

THE ETIOLOGY OF HEARING IMPAIRMENT IN CHILDREN AND ADULTS WITH AN ASSOCIATED MENTAL HANDICAP AT AN INSTITUTE FOR THE DEAF

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Few studies have been directed to the causes of deafness in deaf, mentally handicapped children and adults, while much research has been directed to the causes of deafness in children with a normal intelligence. The causes of deafness in the mentally handicapped are therefore less well known.

Of the 500 profoundly deaf children and adolescents at an Institute for the Deaf 120 have a mild to moderate mental handicap with an IQ between 40 and 75. The mean hearing impairment is 100 - 110 dBHL.

This study was aimed towards the causes of hearing loss in this group of 120 subjects. Thorough anamnestic evaluation, retrospective investigation of pre-existing records, otolaryngological, audiological, paediatric and ophthalmological examination were performed.

The results show a lower prevalence of hereditary causes and a much higher prevalence of pre- and perinatal causes in comparison with the causes of deafness in persons with a normal intelligence.

VESTIBULAR FINDINGS IN PROFOUNDLY HEARING IMPAIRED CHILDREN

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The involvement of vestibular apparatus in profound hearing impairment is rarely reported in children. In this study we analyse the possible relationship between a severe or profound congenital hearing loss and motor development and function in a pediatric group referring to our audiological department during the years 1992-94.

The study was ruled out in two parts: the first phase consisted in a questionnaire based on the one proposed by Moller (1996) to evaluate some aspects of balance development and control in 154 children underwent electrophysiological evaluation of auditory function (ECochG, ABR) and in a group of 10 normal-hearing children without balance disorders, as control group (aged from 3 to 10 years). In the second part of the study, we compared the findings of the vestibular tests performed in 14 profoundly deaf children and 10 normal-hearing children of the same age group.

The control group presented a normal labyrinthine function; 4 out of 14 of the hearing impaired children presented a significant labyrinthine hyporeflexia. The evaluation of the results of the balance questionnaire showed a low incidence of vestibular disorders in profoundly hearing impaired children. In the cases presenting a labyrinthine involvement, a correlation with the Moller questionnaire concerning balance control abilities was found.

In conclusion, the Moller questionnaire demonstrated high specificity and sensibility in identifying balance disorders in children.

A NOVEL MUTATION IN α -TECTORIN GENE ASSOCIATED WITH AUTOSOMAL DOMINANT NON-SYNDROMIC HEARING LOSS

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Mutations in the zona pellucida domain of α -tectorin gene, a major component of the tectorial membrane, have been identified as responsible for stable, severe, mid-frequency hearing loss in two families, one from Belgium (DFNA12) and one from Austria (DFNA8).

In a French family with high frequency, mild to moderate hearing loss, a missense mutation (C1619S) was identified in the zonadhesin-like domain of the α -tectorin gene. This mutation abolishes the first of the vicinal cysteines present in the D4 von-Willebrand factor repeat.

These results suggest that vicinal cysteines are involved in tectorial membrane matrix assembly, and that mutations in distinct domains of α -tectorin gene are responsible for various phenotypes.

A PATIENT DATABASE APPLICATION FOR HEREDITARY DEAFNESS EPIDEMIOLOGY AND CLINICAL RESEARCH: AN EFFORT FOR STANDARDIZATION IN MULTIPLE LANGUAGES

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One of the most challenging and neglected issues in medicine is healthy recording of the data obtained from the patients. "European Workgroup on Genetics of Hearing Impairment" which has been working since 1996 proposed a few questionnaires to collect data regarding the phenotype, ENT findings, audiological examination findings, and other special investigations. In this study, a computerized patient database application written in Delphi 3.0 for Windows for recording the patients with hearing problems has been presented. Delphi is by far one of the most popular and powerful visual programming tools on the market. The application has a modular form including identity information, genetic condition, proband query, audiology and vestibular tests, phenotype, pedigree, special examinations which allows data entry on all these issues. It has been developed by using the guidelines of Hereditary Deafness Epidemiology and Clinical Research (H.E.A.R.) and by the experience gained within the last four years by this research team. The target population of the program is the ENT clinicians, audiologists, epidemiologists, geneticists and researchers on the field. The main idea is to create a program serving the needs of both the daily routine work and research purposes and to distribute this program to the above mentioned specialists, make them try it as beta testers and find a standart and/or better way to collect data. For this reason, the program is aimed to be multilingual and currently available languages are English, German and Turkish.

HETEROGENEITY OF USHER SYNDROME TYPE I

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Usher Syndrome (US) is an autosomally inherited disorder characterized by sensorineural hearing impairment and progressive retinitis pigmentosa. Usher Syndrome is both clinically and genetically heterogeneous. Clinical heterogeneity has been suggested on the basis of variation in the severity and progression of the hearing impairment, age of retinal degeneration onset, and the presence of additional clinical findings. There are three clinical types: Usher I is most severe subtype with congenital profound deafness, prepubescent onset of retinitis pigmentosa, and absent vestibular responses. Usher II is marked by a congenital sloping audiogram, a later onset of retinitis pigmentosa in the second decade of life, and normal vestibular responses. Usher III is categorized by a progressive hearing loss with variable retinitis pigmentosa and vestibular responses. At least nine genes have been identified for Usher Syndrome: six for Usher type I (USH1 A-F), two for Usher type II (USH2 A-B) and one for Usher type III. However, only two genes have been identified: an unconventional myosin, Myosin VIIA and a novel gene, Laminush, which are responsible for USH1B and USH2A respectively. In our analysis of 155 Usher I patients, we estimate that we detected the pathologic mutation in approximately 75% of USH1B chromosomes as predicted by linkage analysis. Less than 10% of USH1B patients were estimated to be missed by this screening. Furthermore, the analysis of our combined mutation and linkage data of patients with Usher I phenotype revealed approximately 40% of non-USH1B families. This proportion is greater than we had previously estimated from linkage analysis alone. The proportions of the other Usher I subtypes are currently being determined and will be reported.

UNRAVELING THE MOLECULAR BASIS OF HEREDITARY DEAFNESS IN MICE AND HUMANS

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The intricate structure and multiple cell types of the inner ear requires a range of proteins with different functions, including structural integrity, neuronal innervation, and mechano-electrical transduction. Defects in any one of these proteins can result in deafness, the most common form of sensory impairment in humans. Knowledge about the molecular basis of deafness is unraveling rapidly with the identification of over ten genes involved in human non-syndromic hearing loss (NSHL). Nevertheless, sensorineural hearing loss has been difficult to study in humans due to the inaccessibility of the sensory organs of the ear.

The inner ear of all mammals has similar metabolic, physiological and neural characteristics, allowing the mouse cochlea to be used as a model to study human auditory function. Mutations in the myosin VI gene cause deafness in Snell's waltzer mutant mice, and our laboratory is studying the formation and structure of the sensory hair cells in these mutant mice, identifying proteins that interact with myosin VI, and studying the developmental role of myosin VI in the inner ear. Our ongoing research on genes known to be involved in genetic hearing loss in the Israeli hearing impaired population will be presented, including, (1) POU4F3, the gene responsible for DFNA15, an autosomal dominant form of progressive deafness and encoding POU4F3, a POU-domain transcription factor and (2) GJB2, the gene responsible for a high proportion of autosomal recessive hereditary deafness and encoding connexin 26, a gap junction protein.

IDENTIFICATION OF INTRACELLULAR CHANGE OF PROTEIN KINASE C (PKC) IN IMMORTALIZED CELL LINE FROM ORGAN OF CORTI

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The cells of the organ of Corti are not able to regenerate and to resume the auditory function. However, in vivo and in vitro studies have documented mitotic activity in immature hair cells of young mammals vestibular epithelia after ototoxic or acoustic insults.

Isolated hair cells do not normally survive in culture, unless they are co-cultivated with hair cells supports and neurons. The cell cycle mechanisms regulating the cessation of cell division, as well as those influencing regeneration, are largely unknown.

An immortalized cells lines (OCK-3) has been developed from the organ of Corti of transgenic mice (Immortamouse™ H-2Kb-tsA58, Charles Rivers Laboratories, Wilmington, MA), encoding a thermolabile protein virus of the SV40, the large T antigen. When these cells grow at the permissive temperature, at 33°C, they are driven to indefinitely proliferate by the oncogene product; on the other hand, when the temperature is raised to the non-permissive level of 39°C, the large T antigen is not produced and the cells cease to divide.

To investigate the cellular mechanisms involved in both drug-induced ototoxicity and in neurotrophic factor protection, we have employed OCK-3 cells treated with gentamicin and BDNF (brain derived nerve factor), focusing on PKC expression and intracellular distribution.

Our observations indicate that different isoforms of PKC are involved in programmed cell death or in the protection of this cell line.

AETIOLOGY OF HEARING IMPAIRMENT IN TWO BIRTH-COHORTS IN SOUTHERN ITALY

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The first aim of this study is to afford a share to European epidemiologic data collection on hearing impairment (HI); but the ultimate aim is the achievement of thorough epidemiologic list concerning HI in the province of Benevento.

In fact the study only includes patients permanently resident in this province at the time of the data collection (november 1998). The province is a local area of interior southern Italy, whose territory measures 2.070 Km² and includes a population of 293.026 (Istat 1991). We particularly studied and classified patients born from 1975 to 1979 (cohort A) and from 1985 to 1989 (cohort B), suffering from a permanent HI >50 dB HL for the better hearing ear averaged across 0.5-4 KHz.

According to guideline advised by Study Group on Epidemiology of Genetic HI (Parving A. et al., 1996) the patients were classified in relation to aetiology of HI. Moreover we have added the item "postnatal infections" which includes the early-acquired infections responsible for speech defects.

Cohort A collect 28 patients, 17 males and 11 females; the total of cohort B includes 34 patients (16 males and 18 females). Cohort A includes 21% of hereditary forms (83% non syndromic, 17% syndromic). Foetal infections, all represented by rubella disease, show a value of 25%. Perinatal complications are 7%, while meningitis, ototoxicity and various value is 4% each. Postnatal infections (measles, parotitis, viral infections) are 21%; the patients with unknown aetiology are 14%.

