failure[1)]. For the establishment a definite CF diagnosis proof of CFTR dysfunction is also required, more commonly through the so-called "sweat chloride test"[2)]. However, more recently other tests, like measurements of chloride secretion in rectal biopsies, have been validated as robust biomarkers to prove CFTR dysfunction[3)4)]. Newer assays like those using swelling in intestinal organoids[5)] or primary cultures of nasal cells are being developed but these still require further validation or development to be used in diagnosis[6)].

Major clinical advances in treating CF symptoms (with mucolytics, antibiotics, etc) have significantly increased survival beyond the second decade (~25 years in Europe). Notwithstanding, CF is still a life limiting condition. However, to further increase CF patients life expectancy, CF needs to be treated beyond its symptoms i.e., through treatments addressing the basic defect associated with CFTR gene mutations[7)]. So far ~1,900 CFTR mutations were reported[8)], but one single mutation, F508del remains the most common one, as it occurs in ~85% of CF patients worldwide in at least one allele[9)] and is associated with a severe clinical phenotype. Despite that most of efforts are focused on correcting the F508del-CFTR which causes intracellular retention of the mutant channel at the endoplasmic reticulum (ER), several additional strategies are emerging to rescue other (rarer mutants) which, in some populations, also have high prevalence. To apply such strategy CFTR mutations are thus classified into six main functional categories[7)], namely: Class 1) these are often mutations generating premature stop codons (e.g., R1162X) which prevent protein production; Class 2) this class includes F508del and they prevent traffic to the cell surface due to intracellular retention and premature degradation,; Class 3) these are mutants, like G551D, that cause impairment in the channel gating (i.e., decreased open probability); Class 4) these mutants have substantially reduced flow of anions through the CFTR channel (e.g., R334W); Class 5) here are include mostly alternative splicing mutants (e.g., 3272-26A>G) which allow synthesis of some normal CFTR mRNA (and protein), albeit at very low levels; and Class 6) these mutants impair the plasma membrane stability of CFTR (e.g., c.120del23[10)] or membrane-rescued F508del).

New therapies aiming the correction of defective CFTR in a mutation-specific manner, are also expected to be extended to mutations in the same functional class. However, in order to extend the existing approved therapies (e.g., Ivacaftor for G551D and other class III mutants) to more CF patients with additional (rarer) mutations in an effective and expedite way, it is crucial to pre-assess how these compounds rescue each CFTR mutation. This specific response to drugs can be achieved by using the same functional CFTR analyses already in use in diagnosis which employ patients' tissues *ex vivo*.

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COMPARATIVE ANALYSIS OF CFTR GENE POLYMORPHISMS BETWEEN CHRONIC BRONCHITIS AND HEALTHY CHINESE POPULATION

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CFTR protein contributes to the regulation of normal physiological functions in many tissues, such as airways, sweat glands, pancreas cells, bile ducts and genital ducts. Severe manifestations of respiratory system may develop into chronic bronchitis with CFTR gene mutation. To identify the role of CFTR variations in the occurrence of respiratory diseases in Chinese people, a total of 68 chronic bronchitis patients, and 117 healthy subjects were included in this study. The Tn-TGm haplotype was sequenced and the CFTR variant M470V was detected using restriction fragment length polymorphism to find the susceptibility to chronic bronchitis. The T7 allele frequency in the chronic bronchitis group was lower (88.2%) than that observed in the control group (93.6%), and the T5 allele was the second most common haplotype observed in patients of the study, with a control group frequency of 4.3%, and 8.8% in the chronic bronchitis group. The frequency of the V allele in the chronic bronchitis group (61.0%) was not significantly different from that observed in the control (56.0%). The T7-TG11 allele was the major haplotype in all subjects, 57.7% in control group and 50.4% in chronic bronchitis. The frequency of the T5-TG12 allele in chronic bronchitis (7.4%) groups was significantly more common than in the frequency observed in the control group (3.0%). T7-TG11-V470 was the primary haplotype observed ubiquitously throughout the study, with frequencies of 36.8% in the control group, and 34.6% in the chronic bronchitis group. The frequency of T5-TG12-V470 in chronic bronchitis (6.6%) was significantly higher than that observed in the control group (1.3%). In summary, the presence of the T5-TG12 haplotype of the CFTR gene is likely to play a key role in the development and progression of respiratory conditions, such as chronic bronchitis.

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GENETICS OF PANCREATITIS IN JAPAN

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Chronic pancreatitis (CP) is a progressive inflammatory disease in which pancreatic secretory parenchyma is destroyed and replaced by fibrous tissue, eventually leading to malnutrition and diabetes. Alcohol is the leading cause in Western countries, but genetic factors are also implicated. Since the identification of mutations in the cationic trypsinogen (*PRSS1*) gene as a cause of hereditary pancreatitis in 1996, we have seen great progress in our understanding of the genetics of pancreatitis. It has been established that mutations in the genes related to the activation and inactivation of trypsin(ogen) such as *PRSS1*, serine protease inhibitor Kazal type 1 (*SPINK1*) and chymotrypsin C (*CTRC*) genes are associated with pancreatitis. In 2013, carboxypeptidase A1 (*CPA1*) was identified as a novel pancreatitis susceptibility gene. Endoplasmic reticulum stress in pancreatic acinar cells resulting from the mis-folding of mutated pancreatic enzymes has been shown to act as a novel mechanism underlying the susceptibility to pancreatitis. In Japan, the nationwide survey revealed 171 patients (96 males and 75 females) with hereditary pancreatitis in 59 families based on the European Registry of Hereditary Pancreatitis and Familial Pancreatic Cancer criteria. Because about 30% of families with hereditary pancreatitis do not carry mutations in any of the known pancreatitis susceptibility genes, other yet unidentified genes might be involved. Next generation sequencing (NGS) is becoming standardized, reducing the cost of DNA sequencing and enabling the generation of millions of reads per run. NGS is especially useful to analyze the large genes such as *CFTR* which has 27 exons. Among 160 patients with CP, we could identify 10 non-synonymous variants including 2 novel ones [c.A1231G (p.K411E) and c.2869delC (p.L957fs)] and 6 synonymous variants including 3 novel ones in the exonic regions. Comprehensive analysis by NGS will be a promising strategy to identify novel pancreatitis-associated genes and further clarify the pathogenesis of pancreatitis.

