Pharmacogenetics and voice related personalized medicine

IFOS 2013 Seoul

Mette Pedersen MD Dr. Med. Sci.
Ear-nose-throat specialist h.c.
FRSM
The medical center

Østergade 18
1100 Copenhagen, Denmark
phone +45 33 15 96 00/
+45 33 13 77 00
Fax +45 33 13 77 05

Student co workers:
Philip A. Andersen
Sanila Mahmood
Anders Jønsson

Genetics, pharmacogenetics and personalized medicine in laryngology

- **Introduction, based on suggestions by Pais Clemente:**
- Genetics and immunology are important factors for the Medicine in the XXI Century. Practicing medicine before the appearance of the symptoms is the great challenge for the generations now and ahead. The human genome sequence has been completed and is under investigation. The **integration** with genetics holds a great potential value for a better understanding of the complex relationship between static genetic pre-disposition and **dynamic environmental factors** and its consequences for health maintenance, disease development and personalized treatment.

- **Purpose:** Although some progress has been made in complex diseases, such as metabolic syndrome, cardiovascular-,cancer diseases and others, **genetics of voice related disorders remain quite unknown for most of voice specialists and scientists.** Technological advancements have been profuse but must be considered as the foundation of current and future progress on voice.

- **Methods: Modern sequencing platforms**, microarrays, high-through put detection technologies, gene transcript profiling, quantitative multiplexed proteomics and nutrients/metabolite analysis should be the key tools achieving the developments in personalized treatment and predicting the rehabilitative process in voice. Establishing and managing databases are further tools to retrieve, visualize, validate, interpret and cross-correlate this data. Examples of a few or single cases are given of the literature, a great challenge is ahead for evidence based research.

- **Keywords:** Genetics, pharmacogenetics, personalized medicine

Overview

voice diagnostics and therapy related genetics, pharmacogenetics and personalized medicine – our own studies

Tissue engineering, cellular actions, micro array and others

• • •

Organization of personalized medicine in voice disorders

Genetic treatment aspects of upper airways

- life style, training, education antihistamines, cortisone and adrenalin (our own research that lead to focusing on genes and personalized medicine)

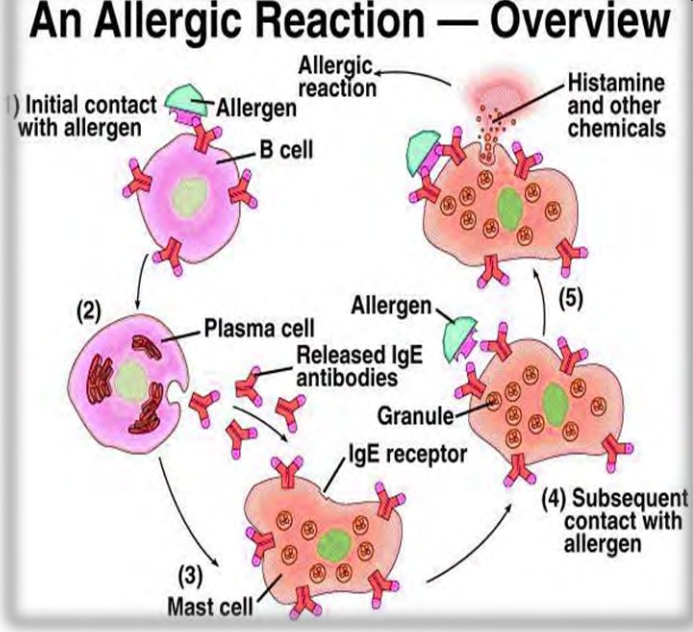
- Pedersen M, Eeg M, (2012) **Does treatment of the laryngeal mucosa reduce dystonic symptoms?**

  Pedersen M., Eeg M. (2012). Does Treatment of the laryngeal mucosa reduce dystonic symptoms? A prospective clinical cohort study of mannose binding lectin and the other immunological parameters with diagnostic use of phonatory function studies. *Eur Arch Otorhinolaryngol*. 269(5). 1477-1482

- Pedersen M, Eeg M (2012) **Laryngopharyngeal Reflux**

  Pedersen M (2012) Laryngopharyngeal Reflux – A Randomized Clinical Controlled Trial. Otolaryngol S1:004. doi:10.4172/2161-119X.S1-004

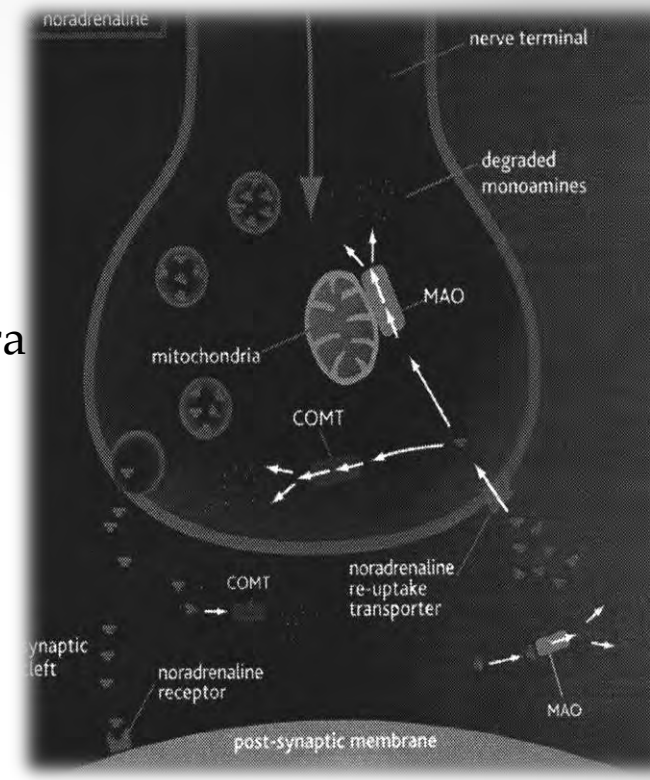
Treatment aspects



Antihistamines :

It is generally accepted scientific evident that these Hl histamine antagonists work by inhibiting the release of histamines on tissues.
In the event of an exaggerated response to allergens and infections in the body, histamine will be released by granulocytes such as basophiles and mast cells.
These kinds of cells are part of **the innate immune system** (see picture) and can be called the first element of immune defense.
On these cells are Fc receptors, which release histamine upon activation. This happens when the IgE antibody attaches to the receptor.
Therefore, **it may require different doses**, as there may be different densities of receptors. When a B-cell is in contact with a allergen such as pollen the cell will proliferate to a plasma cell and begin to release large amounts of IgE antigens into the tissue.