Inheritance is the most common cause of HI in cohort B, with 35% of inherited HI (91% non syndromic, 9% syndromic). Perinatal complications is 12% and postnatal infections value is 15%. Rubella and ototoxicity is 6% each; meningitis, craniofacial abnormality (Goldenhar syndrome) and various (cerebral tumor) value is 3% each. At the end there is 17% of unknown forms. The global datum shows an estimated prevalence of 1,2/1.000 in cohort A and 1,8/1.000 in cohort B.

According to the specific protocol for aetiological evaluation (Parving A. et al., 1998), the high incidence of HI with unknown aetiology demands a careful investigation.

PROGRESSIVE COCHLEOVESTIBULAR IMPAIRMENT CAUSED BY A POINT MUTATION IN THE COCH GENE AT DFNA9

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We examined auditory and vestibulo-ocular functions in a Dutch family with hereditary sensorineural hearing impairment (SNHI), caused by a 208C T mutation in the COCH gene located in chromosome 14q12-q13 (DFNA9), which showed an autosomal dominant pattern of inheritance with full penetrance. We were able to evaluate fifteen of the 16 genetically affected persons. They all developed hearing and vestibular impairment symptoms in the fourth to fifth decade and, in many cases, also cardiovascular disease. We derived the gross characteristics for the hearing impairment in this trait from linear regression analysis of individual longitudinal data (n=11) of age corrected hearing thresholds and scatterplots of all data available. At the low frequencies (0.25-2 kHz), it started at the age of about 40 and showed an average annual progression of approximately 3 dB. In two exceptional cases, annual progression attained levels of up to approximately 20 dB. At the high frequencies (4-8 kHz), the average threshold increased from about 50 dB at the age of 35 to about 120 dB at the age of 75 (1.8 dB annual threshold increase). All affected individuals tested showed normal ocular motor functions. The patients aged over 46 years showed absence and in one case severe impairment of the vestibulo-ocular reflex and enhancement of the cervico-ocular reflex, whereas those aged 40-46 years showed either severe bilateral vestibular hyporeflexia or unilateral caloric areflexia. These findings suggest a gradual development of vestibular areflexia.

MOUSE MODELS FOR HUMAN HEARING IMPAIRMENT

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The mouse is an important model organism for the study of human genetic disease and mouse deaf mutants have played a pivotal role in the identification of genes involved in hearing impairment and in elucidating the role of critical molecules involved in the process of auditory transduction. The shaker1 (*sh1*) mouse mutant that is encoded by the myosin VIIA gene is a paradigm of the utility of mouse models to identify genes involved in human hearing impairment and their role in the sensory neuroepithelia. Myosin VIIA appears to be involved with the proper development and positioning of stereocilia at the apical hair cell surface. Mutations in myosin VIIA underlie Usher syndrome type 1 in the human population, as well as recessive (DFNB2) and dominant (DFNA11) forms of non-syndromic deafness. Recently, we have also shown that myosin VIIA mutations can lead to atypical Usher syndrome. The phenotypic heterogeneity arising from myosin VIIA mutations is very suggestive that genetic background effects have some role to play in determining the development and severity of non-syndromic and syndromic hearing loss. However, in order to make further progress in identifying the critical molecules involved in auditory transduction and their epistatic interactions, it will be important to undertake new programmes both in mutant generation and the identification of modifier genes. We clearly do not possess mouse mutations at all loci involved in hearing impairment; nor is it likely in either human or mouse that mutations are to be found at all the critical loci involved in auditory transduction. An EC consortium has embarked upon a major programme utilising chemical mutagenesis to recover and map a large number of new mouse deafness mutations. The availability of a wider range of mouse mutations will also assist us in exploring epistatic interactions between loci and dissecting the underlying pathways. Moreover, it will be necessary to undertake direct genetic approaches to the identification of genetic modifiers to known deafness loci. We have embarked upon a programme to use mutagenesis approaches to identify modifiers of the myosin VIIA locus. These and other programmes in mouse genetics will help us to realise our ultimate goal of charting the pathways of neuroepithelial development and function.

IS THE MICROTIA GRADE AN INDICATOR OF EXTERNAL AND MIDDLE EAR DEVELOPMENT?

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Microtia is a congenital malformation characterized by total or partial absence of the whole auricle or any of its components; it is caused by genetic disorders, chromosomal defects, intrauterine infections or environmental teratogens.

Microtia may be bilateral and often associated with other congenital malformations.

The purpose of our study is to determine wheter external and eventual middle and inner ear defects in microtia are related, in order to give useful prognostic and therapeutic informations.

We studied thirty children, nineteen males and eleven females; eleven patients were younger than seven months. Classification criteria of auricular deformities were those described by Weerda; we found thirty-two percent first degree microtia, thirty-nine percent second degree microtia and twenty-nine percent third degree microtia.

In unilateral cases right side was more frequently affected; in eleven cases microtia was bilateral; in ten case the auricle anomaly was associated with other congenital malformations.

All patients, mostly affected by conductive hearing loss, were evaluated by high-resolution CT; sixteen patients, aged between twelve days and four years, were examined with "spiral" CT. We found stenosis of the external auditory canal only in patients with first or second-degree microtia; conversely, atresia of the external auditory canal was present in second-degree microtia only and in all cases of third-degree microtia. Among the anomalies of the middle ear, the most frequent were reduction of the tympanic cavity and dysplasia of the malleo-incudal joint; the frequence of these anomalies was directly proportional to the degree of microtia. Inner ear malformation were diagnosed less frequently, without evident correlation with microtia degree.

Our results have demonstated a high correlation between degree of microtia and frequence of external and middle ear dysplasias, probably because of a common embryological origin.

We perform CT in children with microtia even from the neonatal period, in order to define early ear malformation and to give to the clinician useful informations not only for planning the treatment, but also for dealing with the child and his parents.

Spiral CT is a feasible method for rapidly evaluating the temporal bone in pediatric patients; it allows a reduction of motion artifacts and time of sedation. Additionally, we can reduce x-ray exposure using low-dose techniques and image reconstructions.

A CONCRETE EXPERIENCE IN THE FIELD OF EVALUATION AND ASSESSMENT AT THE DIAGNOSTIC CENTRE OF THE LEGA DEL FILO DORO

Data from three years study of deaf people with complicating disorders:

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The Lega del Filo d'Oro, Osimo, Italy, is an association whose aims are the assistance, rehabilitation and, wherever possible, social integration of deafblind and multi-sensory impaired children and adults in their natural, family environment.

There is a rehabilitation centre, which includes a diagnostic centre where global interdisciplinary and multidisciplinary assessments are carried out. Professionals in the medical, social, educational and psychological fields are involved in making these assessments so that a complete picture is obtained of a person's needs and capabilities.

From 1996 to 1998, 195 people had their first contact with the diagnostic centre, 124 males and 69 females with a minimum age of 11 months and a maximum of 76. They came from 20 regions of Italy. 80 of these people had hearing loss in both ears, 47 of them men and 33 women. The minimum age of the males was 4 years and the maximum 76. For the females the minimum was 11 months and the maximum 70.

On the basis of clinical documentation and diagnosis of our 80 cases, we have produced the following summary of the findings relating to the causes of hearing loss:

1. Rubella
2. Usher's syndrome
3. Premature birth
4. Encephalitis
5. Down's syndrome
6. Post-traumatic
7. Meningitis
8. Charge
9. Kearns- Seyre
10. Citomegalovirus
11. Marquardt-Loriaux
12. Unspecified cause

Of the above mentioned 80 people 24 were totally deaf and blind, 19 deaf with motor problems, mental retardation, or epilepsy, 37 multi-sensory impairment with additional deficits.

The objective of the hearing assessment is to find out if there is any residual hearing and if so, how the person uses it in daily life. 32 people used verbal language, sign language and/or fingerspelling and 48 non-verbal communication. The latter included natural gestures, pictograms, and objects of reference.

BRAIN AND INNER EAR MAGNETIC RESONANCE OF PATIENTS WITH USHER SYNDROME

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Usher syndrome is a genetic disease consisting of congenital sensorineural hearing impairment and retinitis pigmentosa of variable onset and severity.

Previous studies using magnetic resonance (MR) have shown some patients to have focal and diffuse atrophic changes in the supratentorial brain and cerebellum.

We report some preliminary data of a study that will collect about 100 patients. We performed brain and inner ear MR exams before and after contrast media i.v. administration in 15 patients affected by Usher syndrome (10 females, 5 males - aged 5 to 71 years). We found parenchimal supra- and infratentorial hyperintensities only in one patient aged 71 years, probably referred to malacotic ischemic lesions. The pericerebellar subarachnoid spaces and the fronto - parietal sulci were larger than normal by age in three patients. In all patients cochlea and labyrinth were normal in morphology and signal intensity, with no pathological contrast enhancement. The acoustic and facial nerves were always visible and normal.

AUDITORY ABERRATIONS IN ARNSHL CARRIERS

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With the aim of examining the possibility of detecting asymptomatic carriers of mutant genes causing deafness from their audiometric profiles, a detailed audiometric investigation protocol was carried out in 24 obligate carriers and 12 possible carriers from 12 families in whom ARNSHL was ascertained. The following audiometric tests were carried out: pure-tone audiometry, Békésy and audioscan sweep audiometry tests, acoustic reflex thresholds, auditory brainstem responses and otoacoustic emissions.

In the obligate carrier group, abnormalities were found on all tests. The audioscan test emerged as the most sensitive test. It was abnormal in 54.2% of the obligate carriers. However, its specificity was poor, because of the high incidence of abnormalities in control subjects (43.3%). The sensitivity of the Békésy test was found to be very low (12.5% compared with 3.3% in age-matched controls). Of the remaining tests, the incidence of abnormalities on any single test did not exceed 25% (n=6) in the obligate carrier group, and 25% (n=3) in the possible carriers.