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CFTR VARIANTS IN JAPANESE PATIENTS WITH CHRONIC PANCREATITIS

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Patients with idiopathic chronic pancreatitis, and some with alcoholic pancreatitis, are frequently carriers of mutants of the *cystic fibrosis transmembrane conductance regulator (CFTR)* gene in Europeans and Americans[1)2)]. Approximately half of Japanese patients with chronic pancreatitis exhibit sweat chloride levels over 60 mmol/L, suggesting the underlying CFTR dysfunction in a subset of the patients[3)]. However, cystic fibrosis (CF) is extremely rare in Japanese; the incidence is estimated to be ~1.7/million live births. We have previously shown that polymorphisms of polythymidine and TG dinucleotide repeats at the junction of intron 8 and exon 9 and the M470V variant, together with non-CF causing Q1352H and R1453W variants, are associated with chronic pancreatitis in Japanese[4)]. To further investigate the role of CFTR variants in chronic pancreatitis, we performed genotyping of two primary enzymes, *alcohol dehydrogenase (ADH1B)* and *aldehyde dehydrogenase (ALDH2)* genes, which are involved in alcohol metabolism and hence influence our drinking habits.

Methods: We conducted a sequence analysis of all the exons of the *CFTR* gene in 100 consecutive patients with chronic pancreatitis (75 patients with alcoholic and 25 with non-alcoholic pancreatitis) and 205 control subjects. Single nucleotide polymorphisms (SNPs) in *ADH1B* and *ALDH2* genes were identified by real-time PCR using specific primers. The *ADH1B*1* (Arg48) and *ADH1B*2* (His48) confer low and high enzyme activities, and *ALDH2*1* (Glu487) and *ALDH2*2* (Lys487) active and inactive enzymes, respectively.

Results: None of the patients and controls had CF-causing mutations observed in Japanese[5)]. Nine SNPs were identified that resulted in amino acid change in the CFTR protein, of which the M470V were most common (pancreatitis: 37.6% vs. control: 39.0%). Patients with chronic pancreatitis had significantly ($P<0.001$) higher frequency (20.5%) of these variants (alcoholic 20.7% and non-alcoholic 20.0%) than controls (8.5%). The L1156F (4.7 vs.0.2%)

and Q1352H (7.0 vs. 1.5%) alleles were more frequent (P<0.001) in alcoholic pancreatitis, while the R1453H (10.0 vs. 1.7%) was more frequent (P<0.05) in non-alcoholic pancreatitis than in controls. Allele frequencies of 4 variants, R31C (1.0 vs. 1.2%), E217G (1.5 vs. 1.5%), L548Q (0 vs. 0.2%), and I556V (2.5 vs. 2.2%), were not different between the patients and controls. No significant difference between patients and controls was found in the *ADH1B* genotypes. The *ALDH2*1/*1* genotype (85.3 vs. 46.8%) was more common (P<0.001), but the *ALDH2*1/*2* (14.7 vs. 43.9%) and *ALDH2*2/*2* (0 vs. 9.3%) were less (P<0.001), in alcoholic pancreatitis than in controls. The frequency of *ALDH2*1/*1*, **1/*2*, and **2/*2* genotypes were 36%, 44%, and 20% in non-alcoholic pancreatitis. All the *CFTR* variants were associated with the *ALDH2*1/*1* genotype in alcoholic pancreatitis. In non-alcoholic pancreatitis and controls, however, they were present in other genotypes.

Conclusion: The association of some but not all *CFTR* variants increases the risk of alcoholic and non-alcoholic chronic pancreatitis in Japanese.

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A JAPANESE CASE OF CYSTIC FIBROSIS-ASSOCIATED LIVER DISEASE

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Introduction: Cystic fibrosis (CF) is an autosomal recessive genetic disorder that is characterised by abnormal transport of chloride and sodium across an epithelium, leading to thick, viscous secretions. CF affects most critically the lungs, as well as the pancreas, liver, and intestine. CF is common within the Caucasian population, but less common in the Japanese, affecting about 1 in 1,500,000-2,000,000.

Case Report: We report a case of a Japanese girl with Cystic fibrosis with liver disease (CFLD). She was the second child of Japanese nonconsanguineous parents. The family history was unremarkable. She had a history of abnormal liver tests since she was six months old. She also had had recurrent bronchitis and sinusitis. At the age of 9 years, she was referred to our hospital due to liver disease of an unknown cause. Her weight was 23.5 kg (-1.2 SD), and her height was 125.0 cm (-1.6 SD). The liver was palpable 4 cm below the costal arch. The spleen was enlarged to 2 cm below the left costal margin. Hematological tests showed: hemoglobin 13.4 g/dL, white blood cell count 10,300/μL, platelets 144,000/μL. Serum liver tests showed: total bilirubin 0.5 mg/dL, conjugated bilirubin 0.2 mg/dL, aspartate aminotransferase 118 IU/L, alanine aminotransferase 145 IU/L, gamma-glutamyltranspeptidase 332 IU/L, prothrombin time 71%. Abdominal contrast-enhanced CT showed an irregular surface of the liver, splenomegaly, and atrophic pancreas. Gastroesophageal varices were not detected. Hepatic biopsy specimen showed focal biliary cirrhosis. Sweat chloride concentration was 117 mmol/L. Genetic testing revealed heterozygosity for the CFTR gene mutation.

Discussion: Although there have been few reports of CFLD in Japan, focal biliary cirrhosis can progress to multilobular cirrhosis with portal hypertension. For those patients with end-stage liver disease, liver transplantation has emerged as the procedure of choice.
Conclusion: In the case of liver disease with chronic bronchitis, CFLD should be considered.

A CASE OF CYSTIC FIBROSIS DIAGNOSED IN ADULTHOOD

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Cystic fibrosis is rare in Japan as in other Asian countries. It is well known that cystic fibrosis is caused by genetic abnormalities, such as CFTR mutations. The most common genetic mutation, F508del, in Europe is rarely identified in Japan. These differences in genetic abnormalities between these ethnics groups can affect the diagnosis. We encountered a case of cystic fibrosis in adulthood that was diagnosed by sweat test rather than by genetic testing. We report this case in order to discuss the problem of diagnosing cystic fibrosis in Japan.

