Pharmacogenetics and voice related personalized medicine

PACIFIC VOICE CONFERENCE 2013 WORKSHOP

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ABSTRACT 1

Overview of literature of voice related genetics, pharmacogenetics and personalized medicine

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 of the beginning of this century and for the generations 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.

Although some progress has been made in complex diseases, such as metabolic syndrome, cardiovascular, cancer 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.

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. A great challenge is ahead.

Treatment aspects of upper airways - lifestyle, education- antihistamines, cortisone, adrenalin and others

- Pedersen M, Eeg M (2012) **Laryngopharyngeal Reflux** – A Randomized Clinical Controlled Trial, Otolaryngology,OMICS publishing group

- Pedersen M, Eeg M, (2012) **Does treatment of the laryngeal mucosa reduce dystonic symptoms?** A prospective clinical cohort study of mannose binding lectin and other immunological parameters with diagnostic use of phonatory function studies,European Archives of Otorhinolaryngology

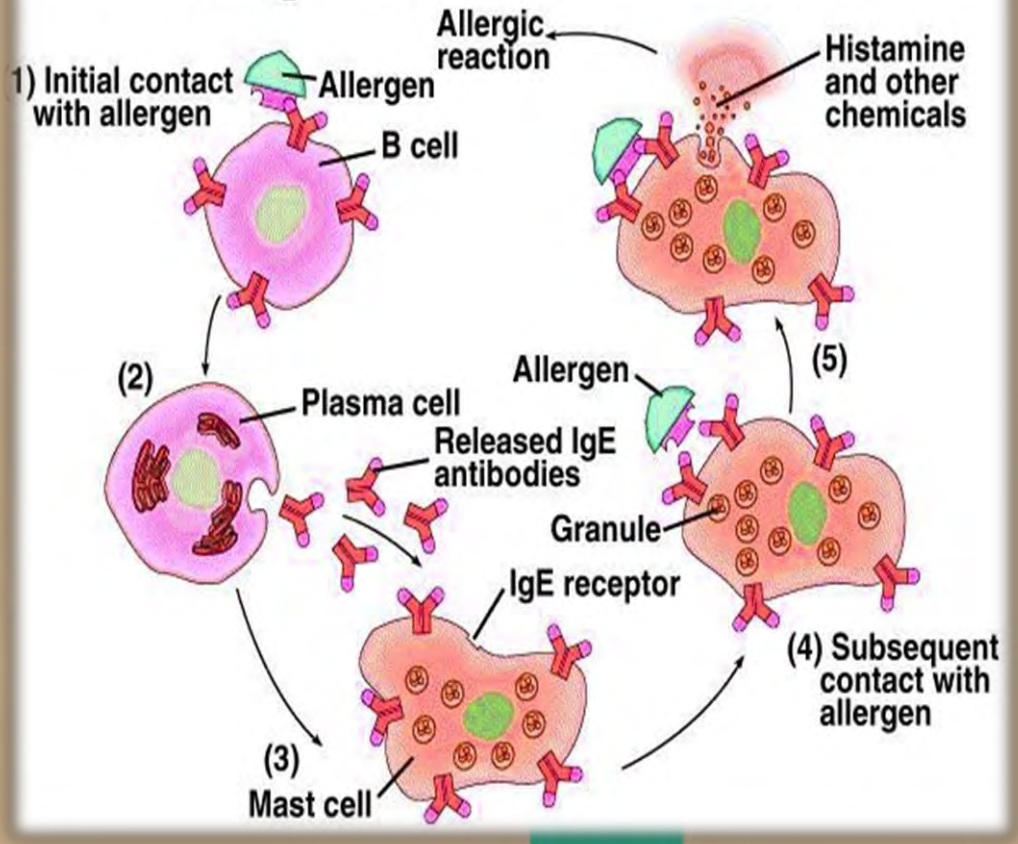
Treatment aspects

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 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 below) 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.

An Allergic Reaction — Overview

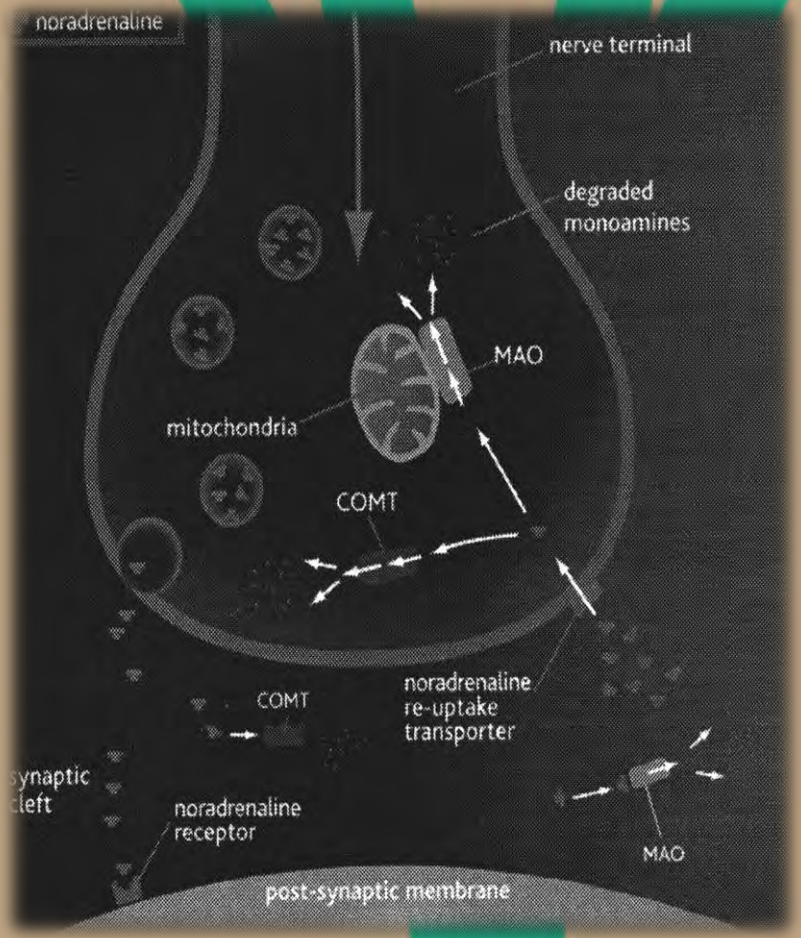


Treatment aspects

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,
raises the-blood sugar concentration, dilation of the pupil.
-**contricts the arterioles** of the mucous membranes and intestines.
-dilates the arterioles in skeletal muscle.

By use of a synthetic adrenaline, also 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 like Bricanyl and other more long-acting products. At the same time this drug can be **allergy-relieving.**

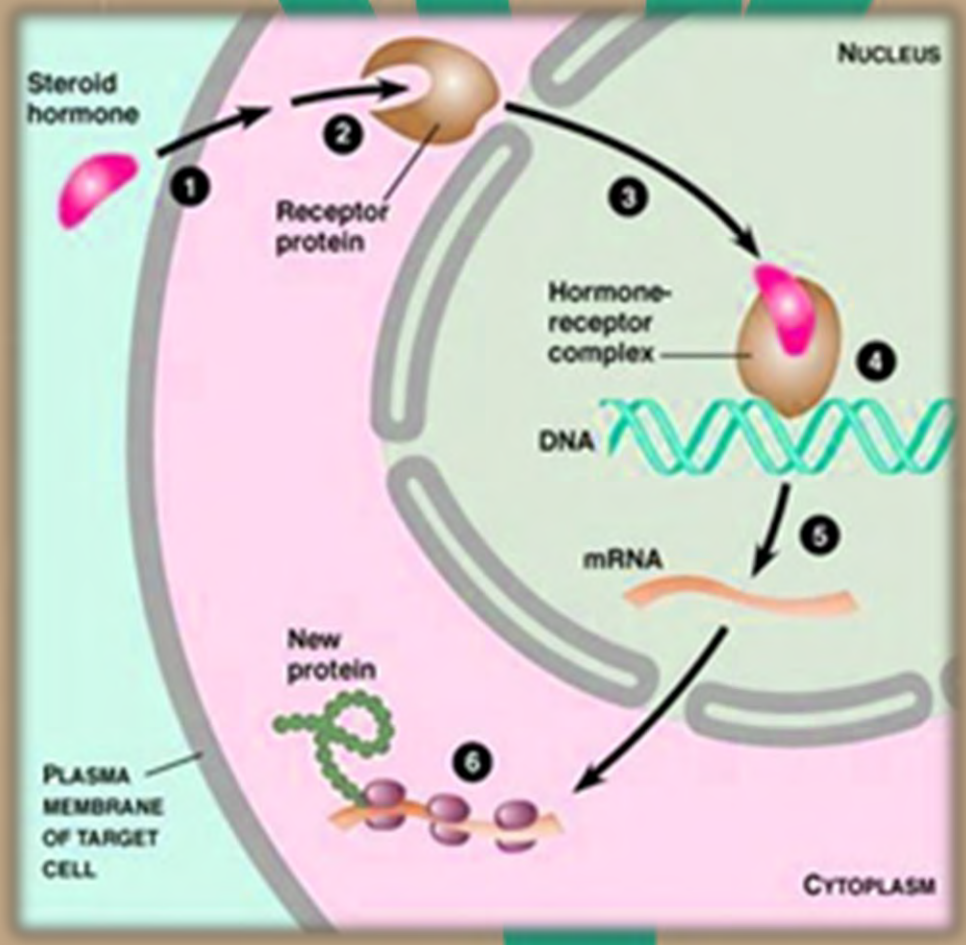

noradrenaline
nerve terminal
degraded
monoamines
MAO
mitochondria
COMT
COMT
noradrenaline
re-uptake
transporter
ynaptic
left
noradrenaline
receptor
MAO
post-synaptic membrane