Ten of the obligate carriers emerged as entirely normal audiotically, 9 had abnormalities on a single test and 5 had abnormalities on two tests. Half (6/12) of the possible carriers had no auditory abnormalities, 4 had an abnormality on a single test, and 2 had an abnormality on two tests. The findings were concordant in parent pairs from 4 families, but discordant in 5. In each of 4 of the families, a common test abnormality was found to segregate in at least one of the parent carriers and one unaffected child. Of the various audiometric tests, OAE deficits emerged as most consistent with the carrier state in families in whom abnormalities were identified: they were abnormal in 5/7 obligate carriers and 3/3 possible carriers of these families, and in 3/5 hearing relatives of one extended family.

The implications of these findings will be discussed in relation to the following: a) identifying distinct phenotypic sub-types in some families with ARNSHL; b) differentiating ADNSHL from ARNSHL and c) the contribution of these findings to the emergence of a standardised investigation protocol.

CLINICAL ANALYSIS OF RECESSIVE NONSYNDROMIC HEARING LOSS CAUSED BY MUTATIONS IN THE CONNEXIN 26 GENE (GJB2).

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The frequency of childhood deafness is estimated at 1/1000. At least half of this hearing loss is genetic and about half of the genetic hearing loss is recessive nonsyndromic. Our goal has been to identify the genetic cause, the relative numbers, and the clinical manifestation of nonsyndromic hearing loss. More than 20 mutations in the connexin 26 (Cx26) gene (GJB2) are associated with DFNB1, a type of autosomal recessive nonsyndromic neurosensory deafness. This form of deafness causes 20% of all childhood deafness and may have a carrier rate as high as 2.8%. Of 68 families with non-syndromic hearing loss, 26 families were either homozygous or compound heterozygous for connexin 26 mutations. One mutation 35delG is responsible for approximately 75-80% of mutations at this gene. The audiologic phenotype observed in individuals who are homozygous or compound heterozygous for Cx26 mutations varied from mild-moderate to profound hearing loss. Some individuals had asymmetric hearing loss. Ten individuals had a progressive hearing loss. These results suggest that other genes or environmental factors may significantly modify the effect of mutations in Cx26. The high occurrence of this type of hearing loss argues for a routine early screening for the 35delG mutation in patients with nonsyndromic recessive hearing loss. (Supported by National Institutes of Health NIDCD grants P01 DC01813-05 and R01 DC02942-02)

PHENOTYPER. PHENOTYPER SOFTWARE FOR NON SYNDROMIC HEARING LOSS CORRELATION GENOTYPE/PHENOTYPE. Study on Connexin 26.

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Phenotype: is the physical manifestation of a set of Genotype sets; specifically, a phenotype is the result of the action(and interaction) of a set of Genotype(s)-(allele pairs). A single Phenotype may be manifested as a variety of different Genotype (s).

This software analyses non syndromic hearing loss.

Phenotype in non syndromic hearing loss.

1. Preface. This program intended to help: Phenotype may currently be characterized by the following pathway
2. Origin
3. Disclaimer

PHENOTYPER DESIGN

1. General information
 - 1.1. Overview of Phenotype Designer
 - 1.2. Software and hardware requirements
 - 1.3. Excel experience
 - 1.4. About Phenotype Designer
2. How to use Phenotype Designer
 - 2.1. Input from the Phenotyper software
 - 2,1.2 An example of a Phenotyper file
 - 2.2. Creating and exporting a table in Phenotyper
 - 2.2.1 Read new Phenotyper file button
 - 2.2.2 Inspect Phenotyper file button
 - 2.3 Initialize new Phenotyper file button
 - 2.3. 2.Menu workset
 - 2.4. Analyze workset

1.1 Overview of Phenotype Designer

The Hereditary Hearing loss Homepage (Van Camp and Smith,1998) provides updated information on the gene locations and the mutations of these genes.However, by its nature it cannot provide all the information necessary for geneticists and audiologists to determine the pattern of genotypes already described.

At the present time, the question of the relationship between different mutations of a particular gene and the resulting phenotype remains unclear, and the program Phenotyper it's the scientific contribution of this work.

- The first problem of the Phenotype software is the the variety of different Genotype(s) in genetic hearing loss.
- The second problem is the the inability of the pc program output to produce genotype/phenotype correlation.
- A third problems involves the checking of Mendelian inheritance of the different phenotypes.

To resolve all these problemes, we developed new software for data analysis, the Phenotyper Designer software This module replace incomplete procedures in Genotyper, and offer new possibilities for automated data analysis.

IDENTIFICATION OF THE CAUSAL GENE (*COCH*) FOR AUTOSOMAL DOMINANT NON-SYNDROMIC HEARING LOSS AND VESTIBULAR DEFECTS (DFNA9) AND A NEW LOCUS FOR MID-FREQUENCY HEARING LOSS AT 6P21 (DFNA21)

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We studied Dutch families with autosomal dominant non-syndromal progressive sensorineural hearing loss. In family W98-011, patients experience a late-onset high frequency hearing loss and vestibular impairment. We mapped the underlying gene defect by linkage analysis to a 11 cM region overlapping the DFNA9 interval on chromosome 14q12-q13. Clinically, the Dutch family differs from the original DFNA9 family by a later age at onset and a more clearly established vestibular impairment. A gene that is highly and specifically expressed in the human fetal cochlea and vestibule, *COCH* (previously described as *Coch5B2*), maps in the DFNA9 critical region. Sequence analysis of the 12 exons revealed a 208C>T mutation in exon 4 of the *COCH* gene resulting in a Pro51Ser substitution in the predicted protein in all affected individuals of the family but not in unaffected family members and 200 control individuals. The same mutation was also identified in three apparently unrelated families with a similar phenotype suggesting the presence of a Dutch founder mutation. The function of *COCH* is unknown but several characteristics of the protein point to a structural role in the extracellular matrix.

In family W97-056 the age at onset varies and most patients show an impairment in the midfrequencies. Linkage analysis excluded the DFNA1-15 and DFB1-19 loci. A subsequent whole genome scan employing 150 DNA markers revealed linkage telomeric to the DFNA13 locus at 6p21, which recently was repositioned farther centromeric, between the markers D6S1666 and D6S1285 (R. Smith, personal communication). Assuming complete penetrance in this family, the new locus (tentatively designated DFNA21) resides between D6S260 and D6S299. The maximal lod score using marker D6S1663 was 4.4. One affected individual did not carry the haplotype that was consistently encountered in affected family members, suggesting the presence of a phenocopy. Further haplotype analysis is underway to refine the critical region.

In a small 3-generation family (W97-061), the transmission of the gene defect was compatible with X-linked or autosomal dominant inheritance. In general, males showed a more severe hearing loss. We excluded the entire X-chromosome and the DFNA1-3 and DFNA5-15 loci for linkage and found cosegregation of the gene defect with the DFNA4 locus at 19q13. The maximal lod score is 2.41 for the markers APOC2 and D19S416.

This research was supported by the Netherlands Organization for Scientific Research (NWO), grant 901-04-205.

EPIDEMIOLOGY OF HEARING IMPAIRMENT IN EUROPE

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The epidemiology of hearing impairment is an essential component to both service planning and research strategy and priority. Without good information, both at a global and also at a local level decisions are often made using prejudice, out of date notions or inapplicable data. The descriptive epidemiology of hearing impairment in Europe is slowly evolving.

We know a fair amount about the prevalence of adult acquired hearing impairment in certain countries eg UK, Italy. However, considerably less is known about the incidence of hearing impairment and deafness, and the natural history of these conditions is fairly notional. We assume that there are only a few people who have sudden onset hearing impairment in their 40s and 50s and that most people's hearing deteriorates slowly over a decades. There are not many clues as to the exogenous factors associated with change over time, and whilst some dominant genes have been associated with late-onset deafness there is as yet no idea of how extensive their impact might be in the general population. Factors associated with hearing impairment *per se* rather than rate of change do not account for much variability once age and noise immission have been controlled. Thus it may make some sense to ask whether the variability within these factors may be attributable to a genetic ageing or noise susceptibility factor. The genetic epidemiology is evolving, but population studies in this area have a long gestation period. I know of 2-3 studies world-wide who are either studying this or at the point of setting up a study.

More work has been done on the descriptive epidemiology of childhood hearing impairment, especially the permanent types of deafness as opposed to glue ear. The work that has been done in the UK, Denmark, Estonia and other countries in Europe suggests that there are indeed substantial differences between the prevalences of hearing impairment and deafness in the different countries. Also the risk factors are different in the different countries. Our group has been looking at the specific aetiologies of the children who have been ascertained in UK studies. There seems to be a difference between the cases reported from clinic series and the population samples that we have ascertained in terms of the specific genetic background to the population.

The work of the sub-group highlight the fact that a greater awareness is needed of the epidemiology of hearing impairment, and that standard definitions ought to be used in all future studies, especially prospective studies. The priorities for future research in the human genetic epidemiology of deafness will be discussed with emphasis given to mid- and long-term research goals as a basis for more general discussion.

PREVALENCE OF OTOSCLEROSIS IN A NON-SELECTED SERIES OF TEMPORAL BONES

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Otosclerosis affects the bone homeostasis of the labyrinth resulting in abnormal resorption and redeposition of bone. The prevalence of histological otosclerosis has been studied by various authors on laboratory's collections. However, the present study is the first based on a non-selected series of temporal bones. During a one-year period, 118 consecutive pairs of temporal bones of deceased patients at a general hospital were collected to determine the prevalence of otosclerosis. Although histology remains the golden standard for evaluation of otosclerosis, the combined use of temporal bone macroslices and microradiography is proposed to detect otosclerotic lesions in a more convenient and less time consuming way. The temporal bones which were suspect of having otosclerosis with these techniques, were further analysed by conventional histology. 2.5 % of the 236 temporal bones (or 3.4 % of patients) studied demonstrated histological otosclerosis.