[Case presentation]

A 25 -year-old man came to us with complaints of productive cough and shortness of breath. He had previously been admitted for pneumonia before coming to our hospital. His grandmother had had a pancreatic disorder, although she was currently healthy. No problems were noted at his birth, although he had suffered from bronchial asthma since attending elementary school. He had no history of smoking and took no herbs. He was studying at a vocational school. Some medications for asthma was prescribed, including inhaled glucocorticoid, theophiline, leukotriene modifiers, and a beta 2 stimulant. On physical examination, his breath sounds were diffusely decreased and wheeze was heard at his lung bases. On chest X-ray and CT images, remarkable over-inflation and bronchiectatic changes were identified. Pulmonary function testing revealed severe obstructive and restrictive dysfunction. Blood chemistry result showed mildly elevated C-reactive protein and normal serum pancreatic enzyme levels. He had no nutritional problems. *Pseudomonas aeruginosa* was detected on sputum culture. He had been suffering from recurrent pneumonia and had been treated with antibiotics. His respiratory condition had gradually deteriorated and pneumothorax had occurred two years later. We conducted a sweat test to determine the cause of his respiratory dysfunction. The sweat chloride concentration was determined at the Department of Human Nutrition, Nagoya University Graduate School. His mean sweat chloride concentration was 112mM. This suggested that his respiratory dysfunction was caused by cystic fibrosis. Further examinations were performed to confirm a diagnosis of cystic fibrosis and his blood sample was sent to the Department of Human Nutrition, Nagoya University Gradual School to examine for any genetic abnormalities. No genetic mutations were found in his blood sample. His clinical findings and the results of his sweat test were consistent with cystic fibrosis. We began treating him with inhaled tobramycin (TOBI, Novartis Pharmaceuticals Japan) and Dornase alfa(Pulmozyme, Chugai Pharmaceutical Co., Ltd., Japan).

A sweat test is a reliable test for diagnosing cystic fibrosis. One study reported that compatible symptoms and a high sweat chloride concentration of > 60 mmol/l were sufficient to diagnose cystic fibrosis[1]. Many adult cases of chronic sinopulmonary disease have been dealt with as being of unknown etiology in Japan because cystic fibrosis is so rare that genetic testing and sweat tests are not performed for screening in clinical practice. We think that there are many under diagnosed cases of cystic fibrosis in Japan. We have reported on a case of suspected cystic fibrosis in adulthood.

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INFANTILE-ONSET CYSTIC FIBROSIS PRESENTING WITH LIVER FAILURE

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Introduction: Cystic fibrosis (CF) is an autosomal recessive disorder which is characterized by dysfunction of Cl^- channel found on the surface of the pulmonary epithelial cells and other organs. Its incidence is quite low in Japan because of the lack of common mutations due to the founder effect. Hepatobiliary disease is a well known complication of CF, however clinical signs usually appear later in life because cirrhosis develops over a period of years. Here we describe a Japanese infant with CF whose initial presentation was hepatic failure.

Case report: A 3-months-old boy born to non-consanguineous Japanese parents was admitted to our hospital. He was found to have feeding difficulty and failure to thrive along with anemia, massive ascites, hypoproteinemia and coagulopathy. Contrast-enhanced computed tomography showed heterogenous density and morphological abnormalities of the liver. Histopathology of the liver showed periportal fibrosis, cholestasis, macrovesicular steatosis, disappearance of interlobular bile ducts, and proliferation of the bile ducts, consistent with the diagnosis of CF. Thereafter, he experienced repeated episodes of respiratory infections associated with fatty stools, further supporting the possible diagnosis of CF. Infantile-onset CF was eventually diagnosed by the sweat chloride test and genetic analysis identifying compound heterozygote of ΔF508 and Q1042Tfs5* at 4 months of age. Despite intensive respiratory support and antibiotic therapy, he died of respiratory failure at the age of 7 month.
Discussion: CF is rare in Japan and its diagnosis is not easy in Japan because of the limited access to the standard sweat test. In this particular case, the diagnosis was even more difficult because CF-associated hepatic failure rarely develops during infancy. Our case, however, illustrates the need to take CF into consideration for the differential diagnosis of hepatic failure even if it developed in infancy.

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TWO CHILDHOOD CASES OF CYSTIC FIBROSIS IN JAPAN

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Cystic fibrosis (CF) is the most common inherited disease among Caucasians, but it is rare in Japanese populations. We experienced 2 Japanese children with CF in Kagoshima prefecture, Japan around the same time.

Case 1 patient was 1-year-old boy. He developed meconium ileus on the first day of birth. He had been showing mild elevation of serum hepatic enzyme from the newborn period. He was admitted to our hospital to further examine the cause of liver dysfunction at 8 months of age. He was then affected by lower respiratory tract infections several times and also presented with steatorrhea during hospitalization. His sweat chloride concentration was 127mmol/l. Therefore, he had a diagnosis of CF. Then genetic screening was performed and CF transmembrane conductance regulator (CFTR) gene mutations were found: her one allele showed deletion of exon16, exon 17a, exon 17b and another showed deletion of exon 1 in the CFTR transcript. He responded to supportive care and is doing well at present day.

Case 2 patients was 2-year-old girl. She had been admitted to hospital 15 times because of asthmatic bronchitis. She was affected with distal intestinal ileus and was referred to our hospital for purpose of operation at 1year and 10 months of age. CF was suspected because of her symptoms and medical history. Genetic test was performed and she had CFTR gene mutations: both alleles showed deletion of exon16, exon 17a, exon 17b. Therefore she was given a diagnosis of CF. However she died at 2 years and 1 month of age due to acute pneumonia, followed by respiratory failure.

Even in Japan, patients who develop recurrent airway infections should be considered as possibly having CF.

THE FIRST CASE OF LIVING DONOR LUNG TRANSPLANTATION FOR CYSTIC FIBROSIS IN JAPAN; 12 YEAR'S FOLLOW-UP WITH MULTIPLE COMPLICATIONS

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Cases of living donor lung transplantation are rapidly increasing in Japan since 2,000. Long-term outcome is significantly superior in Japan to other countries. For this reason, it became one of the ideal therapeutic options for end-stage respiratory illnesses. On the other hand, various complications are observed with long-term survival, posing novel problems to maintain patient's well-being. Twelve years ago, we performed living-donor lung transplantation for CF for the first time in Japan. During the long years of follow-up, we have experienced significant complications in multiple organs. Potential problems of the procedure will be discussed in this symposium based on our experience.

The patient is a 38 years old female. She started to have productive cough 50 days after birth, followed by recurrent episodes of lower respiratory tract infection. She was diagnosed to have CF by sweat test at the age of 11. Thereafter, she had been followed up with supportive therapy for the respiratory symptoms. Her respiratory functions deteriorated rapidly with progression of bronchiectasis and repeated episodes of severe lung infection. She started to have home oxygen therapy at the age of 23. Mechanical ventilation was started at the age of 25 when severe lung infection led to acutely progressive respiratory failure, while she was on the waiting list for cadaveric lung transplantation.

She received living donor lung transplant from her parents at 25 years and 9 months. The respiratory function improved rapidly and it was maintained well for the first 6 years. However, her renal functions deteriorated and chronic obstructive lung disease progressed slowly over time, after long years of immunosuppressive therapy. During the follow-up, many problems surrounding the procedure have been highlighted, including the large medical cost, kidney dysfunction caused by immunosuppressive therapy and episodes of acute rejection due to poor adherence to medication. Significant role of regular IVIG therapy for the prevention of respiratory infection will also be discussed.