Treatment aspects

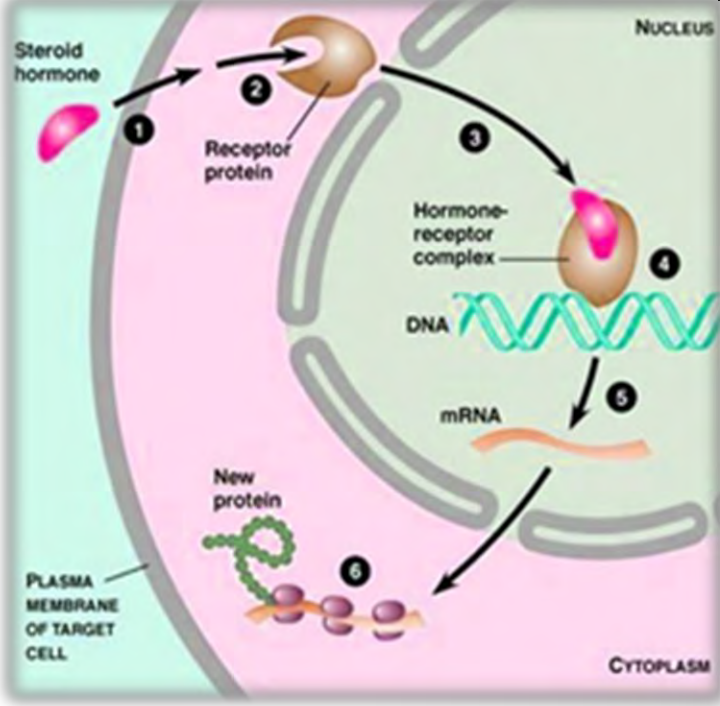


Adrenalin:

Adrenaline is produced in the adrenal gland and is a stress hormone that is released during situations that require extra attention to the body. In such cases where adrenaline is released into the blood
the following happens:
-increased heart rate,
larger displacement of the heartbeat,
raising of the-blood sugar concentration, dilation of the pupil.
-**constriction of the arterioles** of the mucous membranes and intestines.
-dilatitation of the arterioles in skeletal muscle.
By use of a synthetic adrenaline, called ephedrine, it can promote the same effects in the body. For example, there will be **a contraction of the arterioles in the nose and throat** (the small blood vessels) or a **dilation of the bronchi** in the lungs. The effect of bronchodilators is used in products in Astra products and Glaxo products with several short and long acting products. Therefore these drugs can be **allergy and infection-relieving.**

Treatment aspects

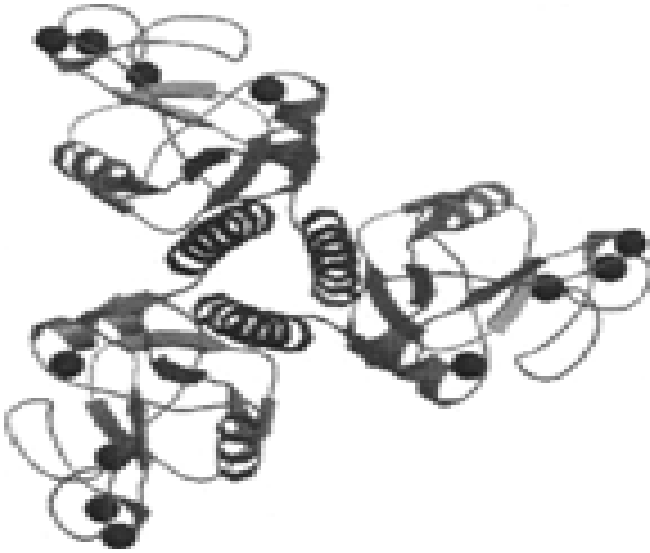
Cortisone :



Cortisone is produced by the body, synthesized from cholesterol in the adrenal gland. All derivatives of cortisone, used for medicinal purposes, are called glucocorticosteroids.
Glucocorticoide effect at the cellular level functions by binding to cytoplasmic receptor proteins (Receptor chaperonin complex), which then **binds to the cell nucleus DNA.**

This has an effect on transcription and translation processes, which will affect the cell's impact on the surrounding tissue. Tissue contains different amounts of these receptors which is why glucocorticoids work differently in different tissue.
For example, a regulation of inflammation is seen in mucous membranes and also in upper airway tracts. The binding of DNA happens by a phosphorylation of the hormone receptor complex.

Treatment aspects



Mannose Binding Lectin:

Is mannose binding lectin related to voice?

It is a problem not to have enough because it **probably indirectly inhibits secondary genetic disorders**.

Mannose-binding lectin is the primitive immune system

This protein is a very important component of the innate immune system (the first defence) because the molecule is designed to recognize carbohydrate patterns on the surface of pathogens (bacteria and viruses, etc.).

Therefore, this molecule has a major influence in the fight against an infection already in the early stages.

Approximately 4% of the Danish population has a mannose-binding lectin <100 μg/l. It is considered low when it is under 500 μg/l.

Tanja the first treated dystonia patient in the cohort study

- Tanja had low MBL and was eager to have other dystonia patients examined for eventual mannose binding lectin (MBL)
- We made a prospective cohort study of **55 patients comparing patients with and without MBL reduction**
- We tried to identify other upper airway related disorders in our systematic search for a reason, including genetic lactose and gluten intolerance, food and inhalation allergy – without positive findings

Pedersen M., Eeg M. (2012). Does Treatment of the laryngeal mucosa reduce dystonic symptoms? A prospective clinical cohort study of mannose binding lectin and the other immunological parameters with diagnostic use of phonatory function studies. *Eur Arch Otorhinolaryngol*. 269(5). 1477-1482

Pedersen M., Eeg M. (2012). Does Treatment of the laryngeal mucosa reduce dystonic symptoms? A prospective clinical cohort study of mannose binding lectin and the other immunological parameters with diagnostic use of phonatory function studies. *Eur Arch Otorhinolaryngol.* 269(5). 1477-1482

Genetic immune system

Mannose binding lectin as part of the genetic immune system was not found directly involved in dystonia in this study of 55 patients, even if there was effect with treatment with the antihistamine: fexofenadin and local cortisone inhalation in the throat