The outcome of this prevalence study will be compared with earlier reports that were carried out on laboratory's collections. These studies were likely biased by the presence of hearing loss or other otological diseases. The present results will also be correlated with the clinical observations in today's practice.

CLINICAL FEATURES OF THE PREVALENT FORM OF CHILDHOOD DEAFNESS, DFNB1, DUE TO A CONNEXIN26 GENE DEFECT: IMPLICATIONS FOR GENETIC COUNSELLING

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DFNB1, an autosomal recessive form of deafness due to connexin26 gene (*Cx26*) mutations, has been recently shown to be one of the most frequent hereditary defects in humans. To date, no clinical characterisation of the DFNB1 inner ear defects has been reported, which prevents the provision of prognostic information and proper genetic counselling.

We enrolled 140 children from 104 families affected by sensorineural deafness with various degrees of hearing loss in a prospective study. The children either belonged to a family affected by autosomal recessive deafness (DFNB family) or represented sporadic cases. They were examined for mutations in the 5' non coding exon and in the coding region of *Cx26*. Audiometric and radiological features were investigated and compared in deaf children with and without *Cx26* mutations.

Cx26 mutations were present in 43 (49%) of the 88 prelingually affected families *versus* none of the 16 families with postlingual forms of deafness ($p < 0.01$). The inner ear defects of 54 prelingually deaf children carrying biallelic *Cx26* mutations were compared with that of 57 prelingually deaf children without *Cx26* mutations. DFNB1 deafness was found to vary from mild to profound, associated with sloping or flat audiometric curves and a radiologically normal inner ear. The hearing loss was not progressive in 69% of the cases. Frequent variations in the severity of deafness between sibs were observed.

The characteristic audiometric and radiological features of DFNB1 described here should be the reference used to guide the order of *Cx26* molecular diagnostic tests for deaf children with a compatible phenotype. Prognostic information can now be given to families: the hearing loss of the DFNB1 form of deafness is non progressive in most cases, at least up to young adulthood. A major element for genetic counselling is that the severity of the hearing loss due to DFNB1 is extremely variable and cannot be predicted, even within families.

AUDIOLOGICAL EVALUATION OF PATIENTS WITH DELETION 22Q11

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Deletion 22q11 (del22) is one of the most frequent causes of genetic syndromes. The majority of the cases of DiGeorge and Velo-cardio-facial syndromes are due to del22. These conditions are considered to be developmentally related to neural crest anomalies influencing the differentiation of the branchial arches, including the precursor tissue of the ear. In addition, the UFD1L gene, an ubiquitination gene being expressed during embryogenesis in the inner ear primordia, has been identified in the 22q11 critical region. The aim of the study was to evaluate the prevalence of hearing loss in del22 syndrome. Audiometric evaluation was performed in 27 children observed at our hospital from 1997 to 1998. Results were related to clinical history, frequency of upper respiratory tract infections and immune status. Sensorineural deafness was found in 4/27 (15%) patients, conductive deafness in 12/27 (45%), and normal hearing in 11/27 (40%). Interestingly, 3 of the patients with sensorineural deafness had cerebral lesions due to neonatal distress, to hydrocephalus, and to post-surgical ischemia each in one. The prevalence of speech delay, upper respiratory tract infections and low CD3 was higher among patients with conductive deafness, although the difference was not statistically significant. In conclusion, hearing loss was documented in 60% of the analyzed patients, and must be included among major clinical features of del22. Audiological evaluation is recommended in patients with del 22, in order to reduce the risk of speech deficit.

BRANCHIO-OTO-RENAL DYSPLASIA AND LOWER LIMB HYPERTROPHY: A CASUAL FINDING?

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BOR Dysplasia (MIM # 113650) is an autosomal dominant disorder with variable clinical manifestation affecting branchial, renal and auditory development. Recently a genomic locus for BOR syndrome at chromosome region 8q13.3 was identified. The human homologue (EYA 1) of the *Drosophila* eyes absent gene, has been found mutated in all kindreds with BOR syndrome studied to date. Mutations of the same gene have also been found in other two families with branchio-otic anomalies (BO dysplasia or BO syndrome).

We report 3 additional cases: mother and two siblings (a male and a female).

The probandus was the *mother* who presented right preauricular pit, and omolateral cervical fistula. She had mixed hearing loss (more severe on the right) associated with a bilateral Mondini-type cochlear malformation. She had a triplet at the 32nd week of gestation as a result of assisted fecondation.

The first born, a female, had none of clinical manifestations of BOR dysplasia and normal Auditory Brainstem Responses (ABR). Actually (5,5 years old) she presents an adequate growth and regular acquired developmental milestones.

The second one, a male (weight 1,810 g, length 40 cm, OFC 31 cm), had bilateral ear structural abnormalities (more severe on the left) with left Hemifacial Microsomia. The right ear duct was absent; there was only a pit in the inner canthal border. ABR suggested bilateral mixed hearing loss. The CT scan showed bilateral hypoplasia of both cochlea and lateral semicircular canal, and bilateral dysplasia of stapes, incus and malleus.

Renal ultrasonography showed a mild left hydronephrosis. Furthermore he presented macrodactyly of 3rd, 4th, 5th digits and cutaneous syndactyly between 3rd, 4th digits of the left hand and *hypertrophy of the lower left limb*. (So far the latter anomaly has not been described in BOR dysplasia).

Actually he presents adequate growth and mild speech delay, in spite of hearing aid performed since one year old. The karyotype was also performed on fibroblastic cultures obtained from the hypertrophic limb.

The last born, a female (weight 1,000g, length 40 cm, OFC 27 cm) had cup-shaped ear with preauricular pit, tragal tag and discharging cervical fistula on the right. ABR suggested mild mixed hearing loss. Renal ultrasonography was normal. The CT scan showed bilateral hypoplasia of the cochlea and lateral and posterior semicircular canals and vestibular dilatation. Actually she is 18 kg of weight and 110 cm of length.

DNA analysis regarding all those patients are in progress at the W.J.Kimberling laboratory (Omaha – NE, USA).

The association of the gigantism with BOR dysplasia is discussed.

A COMPARISON OF EAR MALFORMATIONS IN GENETIC AND EXOGENETIC SYNDROMIC PATIENTS.

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Ear malformations could be isolated or combined with other deformities of the skull, face and upper vertebral column. The reason for these syndromic deformities could be a genetic defect, a teratogenic insult or unknown factors. The present study was undertaken to compare syndromic patients with ear malformations to get a better understanding of conceivable background factors.

All syndromic patients who underwent rehabilitation for ear malformations between 1970 and 1998 were investigated. The patients underwent clinical and roentgenological examinations, audiological investigations, and surgical exploration. The degree of ear malformation was determined as were the details of the other structures of the temporal bone. Hereditary and exogenic background factor were also ruled out, and pedigrees calculated.

There were 32 patients with hemifacial microsomia, 29 with mandibulofacial dysostosis and seven with thalidomide-induced ear malformations. Fifteen out of 55 patients could be shown to have a genetic cause for the malformation and seven could be proven to be caused by a teratogen. Malformations of the external ear, ear canal, middle ear, zygoma, maxilla, mandible, and lower eye lid were prominent features of all syndromes. Facial nerve and 6th cranial nerve paralysis were only seen in patients with hemifacial microsomia and in thalidomide-induced syndrome, indicating that an exogenic factor could be responsible for a disturbed cranial nerve function.

Comparing the deformities with an animal model in which the malformations depends on disturbed migration of neural crest cells during early embryogenesis, the critical time for a similar process in humans would be between the 20th and 29th days of pregnancy.

It is concluded that disturbed neural crest cell migration could be one factor of importance for ear malformations in syndromic patients.

The present study was supported by grants from the Magnus Bergvall Foundation, the O.E and Edla Johansson Scientific Foundation, the Swedish Dental Association, Håselhandikappades Riksförbund, the Sven Jerring Foundation, the Håsel Scientific Foundation, the Swedish Medical Research Council, project number B93-17X-10397-01A, the Samariten Foundation, and the First of May Flower Foundation.

RECESSIVE HEARING IMPAIRMENT IN THREE BIRTH COHORTS IN WESTERN SICILY -

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This study is an epidemiological investigation of congenital hearing impairment in western Sicily. Three birth cohorts of patients were examined at the Department of Audiology, University of Palermo, Italy. The first included 1293 subjects born between 1975-1979, the second cohort included 1276 subjects born between 1985-1989, and the third cohort included 1225 subjects born between 1990-1994. All the subjects suffered from a permanent hearing impairment defined as >40 dB HL in the better hearing ear. The prevalence of the inherited hearing impairment was studied in the three cohorts. In all the cohorts, inheritance was the most common cause of congenital hearing impairment and transmission was recessive. In addition, among the subjects diagnosed with recessive hearing impairment the proportion of those who had consanguineous parents was calculated. A comparison between recessive hearing impairment / consanguinity rate in the three birth cohorts indicated significant differences.

HIGH RESOLUTION AUDIOLOGICAL DESCRIPTORS OF THE USHER SYNDROME CARRIERS

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Objective :The scope of the study was the definition of methodologies which can be used in the “audiological marking” of Usher syndrome carriers.

Methods: The target population were 11 Italian Usher families from which 14 Usher cases and 11 carrier cases were tested. Two methodological approaches were followed: (I) Audioscan measurements in the 1.0 – 8.0 kHz range; and (II) Recordings of $2F_1 - F_2$ distortion product otoacoustic emissions (DPOAEs) with two asymmetrical protocols ($L_1= 75 / L_2= 65$ dB pe SPL ; $L_1= 60 / L_2 = 50$ dB pe SPL ; frequency ratio of both protocols = 1.22) which are known to identify more accurately hearing impairments. The collected data were compared by parametric and non-parametric statistics with normative data from established audioscan and DPOAE databases. For the former a test range of 2.0 – 5.0 kHz (resolution 0.1 kHz) was used while for the latter DPOAE signal to noise ratios (S/N) at the 1.5-5.0 kHz interval were employed.