EFFECT OF AEROSOLIZED DORNASE ALFA AND TOBRAMYCIN TREATMENT ON LUNG DISEASE AND QUALITY OF LIFE IN A JAPANESE CYSTIC FIBROSIS PATIENT

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Introduction: Cystic fibrosis (CF) is an autosomal recessive disease that, while rare in Japan, has a poor prognosis. Pulmonary lesions are the main cause of death in over 95% of CF patients. Aerosolized tobramycin is a standard of care for chronic pneumonia caused by *Pseudomonas aeruginosa* infection in patients with CF. Inhalation of the enzyme dornase alfa reduces sputum viscosity and improves lung function and survival in patients with CF. Both these drugs, which were approved in 2012 in Japan, are easy to use at home. Here, we describe the combined treatment of dornase alfa and tobramycin in a CF patient who had previously been repeatedly hospitalized due to *P. aeruginosa* pneumonia.

Case: A 22-year-old man. Since infancy, he had been repeatedly hospitalized for pneumonia. Finger clubbing was noted at age 6, and he was presumed to have CF at age 14. Genetic diagnosis performed at age 21 detected mutation in E217G and polymorphisms in TG12-M470V, indicating CF affecting only a single organ (the lungs). At age 22, the patient again developed *P. aeruginosa* pneumonia and intravenous administration of an antimicrobial agent did not alleviate fever or breathing difficulty. Administration of dornase alfa plus tobramycin inhalation relieved the patient’s fever and reduced sputum viscosity and breathing difficulty. As a result, he could be treated as an outpatient.

Conclusion: Combination of the two drugs prevented the aggravation of *P. aeruginosa* pneumonia in a patient with CF, and enabled home-based treatment to improve his quality of life.

A CASE OF CYSTIC FIBROSIS IN A 9-YEAR-OLD JAPANESE CHILD

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Cystic fibrosis (CF) is very rare in Japan. We present a case of CF in a Japanese girl. She underwent laparotomy for meconium peritonitis at birth and had repeated hospitalization due to dehydration during infancy. At the age of 3, she suffered from intractable cough at night which gradually became worse. At the age of 6, she was referred to our hospital. Plain chest radiography revealed diffuse nodular infiltrates and chest CT demonstrated multiple areas of bronchiectasis and mucous plugging. Blood test showed increased amylase levels. The chloride concentration of insensible sweat collected from thumb was 114.3mEq/L (normal value <40 mEq/L). Hence, CF was diagnosed. After a comprehensive search for mutations in the cystic fibrosis transmembrane regulator (CFTR) gene, the patient was found to carry L441P mutation in a maternal allele. The patient received intensive treatments with internal medicine, inhalation and physical therapy but their lung function deteriorated. She is planning to receive lung transplantation from their parents. Although the incidence of CF is very low in Japan, the possibility of CF should be suspected in patients with unexplained intractable cough and recurrent pulmonary infections. Sweat chloride test is useful for screening and the research for CFTR gene mutation is required to confirm the diagnosis.

A CASE OF CYSTIC FIBROSIS DIAGNOSED 20 YEARS AFTER FIRST DIAGNOSIS OF DPB

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A 39-year-old women with productive cough, bloody sputum and low grade fever was diagnosed as diffuse panbronchiolitis (DPB) at the age of 19. Then, she was treated with low-dose macrolide therapy. Since she was 28 years old, her FEV 1.0 was decreased so rapidly, by 160 ml per year. She was admitted for bronchopneumonia at the age of 35.With type II respiratory failure requiring mechanical ventilation, She was underwent a tracheotomy, and started Home Oxygen Therapy. She repeated admission for respiratory failure. When she was 36 years old, she started Home mechanical ventilation. Next year she was listed on recipients of lung transplantation. She was admitted for pneumothorax at the age of 39. During this admission, she was diagnosed as Non-Classical Cystic Fibrosis. Dornase alfa (Pulmozyme®) and Tobramycin Solution for Inhalation (TOBI®) were started, but she suffered multiple relapses of pneumothorax. Her pneumothorax was intractable, we couldn't remove the drains. After long-term admission for about 5 months, she finally selected as the recipient of lung transplants from a brain dead donor. This case had been diagnosed as DPB for 20 years. She had only sinusitis in her childhood, without other features of CF. All exons of the CFTR gene were directly sequenced but no mutation was detected. Sweat chloride level was intermediate (47 mmol/L) and fecal elastase level was normal (625 μg/g). She didn't fulfill the diagnostic criteria of CF, but CFTR transcripts level of nasal mucosa was decreased to 10% of healthy subject, eventually she was diagnosed as Non-Classical Cystic Fibrosis. It suggests that there are some Japanese patients, even without the diagnosis of CF, might have the same abnormal CFTR transcripts level.

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A CASE OF CYSTIC FIBROSIS IN A 7-YEAR-OLD GIRL

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Respiratory tract infections are major causes of morbidity and mortality in patients with cystic fibrosis. Protection against exposure to MRSA, *P. aeruginosa*, *B. cepacia*, and other resistant gram-negative organisms is essential because of advancing in severity. We report a 7-year-old girl of cystic fibrosis who repeats hospitalization for bacterial pneumonia. The patient is a first child of healthy, Canadian and Japanese parents. She was born at term following an uncomplicated maternal pregnancy, labor, and delivery and the birth weight was 3776g. Her uncle died of pneumonia at 5 years old. At the age of 3 months, she was admitted to a hospital because of intractable cough and poor feeding, which required tube feeding. She was in fairly good health for about 3 years. At age of 3 years and 9 months she was suffered from pneumonia and admitted to her community hospital. Pulmonary CT scan indicated hyperinflation and bronchiectasis, which suggested diagnosis of CF. She was referred to the Aichi Children's Health and Medical Center at 4 years and 11 months of age. Sweat chloride measurements by pilocarpine iontophoresis and a CFTR genetic test by the PCR method and MLPA analysis was performed, at Nagoya University. Her sweat chloride concentration was 59.6mEq/L, and CFTR genetic analysis showed a compound heterozygous mutation of deletion 16-17 b derived from her mother and deltaF508 mutation derived from her father. Because her father was transferred, she came to our hospital at 5 years and 5 months of age. At age of 5 years and 11 months she was first admitted to our hospital due to *Staphylococcus* pneumonia. At age of 6 years she was hospitalized with meconium ileus twice, *S. aureus* and *H. influenzae* pneumonia 7 times, and otitis media once. At age of 7 years, she was hospitalized with pneumonia 4 times. Now she takes carbocystein, tulobuterol, pancrelipase, lactulose and continuous inhaled Dornase alfa, DSCG, procaterol every day. In addition, she received chest physical therapy during hospitalization but her lung function deteriorated. She is planning to receive lung transplantation from her parents.