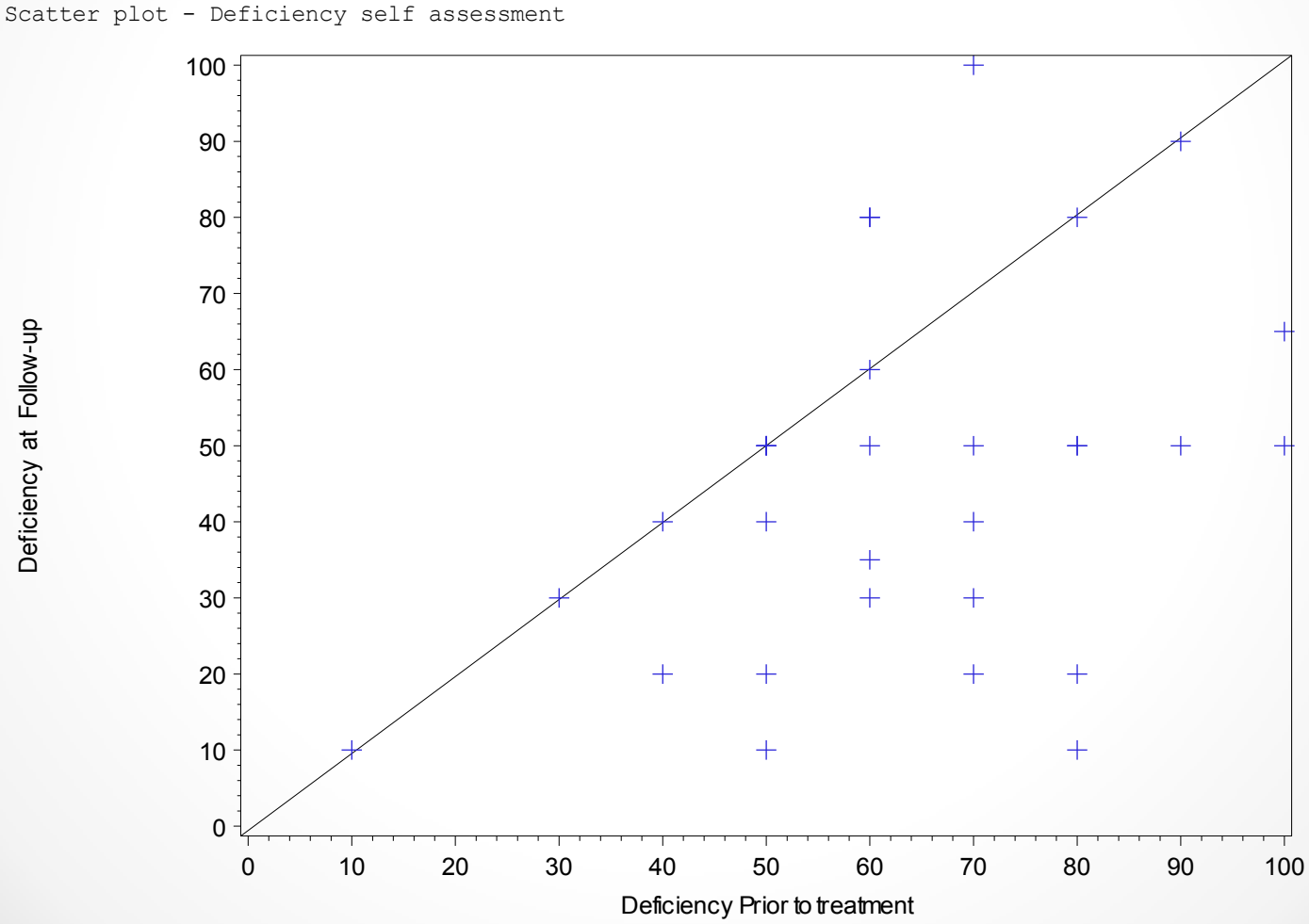
	1st consultation			2nd consultation			Change (2nd-1st consultation)			
	N	Mean	Std	N	Mean	Std	N	Mean	Std	p-value
All dystonia patients symptoms and QoL	55	2.71	0.60	49	2.35	0.63	49	-0.35	0.72	**0.0003*** **
MBL<500 µg/L	26	2.69	0.62	22	2.32	0.57	22	-0.36	0.73	
MBL>500 µg/L	21	2.67	0.58	20	2.30	0.73	20	-0.40	0.75	
MBL<500µg/L vs MBL>500 µg/L										**0.90§**

§: Test in the linear statistical model where MBL is included as a fixed effect and baseline is included as a covariate.
*****: Statistically significant on a 0.1% significance level**

Pedersen M., Eeg M. (2012). Does Treatment of the laryngeal mucosa reduce dystonic symptoms? A prospective clinical cohort study of mannose binding lectin and the other immunological parameters with diagnostic use of phonatory function studies. *Eur Arch Otorhinolaryngol.* 269(5). 1477-1482

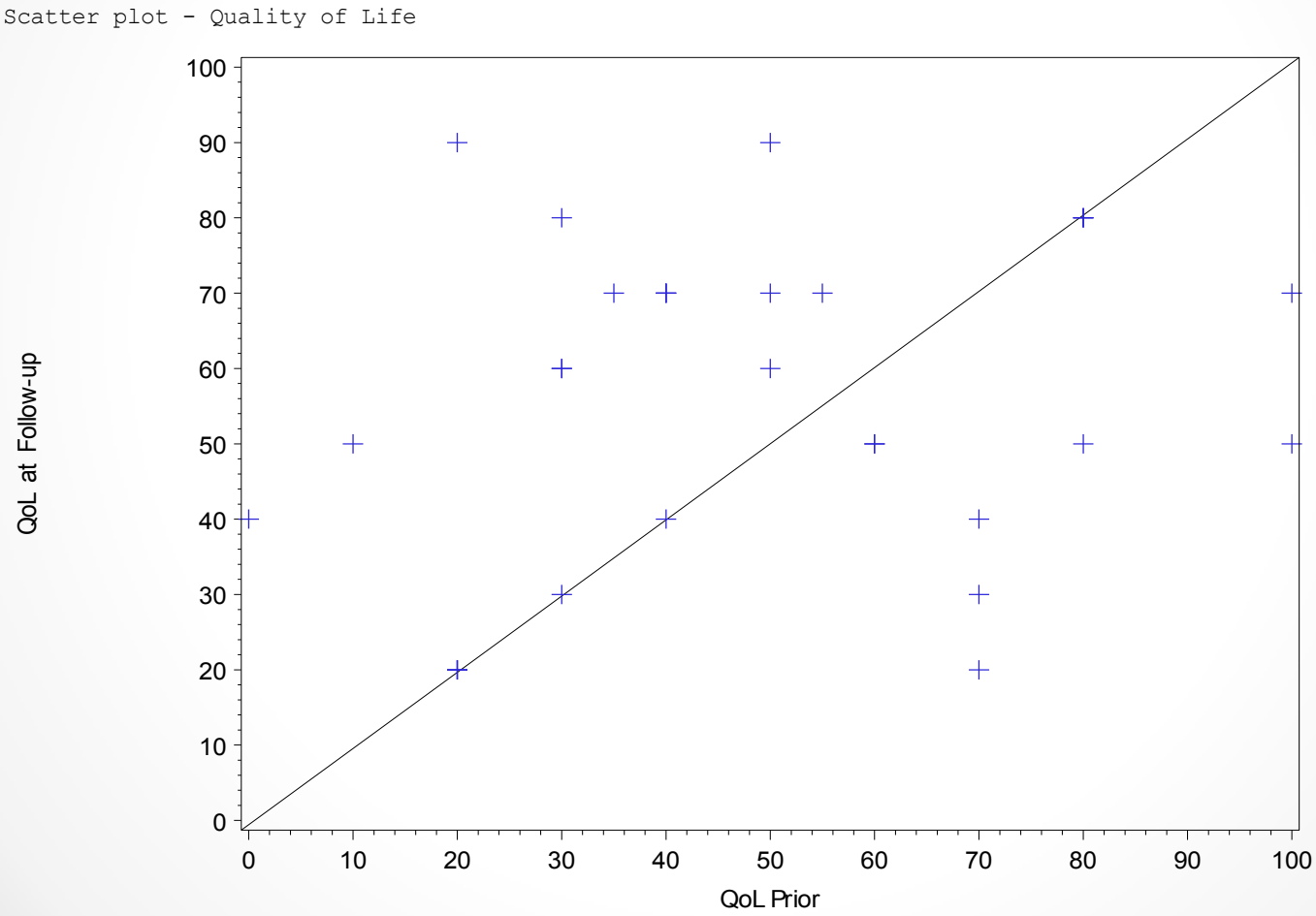
Symptom reduction in 55 patients with dystonia

(absissa deficiency prior to treatment ordinat deficiency at follow-up)



Quality of life in 55 patients with dystonia

(absissa QoL prior ordinat Qol at follow-up)