Results : (I) Significant differences at a $p= 0.001$ were found between the hearing threshold of the normal and the Usher carrier population at the audiometric frequencies 2.0, 2.5, 3.0 – 4.0 , 4.5 and 5.0 kHz. These differences though maybe biased by the small sample size of the carriers group and the differences of age which contribute to the threshold elevation. The carriers group presented an audiometrical profile at the 3.0 – 4.0 range in which a threshold elevation of 10 – 15 dB (in relationship to audioscan nearby frequencies) were present. This pattern was not observable in the corresponding data from the normative database. (II) no significant differences were found in the comparison of the DPOAE data from the 75 / 65 dB SPL protocol, but significant differences at a $p= 0.01$ were found at the DPOAE frequencies 3.0, 4.0 and 5.0 kHz of the 60 / 50 dB SPL protocol. Since the 2.0 – 3.0 adult DPOAE frequencies are known to coincide with standing wave interference, all $2F_1 - F_2$ points were also validated in terms of their corresponding phase delays. By the latter criteria no $2F_1 - F_2$ point was found to be an artifact. The correlation between the auditory threshold and the DPOAE S/N ratios at the common audioscan and DPOAE frequencies, where threshold alteration in the carriers group were observed, was found to be low (range: 35 – 42 %). It is plausible that this estimate is influenced by the fact that the DPOAE protocols used few frequency points per octave.

Conclusions: Low threshold elevations (10 – 15 dB) in the 3.0 – 4.0 kHz audioscan zone and S/N alterations in the 3.0 – 5.0 kHz DPOAE frequencies may represent an audiometric marker for the Usher carriers. In terms of clinical predictive power both methodologies need to be used so that one methodology validates the other. It is important though to define with precision the possible biasing effects caused by the variation of the age factor of the Usher carriers group

CONNEXIN 26 GENE MUTATION SCREENING IN FAMILIAR AND SPORADIC CASES OF NON-SYNDROMAL, CONGENITAL SENSORINEURAL DEAFNESS

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Studies have revealed that ~50% of all cases of familial recessive deafness and ~10% of all sporadic cases are caused by mutations in the Cx26 gene. To date, 21 different mutations have been identified in the coding sequence of the CX26 gene, including conserved amino acid substitutions, base transversions and small deletions/insertions introducing frameshifts causing premature peptide truncation. However, it has been shown that a single common deletion mutation, 35delG, predominates and accounts for the majority (~70%) of Cx26 mutant alleles. We have continued to screen both familial (n=49) and sporadic cases (n=22) of non-syndromal, congenital sensorineural deafness for the common 35delG mutation. In addition, we have also mutation screened the entire coding sequence of the CX26 gene in individuals heterozygous for 35delG mutation who are thus presumed to be compound heterozygotes. These studies have identified a further 3 novel mutations. However, in a significant proportion of these individuals, DNA sequence analysis of the entire coding region of the second Cx26 allele has failed to reveal any mutations. Therefore, we have performed additional mutation screening of exon 1 and promoter sequences extending 1.5kb upstream of the transcription start site. We have identified 4 sequence variants within this promoter region of the CX26 gene - the functional significance of these sequence variations is currently under investigation.

A FKH10 ^{-/-} MUTANT MOUSE – AS A TOOL TO DISECT MORPHOGENETIC EVENTS IN INNER EAR DEVELOPMENT.

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At day 9.5 post coitum (p.c.) *fkh10* is exclusively expressed in the otic vesicle. *Fkh10* is a member of the forkhead family of winged helix transcriptional regulators. Histological examination of *Fkh10*^{-/-} mice reveals a severely malformed inner ear. The bony compartment, in which the inner ear resides, has a very different shape as compared with wild type animals. The entire cochlea and vestibulum are missing in *Fkh10*^{-/-} mice – the inner ear is replaced by a large irregular and communicating cavity. These findings are consistent and typical for the eleven *Fkh10*^{-/-} mutants that we have examined while normal inner ear structures were found in the four examined *Fkh10*^{+/-} mice. These structural malformations are in agreement with the behaviour tests, in which all *Fkh10*^{-/-} mice failed to elicit a Preyer reflex and displayed a pathological reaching response – as signs of cochlear and vestibular dysfunction. These findings bear a striking resemblance with what has been reported for the kreisler mouse, with the exception that we have not seen, in *Fkh10*^{-/-} mice, any inner ear structures protruding into the cranium as is commonly the case in kreisler mutants. The kreisler gene (*kr*) and the homeo box A1 gene (*Hoxa1*) are not only necessary for proper development of the inner ear, kreisler mutants also lack rhombomere 5 (r5) and part of r6 and *Hoxa1*^{-/-} mice have markedly reduced r4 and r5 whereas *Hmx3*^{-/-} mice show an isolated defect in formation of the vestibular apparatus. In a hypothetical pathway, regulating otic vesicle development, this would place *Fkh10* downstream of *kr* and *Hoxa1* and upstream of *Hmx3*.

THE WINGED HELIX TRANSCRIPTION FACTOR FKH10 IS REQUIRED FOR NORMAL DEVELOPMENT OF THE INNER EAR

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Fkh10 is a member of the forkhead family of winged helix transcriptional regulators. Forkhead genes are instrumental during embryogenesis in mammals, in particular during development of the nervous system¹⁻⁵. Here we report that mice with a targeted disruption of the Fkh10 locus exhibit circling behaviour, pathological swim test and abnormal reaching response - all common findings in mice with vestibular dysfunction⁶. These animals also fail to elicit a Preyer reflex, in response to a suprathreshold auditory stimulation, as seen in mice with profound hearing impairment^{7,8}. Histological examination of the inner ear reveals a gross structural malformation of the vestibular part of the inner ear as well as the cochlea. These structures have been replaced by a single irregular cavity in which neither proper semicircular ducts nor cochlea can be identified. We also show that at day 9.5 post coitum (p.c.) Fkh10 is exclusively expressed in the otic vesicle. These findings, implicate Fkh10 as an early regulator necessary for development of both cochlea and vestibulum and identify its human homologue FKHL10 as a previously unknown candidate deafness gene at 5q34.

EDUCATIONAL OUTCOME AND EMPLOYMENT OF FINNS WITH PRELINGUAL GENETIC VS. NON-GENETIC HEARING IMPAIRMENT — A 15-YEAR FOLLOW-UP

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According to the very rare outcome studies available, prelingual hearing impairment (HI) has a strong negative effect on educational outcome and employment status in adulthood. This study aimed to investigate the late outcome of genetic vs. non-genetic HIs. The study sample consisted of 51 hearing impaired children born in 1965–1979 and followed up at the Oulu University Hospital. The hearing impairments were graded according to the recent EU proposal. The data on these 7 mildly, 17 moderately, 13 severely and 14 profoundly hearing impaired children were collected retrospectively from the patient files. All subjects included in this study had a sensorineural, non-syndromal prelingual hearing impairment with no known associated handicaps. The aetiology was prenatal genetic in 31 % (16/40), prenatal non-genetic (e.g. rubella) in 16 % (8/40), perinatal in 8 % (4/40) and postnatal (e.g. meningitis) in 4 % (2/40), while in 41 % of the cases (21/51) the aetiology remained unknown. 21 of the 51 children belonged to a larger group of children whose hearing ability, risk factors and ascertainment of HI had already been studied extensively earlier. These 21 subjects represented well the larger sample (N= 253) with regard to e.g. aetiology, age at ascertainment, grade of the hearing impairment, main communication mode and school setting.

Speech samples of the 51 children with careful follow-up were recorded when they were aged approximately 9 years (picture naming task; however, the age at the recordings was lower for the moderately hearing impaired children than the others). The intelligibility of the speech of each subject was assessed as the mean percentage of single words correctly identified by groups of students, with 5 listeners in each. Information on hearing, rehabilitation, educational setting, main communication mode and language development was collected from the hospital records.

A mailed questionnaire was used to obtain information on the primary stage educational setting, school achievement, upper secondary and tertiary education, employment history and the present employment status, the current main communication mode and social environment. At the time of the inquiry the subjects were young adults in their mid-twenties. The response rate was as high as 78 % (40/51).

The genetic HIs were more often less severe (median BEHL_{0.5–4 kHz}= 49 dB) compared to the pre-, peri- and postnatal non-genetic (median BEHL_{0.5–4 kHz}= 80 dB) and unknown aetiologies (median BEHL_{0.5–4 kHz}= 81 dB). A hearing impairment was ascertained and a hearing aid fitted at a median age of 4.5 years in the genetic, 3.0 years in the non-genetic and 2.10 years in the unknown aetiology group. All children with a mild to moderate HI communicated with speech at the time of the speech recordings as opposed to the children with severe and profound HIs, who used either mainly speech (33 %), speech and signs (22 %) or Finnish sign language (44 %). Regardless of the aetiology, there was no significant difference in the intelligibility of speech. Generally speaking, the more severe the HI, the less intelligible was the child. Ten of the 12 children with genetic HI had completed their comprehensive school education mainstreamed and 2 in the school for the HI. The figures for the entire sample were in keeping with these results.

The highest educational qualification was the 9-year compulsory comprehensive school for altogether 12/40 (30 %) of the respondents and the upper secondary education for 26/40 (65 %). Only 2 of the 40 (5 %) had qualified from a polytechnic and none from a university. The subjects with genetic aetiology did not differ from the others in their educational qualifications. On an average, those with severe or profound HI qualified at a lower level than the others. Compared with the nation-wide statistics on age peers, the figures for no education after the compulsory level (i.e. comprehensive school) was twofold among the hearing impaired subjects. Every fourth of them had had no occupational training, while 75 % had qualified for some occupation (8 young adults even for two or three). At the present, 40 % of the respondents belonging to the labour force were unemployed as opposed to 17 % of all the 25 to 29-year-olds in Finland. Eight of the 40 were still studying and 2 housewives were at home with the children. The subjects with genetic HI did not differ from the others by employment status.