IMPROVEMENT OF GROWTH RETARDATION IN A CHILD WITH CYSTIC FIBROSIS TREATED WITH DORNASE ALPHA AND TOBRAMYCIN INHALATION

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Poor linear growth and inadequate weight gain are very frequent problems in cystic fibrosis (CF) children. The growth failure is usually the result of several interacting causes. The most important factors are under nutrition or malnutrition, chronic inflammation, lung disease, and corticosteroid treatment. There are however patients whose clinical condition is not severe enough to be held accountable for this phenomenon. We aimed at describing a patient with CF who showed growth delay without being affected by severe pulmonary disease or malabsorption, showed a drastic catch-up growth after therapy with inhalation of dornase alpha and tobramycin. The index female patient was initially presented at the age of 11 months with a history of recurrent bronchitis manifested by severe cough, wheeze and dyspnea, which were cause to malnutrition. The diagnosis of CF was suspected by clinical findings and ethnic back ground of both of mother and father as Japanese-Spanish. The sweat test showed the elevated chloride level of 156 mEq/L and genetic testing revealed a compound heterozygous mutation of CFTR gene for 609delCA and 1756G/T.

After the confirmed diagnosis as CF, she had been treated following the clinical guidelines for care for children with CF including nutritional therapy with pancreatic enzyme and multivitamin, airway clearance with inhalation of bronchodilator, mannitol and corticosteroid, infection control with clarythromycin, and anti-biliary congestion with ursodeoxycholic acid. As improving nutrition and respiratory status, her body height and weight was gradually increased by the age of 5 years, in which height SDS and BMI-SDS increased from -3.39 to -0.98, and -2.17 to 0.46, respectively. However, she showed decreasing height velocity from the age of 5 to 7 years. Her respiratory condition was stable enough not to need hospitalization for distinct respiratory infection. Her nutrition sate was also good although BMI-SDS was decreased to -0.21 at the age of 7 years. Hormonal analysis revealed normal thyroid status and low serum IGF-1 levels (34 ng/ml, <-2.0SD) with normal response GH levels in several GH provocation tests. At the age of 7 years, she started additional therapy with dornase alpha and intermittent use of tobramycin and discontinued inhalation of corticosteroid. After this new therapy regime, height was increased from -2.33 to -1.96 SD with no significant changes of BMI-SDS, serum IGI-1 level and respiratory function test. Although nutritional status strongly influences pulmonary health and catch-up growth observed after diagnosis among CF patients, several factors are involved in deficit in length/height and weight to be seen around pubertal period. Although this presenting case did not show distinct improving respiratory function or nutritious status, the new therapy regimen could help to maintain pulmonary function and energy expenditure for pubertal development.

A CASE OF 37 YEARS OLD FEMALE CYSTIC FIBROSIS, 9 YEARS FOLLOW-UP

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In 2005, a 28 years old female was referred to our hospital because of the chronic respiratory infection. In her past medical history, she had been suffered from recurrent high fever in the childhood and took treatment of pneumonia once a year. She also had chronic sinusitis. She was allergic to minocycline, and denied any typical family medical history. At the first medical examination, sputum culture showed Methicillin-susceptible *Staphylococcus aureus* (MSSA) infection. Chest X-ray and CT revealed bronchiectasis of bilateral upper lung field and infiltration in the left middle lung. She was suspected cystic fibrosis (CF) and referred to the 2nd department of internal medicine, Nagasaki University. The sweat test showed elevated amount of chloride to 60mEq/l. Furthermore, BT-PABA (para-aminobenzonic acid) test was performed to reveal slightly diminished level of PABA in urine to 69.8%. Finally, CFTR gene was examined and mutation of R347H on exon 7 was determined. Therefore, she was diagnosed as CF. To control lung infections, Azithromycin (AZM), expectorant, inhalation of hypertonic saline and bronchodilators were started. In 2008, AZM was switched to clarithromycin because of the adverse effect of AZM. She had been taken cefditoren pivoxil to treat frequent exacerbation of MSSA infection because of the allergy to penicillin and quinolone. However bronchiectasis of left upper lung field is deteriorated gradually. In 2013, further examination of CFTR gene was performed and deletion of exon16, 17a and 17b on the other allele were detected. We will present the clinical course and current problem of this case.

A JAPANESE INFANTILE CASE OF CYSTIC FIBROSIS PRESENTING PSEUDO-BARTTER SYNDROME CAUSED BY H1085R AND Y563H COMPOUND HETEROZYGOSITY

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[Background] Cystic fibrosis (CF) is a very rare hereditary disease in the East Asian area, including Japan. CF shows an autosomal recessive inheritance pattern and is usually diagnosed by the presence of meconium ileus and frequent respiratory tract infections. Herein, we report a Japanese infantile case of CF. He exhibited marked electrolyte abnormalities, metabolic alkalosis, and weight loss. We initially suspected hereditary salt-losing renal tubular dysfunction disorders such as Bartter syndrome. However, there was no chloride (Cl) loss into the urine. He showed excessive sweating and Cl in his sweat was beyond the normal range. CFTR gene analysis of this patient revealed H1085R and Y563H compound heterozygous mutations.

[Case] A seven-month-old boy had experienced no remarkable events, such as meconium ileus, during the perinatal period. He had no family history of CF or parental consanguinity. He was evaluated by his family doctor for persistent diarrhea, vomiting, weight loss, and poor appetite. He showed marked electrolyte abnormalities such as Na 115 mEq/l, Cl 57 mEq/l, K 2.3 mEq/l, and metabolic alkalosis of HCO_3 46.5 mmol/l, high renin activity >20ng/ml/h, and an aldosterone level of 2370 ng/dl. He was referred to our hospital for further examination and treatment. His electrolyte balance normalized and his weight returned to the preclinical level with fluid therapy. His blood pressure was within normal range. Discontinuation of fluid therapy caused the same electrolyte abnormalities to recur. At first, we suspected a hereditary salt-losing renal tubular dysfunction disorder such as Bartter syndrome. However, as there was no Cl loss into his urine, salt-losing renal tubular dysfunction was ruled out. Excessive sweating prompted us to measure Cl in his sweat twice on different days. The measured Cl levels in his sweat were very high at 91 mEq/l and 109 mEq/l, respectively. CFTR gene analysis of this patient revealed H1085R and Y563H compound heterozygous mutations and confirmed the diagnosis of CF. He has since received NaCl supplementation and shown neither electrolyte abnormalities nor metabolic alkalosis.

[Conclusion] CF patients in Japan with marked electrolyte abnormalities, as in this case, may not be adequately diagnosed. In our present case, excessive sweating prompted measurement of Cl in his sweat and resulted in the diagnosis of CF. When we encounter electrolyte abnormalities, such as with a hereditary salt-losing renal tubule dysfunction disorder, we need to differentiate salt-losing renal tubule dysfunction disorders from CF by measuring Cl in both urine and sweat even though CF is rare in Japan.

