1.1.8 Genetics

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Abstract and introduction

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 predisposition 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 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.

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 (few or single) cases are given of the literature, a great challenge is ahead for evidence based research.

Literature overview

Voice diagnostics and therapy related genetics, pharmacogenetics and personalized medicine, tissue engineering, cellular actions, organization of genetics and personalized medicine in voice disorders are presented.

Genetic treatment aspects of upper airways

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 or provoked by e.g. infections in the larynx. A difficulty of diagnosis 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.

Our own research lead to focusing on genes and personalized medicine, because lifestyle, training and education seemed to be important in some patients and pharmacological treatment in others e.g. antihistamines, cortisone and adrenaline [1, 2].

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

Pharmacogenetics 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 practices being tailored to individual patients [3].

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 [4]. Computing techniques should be discussed for genetics, pharmacogenetics and voice related personalized medicine. [5].

Which priority should we make for diagnosis? E.g. 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 are 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 [6].

Another example is a 6-year-old boy with the floating-Harbor syndrome (F-HS). 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. It is proposed that the exceptionally high-pitched voice and supernumerary upper incisor are additional. The elevated gliadin antibody levels suggest celiac disease, which has been described in three out of the 15 previously reported F-HS patients. The facial features and delayed speech development are very characteristic. The patient is a sporadic case like all the F-HS cases so far [7].

The roles of biological cells self-regulation have to be analyzed. Simulations have been used to gain basic insights into the inflammatory response baseline, gene-knockout, and drug-treated experimental with animals. These simulations transcend typical cytokine networks by associating inflammatory processes with tissue/organ impacts via tissue damage/dysfunction. Translational Systems Biology aims to unify mechanisms described in the scientific literature using methods and tools developed by the computational and systems biology communities [8].

Bioreacter induced vibrational stimulation is described. The results show that vocal fold-like vibrational stimuli are 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 [9].

There is a relationship between neurological speech/voice disorders and genetics. Etiologically dystonia, the neurological disorder dystonia, divides into major categories. 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 there is impaired effect of chaperone protein [10].

Genetic speech and language deficits include 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 lysosomal enzyme targeting pathway, has been reported to cause stuttering. Comparative studies have shown that genetic variations of FOXP2 transcription factor are 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 [11].
Genetic tissue engineering, cellular actions, microarray are discussed (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. Fibroblast synthesis and function are related to vibratory microstructure 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. Vibratory stimulation on ECM gene expression and synthesis of fibroblast in hyaluronic acid hydrogels with approximate viscoelastic properties of vocal mucosa was focused upon. 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 [12].
The human elastin gene (ELN) is responsible for the generation of elastic fibers 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 acoustical characteristics of voice quality for individuals with SVAS/WS, indicates that their voices is significantly abnormal. This supports that heterozygous ELN abnormalities possibly influence vocal fold biomechanics negatively [13]. Understanding of the elastin genetic function of the vocal folds is necessary with potential differential therapeutic aspects. Results suggest that Eln requires two functioning alleles for normal structural development of the vocal fold lamina propia [14].
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. A chitosan-collagen based scaffold is anticipated to accelerate fibroblast and endothelial cell in growth from underlying host tissue after grafting [15].
A holistic approach e.g. human lymphocytes and leucocytes analysis is often necessary for the basic advice for personalized treatment of voice. 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' characteristics, from which testable hypotheses can be generated and validated on the small number of actual patients available [16].
Many aspects of stem cells have been studied- till now without clinical applications in laryngology, *but the research field is necessary* [17].
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 parameters, fundamental frequency and phonetograms [18].
DNA concordance is presented in a twin pair with hoarseness [19].
RNA segment studies of vocal cord carcinoma: this is an important area where the role of genetic RNA interference must be taken into account. The method: to take copies of a small interfering RNA segment directed against the HuR gene and transfect it into Hep-2 cells, using LipofectamineTM 2000 [20].
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 [21].
The following is 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 were giving experimentally surgically induced modal or raised intensity phonation for 30 minutes. The results provide preliminary data on the effects of raised intensity phonation on inflammatory mRNA expression in the 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 [22].

Can the transcripts be reproduced: the following study has 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 of defence mechanisms of mucin gene profiles in normal laryngeal epithelium compared with patients with reflux attributed laryngeal injury or disease. Reverse Transcription Polymerase Chain Reaction (RT-PCR) was performed to establish the mucin gene profile [23].

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 upper airway infections [24].

It is so important that we follow the myopathy 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. Different disease related haplotype signatures have provided evidence that there were two independent mutational events at the same position in MATR3 [25].

Virus infection associated with a genetic syndrome is dangerous. Normally asymptomatic to mild, those infections tend to take a more severe course in immune compromised patients. 22q11 deletion syndrome (22q11DS) represents a common genetic disorder causing velopharyngeal dysfunction, provoked by infection. It is proposed that personalized medicine for hoarseness is more effective if pharmacogenetic problems have been solved. 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. 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 [27].

299 different voice related syndromes were presented. 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 [28].

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. Most Turner Syndrome women exhibit a higher frequency of pitched voice than non-TS women [29].

Up regulation of disease preventing genes: lifestyle can have a profound impact on the health situation for a number of diseases. This is done by up regulating disease preventing genes and down regulating disease promoting genes [30].

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. 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 [31, 32].

Conclusion

Inter individual 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 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 [33].

There is a need for enhanced genetic understanding of voice. This understanding must be documented with randomized controlled trials based on reviews. In our first Cochrane review: Vocal Chord Nodules [34] and our second Cochrane review: Acid Reflux Treatment for hoarseness [35], no evidence of treatment effect was documented.

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