Treatment aspects

Cortisone

Cortisone is produced by the body, is synthesized from cholesterol in the adrenal gland. All derivatives of cortisone, used for medicinal purposes, are called glucocorticosteroids.
Glucocorticoides 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 respiratory tracts. Products containing these substances include Innovair, Pulmicort og Flixonase. The binding of DNA happens by a phosphorylisation of the hormone receptor complex.


Steroid hormone
1
2
Receptor protein
3
Nucleus
Hormone-receptor complex
4
DNA
5
mRNA
New protein
6
Plasma membrane of target cell
Cytoplasm

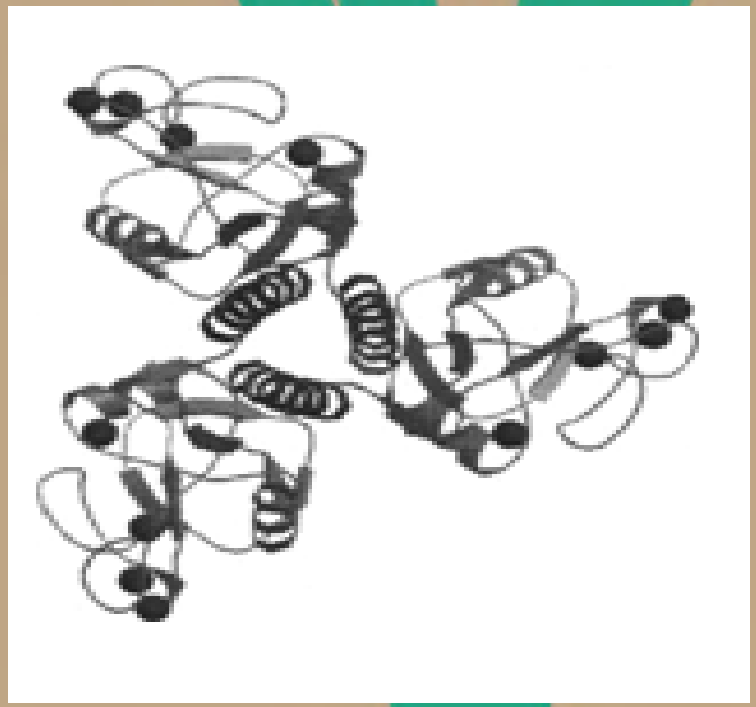
Treatment aspects

Mannose Binding Lectin

- Voice related genetical aspects of mannose binding lectin.It is a problem not to have enough because it **probably indirectly inhibits secondary genetic disorders**.

- Mannose-binding lectin, **the primitive immunesystem**. This protein is a very important component of the innate immune system (the first defense) 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.



Tanja
the first treated dystonia patient

- Tanja 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 lactose and gluten intolerance, food and inhalation allergy – without positive findings

Genetic immune system

Mannose binding lectin as part of the genetic immune system is not directly involved in dystonia

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

- 55 dystonia patients aged approx. 55 years in observational cohort study for 8 months
- 15 males and 41 females with dystonia-related symptoms of chronic laryngitis for an average of 13 years
- Systematic blood test showed that **47% patients had reduced function of the innate immune system**.
- Laryngitis complaints included: sore throat, dysphonia and mucosal complications.
- Objective techniques were used: high-speed digital imaging of the vocal folds, kymography, EGG and acoustics.
- **Patients were placed on local steroid and antihistamines** with adjuvant lifestyle corrections.
- All cases showed elimination or significant clinical reduction of symptoms for "laryngitis", the spasms clearly seen on high speed films.
- All consecutive cases showed various responses with regard to their underlying etiology.

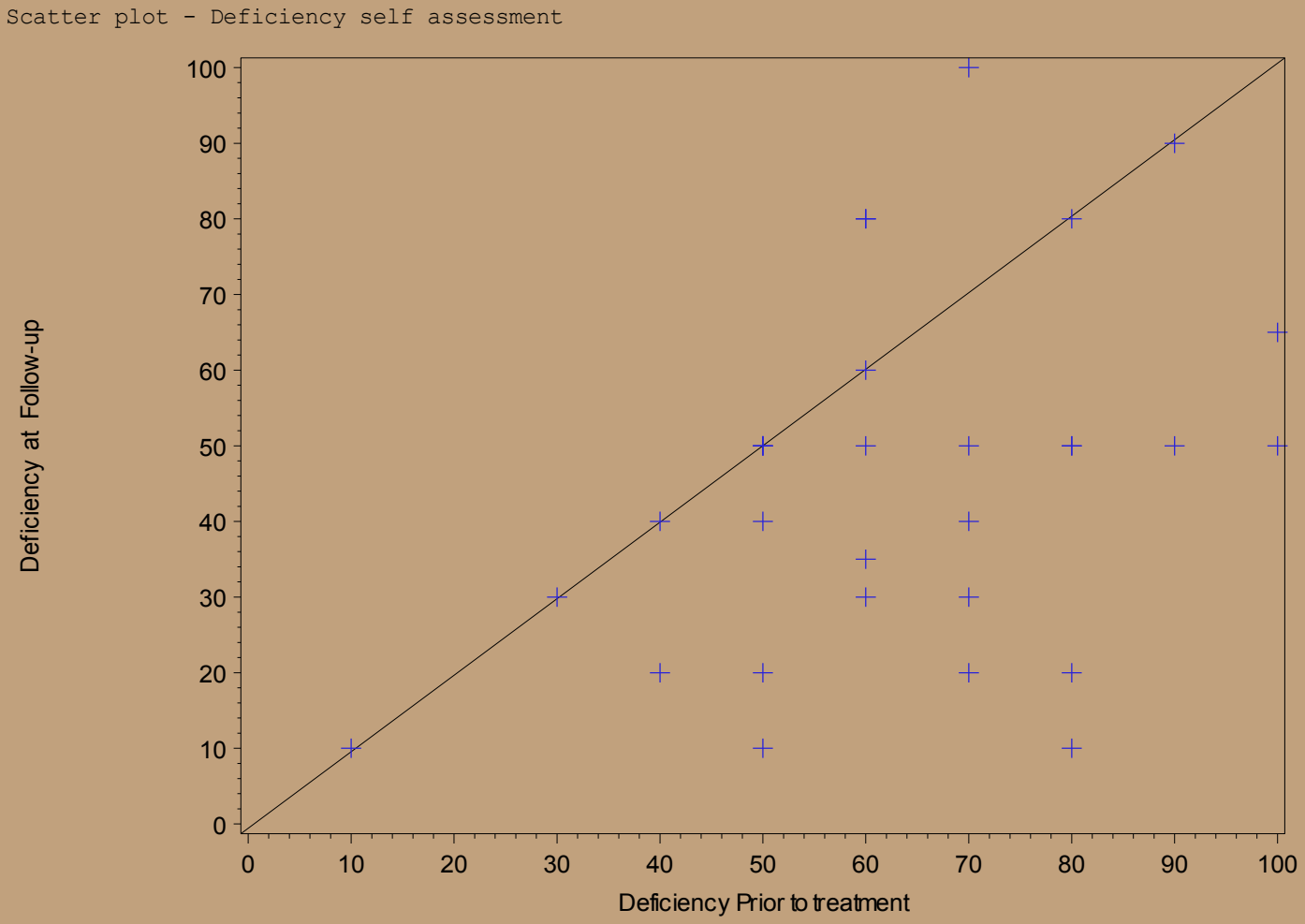
- **Namely that dystonia severity was affected as a function of the treatment of the upper respiratory component, the larynx, including the vocal folds**.