PULMONARY HYPERTENSION IN A JAPANESE PATIENT WITH CFTR-RELATED BRONCHIECTASIS: A CASE REPORT WITH AUTOPSY

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Background: Pulmonary hypertension (PH) is associated with advanced pulmonary disease in adult cystic fibrosis (CF) patients. PH is also reported to be one of predictive factors for their survival. We describe a Japanese case with cystic fibrosis conductance regulator (CFTR)-related bronchiectasis developing severe PH with autopsy findings.

Case presentation: A 32-year-old man with the diagnosis of CFTR-related bronchiectasis had received treatment for chronic airway diseases since 16 years old. His brother also showed the similar bronchiectasis with the same pattern of *CFTR* gene mutation. He complained palpitation on exertion for 2 months and was admitted to our hospital due to hypoxemia with hypercapnia. Chest computed tomography showed severe bronchiectasis especially in bilateral upper lobes. We treated respiratory failure with noninvasive positive pressure ventilation, however it was not improved. Because he presented severe PH with estimated pulmonary artery pressure (PAP) of 90 mmHg by echocardiogram, which was confirmed by right heart catheterization, the combination therapy with sildenafil and bosentan was applied. One week after the treatment, estimated PAP was decreased to 65 mmHg and respiratory failure was temporarily improved. Finally he died 1.5 months after the admission, because of recurrent exacerbations of pulmonary infection and right heart failure. Autopsy findings suggested that pulmonary artery narrowing was related to the destruction of lung parenchyma.

VITAMIN C DEFICIENCY EXACERBATES RESPIRATORY FUNCTION AND EMPHYSEMA IN EPITHELIAL NA+ CHANNEL-OVEREXPRESSING MICE

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Oxidative stress, chronic inflammation, mucus overproduction and obstruction in airway are pathophysiological characteristics of severe lung diseases including cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD). Our preliminary microarray analysis using the lung tissue of CF- and COPD-like murine models (βENaC-transgenic mice) suggested an imbalance between oxidants and antioxidants. Vitamin C (VC), or ascorbic acid, is one of the strongest water-soluble anti-oxidants. Because plasma concentration of VC decreased significantly with age in CF and COPD patients and VC prevents cigarette smoke-induced inflammation in mice, VC may have beneficial effects on CF and COPD. In the present study, we sought to investigate whether endogenous VC affects pulmonary phenotypes of βENaC-transgenic (Tg) mice, including mucus hypersecretory, airway inflammatory and emphysema-like phenotypes. We first crossed βENaC-transgenic mice with senescence marker protein-30 (SMP30) knockout (KO) mice, which has been shown unable to synthesize VC due to the genetic disruption of gluconolactonase (i.e., SMP30). We further utilized βENaC-Tg male and female mice with SMP30 KO background (male: ENaC-Tg-SMP30 Y/-, female: ENaC-Tg-SMP30 -/-) deprived of VC for 8 weeks. Consistently, VC depletion increased the expression of oxidative stress-related genes in the lung tissue and of H_2O_2 level in plasma in ENaC-Tg-SMP30 KO mice. More importantly, VC depletion increased inflammatory status in lung tissue and exacerbated pulmonary emphysema that resulted in a significant decrease in FEV0.1/FVC, a marker of airflow obstruction during expiration possibly due to increased oxidative stress. Thus, our results demonstrate that VC plays an important role in the pathogenesis of CF and COPD in murine models and support the idea that ENaC-Tg-SMP30 KO lines may be one of the ideal CF and COPD models that mimic

characteristics of human patients with lower VC level.

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ABERRANT SPLICING OF ZINC TRANSPORTER ZIP2 CAUSES MUCUS HYPERSECRETORY PHENOTYPE IN CF AIRWAY EPITHELIAL CELLS

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CF is mainly characterized by pulmonary obstruction caused by chronic mucus hypersecretion and inflammation, that ultimately lead to death from respiratory failure. Identification of novel factors that control the CF phenotypes has been an important issue. One of such candidates is zinc ion (Zn^{2+}), an important element for activity of many proteins and cellular signaling, because of the reports that show its involvement in the pathogenesis of obstructive lung diseases like CF. Our preliminary microarray analysis focusing on zinc-related genes suggest that Zrt-Irt-like Protein 2 (ZIP2), a member of the SLC39A family that is expressed at plasma membrane and transports zinc ion into the cells, seems to be selectively dysregulated in airway specific βENaC-transgenic mice, a mice model that exhibits CF-like pulmonary phenotypes. To better understand the mechanism responsible for ZIP2 dysregulation and the relationship between ZIP2 dysregulation and pulmonary phenotype of CF, we first compared the expression levels and patterns of ZIP2 genes in human non-CF bronchial epithelial 16HBE14o- cells, primary airway epithelial cells and cell line (CFBE41o-) derived from CF patients, and β/γENaC-overexpressing CF-like airway epithelial cell line (β/γENaC-16HBE14o-). Importantly, increased expression of the novel splicing isoform of ZIP2 that contains additional exon inserted between the exons 1 and 2 was observed in CF-related cells, which causes a frameshift that results in a premature stopcodon

and deletion of most of the C terminus of ZIP2 (ΔC-ZIP2). Inverse correlation of expression between normal ZIP2 and ΔC-ZIP2 genes was confirmed. Moreover, in addition to decreased ZIP2 protein expression in CF-related cells, predominant existence of the cellular subset with lower zinc concentration in β/γENaC-16HBE14o- was observed. Notably, reduction of intracellular zinc concentration with a zinc chelator TPEN up-regulated MUC5AC gene expression in non-CF cells, suggests an important role of zinc concentration in the regulation of mucus hypersecretory phenotype. Finally, primary tracheal epithelial cells isolated from a model of CF lung disease (βENaC-transgenic mice) also expressed ΔC-ZIP2 gene transcript, suggesting its conservation within and between human cells and mouse tissue. Thus, our finding demonstrates that aberrant splicing of ZIP2 is a critical determinant factor that causes mucus hypersecretory phenotype in CF and CF-like airway epithelial cells.