VSSolutions - Generated 30MAR11/scatter qol.cgm

Laryngopharyngeal Reflux – A Randomized Clinical Controlled Trial

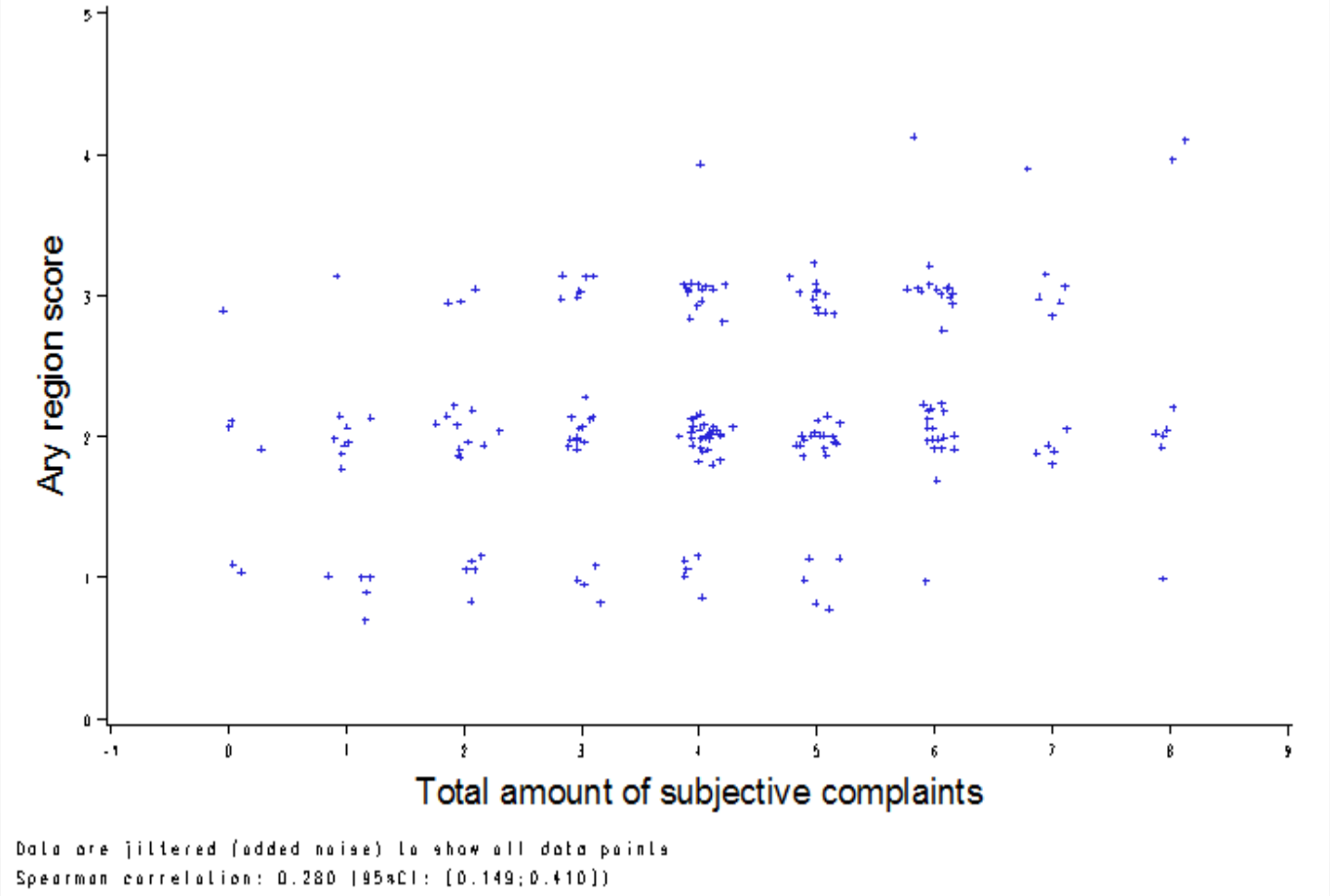
(referring to our Cochrane review 2006).

The following treatments of Laryngopharyngeal Reflux (LPR) were compared: lifestyle advice, lifestyle advice combined with proton pump inhibitor, and life style advice combined with proton pump inhibitor and alginate, based on subjective complaints and documented with high speed films with minimum 2000 frames per second.
(Lifestyle advise referring to gastrooestophageal reflux)

A total of 237 patients were randomized based on the planned statistical power calculation of 90%
to detect a difference of 20%, under the assumption that the true difference was 5% and that the standard deviation was 25%

- Pedersen M (2012) Laryngopharyngeal Reflux – A Randomized Clinical Controlled Trial. Otolaryngol S1:004. doi:10.4172/2161-119X.S1-004

Figure 1: Scatter plot of the total amount of subjective complaints (abscissa) versus oedema of the inter-arytenoid region, week 2 (ordinate). Data are filtered (added noise) to show all data points. (Spearman Correlation 0.280 | 95% CI [0.149:0.410]).



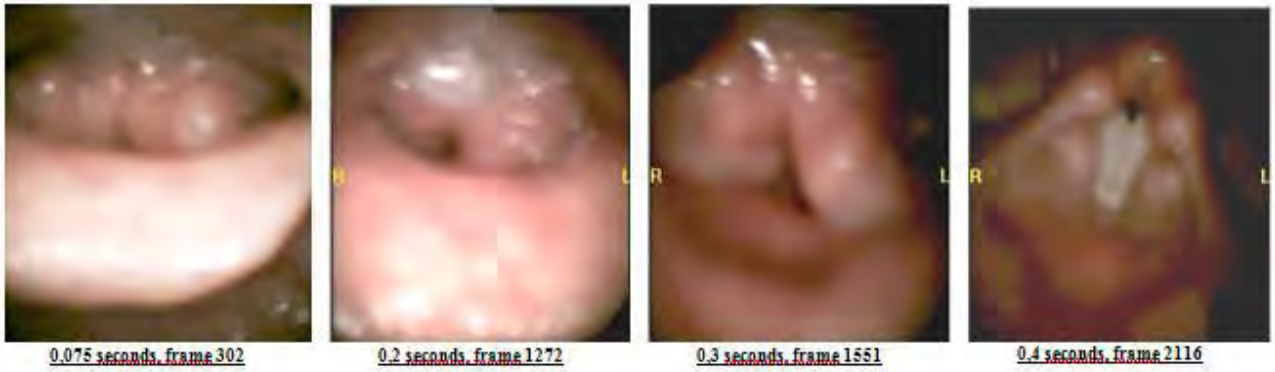
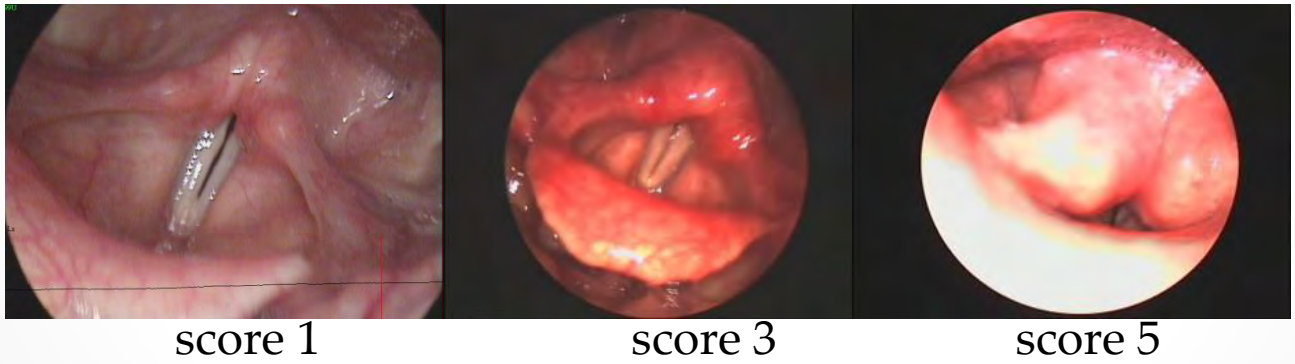


Figure 1: Frames taken from a high-speed video set on recording 4000 pictures per second. The picture, at frame 1272 shows the appearance of mucus in the larynx, and shortly after (frame 1551) it is reduced. On frame 2116, the mucus has disappeared. The process took a total of 0.2 seconds.