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

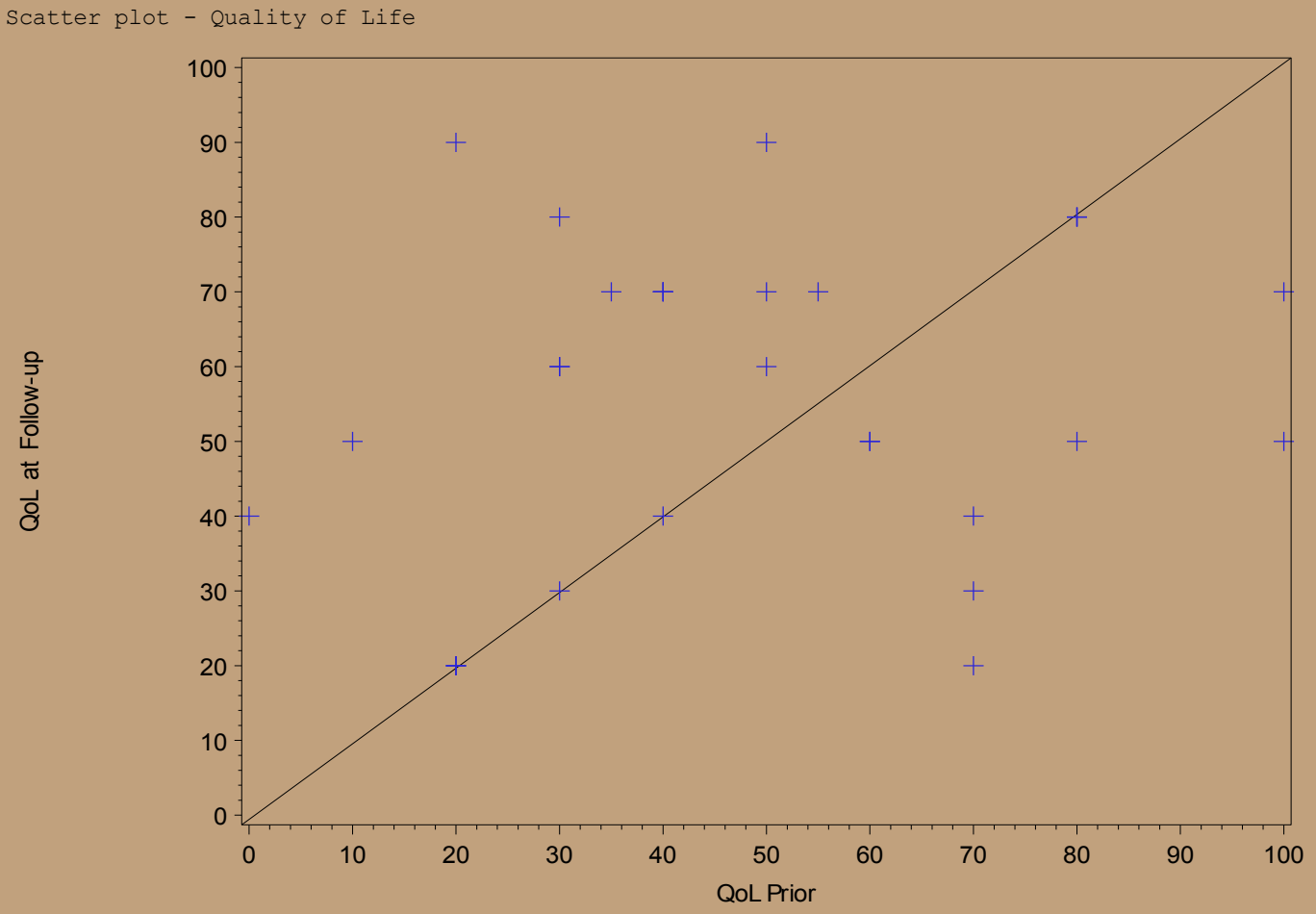
	1st consultation			2nd consultation			Change (2nd-1st consultation)			
	N	Mean	Std	N	Mean	Std	N	Mean	Std	p-value
All dystonia patients symptoms and QoL	55	2.71	0.60	49	2.35	0.63	49	-0.35	0.72	0.0003***
MBL<500 µg/L	26	2.69	0.62	22	2.32	0.57	22	-0.36	0.73	
MBL>500 µg/L	21	2.67	0.58	20	2.30	0.73	20	-0.40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

Symptom reduction in 55 patients with dystonia



Quality of life in 55 patients with dystonia



ABSTRACTS 2

Speech therapy, aspects of literature of genetics, pharmacogenetics and personalized medine

One of the problems of voice related genetics, pharmacogenetics and thereafter person related treatment is the necessary combined approach of **voice, respiration and swallowing interference in the larynx.**

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

A third aspect 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.
Most important is up-regularity of knowledge of disease preventing (e-?)genes necessary in the future based on visualization, validation, integration and cross-collaboratoratorial scientific maneuvers.

Education

How do we educate in our own field?

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*

- Pharmacogenectics is suffering from lack of integration in to **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.
- Healthcare professionals, policy makers and patients need to have the knowledge for making educated treatment decisions.
- **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*

Genetics and mucosal function

To follow research of the mucosal immune system in necessary in our field

Parham P. (2009). *The immune system*. New York: Garland Science

- **<u>Defects in genes cause immunodeficiency diseases</u>**

  which manifest themselves by enhanced susceptibility to infection or autoimmunity.

- Numerous inherited immunodeficiency diseases have been correlated with susceptibility to particular classes of pathogens.
- The cause of these diseases is the defect protein or glycoprotein encoded by the given mutation in the gene
- Altered gene expression pattern in macrophages is seen in response to interferon-*y* binding to its receptor
- Recessive and dominant mutations in the interferon-*y* receptor cause diseases of differing severity

Parham P. (2009). *The immune system*. New York: Garland Science

Genetics in laryngology

Understanding of genetics in laryngology is probably not as specific as in otology which the refered book is focusing upon

Alford R.L., Sutton V.R. (2011). Nonsyndromic Hereditary Hearing Loss. *Adv Otorhinolaryngol.* 70. 37-42

The etiology of hereditary hearing loss is extraordinarily complex

- More than 400 genetic syndromes are associated with hearing loss
- More than 140 genetic loci are associated with hearing loss
- Advances in DNA sequencing and the rapid decline in the cost of sequencing presage the availability of testing that can identify the etiology in the majority of cases of genetic hearing loss.
- Until genetic testing of hearing loss is clinically available and cost-effective, infectious exposures and patient and family medical history will continue to be important to effort directed toward etiologic diagnosis

Alford R.L., Sutton V.R. (2011). Nonsyndromic Hereditary Hearing Loss. *Adv Otorhinolaryngol.* 70. 37-42

Applications of genetics

Applications of genetics and tissue engineering in the practical voice therapy

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

- 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

Genetics, personalized vocal exercise

Development of a personalized vocal exercise (or) rest program, documented with measuring of inflammation and healing

Li N.Y.K., Verdolini K., Clermont G., Mi Q., Rubinstein E.N. (2008). A Patient-Specific *in silico* Model of Inflammation and Healing Tested in Acute Vocal Fold Injury. *PLoS ONE.* 3(7)

- The development of personalized medicine is a primary objective of the medical community and increasingly also of funding and registration agencies
- Modeling is generally perceived as a key enabling tool to target this goal.
- Agent-Based Models (ABMs) have previously been used to simulate inflammation at various scales up to the whole-organism level
- **Subject-specific simulations also predicted different outcomes from behavioral treatment regimens to which subjects had been exposed**

Li N.Y.K., Verdolini K., Clermont G., Mi Q., Rubinstein E.N. (2008). A Patient-Specific *in silico* Model of Inflammation and Healing Tested in Acute Vocal Fold Injury. *PLoS ONE.* 3(7)

Randomized control trials reference

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

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

- 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 highest classification accuracy was achieved when using the single classifiers and genetic search based aggregation exploiting the space the class a posteriori probabilities.
- **The combination of both multiple feature sets characterizing a single modality and the total modalities allowed to substantially improve the classification accuracy**, if compared to the highest accuracy obtained from a single feature set and a single modality.