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GLP-1 RECEPTOR AGONIST EXTENDIN-4 EXACERBATES MUCUS HYPERSECRETORY PHENOTYPE IN EPITHELIAL NA^+ CHANNEL-OVEREXPRESSING CELLS AND MICE

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CF is mainly characterized by pulmonary obstruction caused by chronic mucus hypersecretion and inflammation, that ultimately lead to death from respiratory failure. Identification of novel factors that control the CF pulmonary phenotypes is an important issue for the better treatment of CF patients. Glucagon-like peptide-1 (GLP-1) is a gastrointestinal hormone that mainly acts as a stimulator of glucose-mediated insulin production by pancreatic beta cells. Recent reports suggest pleiotropic effects of GLP-1 agonist on multiple organs, and decreased expression of GLP-1 in patients with CF, whereas little is known about the effect of GLP-1 in lung pathophysiology. Here, we showed that intratracheal treatment of airway specific βENaC (epithelial Na+ channel β subunit)-transgenic mice, the model of CF airway disease, with GLP-1 receptor agonist Exendin-4 (10 pmol/day, 2 weeks) significantly up-regulates mucin gene expression in lung tissue. Moreover, Exendin-4 significantly increased the alveolar mean linear intercept (MLI) and decreased FEV0.1% (FEV0.1/FVC; forced expiratory volume in 0.1 second/forced vital capacity), a marker of airflow obstruction during expiration, in the βENaC-Tg mice. Notably, Exendin-4 treatment also increased mucin expression in β/γENaC-overexpressing 16HBE14o- cells, and the effect was possibly induced by p38 MAP kinase pathway. Despite observations of Exendin-4-dependent mucin up-regulation in WT mice and parental 16HBE14o- cells, exacerbation was not observed. Taken together, the studies demonstrate that Extendin-4 specifically exacerbates the

pulmonary phenotypes of βENaC-Tg mice at least partly *via* increasing mucin expression, and our data may caution against the clinical use of GLP-1 agonist in CF-related diabetes (CFRD).

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INCREASED IL-17C PRODUCTION BY THE TLR3 LIGAND POLY(I:C) IN PRIMARY CYSTIC FIBROSIS AIRWAY EPITHELIAL CELLS

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CF is characterized by chronic inflammation that ultimately lead to death from respiratory failure. Overproduction of IL-8, a key chemokine that is crucial for an induction of neutrophil-dominated inflammation, has been considered as one of the important hallmarks of CF airway. Thus, identification of novel pathways underlying IL-8 induction could provide novel drug targets for prevention and treatment of CF. Importantly, increased expression of IL-17A, a cytokine that was shown to be primarily produced by a CD4+ T cell subset, T helper 17 (Th17) cells, was shown in BALF and sputum from bacterially infected CF patients, while we have recently shown that IL-17A is a critical factor in increasing IL-8 expression in bacteria-infected CF airway (Mizunoe *et al*, *J Phamacol Sci* 2012). Moreover, recent studies suggest that airway epithelial cells produce IL-17C, another IL-17 member that is produced by epithelial but not Th17 cells, which contributes to production of proinflammatory cytokines such as IL-8 in an autocrine manner. However, the regulatory mechanism responsible for IL-17C expression in both non-CF and CF airway epithelial cells is still largely unexplored. The present study sought to determine how IL-17C expression is regulated during pathogenic infection in CF airway. Among the tested ligands of toll-like receptors (TLRs) that mimic infection signaling, poly(I:C), a synthetic analog of viral double-stranded RNA that works as a ligand for TLR3, strongly induced IL-17C gene expression and secretion in primary airway epithelial cells derived from non-CF (NHBE) and CF (DHBE-CF) subjects. Maximum induction of IL-17C gene expression and secretion was observed at 24 hrs post-treatment in both cells, and the induction was mainly through NF-κB and partially through MAPKs (ERK, JNK, p38). Importantly, the poly(I:C)-induced IL-17C up-regulation was strongly enhanced in DHBE-CF cells. Moreover, TLR3 expression and poly(I:C)-induced up-regulation of IL-8 and IFNβ were also higher in DHBE-CF cells,

suggesting that the enhancement of poly(I:C)-dependent IL-17C induction appears to be the result of increased TLR3 expression in CF airway epithelial cells. Thus, these findings firstly provide the idea that poly(I:C) signal is a critical pathway in accelerating IL-17C expression in CF airway, which may imply that the TLR3-IL-17C axis is crucial for exacerbating viral infection-associated inflammation in CF lung disease.

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A HOMOLOGY MODELING OF HUMAN CFTR

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Over 1,900 mutations or polymorphisms have been found in *CFTR* gene. However, the severity of dysfunction and clinical consequences are known for only a subset of CFTR mutants. Molecular modeling may help predict the severity of dysfunction of CFTR mutants. At present, the most reliable method to predict the tertiary protein structure is the 'homology modeling' which uses homologous template-structures analyzed by NMR or X-ray crystallography. In this study we have tried the homology modeling of CFTR in the closed state. Pairwise sequence alignment was performed by EMBOSS web server (http://www.ebi.ac.uk/Tools/psa/emboss_water/). All calculations for homology modeling were performed using Discovery studio version 3.5 (Accelrys Inc., San Diego, CA). The sequence alignment was performed using mouse P-glycoprotein (PDB code: 3G5U for TMD1 and TMD2), a synthetic protein (PDB code: 1XMI for NBD1), and a fusion protein of human CFTR (PDB code: 3GD7 for NBD2) as template-structures[1)]. After the superimposition, energy minimization and equilibration were performed for conformational refinement. The model lacks R domain (residues 638-843), N-terminal (residues 1-56) and C-terminal (residues 1428-1480) regions. TMD2 and TMD1 in 3G5U (mouse P-glycoprotein) were used as templates for modeling of TMD1 and TMD2 respectively (TMDs were exchanged) according to a previous work[1)]. The whole structure of CFTR in the inward-facing conformation (closed state) was similar to that of multidrug resistance 1a.

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OPTIMIZATION OF A MATHEMATICAL MODEL OF ION TRANSPORT BY PANCREATIC DUCT CELL

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Pancreatic duct cell produces isotonic fluid secretion containing ~140 mM HCO_3^-. We have been constructing a mathematical model of pancreatic duct cell that various ion transporters, channels, and pumps are allocated in the basolateral and apical membranes by using MATLAB/Simulink. In the present study, we have tried to optimize the permeability of several transporters/channels/pumps at one time using an algorithm "fminsearch" which is based on the Nelder-Mead method. The permeability values to be optimized included those of Na^+-K^+ pump, K^+ channel, NBC1 1Na^+-2HCO_3^- cotransporter, and AE2 1Cl^--1HCO_3^- exchanger in the basolateral membrane, and of CFTR anion channel (P_{HCO3-}/P_{Cl-}) was set at 0.4) and SLC26A6 1Cl^--2HCO_3^- exchanger in the apical membrane. The values were optimized to reproduce the published experimental data of interlobular ducts isolated from guinea-pig pancreas. The data included (1) intracellular pH, [Cl^-], and membrane potential in the resting and cAMP-stimulated ducts luminally-perfused with low HCO_3^- (25 mM HCO_3^--125 mM Cl^-) or high HCO_3^- (125 mM HCO_3^--25 mM Cl^-) solution and (2) the maximal rate of fluid secretion (3.5 nl/min/mm^2 epithelium) and fluid [HCO_3^-] (140 mM) into the closed ducts. The standard errors of experimental data were set as acceptable variation ranges for optimization. The permeability values of transporters were successfully optimized to reproduce HCO_3^- secretion and intracellular parameters within acceptable ranges.