To sum up, our subjects, who were followed up for 15 years, did not qualify educationally as high as their hearing peers, but unexpectedly so many of them, nevertheless, had acquired some formal occupational education. Despite this, the unemployment rate was more than double among the hearing impaired compared to the population aged 25 to 29 years in Finland. According to this study on non-syndromic prelingual hearing impairment, it is the grade rather than the aetiology of the HI that affects the late outcome.

TECHNICAL KNOCKOUT, A DROSOPHILA MODEL OF HUMAN MITOCHONDRIAL DEAFNESS

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Many deafness-associated mitochondrial DNA mutations affect components of the mitochondrial translation system. Understanding the developmental defects and pathogenic processes associated with these mutations is hampered by the lack of an animal model, in part caused by the difficulties in manipulating metazoan mtDNA. In order to circumvent this, we have been studying a *Drosophila* mutant (*tko* or *technical knockout*) that has a defect in a key (nuclear-coded) component of the mitochondrial translational apparatus, and which shows a phenotype strongly reminiscent of sensorineural deafness in humans. The mutant fly is bang-sensitive, i.e. suffers temporary paralysis in response to mechanical vibration. Other labs have traced this phenotype to a failure of signaling from mechanoreceptor cells, which may parallel aspects of deafness in humans. The *tko* mutant fly also shows pronounced developmental delay. We have used P-element transgenesis to demonstrate that a point mutation in a conserved leucine residue of the protein encoded by the *tko* gene (mitoribosomal protein S12) is responsible for the mutant phenotype described. Modeling in bacteria suggests that the mutation impairs the assembly of the protein into functional ribosomes. Further exploitation of this model, using the range of molecular genetic techniques available in *Drosophila*, will enable us to establish the precise developmental consequences of defective mitochondrial protein synthesis, and understand better the pathogenic processes associated with mtDNA mutations in humans. The system also offers great promise for modeling other types of mitochondrial translational defect.

MOLECULAR PHENOTYPES OF DEAFNESS-ASSOCIATED MITOCHONDRIAL TRNA MUTATIONS

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Many different point mutations in mitochondrial tRNA genes are associated with syndromic disorders in which sensorineural deafness is a cardinal feature. These include the np 3243 mutation in tRNA-leu(UUR), plus two mutations (np 7445 and np 7472) in tRNA-ser(UCN). We have studied the biochemical and molecular genetic effects of these mutations in model systems in cell culture, mainly using *rho-zero* cybrid cells. Both of the deafness-associated tRNA-ser(UCN) mutations affect the steady-state levels of the tRNA, in one case acting certainly at the level of RNA processing. Aminoacylation does not seem to be specifically impaired, and effects on mitochondrial translation are minimal. However, in combination with a slightly reduced mtDNA copy number (which of itself produces no phenotype), homoplasmy for the np 7472 mutation does result in modestly impaired growth on a substrate imposing a requirement for mitochondrial respiration and a small drop in the activity of fully assembled complex I. The np 3243 mutation blocks tRNA-leu(UUR) aminoacylation almost completely, but mitochondrial protein synthesis is grossly impaired only when the mutation is present at very high relative levels (>98%). In cybrids with high levels of mutant, the balance of differently modified isoforms of the other leucyl-tRNA is altered compared with control cells, suggesting that one of these isoforms may be able to compensate, at least partially, for the missing tRNA. This is further supported by the identification of a heteroplasmic anticodon mutation in tRNA-leu(CUN), that restores np 3243 mutant cells to a normal or almost normal respiratory phenotype, in at least two nuclear backgrounds. By contrast, over-expression of two nuclear genes for the mitochondrial translation system, the mitochondrial elongation factor EF-Tu, and a homologue of the yeast mitochondrial leucyl-tRNA synthetase, are both unable to complement the effects of the np 3243 mutation in cell culture. Our studies have also shed light on the mechanisms of mtDNA segregation in cells heteroplasmic for disease-associated mutations. Surprisingly, heteroplasmy can be stably maintained for long periods, but this stability can also be abruptly abrogated, by an as yet unidentified stimulus, leading to rapid mitotic segregation. Understanding these phenomena has potentially great importance for explaining the tissue-specificity and progression of mtDNA disease.

X-LINKED DEAFNESS TYPE 3 (DFN3): THE INS AND OUTS OF *POU3F4*

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X-linked deafness type 3 (DFN3) is the most frequent X-linked form of deafness. Male patients have a fixed stapes and show a cerebrospinal fluid/perilymph gusher upon stapedectomy. Most patients suffer from sensorineural and conductive deafness. Computed tomography scanning reveals temporal bone defects, i.e. an enlargement of the inner auditory canal and/or an abnormal wide communication between the inner auditory canal and the cochlea.

The gene involved in DFN3 encodes a POU domain transcription factor, *POU3F4*. Mutation analysis in 33 unrelated DFN3 patients has revealed 29 mutations which can be grouped in five classes. i) The first class of mutations affect the *POU3F4* directly. Thus far, 2 *POU3F4* spanning deletions, 7 protein truncating mutations, and 9 missense mutations were identified. One missense mutation is situated in the POU-specific domain; 8 are conspicuously clustered in the POU homeodomain, in particular in the $\alpha 3$ -helix. Possibly, missense mutations outside the POU domain result in a different (dominant) phenotype or are not compatible with life. ii). The second class of mutations consists of 8 deletions overlapping a 10 kb genomic segment situated 900 kb proximal to the *POU3F4* gene. iii). One DFN3 associated deletion spans both the *POU4F4* gene as well the frequently deleted proximal region and measures 5 megabases. iv). A fourth type of mutation is a 150 kb duplication/3 Mb paracentric inversion dislocating the *POU3F4* gene away from the proximal DNA element. v). Finally, in one patient we observed a 800 kb deletion residing between the *POU3F4* gene and the proximal DNA element. Most likely, as yet unidentified regulatory elements are located in the deleted region. Thus far, no mutations were found in 4 patients with the typical features of DFN3.

"Zoo-blot" analysis showed that at least 4 DNA elements in the proximal DNA region, spread over 50 kb, are evolutionary conserved. We sequenced the commonly deleted 10 kb region and a 3 kb homologous region of mouse DNA and found 80% nucleotide sequence similarity over a region of at least 2 kb. Extensive cDNA library screening, reverse-transcription PCR analysis as well as the search for protein motifs did not reveal a hint for the presence of a transcribed sequence. Most likely, the proximal DNA element contains a 'locus control region'. If so, the distance between the presumed regulatory DNA element and the *POU3F4* gene is the largest known in mammals.

This study was supported by the Netherlands Organisation for Scientific Research (NWO), grant 901-01-173.

THE GERMAN REGISTRY FOR HEARING LOSS IN CHILDREN: ASSOCIATED ANOMALIES

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In 1995 the German Registry for Hearing Loss in Children started collecting medical data of children with permanent hearing loss. Until January 1999, more than 2500 children have been registered throughout Germany. At the moment, 83 centres are providing about 100 additional cases per month. Information about probable causes of hearing loss, associated anomalies, and a possible connection with a syndrome is part of the questionnaire used for data collection.

In 178 of the 2515 patients (7 %), the reporting centre diagnosed or presumed a syndromic disease. From 353 patients (14 %), craniofacial anomalies have been reported, thereof 43 cases with cleft lip and/or palate, 52 cases of stenosis or atresia of the outer ear canal (22 unilateral).

Methods

2515 records in the data base have been evaluated using ACCESS 2.0 and EXCEL 5.0 tools. Fields containing free text information have been exported and evaluated manually. The results have been compared with data from the literature.

Results

In 7 % of the patients a syndrome was diagnosed or presumed. In 353 patients (14 %), craniofacial anomalies have been reported, Altogether, in 652 cases (25.9 %) associated anomalies or diseases were diagnosed. The most frequent syndromes are trisomy 21, Waardenburg syndrome, mucopolysaccharidoses, Usher syndrome, Goldenhar syndrome, CHARGE association, Turner syndrome, BOR, Treacher-Collins (Franceschetti) syndrome, Pendred syndrome (table 1). The most common craniofacial anomalies are abnormalities of the pinnae, stenosis or atresia of the outer ear canal, cleft lip and/or palate. Anomalies outside the craniofacial area have been reported most frequently as cardiac (58 patients) or renal anomalies (19 patients), abnormalities of hands and/or feet (25 patients).

In 148 of the 652 patients with associated anomalies (22,7 %) the hearing loss is probably acquired. The most common of the identified causes are peri- and postnatal complications (42 patients), meningitis (27 patients), prenatal rubella (20 patients) and prenatal CMV infection (12 patients).

The probable causes of hearing loss in all patients and in patients with associated anomalies are shown in table 2a and table 2b.

Table 1: The most Frequent Syndromes in the German Registry for Hearing loss in Children

Syndrome	No of Cases (+ suspected)
trisomy 21	21 (+1)
Waardenburg syndrome	8 (+1)
Usher syndrome	7 (+1)
Mucopolysaccharidosis	7
Goldenhar syndrome	6 (+1)
CHARGE association	5 (+1)
Treacher-Collins (Franceschetti) syndrome	4
Branchio-oto-renal syndrome (BOR)	3
Turner syndrome	3
Pendred syndrome	2 (+4)

Table 2a: Probable Cause of Hearing Loss
(n = 2515 records)

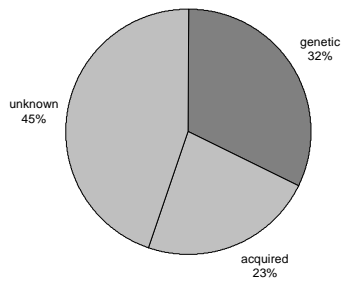


Table 2b: Probable Cause of Hearing Loss
(652 records of patients with associated anomalies)



Discussion

The frequency of associated anomalies is compatible with data from other sources [1]. Most of the syndromes in table 1 are also listed in: "Ten Syndromes Most Commonly Associated with Hearing Loss" published by the Research Registry for Hereditary Hearing Loss via WWW [2].