Grading of irritation of the arytenoids into grade 1-5
The grading was presented at MAVEBA 2007
Acoustically, a difference was found only between grade 1 and grade 2-5

Pedersen M (2012) Laryngopharyngeal Reflux – A Randomized Clinical Controlled Trial. Otolaryngol S1:004. doi:10.4172/2161-119X.S1-004

Speech therapy, *aspects of literature* of genetics, pharmacogenetics and personalized medicine

(It is important to look at the presented referrences in red)

One of the problems is the necessary **combined approach of voice, respiration and swallowing interference in the larynx.**

Another problem is that many known genetic deficiencies are related to e.g**. infections in the larynx.**

A difficulty is the **central nerve system and its relation to periphery genetic voice disorders**, around 300 different syndromes being isolated. Phenotypes can be difficult to differentiate based on heterogeneity and polymorphism.

Education

How do we change lifestyles - train and educate in our own field?

- **Pharmacogenectics is suffering from lack of integration into clinical practice – also in the voice related area**
- Lack of technical knowledge and awareness could cause severe obstructions on the road to implementing personalized medicine into medical practice.
- **Personalized medicine is a medical model emphasizing in general the customization of health care, with all decisions and practices being tailored to individual patients**

Frueh F.W., Gurwitz D. (2004). From pharmacogenetics to personalized medicine: a vital need for educating health professionals and the community. *Pharmacogenetics . 5(5). 571-579*

Applications of genetics

Applications of genetics and tissue engineering in the practical voice therapy

- **Due to advances in genomics and tissue engineering, new tools and methods are available in voice research**
- Microarray analysis has greatly hastened the development of biochips
- Gene expression profiles, a fundamental part of biochip development, are now commonly performed in some voice laboratories
- Tissue engineering initiatives have led to the ability to grow and work with laryngeal fibroblasts
- **Due to the extreme conditions that vocal fold fibroblasts tolerate, engineering living lamina propria of vocal folds is challenging**

Gray S., Thibeault S., Tresco P. (2003). Witnessing a revolution in voice research: genomics, tissue engineering, biochips and what's next!. *Logoped Phoniatr Vocol.* 28. 7-13

Randomized control trials reference

Which computing techniques should we use for genetics, pharmacogenetics and voice related personalized medicine? And how should we make randomized controlled trials?

- To characterize colour, texture, and geometry of biological structures seen in colour images of vocal folds, feature sets are used.
- Twelve feature sets are used to obtain a comprehensive characterization of a voice signal. Answers to 14 questions constitute the questionnaire feature set.
- The data represented by multiple feature sets were categorized into the healthy, nodular and diffuse classes. The effectiveness of single classifiers as well as committees of classifiers was studied.
- **The combination of both multiple feature sets characterizing a single modality and the total modalities allowed to substantially improve the classification accuracy**.

Verikas A., Gelzinis A., Bacauskiene M., Hållander M., Uloza V., Kaseta M. (2010). Combining image, voice, and the patient's questionnaire data to categorize laryngeal disorders. *Artificial Intelligence in Medicine.* 49. 43-50

Hyalinosis cutis et mucosa

Hyalinosis of skin and mucosa, is a rare autosomal recessive disorder

- **It is characterized by deposition of hyaline material around the basement membrane of the skin, mucous membranes and around skin and brain vasculature.**

- Typical symptoms: hoarseness, infiltration of the mucous membranes and papular verrucae skin changes.

- Mutations within the extracellular matrix protein gene (ECM-1) are the underlying defect.

- **Hoarseness remains for the rest of life. Skin changes usually appear in the first two years of life, rarely later.**

Vago B., Hauser I., Hennies H.C., Enk A., Jappe U. (2007). Hyalinosis cutis et mucosae. *JDDG*. 5. 401-405

Floating-Harbor syndrome

All cases of this syndrome have the triad of short stature, expressive language delay and a triangular face with a prominent nose and deep set eyes.

- A 6-year-old boy with the floating-Harbor syndrome (F-HS) is described
- It is proposed that his exceptionally **high-pitched voice** and supernumerary upper incisor are additional. The **elevated gliadin antibody levels** suggest coeliac disease, which has been described in three out of the 15 previously reported F-HS patients
- His facial features and delayed speech development are very characteristic but his shortness (-3 SD) is milder than usual in the syndrome
- The patient is a **sporadic case** like all the F-HS cases so far.

Ala-Mello, S; Peippo, M. Two more diagnostic signs in the Floating-Harbor syndrome. Clinical dysmorphology 5. 1: 85-5. (Jan 1996)

Self-regulation of biological cells

Biology and self regulation of inflammation and healing of vocal folds – functional genetics and clinical trial aspects. The role of biological cells self regulation have to be analyzed

- Simulations have been used to gain basic insights into the inflammatory response under baseline, gene-knockout, and drug-treated experimental animals
- These simulations have converged with other systems´ biology approaches to aid in the design of new drugs or devices geared towards modulating inflammation
- **These simulations transcend typical cytokine networks by associating inflammatory processes with tissue/organ impacts via tissue damage/dysfunction**
- This frame work has now allowed us to suggest how to modulate acute inflammation in a rational, individually optimized fashion
- **Translational Systems Biology aims to unify mechanisms described in the scientific literature using methods and tools developed by the computational and systems biology communities.**

Vodovotz Y., Constantine G., Faeder J., Mi Q., Rubin J., Bartels J., Sarkar J., Squires R.H. Jr., Okonkwo D., Gerlach J., Zamora R., Luckhart S., Ermentrout B., An G. (2010). *Immunopharmacology and immunotoxicology.* 32(2). 181-195

Bioreactor induced vibrational stimulation

A mean of harvesting the secreted matrix as a bulk biomaterial by removing the polymer foam using an organic solvent.

- Using the system human derived laryngeal fibroblasts were subjected to vibrational stimuli for 1-21days. Cytokine production, matrix protein accumulation, and construct materiel properties were assessed with DNA microarray, enzyme linked immunosorbent, indirect immunofluorescent, and uni-axial tensile assays respectively.

- The results show that **vocal fold-like vibrational stimuli is sufficient to influence the expression of several key matrix and matrix-related genes**, enhance the secretion of the profibrotic cytokine TGFβ1, increase the accumulation of the extracellular matrix proteins, fibronectin and collagen type 1, as well as enhance construct stiffness compared to non-stimulated controls.

- **The results demonstrate that high frequency substrate vibration, like cyclic strain, can accelerate matrix deposition from human derived laryngeal fibroblasts.**

- The study supports the notion that preconditioning regimens using human cells may be useful for producing cell derived biomaterials for therapeutic application.

Wolchok J.C., Brokopp C., Underwood C.J., Tresco P.A. (2009). The effect of bioreactor induced vibrational stimulation on extracellular matrix production from human derived fibroblasts. *Biomaterials.* 30. 327-335

Relations neurological/speech/voice genetic disorders

Dystonia is twisting movements. They are frequently repetitive and often progress to prolonged abnormal postures.

- The dystonic movements are involving the same group of muscles: agonist and antagonist muscles contract simultaneously to produce these movements.