EXPRESSION AND FUNCTION OF CFTR MUTANTS FOUND IN JAPANESE CF PATIENTS

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We investigated expression and function of CFTR mutants found in Japanese CF patients, M152R, E267V, R347H, L441P, Y517H, T633P, R1066C (Pilipino-origin), T1086I and T1220I. We transfected the CFTR mutants to CHO cells and evaluated their expression and function using western blotting and whole-cell (WC) clamp technique.

R347H-, T633P- and T1220I-CFTR showed a WC current comparable to WT-CFTR. L441P- and R1066C-CFTR showed a smaller but significant WC current than WT-CFTR. However, we have not detected significant current on Y517H- and E267V-CFTR whereas M152R- and T1086I-CFTR showed a minimal WC current. In the western blotting, R347H- and T633P-CFTR showed the mature C band which intensity was higher than that of the premature B band. R1066C-CFTR showed significant B band which signal intensity was higher than that of the C band whereas E267V- and T1086I-CFTR showed minimal signal intensities for both B and C bands.

These results are generally consistent with the phenotype in the Japanese CF patient with each mutation.

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劇薬 処方せん医薬品 注意ー医師等の処方せんにより使用すること

＊効能又は効果：嚢胞性線維症における緑膿菌による呼吸器感染に伴う症状の改善

【禁忌（次の患者には投与しないこと）】
本剤の成分並びに他のアミノグリコシド系抗生物質又はバシトラシンに対し過敏症の既往歴のある患者

効能又は効果

嚢胞性線維症における緑膿菌による呼吸器感染に伴う症状の改善

〈効能又は効果に関連する使用上の注意〉

（1）6歳未満の小児における有効性及び安全性は確立していない。
（2）1秒量（FEV_1）が予測正常値に対し＜25％又は＞75％の患者、バークホルデリア・セパシア感染を合併している患者における有効性及び安全性は確立していない。

用法及び用量

1回300mgを1日2回28日間噴霧吸入する。その後28日間休薬する。これを1サイクルとして投与を繰り返す。

〈用法及び用量に関連する使用上の注意〉

（1）本剤を吸入以外の経路で投与しないこと。
（2）可能な限り12時間間隔で投与し、少なくとも投与間隔を6時間以上あけること。
（3）本剤の投与には、原則としてパリ・LC プラスネブライザー及びプロモエイドコンプレッサーを使用する。なお、コンプレッサーは、パリ・LC プラスネブライザーに装着した際に、流量4～6L/分又は圧力110～217kPaが得られるコンプレッサーを使用することも可能である。〔外国の臨床試験においては、パリ・LC プラスネブライザーが使用されており、これ以外のネブライザーを使用した場合の有効性及び安全性は確認されていない。〕
（4）患者が気管支拡張薬等の吸入及び肺理学療法を必要とする場合は、本剤の呼吸器における作用を確実にするために、これらの治療を行った後に本剤を投与することが望ましい。

使用上の注意

1.慎重投与（次の患者には慎重に投与すること）

（1）第8脳神経障害のある患者又は第8脳神経障害が疑われる患者
（2）腎機能障害のある患者又は腎機能障害が疑われる患者
（3）パーキンソン病や重症筋無力症等の神経筋障害のある患者又はこれらの障害が疑われる患者

2.重要な基本的注意

（1）吸入薬の場合、薬剤の吸入により気管支痙攣が誘発される可能性があるので、異常が認められた場合には投与を中止し、適切な処置を行うこと。
（2）注射用アミノグリコシド系抗生物質製剤を投与した患者において、**眩暈、耳鳴、難聴等の第8脳神経障害**が発現したとの報告があるので、第8脳神経障害が疑われる患者又は発現する可能性が高い患者には、**聴覚検査**を実施することが望ましい。
（3）第8脳神経障害又は腎機能障害が認められた場合には、**血中濃度が2μg/mL以下**に低下するまで本剤の**投与を中止**すること。
（4）注射用アミノグリコシド系抗生物質製剤と併用する場合には、トブラマイシンの血清中トラフ値をモニタリングすることが望ましい。

3.相互作用

併用注意（併用に注意すること） 腎毒性及び聴器毒性を有する薬剤：バンコマイシン、エンビオマイシン、白金含有抗悪性腫瘍剤（シスプラチン、カルボプラチン、ネダプラチン）等、ループ利尿剤：フロセミド等、マンニトール、腎毒性を有する薬剤：シクロスポリン、タクロリムス水和物、アムホテリシンB、セファロチンナトリウム、ポリミキシンB等、筋弛緩剤：A型ボツリヌス毒素等

4.副作用

本剤は、国内臨床試験は実施していない。
外国で実施された第Ⅲ相臨床試験において、本剤投与258例中121例（46.9％）に副作用が認められた。主な副作用は、咳嗽61例（23.6％）、咽頭炎31例（12.0％）、鼻炎27例（10.5％）、胸痛18例（7.0％）、喀血17例（6.6％）、喀痰増加17例（6.6％）、味覚異常16例（6.2％）、肺機能検査値低下16例（6.2％）、発声障害15例（5.8％）、肺障害（ラ音）13例（5.0％）、無力症13例（5.0％）等であった。 （承認時までの集計）

（1）重大な副作用

1）**急性腎不全**（頻度不明）： 注射用アミノグリコシド系抗生物質製剤を投与した患者において、急性腎不全等の重篤な腎障害が発現したとの報告があるので、定期的に検査を行うなど観察を十分に行い、異常が認められた場合には、投与を中止し適切な処置を行うこと。
2）**第8脳神経障害**（頻度不明）：注射用アミノグリコシド系抗生物質製剤を投与した患者において、眩暈、耳鳴、難聴等の第8脳神経障害が発現したとの報告があるので、観察を十分に行い、このような症状があらわれた場合には投与を中止することが望ましいが、やむを得ず投与を続ける必要がある場合には慎重に投与すること。

承認条件

日本人での投与経験が極めて限られていることから、再審査期間中は、本剤投与症例全例を登録して安全性及び有効性に関する製造販売後調査を実施すること。その中で、長期投与時の安全性及び有効性について十分に検討すること。

※その他の使用上の注意等は添付文書をご参照ください。
「禁忌を含む使用上の注意」の改訂に十分ご留意ください。

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