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 cutis and mucosae, is a rare autosomal recessive disorder

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

- **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

- **We must dig down into syndroms to understand what voice really is, if we want to treat effectively**

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

- 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 diagnostic signs of F-HS
- 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

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

- A systems-based understanding of inflammation is necessary for efficient development of drugs and devices, for streamlining analyses at the level of populations, and for the implementation of personalized medicine
- Simulations have been used to gain basic insights into the I 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
- **<u>These simulations transcend typical cytokine networks by associating inflammatory processes with tissue/organ impacts via tissue damage/dysfunction</u>**
- 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

Pharmacogenetics

Can this theoretical paper be related to any kind of genetics, pharmacogenetics or personalized aspects or is it invalid? It is not prospective randomized with power calculation. No evidence exists for acoustical measure of pathological voices

Schalling E., Hammarberg B., Hartelius L. (2007). Perceptual and acoustic analysis of speech in individuals with spinocerebellar ataxia (SCA). *Logopedics Phoniatrics Vocology.* 32. 31-46

- Autosomal, dominant, hereditary, neurodegenerative diseases, affecting the cerebellum and cerebellar connections, are increasingly diagnosed as spinocerebellar ataxias (SCA)
- Dysarthria is a motor speech disorder resulting from neurological injury of the motor component of the motor-speech system and is characterized by poor articulation of phonemes
- It is a condition in which problems occur with the muscles that help one talk
- It is unrelated to any problem with understanding cognitive language
- Perceptual analysis indicated that equalized stress, imprecise consonants, vocal instability, monotony and reduced speech rate were the speech parameters that yielded the highest mean perceptual ratings
- A factor analysis of perceptual speech parameters revealed two main factors:
- Factor 1 was associated with articulatory timing and Factor 2 with vocal quality.
- A coustic analysis revealed significantly reduced speech rate during text reading, reduced alternating and sequential motion rates, significantly longer and more variable syllable and pause durations, and significantly higher vocal instability for subjects with SCA compared to control subjects

Schalling E., Hammarberg B., Hartelius L. (2007). Perceptual and acoustic analysis of speech in individuals with spinocerebellar ataxia (SCA). *Logopedics Phoniatrics Vocology.* 32. 31-46

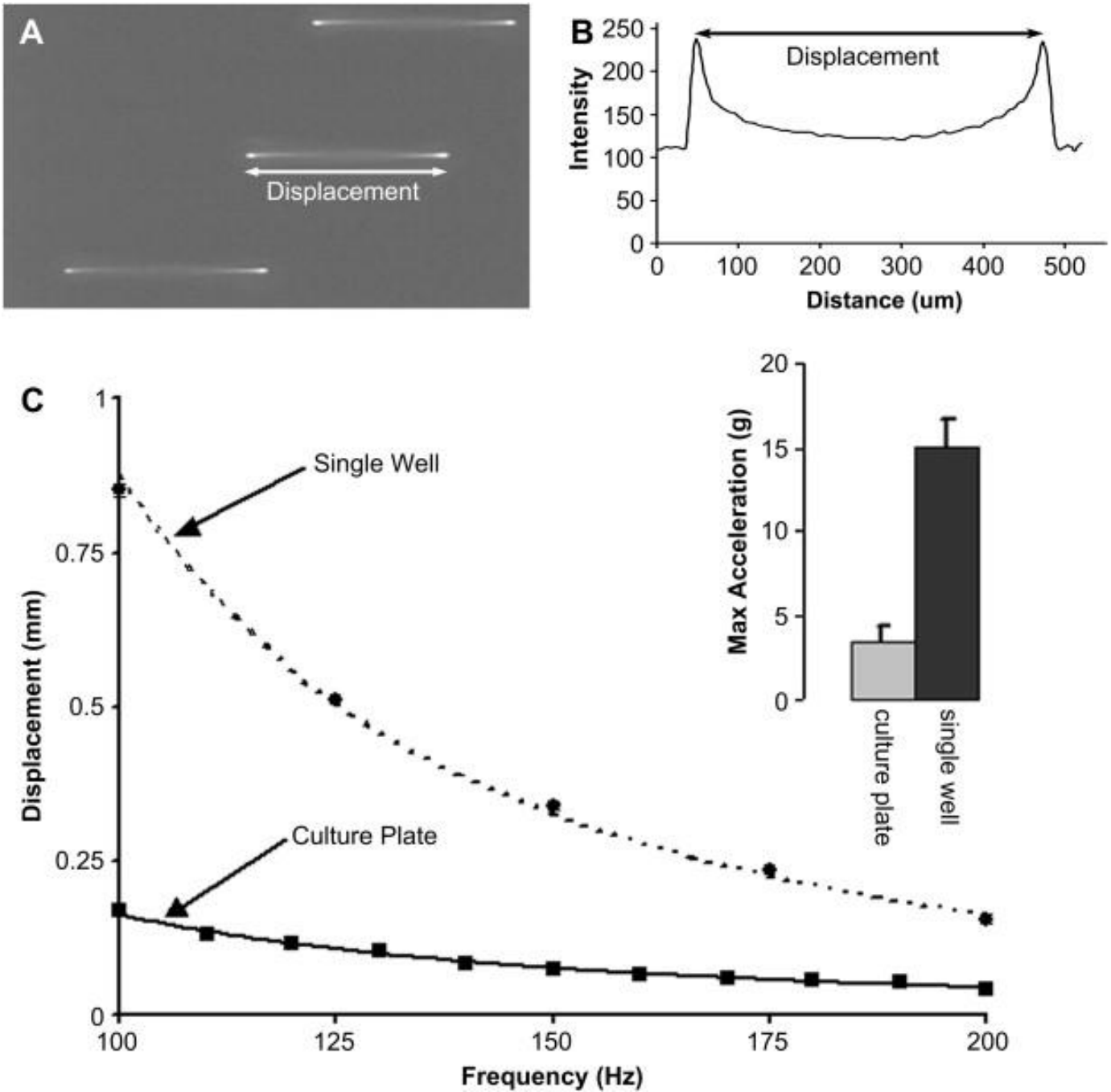
Bioreactor induced vibrational stimulation

To study the affect of mechanical stimuli on human laryngeal fibroblasts, with bioreactors capable of vibrating cell seeded substrates at frequencies and displacements comparable to measured phonation values in human subjects

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

- 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


A
Displacement
B
Intensity
Displacement
250
200
150
100
50
0
0
100
200
300
400
500
Distance (um)
C
Displacement (mm)
1
0.75
0.5
0.25
0
Single Well
Culture Plate
100
125
150
175
200
Frequency (Hz)
Max Acceleration (g)
20
15
10
5
0
culture plate
single well

Relations neurological/speech/voice genetic disorders

There are also in many cases relations between neurological and speech/voice disorders. With the new tool: online segmentation of high-speed films neurological genetic aspects can be analyzed far better

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

- 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

Genetics and speech therapy

Speech and language deficits are varied, including aphasia, stuttering, articulation disorders, verbal dyspraxia and language impairment.

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

- 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 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

Protein production of the genes

An amino acid deletion in 9q region alters the protein production of the gene torsin A. Does this knowledge influence the future?

Sulica L. (2004). Contemporary management of spasmodic dysphonia. *Current Opinion in otolaryngology & Head and Neck Surgery.* 12. 543-548

- The standard of care for spasmodic dysphonia in 2004 remains botulinum chemodenervation for symptomatic management.
- Surgery is best reserved for the rare patient who does not benefit or cannot tolerate botulinum toxin injections.
- Symptom relief in abductor spasmodic dysphonia and dystonia with tremor remains suboptimal.
- **Spasmodic dysphonia is a disorder of the central nervous system rather than the larynx**
- The pathophysiology underlying dystonia is gradually becoming better understood