Literature

- [1] Gorlin RJ, Toriello HV, Cohen MM jr: Hereditary Hearing Loss and its Syndromes. New York Oxford 1995, S. 9
- [2] Research Registry for Hereditary Hearing Loss: Ten Syndromes Most Commonly Associated with Hearing Loss. [http:// www.boystown.org/deafgene.reg/tensyn.htm](http://www.boystown.org/deafgene.reg/tensyn.htm)

A COMPREHENSIVE GENOME-WIDE SCREENING, CLINICAL CHARACTERIZATION AND GENETIC MAPPING OF A SECOND FORM OF BRANCHIO-OTO-RENAL SYNDROME (BOR2).

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Autosomal Dominant branchial anomalies associated with hearing loss and renal anomalies affect at least 2% of profoundly deaf children and have estimated prevalence of 1 in 40,000. The clinical features of Branchio-oto-renal syndrome (BOR) consist of external, middle and inner ear malformations, branchial cleft sinuses, cervical fistulas, mixed hearing loss and renal anomalies. The phenotypic expression of the branchial arch as well as audiologic and renal development can be quite variable, even within the same family.

The first BOR gene has been localized to chromosome 8q13. Recently, EYA1 gene, the human homologue of *Drosophila eyes absent gene*, was identified by positional cloning and mutations have been reported. In this study, we present results of mutation analysis conducted on more than fifty-five BOR families by heteroduplex followed by sequence analysis of sixteen EYA1 exons. Using this approach, we have identified twelve novel mutations. At least 60% of our families, investigated so far, have not shown mutations in the EYA1 gene. It is not clear whether the inability to detect the mutation is due to locus heterogeneity or mutations lie in the non-coding region of the EYA1 gene. The complex clinical symptoms as well as our genetic data suggest that more than one gene is involved in the development of branchiogenic disorders. Therefore, in the present study, we have also performed genetic linkage analysis on multigenerational BOR type families with 8q markers. Our current results indicate that three large BOR type families did not show linkage with 8q markers suggesting the involvement of more than one gene. A genome-wide search is in progress to identify the second genetic locus involved in branchio-otic type syndrome.

The present results, together with mutation screening and genetic linkage study, demonstrate a genetic heterogeneity. Also, our results show that a variety of EYA1 mutations can produce a continuum of disease, ranging from mild to severe clinical phenotype within the same family. Further characterization of EYA1 mutation and identification of other BOR genes will significantly help in defining the spectrum of defects associated with branchial and hearing anomalies.

(Supported by NIH/NIDCD grant #P01 DC01813)

CLINICAL FEATURES OF DFNA14

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A Dutch kindred was studied with low-frequency sensorineural hearing impairment linked to a new locus on chromosome 4p16 (DFNA14). Of the affected persons, 21 (aged 11-75 years) were examined and the most recent audiogram was used for cross-sectional analysis of hearing threshold in relation to age. Suitable serial audiograms were available for a longitudinal analysis in 9 cases; they had been obtained from the age of 6 years onwards and covered a follow-up period from 14 to 36 years. The presumably congenital (offset) component of SNHI was extrapolated or estimated from average values and offset thresholds were found of about 45 dB at 0.25-1 kHz, 25 dB at 2 kHz and 10 dB at 4-8 kHz.

Significant progression in hearing impairment occurred at all frequencies, but could be attributed to presbycusis. The combination of congenital, stationary low-frequency SNHI and presbycusis resulted in an up-sloping audiogram in the first 5 decades of life, which evolved into a flat-type audiogram in the 6th or 7th decade and a down-sloping audiogram at a more advanced age. With few exceptions, vestibular function was intact.

PHENOTYPE OF HEARING IMPAIRMENT LINKED TO DFNA13

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We examined 21 living members of a Dutch family (150 relatives in 5 generations) with autosomal dominant non-syndromic sensorineural hearing impairment (SNHI). They showed linkage to the DFNA13 locus and, with few exceptions, SNHI to a certain degree. Most of the affected persons dated their first hearing impairment symptoms to the 2nd-3rd decade of life, but two affected persons had hearing impairment symptoms from early childhood onwards and one of these had previously been judged to have a delay in language development. Most of the threshold levels differed significantly between the frequencies within each and, at the high frequencies (2-8 kHz), between the two age groups covered by generations IV and III (ages 30-38 y and 58-74 y, respectively); the latter could be attributed to presbycusis.

Correcting for age, the worst or second-worst threshold was usually found at 1-2 and 6 kHz (40 dB) or 8 kHz (50 dB), while the best or second-best threshold in the affected persons was usually found at 0.25-0.5 and 4 kHz (30 dB). Presbycusis presumably from the 4th decade of life onwards caused a change of the typically shaped audiogram ("mid-frequency SNHI with additional high-frequency SNHI") into a downsloping type. Various caloric abnormalities, including bilateral areflexia in one case, were found in 47% of the cases with SNHI.

TRANSGENE EXPRESSION IN THE GUINEA PIG COCHLEA MEDIATED BY THE LENTIVIRUS DERIVED GENE TRANSFER VECTOR

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Studies investigating gene delivery in the cochlea have focused their efforts on viral vectors including adeno associated virus (AAV), Herpes simplex virus (HSV), and adenovirus. These virus derived gene transfer vectors utilize the natural infectivity of the virus to introduce and express exogenous genes inserted within the viral genome. However, no single available viral vector is suitable for the diverse gene therapy applications as each possess characteristics that may not be useful in a particular experimental or therapeutic setting. Moreover, the viral vectors that have been assessed for their utility in cochlear gene transfer are beset with their own drawbacks. These include unstable expression of the transgene, direct cytotoxicity, host-induced immune response as well as relatively non-specific or non-selective transduction of the cell types within the target organ.

In view of these shortcomings associated with the conventional viral vectors, lentivirus, a retroviral vector system based on the human immunodeficiency virus (HIV) was assessed as a gene delivery agent in the cochlea. The lentivirus is capable of integrating into the genome of dividing cells as well as non-proliferating, post-mitotic cells. The replication-defective, lentivirus-derived viral vector has demonstrated efficient *in vivo* gene transfer into terminally differentiated neurons of rat brain without any observable toxicity. Thus, the post-mitotic cochlear neuroepithelia and the spiral ganglion neurons represent suitable targets for lentivirus mediated gene transfer.

The utility of lentivirus as a gene delivery vector in the cochlea was evaluated *in vitro* and *in vivo* through expression analysis of a reporter gene, green fluorescent protein (GFP). Utilizing cochlear explants from neonatal rats, the lentiviral vector transfected spiral ganglion neurons (SGN) and glial cells *in vitro* without associated cytotoxicity. *In vivo* characterization of lentivirus-GFP was assessed by directly infusing the vector into the scala tympani of the guinea pig cochlea via an osmotic minipump. Sections of lentivirus-infused cochlea revealed highly restricted fluorescence pattern limited to the periphery of the perilymphatic space of scala vestibuli and scala tympani; SGN and cells within the endolymphatic space (scala media) including the auditory neurosensory epithelia did not demonstrate transgene expression. The cellular and tissue architecture of the lentivirus infused cochlea was intact and free of inflammation. Transduction of SGN and glial cells by lentivirus *in vitro* but not *in vivo* suggests limited dissemination of the viral vector from the perilymphatic space where it was infused. Restricted transduction of cells within the perilymphatic space by the lentivirus *in vivo* is ideal for secretion of gene products within the perilymph for therapeutic benefit.

OTOLOGICAL MALFORMATIONS IN GOLDENHAR SYNDROME: SURGICAL THERAPY.

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ENT Clinic.University of Siena Chairman: Prof.D.Passäi

The Goldenhar Syndrome is an oculo-auriculo-vertebral dysplasia related to an abnormal development of the first and second branchial arches; this disease is clinically characterized by orbital anomalies, fusion of cervical vertebrae, maxillary hypoplasia and malformations of the external and middle ear.

The genetic disorder of this syndrome is transferred as an autosomal dominant trait. Otological malformations are atresia of external auditory canal, anotia, preauricle cartilaginous ectopiae and anomalies of ossicular chain (conductive hearing loss!).

The clinical case reported by Authors (A.A.) is a child, 8 years old, male, with a hearing loss of the left ear.

The E.N.T. examination: normal otoscopic findings; audiometric tests show conductive hearing loss on the left side, absence of acoustic reflex and C tympanogram.

Radiological study (X rays of the cervical rachis and CT of temporal bones) showed the anomalies of the first and second vertebrae and a fibrous substance wrapping up the ossicular chain; no alterations were found of the hands and feet x-ray

The patient underwent surgery for a sounding tympanotomy under total anaesthesia: stapedotomy with laying piston prosthesis was performed.

Post-surgical follow-up showed an improvement of audiometric tests

In conclusion, the AA advise surgical treatment of the middle ear anomalies in Goldenhar Syndrome, especially if these are on both sides, to improve the hearing and to allow a regular speech and psychological development.

EPIDEMIOLOGY OF MODERATE TO PROFOUND CHILDHOOD HEARING IMPAIRMENTS IN CHILDREN BORN IN NORTHERN FINLAND IN 1983-1992

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The epidemiology of childhood hearing impairments was studied in a clinical series in Northern Finland. Children with permanent moderate to profound hearing impairments ($BEHL_{0.5-4\text{ kHz}} \geq 40\text{ dB}$) resident in the area served by the Oulu University Hospital and born in 1983-92 comprised the study population. Altogether 112 children, 65 boys (58.0 %) and 47 girls (42.0 %), fulfilled the inclusion criteria, and their patient files were retrospectively reviewed. Including all types of impairments, the prevalence was 1.1/1000 live births. The type of impairment was sensorineural in 87.5 % of the subjects, conductive in 6.3 %, mixed in 3.5 % and unknown in 2.7 %. Three aetiological groups were formed, i.e. genetic (Group I), non-genetic (Group II) and unknown (Group III). Group I included 51 children (45.6 %), Group II 19 children (16.9 %) and Group III 42 children (37.5 %). The median $BEHL_{0.5-4\text{ kHz}}$ was 62.5 dB in Group I, 63.8 dB in Group II and 58.8 dB in Group III, and the median ages of ascertainment of the hearing impairment were 2.1 years, 2.5 years and 4.1 years, respectively. The clinical significance of the results and prospective studies will be discussed.