  The speed of the movements varies from slow to shock-like.
- *Etiologically dystonia divides into major categories: primary dystonias, dystonia-plus syndromes, non-degenerative diseases highlighted by dystonia and other features, secondary dystonias and heredodegenerative dystonias*
- Most of the genes are involved in primary or dystonia-plus syndromes.
- The gene *TOR1A* codes for the protein torsinA, found in neurons in the endoplasmic reticulum.
- TorsinA is an ATPase of the heat-shock type, which restores damaged proteins particularly in membranes.
- The mutated TOR1A gene **results in a loss of ATPase activity** and therefore impaired effect as a chaperone protein.

Lisak RP, Truong DD, Carroll WM, Bhidayasiri R, (2007) International Neurology A Clinical Approach, Wiley-Blackwell, A John Wiley & Sons, Ltd, Publication, pp 168

Genetic speech and language deficits are varied

They are including aphasia, stuttering, articulation disorders, verbal dyspraxia and language impairment.

- Many of the disorders cluster in families, suggesting involvement of genetic factors.
- Mutations in genes *GNPTAB*, *GNPTG* and *NAGPA*, all of which are associated with the lysosomalenzyme targeting pathway, has been reported to cause stuttering
- Comparative studies have shown that genetic variations of ***FOXP2*** **transcription factor is important to the development of speech**. A number of candidate genes regulated by this transcription factor has also been identified
- *CMIP* and *ATP2C2* are associated with language disorders.
- Genetic studies of dyslexia proposed *ROBO1*, *DCDC2*, and *KIAA0319* as candidate genes

Kang C., Drayna D. (2011). Genetics of Speech and Language Disorders. Annu. Rev. Genomics Hum. Genet. 12. 145-64

Genetic tissue engineering, cellular actions, microarray (based on e.g. light and electronic microscopy aspects as well as Optical Coherence Tomography)

- Growth factor is used for gene transfer, and stem cell procedures for collection of tissue are already known. A possibility for the voice field is restoration of normal function of voice. Focus has been on fibroblast function and elastin genetic function. • • •

- **Some supplementary aspects will be given of the literature till now, as well as future practical aspects related to voice function.**

Fibroblast synthesis and function related to vibratory micro structure of the vocal cord.

- The special composition and organization in extracellular matrix (ECM) in vocal folds is a critical component in sustaining high frequency vibration.
- Objective: To investigate the effect of vibratory stimulation on ECM gene expression and synthesis of fibroblast in hyaluronic acid hydrogels with approximate viscoelastic properties of vocal mucosa.
- **Results: Indications were found that vibration is a crucial positive factor in restoring ECM structure. It may provide basis for reducing vocal scarring and improvement of voice quality.**

Kutty J, Webb K. Vibration Stimulates Vocal Mucosa-like Matrix Expression by Hydrogel-encapsulated Fibroblasts. J Tissue Eng Regen Med. 2010; 4(1): 62-72.

The human genetic connective tissue studies involve many treatment aspects

- **The human elastin gene (ELN) is responsible for the generation of elastic fibres in the extracellular matrix of connective tissue throughout the body**
- Individuals with Supravalvular aortic stenosis (SVAS) and Williams syndrome (WS) lack one normal ELN allele
- The perceptual and acoustic characteristics of voice quality for individuals with SVAS/WS, indicates that their voice was significantly more abnormal
- These findings supports the possibility that heterozygous ELN abnormalities negatively influence vocal fold biomechanics

Watts C, Awan S, Marler J. An investigation of voice quality in individuals with inherited elastin gene abnormalities. Clinical Linguistics & Phonetics. 2008; 22(3): 199-213.

Understanding of the elastin genetic function of the vocal folds is necessary with potential differential therapeutic aspects

- Used for this study was five **mice,** *four* with heterozygous elastin (Eln) gene Eln delection (Eln+/-) serving as an animal model for the human disease supravalvular aortic stenosis and *one* normal wild-type control (Eln +/+)-
- Vocal folds were obtained from each animal and stained for the protein elastin using histochemical methods.
- Qualitative visual inspections revealed greater staining density for elastic fibers in the Eln+/+ animals
- Quantitative measurements using digital pixel analysis of staining density revealed significant differences between mice with two genotypes, confirming the qualitative findings
- **Results suggest that Eln requires two functioning alleles for normal structural development of the vocal fold lamina propia**

Watts C, Knutsen R, Ciliberto C, Mecham R. Evidence for Heterozygous Abnormalities of the Elastin Gene (ELN) Affecting the Quantity of Vocal Fold Elastic Fibers: A Pilot Study. Journal of Voice. 2011; vol. 25, 2: 85-90.[[

Application of tissue engineering combines cells, scaffolds and engineering to reconstruct defect tissue

- **Collagen is the primary component of the extracellular matrix fibroblast growth attracting keratinocytes and promoting their growth is used for scaffolds support**
- An *ex-vivo* produced oral mucosa equivalent has been constructed and used for reconstruction of oral mucosa defects
- Terada et al. studied the *in vitro* biocompatibility of a chitosan- collagen scaffold. This was done by blending chitosan and tilapia scale collagen with oral mucosa keratinocytes.
- Due to advantageous biochemical properties, such as promotion of cellular infiltration, extracellular matrix production, antimicrobial and hemostatic activities, chitosan based materials are promising for skin regeneration.
- **A chitosan-collagen based scaffold is anticipated to accelerate fibroblast and endothelial cell in growth from underlying host tissue after grafting.**

Terada M., Izumi K., Ohnuki H., Saito T., Kato H., Yamamoto M., Kawano Y., Nozawa-Inoue K., Kashiwazaki H., Ikoma T., Tanaka J., Maeda T. (2012). Construction and characterization of a tissue-engineered oral mucosa equivalent based on a chitosan-fish scale collagen composite. *Journal of biomedical materials research*. 100B (7)

A holistic approach e.g. human lymphocytes and leucocytes analysis is often neccesary for the basic advice for personalized treatment in our field

- Among the major bottlenecks in translation systems biology into systems medicine are the limited number of clinical cases that can be included in randomized trials and the number of genetic, environmental and nutritional variables that cannot be easily accounted for

- Development is necessary of **virtual patient models** that mimic the patients' main characteristics, from which testable hypotheses can be generated and validated on the small number of actual patients available.

Auffray C, Charron D, Hood L. Predictive, preventive, personalized and participatory medicine: back to the future.
Genome Medicine. 2010; 2: 57.

Many aspects of stem cells have been studied- till now without clinical applications in laryngology,

but the research field is necessary

Akama K., Horikoshi T., Nakayama T., Otsu M., Imaizumi N., Nakamura M., Toda T., Inuma M., Hirano H., Kondo Y., Suzuki Y., Inoue N. (2011). Proteomic idientification of diffentially expressed genes in neural stem cells and neurons differentiated from embryonic stem cells of cynomolgus monkey (macaca fascicularis) in vito. *Biochemica et Biophysica Acta.* 1814. 265-276

Estrogens and Androgens

Further understanding has been developed in a stratified analysis of voice development in a choir during puberty (8-18 years),

comparing the adrenal and sexual hormonal development with

pediatric and voice parametres,

fundamental frequency and phonetograms

Pedersen M. (2008). Development of voice in childhood. Springer. ISBN: 978-3-540-69358-1

DNA concordance in a twin pair.

(High speed film, optical coherence tomography and 3 dimensional films could be used in the future)

Two monozygotic twins with vocal fold bowing did both explore genetic and environmental factors. They both underwent surgical intervention and subsequent voice therapy.