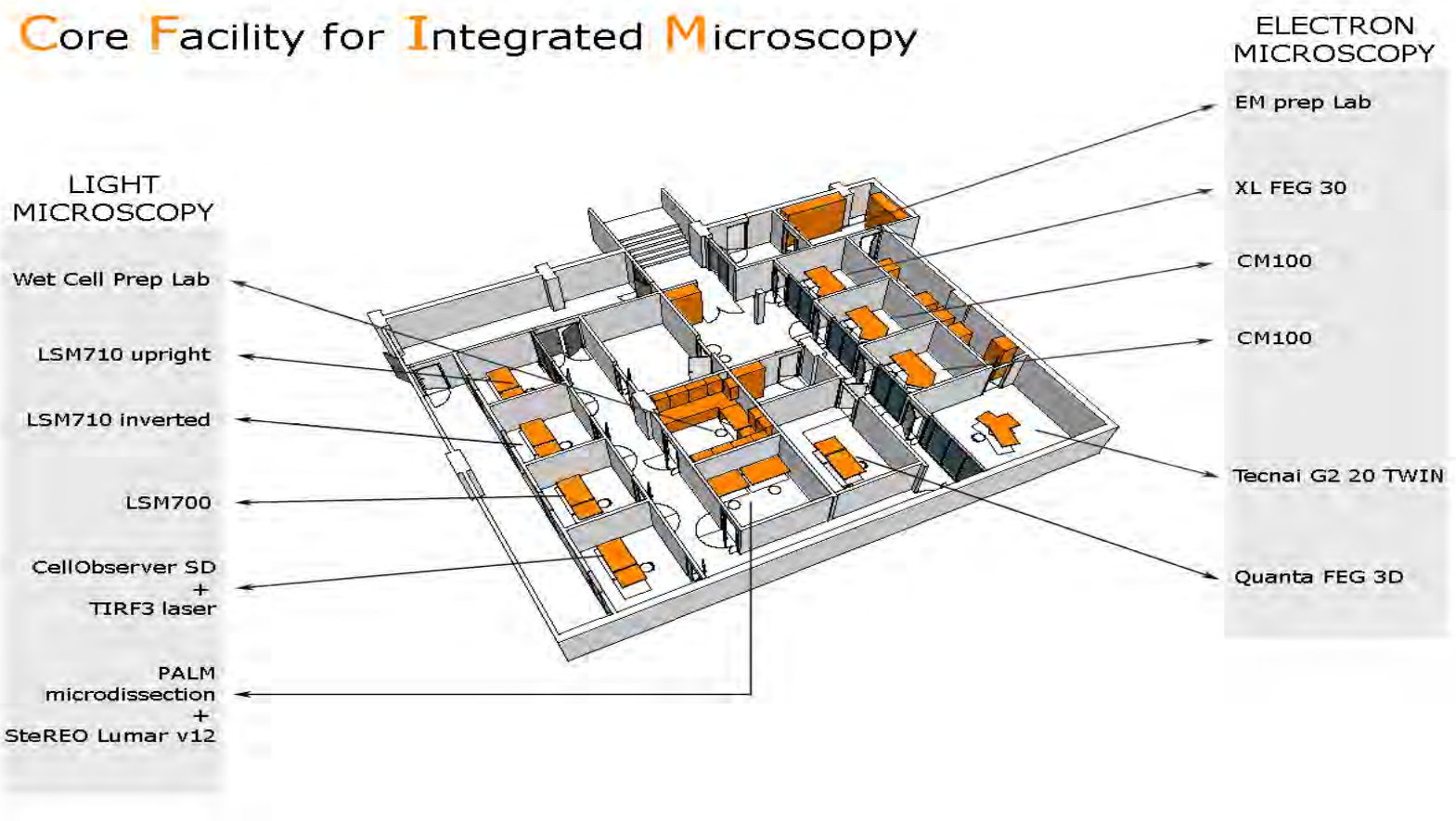
Sulica L. (2004). Contemporary management of spasmodic dysphonia. *Current Opinion in otolaryngology & Head and Neck Surgery.* 12. 543-548

ABSTRACTS 3

Tissue engineering, cellular actions, micro array (light and electronic microscopy aspects)

- Genetic tissue engineering is based on cellular action also in the voice area. Light and electronic scenic procedures can be made
- 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.

The facilities at Panum Institut Copenhagen, for light and electron microscopy



Innate mechanism of voice production

Innate mechanism of voice production. We do not know the role of genes in human innate vocalization

Gaub S, Groszer M, Fisher S, Ehret G. The structure of innate vocalizations in Foxp2-deficient mouse pups. Genes, Brain and Behavior. 2010; 9: 390-401

- **FOXP2 mutations plays a role in severe speech and language disorder in humans.**
- FOXP2 mutations in mice yield abnormal synaptic plasticity and impaired motorskill learning.
- There is no proven connection between FOXP2 mutation and emotional vocalizations and acustic properties in mice.

Gaub S, Groszer M, Fisher S, Ehret G. The structure of innate vocalizations in Foxp2-deficient mouse pups. Genes, Brain and Behavior. 2010; 9: 390-401

Vocal cord micro structure

Fibroblast synthesis and function related to vibratory structure of the vocal cord. How to use the new knowledge -

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 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.

Connective/elastin tissue

The human genetic connective tissue studies involve many treatment aspects. How to get on with structured differentiation to help patients?

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.

- **<u>The human elastin gene (ELN) is responsible for the generation of elastic fibres in the extracellular matrix of connective tissue throughout the body</u>**

- 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 a bnormalities 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.

Elastin gene function in mice

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

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.[[

- There was used 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 +/+)- were used for this study.
- Vocal folds were obtained from each animal and stained for the protein elastin using histochemiacal methods.
- Qualitative visual inspections revealed greater staining density for elastic fibers in the Eln+/+ animal
- 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.[[

Applications of tissue engineering

Tissue engineering combines cells, scaffolds and engineering to reconstruct defect tissue

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)

- **Collagen** is the primary component of the extracellular matrix, these scaffolds support fibroblast growth attracting keratinocytes and promoting their growth.
- 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 ingrowth 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)

Leukocytes analysis / Lymfocytes analysis

Holistic approach e.g. human lymfocytes and leucocytes analysis, how the basic advice for personalized treatment should be made in our field

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

- 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.

Stem cells

Many aspects of stemcells 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.

Growth factor

- Genetics studies include combined approach: how?
- for voice, respiration, swallowing interference
- The use of growth factor for gene transfer.
- Practical application. Definition of patient potentials

Heavner S, Rubin A, Fung K, Old M, Hogikyan N, Feldman E. Dysfunction of the Recurrent Laryngeal Nerve and the Potential of Gene Therapy. Annals of Orology, Rhinology & Laryngology. 2007; 116(6):441-448.

- Injury to the recurrent laryngeal nerve causes vocal fold paresis or paralysis resulting in poor voice quality, and possibly swallowing dysfunction and/or airway compromise.

- Gene transfer may be a useful adjunct to enhance nerve regeneration in the **setting of neurodegenerative disease or trauma.**

Heavner S, Rubin A, Fung K, Old M, Hogikyan N, Feldman E. Dysfunction of the Recurrent Laryngeal Nerve and the Potential of Gene Therapy. Annals of Orology, Rhinology & Laryngology. 2007; 116(6):441-448.

Estrogens and Androgens

Further understanding has been developed in an analysis of voice development during puberty, comparing the 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 Analysis

DNA concordance in a twin pair, how is it related to the voice deficiency, which criteria shall we use in the future: High speed film, optical coherence tomography and 3 dimensional films

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.

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.

Task	Twin 1				Twin 2			
	RTA Mean (Max)	LTA Mean (Max)	RLCA Mean (Max)	LLCA Mean (Max)	RTA Mean (Max)	LTA Mean (Max)	RLCA Mean (Max)	LLCA Mean (Max)
Prephonatory (vowel)								
NPNL	1.2 (4.4)	3.5 (11.8)	28.9 (53.4)	2.2 (5.2)	7.4 (28.4)	34.3 (66.2)	19.9 (33.0)	18.7 (55.5)
Soft	0.7 (1.3)	1.4 (8.6)	11.7 (19.7)	1.9 (3.1)	4.0 (6.5)	20.7 (32.3)	11.7 (28.1)	7.6 (15.3)
Loud	13.9 (59.1)	23.6 (80.6)	23.2 (83.2)	6.4 (30.0)	7.9 (33.8)	22.4 (33.1)	32.1 (55.7)	15.6 (31.5)
Phonatory (vowel)								
NPNL	1.0 (2.1)	2.2 (9.9)	11.7 (29.7)	2.3 (4.2)	12.0 (15.7)	25.6 (56.9)	26.1 (36.5)	11.1 (18.8)
Soft	0.6 (0.9)	0.4 (1.2)	6.8 (17.2)	1.6 (4.4)	15.7 (30.8)	37.5 (64.3)	22.7 (49.2)	14.3 (38.1)
Loud	3.7 (41.3)	5.9 (35.2)	15.4 (37.3)	2.6 (5.3)	14.0 (32.7)	24.9 (37.5)	28.2 (52.1)	18.5 (35.2)
Prephonatory (DDK)	5.9 (47.9)	10.2 (75.5)	15.4 (31.6)	2.8 (4.5)	7.6 (18.2)	8.9 (23.0)	6.5 (12.6)	3.2 (7.2)
Phonatory (DDK)	1.6 (5.3)	2.3 (13.6)	12.6 (35.9)	3.6 (6.4)	13.4 (24.1)	16.3 (25.2)	10.9 (25.1)	5.5 (9.6)

RNA

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

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.

- 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 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, synthesis, biochip

Aspects of personalized medicine, use of fibroblast growth, biochip development for vocal care

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.

- DNA does not directly makes proteins, it is first transcribed into RNA.
- RNA is 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.

- **<u>The use of collagen injection in the vocal cords is a form of protein therapy as the use of botox</u>**

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.

Restoration and normal gene function

Restoration of normal gene function might be a possibility

Hopson K. Genes Signal Late-Stage Laryngeal Cancer, Poor Outsome. Henry Ford News. 14-12-2010: 1-2.

- The study finds genetic **abnormalities of the ESR1 gene and the HIC1 gene** are predictors of late-stage laryngeal cancer and shorter survival, respectively, for patients with the disease

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- Loss of gene function as a result of abnormal methylation of promoter regions of tumor suppressor genes can be reversed by drugs that can restore normal gene function, opening the door to other treatment options for patients with laryngeal cancer

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- A patient in stage 1, and where we find methylation of ESR1, the patient may have underpinnings of a more aggressive tumor with characteristics that are more in line with a stage 3 or stage 4, refining treatment options

Hopson K. Genes Signal Late-Stage Laryngeal Cancer, Poor Outsome. Henry Ford News. 14-12-2010: 1-2.

Inflammatory mRNA, RNA

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)

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.

- 10 rabbits was giving experimentally 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 to 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.

Gene transcript

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

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.

- 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.

Sensomotor integration

Sensomotor integration in mice. Synaptic plasticity: how to measure in humans. Is it possible to correlate from mice to humans, if so, how?

Groszer M, Keays D, Deacon R, Bono J, Prasad-Mulcare S, Gaub S, Baum M, French C, Nicod J, Coventry J, Enard W, Fray M, Brown S, Nolan P, Pääbo S, Channon K, Costa R, Eilers J, Ehret G, Rawlins J, Fisher S. Impaired Synaptic Plasticity and Motor Learning in Mice with a Point Mutation Implicated in Human Speech Deficits. Current Biology. 2008; 18: 354-362

- The most well-described example of an inherited speech and language disorder is that observed in the multigenerational KE family, caused by a heterozygous missense mutation in the **FOXP2 gene**

- Deficits in the learning and production of complex orofacial motor sequences underlying fluent speech and displaying impaired linguistic processing for both spoken and written language

- The FOXP2 transcription factor is highly similar in many vertebrate species, with conserved expression **in neural circuits related to sensorimotor integration and motor learning**

Groszer M, Keays D, Deacon R, Bono J, Prasad-Mulcare S, Gaub S, Baum M, French C, Nicod J, Coventry J, Enard W, Fray M, Brown S, Nolan P, Pääbo S, Channon K, Costa R, Eilers J, Ehret G, Rawlins J, Fisher S. Impaired Synaptic Plasticity and Motor Learning in Mice with a Point Mutation Implicated in Human Speech Deficits. Current Biology. 2008; 18: 354-362

Cystic fibrosis

Interesting for voice deficits

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.

- In adult 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 and voice

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

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.

- 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 VCPDM family of Bulgarian descent and fine mapping of the critical interval.

- Sequencing of positional candidates genes revealed precisely the same nonconvertative 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 two independent mutational event at the same position in MATR3 cause VCPDM.**

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

Virus infection associated with a genetic syndrom is more dangerous. Tests must be carried out in our field, e.g. respiratory syncytial virus, adenovirus and human papilloma virus

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.

- 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 immunocompromised patients.
- **22q11 deletion syndrom** (22q11DS) represents a common genetic disorder causing velopharyngeal dysfunction.
- 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.

D-vitamin receptor study

D-vitamin receptor study. Relevant in our field, for mucosal function as well as risk of cancer development

Liu Z, Calderon J, Zhang Z, Sturgis E, Spitz M, Wei Q. Polymorphisms of vitamin D receptor gene protect against the risk of head and neck cancer. Pharmacogenetics & Genomics. 2005; 15 (issue 3): 159-165

- The vitamin D has potent anti-tumour properties
- Calcitriol is an antiproliferative and prodifferentiation factor for several cell types, including human sqamous cells of the head and neck
- Several polymorphisms of the vitamin D receptor (VDR) gene have been described
- It is hypothesized that the VDR FokI and TaqI polymorphisms are associated with the risk of developing squamous cell carcinoma of the head and neck (SCCHN)
- Case-control study of 719 SCCHN cases and cancer-free controls: **it was shown that VDR FokI and TaqI alleles may protect against SCCHN**

Liu Z, Calderon J, Zhang Z, Sturgis E, Spitz M, Wei Q. Polymorphisms of vitamin D receptor gene protect against the risk of head and neck cancer. Pharmacogenetics & Genomics. 2005; 15 (issue 3): 159-165

ABSTRACTS 4

Organization of personalized medicine in voice disorders

It is proposed that exercises for hoarseness are more effective if pharmacogenetic problems have been solved.

Exercises can also reveal a connection to genetics and potential demands for biological understanding and medical treatment.

Person centered care

Person-centered care (PCC) has become the foundation for practice in many areas of health care provision

DiLollo C., Favreau C. (2010). Person-Centered care and speech and language therapy. *Seminars in Speech and Language*. 31(2). 90-97

- **<u>Research has suggested that providing PCC may improve therapy outcomes, client satisfaction, and perceived quality of care</u>**

- Some research has indicated that speech-language pathologists (SLPs) have a tendency to provide more structured, task-oriented therapies.

- Results from this study indicated no increase in the use of PCC as student clinicians gained clinical experience and suggested that current approaches to clinical supervision and grading may play a role in reducing the amount of PCC provided by student clinicians.

DiLollo C., Favreau C. (2010). Person-Centered care and speech and language therapy. *Seminars in Speech and Language*. 31(2). 90-97

Genetic and environmental effects on vocal symptoms and their intercorrelations

- A total of 1728 twins born between 1961 and 1989 completed a speech, voice and language questionaire.
- Symptoms of strain, hoarseness, voice breaks, throat clearing and coughing when talking as well as a lump in the throat were asked for.
- Genetic effects were moderate,
- whereas the environmental effect seemed to be the most important factor contributing to the presence of vocal symptoms

Nybacka I, Simbergs, Santtila P, Sala E, Sandnabba. J Speech and Language Research. 2012,55, 541-533

Genetic voice quality in monozygotic twins

Genetic voice quality in monozygotic twins. Yes, fine, but how can we use these results?

Lierde K, Ley S, Clement G, Cauwenberge P. Genetics of Vocal Quality Characteristics in Monozygotic Twins: A Multiparameter Approach. Journal of Voice. 2004; vol. 19 no. 4: 511-518.

- The main purpose of this study was to determine the vocal quality characteristics among the 45 monozygotic cotwins (MT)
- It was hypothesized that the vocal characteristics and the overall vocal quality by means of the Dysphonia Severity Index (DSI) will be identical in MT. An additional objective of this study was to determine whether sex and age influence vocal similarities in MT. Subjective and objective assessment techniques determined the vocal quality

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- No significant differences were obtained and most comparisons resulted in significant correlation coefficients.
- It is clear that the perceptual voice characteristics, the laryngeal aerodynamic measurements of maximum phonation time (MPT), the vocal performances, and the overall vocal quality by means of the DSI are similar in MT.
- These vocal characteristics are not influenced either by the subjects' age or sex and **are situated within the normative range of unrelated peers**. To what extent other aspects (environment, anxiety, tension, etc) might play a role in the acoustical dimensions regarding frequency and amplitude perturbation, which were in the normal range, is a subject of further research.

Lierde K, Ley S, Clement G, Cauwenberge P. Genetics of Vocal Quality Characteristics in Monozygotic Twins: A Multiparameter Approach. Journal of Voice. 2004; vol. 19 no. 4: 511-518.

	MPT (s)			I-low (dB)			F-high (Hz)		
	Cotwin 1	Cotwin 2	*r*	Cotwin 1	Cotwin 2	*r*	Cotwin 1	Cotwin 2	*r*
Male	21.4	16.5	**0.87***	62.6	60.7	**0.67***	627.2	669.8	**0.87***
Female	15.8	18.1	**0.58***	61.0	61.5	**0.47***	773.3	762.5	**0.49***
≤ 17 yrs	17.0	18.7	**0.57***	61.9	61.8	**0.45***	754.5	751.8	**0.59***
> 17 yrs	19.3	16.1	**0.87***	61.6	60.5	**0.60***	666.8	693.5	**0.79***

	F0 (Hz)			Jitter (%)		
	Cotwin 1	Cotwin 2	*r*	Cotwin 1	Cotwin 2	*r*
Male	138.2	140.4	**0.94***	0.7	1.1	−0.09
Female	235.3	232.3	**0.43***	1.0	1.3	−0.12
≤ 17 yrs	208.0	206.1	**0.91***	1.0	1.5	−0.19
> 17 yrs	180.0	180.3	**0.95***	0.7	0.9	0.02

	Shimmer (%)			DSI		
	Cotwin 1	Cotwin 2	*r*	Cotwin 1	Cotwin 2	*r*
Male	3.9	5.2	−0.05	0.6	0.4	**0.70***
Female	5.3	5.3	0.22	0.5	0.0	0.26
≤ 17 yrs	4.9	5.6	0.19	0.5	−0.4	0.11
> 17 yrs	4.5	4.9	0.01	0.6	0.8	**0.69***

* Twin intercorrelations (Pearson *r*) are reported for each of the different genders and age groups. Significant *P* values (< .05) are indicated in bold and with.