Monozygosity was confirmed for DNA polymorphisms, with 10 of 10 concordances for Short Tandem Repeat (STR) DNA markers

For both twins, auditory- and visual-perceptual assessments indicated severe bowing, hoarseness, and breathiness. Smaller relative amplitudes were observed for Twin 1.

No consistent voice improvement was observed after surgical interventions

Marked reductions in Voice Handicap Index total scores were observed, coinciding with increased mid-membranous and posterior laryngeal glottal closure. There was not observed any substantive differences in the acoustic measures.

Vocal fold bowing was more servere for Twin 1, but the overall voice improvement was greater for Twin 2

Environmental factor might partially account for the differences, including variability in their responsiveness to behavioral voice therapy.

Voice therapy was useful in improving mid-membranous and posterior laryngeal closure although dysphonia remained severe in both cases

Tanner K, Sauder C, Thibeault S, Dromey C, Smith M. Vocal Fold Bowing in Elderly Male Monozygotic Twins: A Case Study. Journal of Voice. 2008. Vol. 24, No. 4: 470-476.

The role of RNA segment studies of vocal cord carcinoma. This is another important area where the role of genetic RNA interference must be taken into account

- **The method was to take copies of a small interfering RNA segment directed against the HuR gene and transfect it into Hep-2 cells, using LipofectamineTM 2000**
- The effect of the small interfering RNA segment on Hep-2 cell proliferation was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay
- Changes in the expression of the HuR, cyclooxygenase-2 and surviving genes were detected by semi-quantitative reverse transcription polymerase chain reaction analysis
- Concentrations of the HuR, cyclooxygenase-2 and surviving proteins were evaluated using Western blotting
- Expression of the HuR, cyclooxygenase-2 and surviving genes, as indicated by messenger RNA and protein levels, was suppressed by the HuR gene small interfering RNA segment in dose-depend manner.
- **The proliferations indices of all treated groups were significantly lower than those of control larynx cancer groups**

Shen Z, Ye D, Zhang X, Jiang Z, Xiao B, Guo J. Inhibitory effect of HuR gene small interfering RNA segment on laryngeal carcionoma Hep-2 cell growth. The Journal of Laryngology & Otology. 2010; 124: 1183.1189.

Fibroblast growth, biochip development for vocal care

- DNA does not directly make proteins, it is first transcribed into RNA.
- RNA contains a similar message as the DNA and it is more usable by the cell to make a protein.
- Microarray analysis was used to find the genes. The gene environment interaction cellular and tissue protein, the field of proteomics was found relevant to the area of voice disorders.

- **The use of collagen injection in the vocal cords is a form of protein therapy (as the use of botox)**

Gray S, Thibeault S. Diversity in voice characteristics-interaction between genes and environment, use of microarray analysis. Journal of Communication Disorders. 2002; 35: 347-354.

A rabbit study of inflammatory mRNA. We know too little of the central regulation of fundamental voice frequencies, especially the role of genetic differences between the two genders (estrogens and androgens)

- 10 rabbits was giving experimentally surgically induced modal or raised intensity phonation for 30 minutes. There was a control group of five, which received sham surgery.
- Vocal folds were harvested post-procedure and real-time polymerase chain reaction (PCR) was used to investigate mRNA expression of Interleukin-1beta (IL-1B), transforming Growth Factor-beta1 (TGF-B1), and Cyclooxygenase-2 (COX-2)
- One way analysis of variance (ANOVA) tests were used to investigate differences in gene expression across groups. Significant main effects were further examined using Tukeys post-hoc tests ANOVA revealed a significant main effect for IL-1b, TGF-B1, and COX-2

- **The results provide preliminary data on the effects of raised intensity phonation on inflammatory mRNA expression in an in-vivo rabbit model.**
- **Ultimately, this model will be used to investigate clinical observations, such as too-long and too-loud, which are terms frequently used to describe the pathophysiology of dysphonia in patients**

Rousseau B, Abdollahian D, Ohno T, Garret C, Swanson E. Effects of raised phonation on vocal fold gene expression. Otolaryngology-Head and Neck Surgery. 2009; 141 (3S1): 64.

Can the transcripts be reproduced? Here are only three patient and two controls. This is another big study of defence mechanism in the larynx against acid from the stomach. There might be huge genetic differences

- Mucin gene profile in normal laryngeal epithelium was compared with patients with reflux attributed laryngeal injury or disease
- low PH with or without pepsin on mucin messenger RNA levels in vitro
- Reverse Transcription Polymerase Chain Reaction (RT-PCR) was performed to establish the mucin gene profile. Expression of selected mucin was analyzed via real-time RT-PCR.
- **Mucin 2, 3 and 5 transcripts were expressed at reduced levels in patients with reflux attributed laryngeal injury or disease**

Samuels T, Handler E, Syring M, Pajewski N, Blumin J, Kerschner J, Johnston N. Mucin Gene Expression in Human Laryngeal Epithelia Effect of Laryngopharyngeal Reflux. Annals of Otology, Rhinology & Laryngology. 2008; 117(9): 688-695.

Cystic Fibrosis – Interesting for voice deficits

- **In cystic fibrosis patient populations,** gram-negative bacteria, particularly Pseudomonas aeruginosa, frequently **require aggressive therapy** including systematic antibiotics, bronchodilators and airway clearance techniques.

- Aminoglycosides including tobramycin are used frequently to control these chronic airway infections.

Conrad D, Stenbit A, Zettner E, Wick I, Eckhardt C, Hardiman G, Frequency of mitochondrial 12S ribosomal RNA variants in an adult cystic fibrosis population. Pharmacogenetics & Genomics. 2008; 18(issue 12): 1095-1102.

Myopathy of voice

Weakness of voice, how to make future research. It is so important that we follow this genetic research in highly developed centres

- **Distal myopathies represent a heterogeneous group of inherited skeletal muscle disorders**
- One type of adult-onset, progressive autosomal-dominant distal myopathy, frequently associated with dysphagia and dysphonia, has been mapped to chromosome 5q31 in a North American pedigree
- We report the identification of a second large family of Bulgarian descent and fine mapping of the critical interval.

- Sequencing of positional candidates genes revealed precisely the same non convertative S85C missense mutation affecting an interspecies conserved residue in the MATR3 gene in both families.
- **Different disease related haplotype signatures in the two families provided evidence that there was two independent mutational event at the same position in MATR3.**

Senderek J, Garvey S, Krieger M, Guergueltcheva V, Urtizberea A, Roos A, Elbracht M, Stendel C, Tournev I, Mihailova V, Feit H, Tramonte J, Hedera P, Crooks K, Bergmann C, Rudnik-Schöneborn S, Zerres K, Lochmüller H, Seboun E, Weis J, Bechmann J, Hauser M, Jackson C. Autosomal-Dominant Distal Myopathy Associated with a Recurrent Mutation in the Gene Encoding the Nuclear Matrix Protein, Matrin 3. The American Journal of Human Genetics. 2009; 84: 511-518.

Virus infection associated with a genetic syndrome is more dangerous

Examinations must be carried out in our field, e.g. respiratory syncytial virus, adenovirus and human papilloma virus

- Infections with adenoviruses are a common problem in the pediatric population.
- Normally asymptomatic to mild, those infections tend to take a more severe course in immuno compromised patients.
- **22q11 deletion syndrom** (22q11DS) represents a common genetic disorder causing velopharyngeal dysfunction, provoked by infection.
- Necrotizing enterocolitis (NEC) is a frequent gastrointestinal emergency observed in neonatal intensive care units.
- The occurrence of NEC is more prevalent in preterm infants.