Longitudinal twin studies

Longitudinal twin studies should systematically be made for genetic voice disorders in highly develop centres

Dworzynski K., Remington A., Rijsdijk F., Howell P., Plomin R. (2007). Genetic etiology in cases of recovered and persistent stuttering in an unselected, longitudinal sample of young twins. *Am J Speech Lang Pathol.* 16(2). 169-178

- Data from the twins' early development study were employed Parental reports regarding stuttering were collected at ages 2, 3, 4 and 7 years and were used to classify speakers into recovered and persistent groups
- Of 12,892 children with at least two ratings, 950 children had recovered and 135 persisted in their stutter

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- Logistic regression showed that the rating at age 2 was not predictive of later stuttering, whereas rating at ages 3 and 4 were concordance rates were consistently higher for monozygotic than for diszygotic twin pair (with the exception of girls at age 3)
- At 3, 4 and 7 years, the liability to stuttering was highly heritable
- **Stuttering appears to be a disorder that has high heritability and little shared environment effect** in early childhood and for recovered and persistent groups of children, by age 7

Dworzynski K., Remington A., Rijsdijk F., Howell P., Plomin R. (2007). Genetic etiology in cases of recovered and persistent stuttering in an unselected, longitudinal sample of young twins. *Am J Speech Lang Pathol.* 16(2). 169-178

Twin Study

The role of genetics is not evidence based. A high level of quality of studies is demanded

Simberg S, Santtila P, Soveri A, Varjonen M, Sala E, Sandnabba N. Exploring Genetic and Environmental Effect in Dysphonis: A Twin Study. Journal of Speech, Language, and Hearing Research. 2009; 52: 153-163.

- 1728 Finnish twins (555 male; 1173 female) born between 1961 and 1989 completed a questionnaire concerning vocal symptoms and occupation
- The zygosity determination resulted in 125 monozygotic and 108 dizygotic full twin pairs.
- Individuals differences in dysphonia were explained by genetic effects (35%) and nonshared environmental effects (65%).Shared environmental effects were estimated at 0%

- Both genetic and environmental factors have an impact on the etiology of voice problems, the genetic –environment interaction not being statistically significant.
- **Environmental factors, either independently or interacting with genetic factors, seem to play the key role, especially if the person has a voice-demanding occupations**

Simberg S, Santtila P, Soveri A, Varjonen M, Sala E, Sandnabba N. Exploring Genetic and Environmental Effect in Dysphonis: A Twin Study. Journal of Speech, Language, and Hearing Research. 2009; 52: 153-163.

Genetic function in the lower airways

Genetic vocal cord dysfunction, and pharmacological treatment. The upper airways' function is likely to follow the lower airways' function for genetic aspects

Mathur S.K., Busse W.W. (2006). Asthma: Diagnosis and Management. *The Medical Clinics of North America.* 90. 39-60

- Asthma is characterized by a chronic airway inflammation that may lead to airway obstruction, hyper responsiveness, and clinical symptoms of cough, wheeze, and shortness of breath.

- Prevalence, morbidity and mortality of asthma have been increasing worldwide. There is a concern that asthma patients are not always readily identified and may not receive optimum treatment of their disease.

- Comorbidities associated with asthma should be considered, because they often complicate the effectiveness of asthma management.

Mathur S.K., Busse W.W. (2006). Asthma: Diagnosis and Management. *The Medical Clinics of North America.* 90. 39-60

Pharmacogenetic aspects related to the lower airways

Personalized pharmacogenetic treatment of the upper airways. The need is obvious for coordination with advanced knowledge of the lower airway

Green R, Brightling C, Pavord I, Wardlaw A. Management of asthma in adults: current therapy and future directions. Postgrad Med J. 2003; 79: 259-267.

- Asthma is increasing in prevalence worldwide and results in significant use of healthcare resources

- Most patients with asthma can be adequately treated with inhaled corticosteroids, but an **important number of patients require additional therapy** and an increasing number of options are available

- A further minority of patients develop severe persistent asthma which remains difficult to manage despite current pharmacological therapies

Green R, Brightling C, Pavord I, Wardlaw A. Management of asthma in adults: current therapy and future directions. Postgrad Med J. 2003; 79: 259-267.

Predictive and preventive medicine

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

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

- **<u>Displaying the highest level of any functional genetic complex with medical impact</u>**

- Systems biology approach and integrative methodologies will need to unravel the ever growing number of HLA and diseases associations

- HLA, immunogenetics 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.

Dysphonia in many genetic syndromes

Dysphonia is a part of many genetic syndromes and should always be diagnosed e.g. with high speed films to understand voice function better

Nanri T, Utsumi H, Yamada M, Takata Y, Matsumura A, Kougo K, Sekine S, Ogawa D, Toyoda M. Transthyretin Val 107 in a Japanese patient with familial amyloid polyneuropathy. Journal of the Neurological Sciences. 2002; 198: 93-96

- 70-years-old Japanese man with **amyloid polyneuropathy** associated with a Val 107 transthyretin (TTR) mutation is reported
- The patient presented with carpal tunnel syndrome, cardiomyopathy, bulbar palsy, dysphonia and polyneuropathy. DNA analysis of the TTR gene revealed a point mutation responsible for substitution of valine for isoleucine at position 107 of the TTR molecule
- Taken together with reports of patients with the same TTR variant, Val 107 TTR mutation is probably associated with a clinical phenotype characterized by carpal tunnel syndrome, cardiomyopathy, bulbar palsy and dysphonia

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- This case implies a worldwide distribution of the Val 107 TTR mutation with a common clinical phenotype, despite different ethnic background

Nanri T, Utsumi H, Yamada M, Takata Y, Matsumura A, Kougo K, Sekine S, Ogawa D, Toyoda M. Transthyretin Val 107 in a Japanese patient with familial amyloid polyneuropathy. Journal of the Neurological Sciences. 2002; 198: 93-96

Genetics polymorphism

Aspects of genetic polymorphism are also necessary in our area. Especially singers need advice of which gene provocation are most important

Rémen, Thomas; Coevoet, Vincent; Acouetey, Dovi-St/ephanie; Guéant, Jean-Louis; Guéant-Rodriguez, Rosa-maria; et al. Early incidence of occupational asthma among young bakers, pastry-makers and hairdressers: design of a retrospective cohort study. BMC public health 10 (Apr 26, 2010): 206

Occupational exposures are thought to be responsible for 10-15% of new-onset asthma cases in adults, with disparities across sectors
The study population was composed of subjects who graduated between 2001 and 2006 in bakers and hairdressers sectors where they experience exposure to organic or inorganic allergenic or irritant compounds compared with 250 young workers with no specific occupational exposure.

Subjects were invited to participate in a medical visit to complete clinical and lung function investigations, including fractional exhaled nitric oxide (FENO) and carbon monoxide (CO) measurements, and to collects blood samples for IgE (immunoglobulin E) measurements
Markers of oxidative stress and genetic polymorphisms exploration were also assessed. A random sample of 200 “non-cases” (controls) followed a nested case-control design

Rémen, Thomas; Coevoet, Vincent; Acouetey, Dovi-St/ephanie; Guéant, Jean-Louis; Guéant-Rodriguez, Rosa-maria; et al. Early incidence of occupational asthma among young bakers, pastry-makers and hairdressers: design of a retrospective cohort study. BMC public health 10 (Apr 26, 2010): 206

299 different genetic syndromes isolated (voice related)

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

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

- **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 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 communiation 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.

Phenotypes based on genetic heterogenity

Individual studies of syndromas, functional voice defect (phenotypes) based on genetic heterogeneity.

Lierde K, Luyten A, Mortier G, Tihskens A, Bettens K, Vermeersch H. Overall intelligibility, articulation, resonance, voice and language in a child with Nager syndrome. International Journal of Pediatric Otorhinolaryngology. 2011; 75: 270-276.

- The purpose of this study was to provide a description of the language and speech in a 7-year-old Dutch speaking boy with Nager syndrome
- To reveal these features comparison was made with an age and gender related child with a similar palatal and hearing problem
- Language was tested with an age appropriate language test namely the Dutch version of the Clinical Evaluation of Language Fundamentals
- The most striking communication problems with this child were: **expressive and receptive language delay, moderately impaired speech intelligibility, the presence of phonetic and phonological disorders, resonance disorders, a high-pitched voice.**
- The language and the phonological impairment present in the child with Nager syndrome is not part of a more general developmental delay. The resonance disorders can be related to the cleft palate, but were not present in the child with the isolated cleft palate
- According to the results of this study, the speech and language management must be focused on receptive and expressive language skills and linguistic conceptualization, correct phonetic placement and the modification of hypernasality and nasal emission

Genetically well defined disorders

Genetically well defined disorders should be incorporated in our field for understanding of voice function e.g. related to the immune system. A systematic voice related analysis in patients should be made

Stanke F, Becker T, Kumar V, Hedtfeld S, Becker C, Cuppens H, Tamm S, Yarden J, Laabs U, Siebert B, Fernandez L, Macek Jr M, Radojkovic D, Ballmann M, Greipel J, Cassiman J, Wienker T, Tümmler B. Genes that determine immunology and inflammation modify the basic defect of impaired ion conductance in cystic fibrosis epithelia. J Med Genet. 2011; 48: 24-31.

- The cystic fibrosis (CF) basic defect has not been employed so far to support the role of CF modifier genes
- Patients were selected from 101 families with a total of 171 F508del-CFTR homozygous CF patients to identify CF modifying genes
- A candidate gene based association study of 52 genes on 16 different chromosomes with a total of 182 genetic markers was performed.
- Variants at immunologically relevant genes were associated with the manifestation of the CF basic defect
- **The inherited capabilities of the innate and adaptive immune system determine the manifestation of the CF basic defect.**
- A survivor effect, manifesting as transmission disequilibrium at many loci, is consistent with the improvement of clinical care over the last decades, resulting in a depletion of risk alleles at modifier genes
- Awareness of non-genetic factor such as improvement of patient care over time is crucial for the interpretation of CF modifier studies

Stanke F, Becker T, Kumar V, Hedtfeld S, Becker C, Cuppens H, Tamm S, Yarden J, Laabs U, Siebert B, Fernandez L, Macek Jr M, Radojkovic D, Ballmann M, Greipel J, Cassiman J, Wienker T, Tümmler B. Genes that determine immunology and inflammation modify the basic defect of impaired ion conductance in cystic fibrosis epithelia. J Med Genet. 2011; 48: 24-31.

X chromosome in the voice area

The effect of growth factor in the absence of X chromosome in all parts of the body, including the voice?

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

- 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 discribed
- 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 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.

Genetics and vocal learning

Aspects of research of genetics and learning even with different genetics vocal learning is an aspect of its own.

White S. Genes and vocal learning. Brain and Language. 2010; 15: 21.28.

- A rare mutation in the molecule known as FOXP2 discovered in human family seemed to have a mutation in a single gene, that is a evolutionary lynchpin supporting the development of human language.

- Its sequence phylogeny reinforced a Chomskian view that language emerged wholesale in humans.

- Spurred by this discovery, research in primates, rodents and birds **suggests that FOXP2 and other language-related genes are interactors in the neuromolecular networks that underlie subsystems of language.**

White S. Genes and vocal learning. Brain and Language. 2010; 15: 21.28.

Potential gene therapy

Why are genetics and voice not highly related?

[54] Bondy, Carolyn A.; Bondy, C.A. Clinical practice guideline: Care of girls and women with Turner syndrome: A guideline of the Turner Syndrome Study Group. Journal of Clinical Endocrinology and Metabolism 92. 1: 10-25. Endocrine Society. (Jan 2007)

- **<u>The objective of this work is to provide updated guidelines for the evaluation and treatment of girls and women with Turner syndrome (TS)</u>**

- Breakout groups focused on genetic, cardiological, auxological, psychological, gynecological, and general medical concerns and drafted recommendations for presentation to the whole group
- It is suggested that parents receiving a prenatal diagnosis of TS be advised of the broad phenotypic spectrum and the quality of life observed in TS in recent years

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- It is recommended that magnetic resonance angiography be used in addition to echocardiography to evaluate the cardiovascular system and suggest that patients with defined cardiovascular defects be cautioned in regard to pregnancy and certain types of exercise
- It is suggested that caregivers address the prospect of premature ovarian failure in an open and sensitive manner and emphasize the critical importance of estrogen treatment for feminization and for bone health during the adult years

Bondy, Carolyn A.; Bondy, C.A. Clinical practice guideline: Care of girls and women with Turner syndrome: A guideline of the Turner Syndrome Study Group. Journal of Clinical Endocrinology and Metabolism 92. 1: 10-25. Endocrine Society. (Jan 2007)

Genetics and toxic aspects

Toxic aspects of aldehyd and nickel – chromium with genetic sentization

Leikauf, George D. Hazardous air pollutants and asthma Environmental health perspectives 1104 (Aug 2002): 505-26

- Asthma has a high prevalence in the United States, and persons with asthma may be at added risk from the adverse effects of hazardous air pollutants (HAPs)
- Certain HAPs are occupational asthmagens and may interact with critical pollutants in ambient air to exacerbate asthma.
- Based on these observations and past experience with 188 HAPs, a list of 19 compounds that could have the highest impact on the induction or exacerbation of asthma was developed
- Although the ambient levels of these compounds estimated exposures from emissions inventories and limited air monitoring suggest that **especially aldehydes and metals may have possible health risk**

Leikauf, George D. Hazardous air pollutants and asthma Environmental health perspectives 1104 (Aug 2002): 505-26

Disease preventing genes

Up regulation of disease preventing genes

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

- 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 52years 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 upregulating disease preventing genes and downregulating disease promoting genes**

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

Concepts of personalized medicine

- Over the past two decades, great progress has been made in the management of larynx cancer, with multimodality approaches aimed at laryngeal preservation reshaping the treatment landscape

- In the era of chemo radiation, greater focus and attention are now simply on "organ preservation"

- The continued development and integration of new treatment approaches, including organ preservation surgery is tailored to the circumstances of each patient.

Holsinger F. Swing of the Pendulum Optimizing Functional Outcomes in Larynx Cancer. Current Oncology Reports. 2008; 10: 170-175

Genetics of stammering (Genetics endocrinology and metabolism/voice)

Genetically well defined disorders should be incorporated in our field for understanding of voice function e.g. related to the immune system.

A systematic voice related analysis in patients should be made

Speech therapy. The science of stuttering. The Economist. 2011: 76.

Epidermal growth factor

Epidermal growth factor receptor might be a promising therapeutic target

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

- 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

Cancer bio markers

The field of cancer continues to be the leading edge of personalized medicine

3rd Oncology Biomarker Conference. Sessions Biomarker. 2011.

- Cancer biomarkers are **playing an ever-increasing role in the stratification of patient populations**
- The identification of new therapeutic targets, and the development of novel technology
- GTCbio's 3rd Oncology Biomarkers Conference focuses on topics paramount to the continuing progress of biomarker research and development

3rd Oncology Biomarker Conference. Sessions Biomarker. 2011.

Benign and malignant genetic disorders

There are genetic relations between benign and malignant genetic disorders

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

- 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

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- 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

Genetics of chemotherapeutical prevention

Evidence of chemotherapeutical prevention in the larynx as in the lungs? The arach-donic pass way gives some understanding of future aspects

Keith R, Miller Y. Lung cancer: genetics of risk and advances in chemoprevention. Current Opinion in Pulmonary Medicine. 2005; 11: 265-271.

- Linkage analysis has identified a locus on chromosome 6q23-25 that determines susceptibility to lung cancer in families with multiple members with cancer of the lung, throat, and larynx

- Epidemiologic and preclinical studies suggest that chemoprevention of lung cancer is an achievable goal

- Early trials with β-carotene supplementation have revealed a harmful effect

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- As a result of the large number of lung cancer death and at-risk individuals, even modestly effective chemoprevention could save many lives

Keith R, Miller Y. Lung cancer: genetics of risk and advances in chemoprevention. Current Opinion in Pulmonary Medicine. 2005; 11: 265-271.

- Possible future studies of defence mechanisms of acid in the larynx.
- The need is also there for higher genetic understanding of gastro-oesophageal reflux and the influence of lifestyle treatment – not only genetics and pharmacogenetics but certainly personal aspects not yet understood

Hopkins C, Yousaf U, Pedersen M (2006). Acid Reflux Treatment for Hoarseness [Review]. January 2006 in The Cochrane Library Oxford, Wiley publishing. Issue 1.
Pedersen M,McGlashan J (updated 2012). Vocal cord nodules [review]. Oxford, Wiley publishing

Conclusion

- The genomic aspects related to the voice and it´s treatment may be dependent on vocal related techniques with light and electronic microscopy.
- 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 new areas have been suggested in our introduction today – for pharmacogenetics and personalized treatment