Cichocki M, Singer G, Beyerlein S, Zeder S, Schober P, Höllwarth M. A case of necrotizing enterocolitis associated with adenovirus infection in a term infant with 22q11 deletion syndrome. Journal of Pediatric Surgery. 2008; 43: E5-E8.

Organization of personalized medicine in voice disorders

It is proposed that personalized medicine for hoarseness is more effective if pharmacogenetic problems have been solved.

• • •

There is often revealed a connection to genetics. Potential demands are suggested for biological understanding and medical treatment.

A land mark and a model for the development of predictive and preventive medicine.

- Displaying the highest level of any functional genetic complex with medical impact
- **Systems biology approach and integrative methodologies will need to unravel the ever growing number of Human Leukocyte Antigen (HLA) and diseases associations.**
- HLA, immuno-genetics and pharmacogenetics are merging to bring to the individual patient tailored and personalized treatment.
- The role of the HLA system will be consolidated at the forefront of the newer medicine

Charron D. HLA immunogenetics, pharmacogenetics and personalized medicine. Vox Sanguinis. 2011; 100: 163-166.

299 different syndromes voice related, rarely isolated, probably a under researched area.

- **The paper reports the results of a meta analysis with the aim of documenting the occurrence of voice – and resonance disorders in some genetic syndromes.** The analysis studied the occurrence in general and in different etiological subtypes of syndromes and the association with other disorders, in particular clefting, cognitive impairment and hearing problems
- The database for the analysis was a list of 299 different syndromes all of which had been reported to entail communication disorders
- **Disorders of voice and resonance in genetic syndromes are only rarely isolated problems, an under researched area.** Causal mechanisms are not a major factor in determining the presence of the syndromes

Van J. Voice and Resonance Disorders in Genetic Syndromes: A Meta-Analysis. Folia Phoniatrica et Logopaedica. 2004; 56 (issue 2): 83-92.

The effect of growth factor in the absence of X chromosome in Turners Syndrome is active in all parts of the body, *including the voice*

- Objective and subjective voice parameters among **Turner syndrome (TS)** women in relation to genotype, hearing, growth, and previous treatment with growth hormone (GH) and androgen were described
- **Voice function was studied objectively with speech frequency (SFF) and subjectively (questionnaire) in 117 women with TS**
- SFF did not differ between treated and non-treated participants or between patients with a spontaneous versus induced puberty. SFF was dependent on karyotype but not age. Subjective voice change was reported four times more often among treated compared with non-treated TS women
- *GH and androgen normalized SFF and reduced voice and articulation problems where found in adulthood*
- The TS phenotype includes important voice and speech problems, which in turn are associated with hearing problems, although genotypic, *monosomic, and isochromosome patiens have more voice problems and also more high-pitched voices than mosaic patients*
- **Most Turner Syndrome women exhibit a higher frequency of pitched voice than non-TS women**

Wall G, Ohlsson A, Wikland K, Barrenäs. Growth promoting Treatment Normalizes Speech Frequency in Turner Syndrome. Laryngoscope. 2008; 118: 1125-1130.

Up regulation of disease preventing genes

- ***Lifestyle*** can have a profound impact on the health situation for a number of diseases including coronary heart disease, prostate and breast cancer, diabetes, and obesity account for 75% of health-care costs in the US.

- The economic benefit of informing the patients about the health benefits of lifestyle changes is another motivational factor, as lifestyle guidance in some cases has a good or even better effect than surgery or drug administration.

- Perhaps the best example of the great potential of lifestyle change is the INTERHEART study, that involved 30.000 patients from 52 years of age, showing that nine risk factors which could all be modified by changing diet and lifestyle, accounted for 90% of the population attributable risk in men and 94% in women.

- **The genetic understanding of how these changes benefit the patients is expanded these days and it has been shown that lifestyle changes have an advantageous effect on the gene expression, by up regulating disease preventing genes and down regulating disease promoting genes**

Ornish D. Intensive lifestyle changes and health reform. Lancet. 2009; 10; 638-639.

Epidermal growth factor receptor is a very promising therapeutic target

- Cancers of the head and neck and of the lung are associated with high morbidity and mortality rates that have remained relatively unchanged for more than 3 decades
- **It is generally believed that the efficacy of standard therapy regimens has reached a plateau for these cancers.**
- **The discovery of specific aberrant molecular signaling pathways in solid tumors has afforded promising new directions for newer "targeted" cancer therapeutics:**
- The epidermal growth factor receptor (EGFR) shows promise as a therapeutic target.
- Clinical studies have demonstrated that this targeted approach provides clinically meaningful benefit.

Sattler M, Abidoye O, Salgia R. EGFR-Targeted Therapeutics: Focus on SCCHN and NSCLC. TheScientificWorldJOURNAL. 2008; 8: 909-919

There are genetic relations between benign and malignant disorders

- Mortality associated with head and neck cancer has remained unchanged for the past decades
- Gene therapy is a novel treatment approach that potentially advance the treatment of genetic diseases.

- Multiple vector systems have been developed that facilitate the introduction of therapeutic genetic material into cells
- Gene therapy strategies can be classified in 3 groups:
  - **cytoreductive therapy** aimed at directly inducing cell death
  - **corrective therapy** intended to repair genetic defects underlying malignancy
  - **immune modulation** to promote a robust immune response against cancer

Abuzeid W.M., Li D., O'Malley Jr. B.W. (2011). Gene Therapy for Head and Neck Cancer

Personalizing healthcare: from genetics to improving care

Interindividual variability in patients' responses to medicines, including the likelihood of toxicity, is commonly due to differences in their genetics

Ultimately, full personalization of medicines will require a better understanding of the systems of genetic pathways rather than just single gene association

- Overall, greater integration of personalized medicine into routine care will require new clinical trial structures. Patient education will also be needed.
- Increasing pressure on resources will also mean that the value of new drugs will come under greater scrutiny, especially if they are adding to choices rather than targeting real unmet need.
- Personalization will reduce the likelihood of failure in drug development. If personalization means less failure, then market fragmentation becomes less hazardous.

Finlayson A, Godman B, Paterson K, Aston E, Haycox A, Gustasson L, Ali R. Personalizing healthcare: from genetics through payment to improving care? J R Soc Med 2013, 106 : 41-44

There is a need for enhanced genetic understanding of voice. This understanding must be documented with randomized controlled trials based on reviews.

Our first Cochrane review: Vocal Chord Nodules

Pedersen M, McGlashan J (updated 2012). Vocal cord nodules [review]. Oxford, Wiley publishing

Our second Cochrane review: Acid Reflux Treatment for hoarseness

Hopkins C, Yousaf U, Pedersen M (2006). Acid Reflux Treatment for Hoarseness [Review]. January 2006 in The Cochrane Library Oxford, Wiley publishing. Issue 1.

Conclusion

- The genomic aspects related to the voice and it´s treatment may be dependent on vocal related techniques with light and electronic microscopy as well as optical coherence tomography.

- More basic cellular understanding are upcoming, but pharmacological focus on what really happens with different patients and different treatments over time should be focused upon.

- Some genetic areas have been refered in our introduction today – for voice related pharmacogenetics and personalized medicine.

Thank you for your attention

Union of the European Phoniatricians