ETIOLOGIC STUDY OF HEARING LOSS IN CHILDREN BY MEANS OF PERSONAL AND FAMILY HISTORY.

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When a diagnosis of neurosensorial hearing impairment is made in a child, doctors and parents are placed in face of searching an aetiology for the problem, deriving in many speculations.

Many of such cases have a genetic origin, but due to the absence of an easy test to apply daily in the clinic for these patients in order to identify it we have to Manage with personal and family history to achieve an aetiology.

We have made a retrospective study of the clinical history in 91 children diagnosed for a profound and deep hearing loss during the last two decades in our centre. if we consider a possible genetic aetiology in those cases where no risk indicator could be assessed, in accordance with CODEPEH (Spanish commission for early detection of hearing loss) or rather is the family history of hearing loss or syndromes associated with hearing loss, then a genetic origin is predominant over the acquired aetiology (65.9%). we dont observe significative statistical differences in this distribution over time between cases diagnosed before and after 1990.

This pilot study suggests that in our community the genetic aetiology is predominant in the cases of early hearing impairment, therefore when another cause cannot be identified, a genetic study is advised.

A NOVEL MISSENSE MUTATION ILE59ASN OF THE PAX3 IN A FAMILY WITH WAARDENBURG SYNDROME TYPE 1.

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Waardenburg syndrome type 1 (WS1) is an autosomal dominant auditory-facial- pigmentary disorder, with dystopia canthorum, pigmentary disturbances of eyes, hair and skin, and sensorineural hearing loss. We describe here one novel paired domain mutation in the PAX3 gene observed in one Waardenburg syndrome type 1(WSI) family. Most consistently manifested anomalies in three generations of this family are dystopia canthorum, heterochromia iridum, congenital bilateral and unilateral deafness, and depigmented skin areas.

We studied 8 unrelated families with clinical features of WSI. We presumed to search mutations of human PAX3 gene in WSI patients by means of PCR and single stranded conformational polymorphism (SSCP) analyses. All affected and normal family members were screened for changes in exon 1 through 8 of the PAX3 gene.

The SSCP analysis of the exon 2 PCR product revealed a variant band in one proband. The primers used for amplification of this exon was following: upstream - 5' tgt cga gca gtt tca gcg 3' and downstream - 5' cag tct ggg agc cag gag 3'. This PCR product consisted of 409 b.p. which included the intron- exon boundaries. The sequence analysis by Sanger's method confirmed a T-to-A transition at position 176 (ATC(TTC), which led to an Ile59 to Asn substitution at codon 59 in paired domain of PAX3 and was designated as I59N. This single- base substitution abolishes original MboI recognition site. Since two MboI sites exist in the exon 2 PCR product, the enzyme cuts the products corresponding to the wild-type and mutant alleles into two and three fragments, respectively. Heterozygotes for mutant alleles have additional uncut 304 b.p. fragment. By means of restriction analysis we have found this mutation in our proband's grandfather. Unfortunately, proband's father, uncle and cousin were not available for this analysis. To confirm the pathogenical role of this substitution we estimated the presence of this mutation in other probands and in group of healthy persons. We did not find this mutation in 119 healthy persons (238 chromosomes). H. Soejima et al.(1997) described the A-to-T transversion in 175 position from the 59 codon caused in Ile to Phe substitution. In this respect it should be pointed out that two different substitution exist in the same codon and both mutations determined the WSI development. These results will allow us to make a conclusion that 59 codon in the PAX3 is very important for normal function of this protein.

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VESTIBULAR FUNCTION AND DEAFNESS, RESULTS FROM THE EUROPEAN WORK GROUP ON GENETICS IMPAIRMENT

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It is common among deaf to be “clumsy”, which is often treated as a trait of deafness and caused by the hearing loss itself. Relatively few studies in hereditary hearing loss have been conducted to examine the vestibular function. One of the subgroups in the EU consortium is the study group on vestibular involvement and to provide better clinical diagnosis in hereditary hearing disorders. The aim is to form a base for standardised questions and tests. A computerised vestibular test protocol produced by the European Work group on genetics of hearing impairment will be presented. The test protocol comprises questions concerning childhood motor milestones, vestibular symptoms etc. Balance assessment including ENT, posture tests, bithermal and ice-water calorics, rotatory testing with video-oculography etc.

The test protocol has been applied to different hearing disorders and syndromes. Non-syndromatic hearing loss: Thirty-two subjects with severe hearing loss or profound deafness was evaluated. Fifty per cent of all subjects displayed a walking age later than 18 months. In calorics, 35% displayed bilateral vestibular areflexia. All the subjects who displayed absent vestibular responses in the caloric and rotatory tests reported a walking age later than 18 months. Usher syndrome: Type 1 (250 affected) showed bilateral absent vestibular function. Type 2 (200) had normal vestibular function bilaterally. In Type 3 (20), the vestibular function was found to be parallel to the progressive hearing loss with a gradual decrease of the vestibular function.

Refsum syndrome: Five affected displayed various vestibular function. Two affected had normal vestibular function and three cases showed hypofunction. None had bilateral areflexia.

Alström syndrome: Six affected with blindness, diabetes, progressive sensorineural hearing loss displayed progressive vestibular loss as well. The loss of the vestibular function followed the progression of the

hearing loss. From a genetic perspective, vestibular tests can help discriminating between different non-syndromatic deafness with identical audiometric patterns. It is likely that a phenotype reflects an underlying defect which is gene specific. As we have demonstrated previously in Usher syndrome, it is already possible to use vestibular tests as discriminator since a normal vestibular function in a profoundly deaf child excludes most types of Usher syndrome type 1.

CONNEXIN 26: GENOTYPE/PHENOTYPE STUDIES

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DFNB1 was the first locus identified as being responsible for autosomal recessive non-syndromal sensorineural hearing impairment/deafness (ARNSSNHI/D) assigned to the proximal portion of the long arm of chromosome 13 (13q12) by autozygosity mapping. Studies of the segregation of polymorphic DNA microsatellites linked to the DFNB1 locus in families from Australia, New Zealand, Italy and Spain suggested that a gene at the DFNB1 locus was likely to have a role in the hearing impairment/deafness in the majority. Identification of connexin 26 (Cx26) as the gene at the DFNB1 locus allows mutation screening of individuals with NSSNHI/D.

We have screened DNA from 59 consanguineous families of Pakistani origin and from the United Arab Emirates, 72 Caucasian sib-pairs and 104 sporadically affected individuals with congenital or childhood-onset non-syndromal sensorineural deafness for mutations in the Cx26 gene. SSCP analysis and DNA sequencing revealed 4/59 (6.8%) of the consanguineous families of Pakistani origin and from the United Arab Emirates to have a mutation in exon 2 of the Cx26 gene. This included 1 family homozygous for the nonsense mutation 74G→A (W24X), 2 families homozygous for 231G→A (W77X) and 1 family homozygous for 35delG. 29/72 (40.3%) of the sib-pairs were heterozygous or homozygous for a mutation in Cx26, 13 homozygous 35delG/35delG, 14 heterozygous for 35delG/-, 1 compound heterozygote 35delG/310del14 and 1 heterozygous 310del14/-. 11/104 (10.6%) of the sporadically affected individuals were heterozygous or homozygous for a mutation, 5 homozygous 35delG/35delG, 6 heterozygous 35delG/-. One of the sib-pairs and one of the sporadically affected individuals heterozygous for 35delG and two further sporadically affected individuals had a 10 bp deletion in the promoter region of the gene.

We analysed the Pure Tone Audiograms of these individuals classifying the hearing impairment by the shape of the audiogram and determined the average hearing impairment in each ear for comparison between individuals and for symmetry. Serial audiograms, where available were analysed for evidence of progression of the hearing impairment

Individuals with profound hearing impairment were more likely to have an identifiable mutation in the Cx26 gene (χ^2 test, 3 df, $P = 0.001$). While there was no significant differences in the shape of the audiogram or symmetry of hearing impairment between individuals with and without an identifiable mutation in the Cx26, the recurrence of severity of the hearing impairment in affected siblings was more likely to be of similar severity with an identifiable mutation in the Cx26 gene (Students t test, two-tailed, $P = 0.02$). Follow-up of 11 individuals aged 3-17 years with hearing impairment homozygous for the 35delG mutation for periods from 1-10 years showed a change or hearing impairment which averaged 7 dB ranging from 0-29 dB.

(Supported by funding from The European Community, The Hearing Research Trust, The Wellcome Trust and St James's University Hospital Trustees).

MOLECULAR AND CLINICAL EVALUATION OF TWO FAMILIES SEGREGATING THE CX26 MUTANT M34T.

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Mutations in the Connexin 26 gene have been found to be the cause of congenital autosomal recessive, nonsyndromic, neurosensory deafness associated to the DFNB1 locus on chromosome 13q12. A dominant form of deafness, DFNA3, has been mapped to the same region of chromosome 13. This second form of deafness has also been attributed, by some authors, to a mutation of the Cx26 gene, a C →T transition at nucleotide 101 which leads to a methionine to threonine substitution at codon 34 (M34T). The same mutation has been reported to not segregate with the hearing impairment in other families and has been considered as a possible asymptomatic polymorphism.

We present the molecular and audiological evaluation of two families segregating the M34T mutant Cx26. In one family the hearing impairment is found in several members and could be transmitted as autosomal dominant; M34T is the only Cx26 mutation found in the affected individual so far tested. In the second family the hearing impairment is sporadic and the mutation definitely seem to be transmitted as a putative recessive allele. We are currently extending the molecular analysis to other members of the two families in order to identify other carriers and study the possible effects of this mutation on the cochlear function.

CX26 DEAFNESS: MUTATION ANALYSIS AND CLINICAL EVALUATION

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Alterations of the Cx26 gene account for many cases of congenital sensorineural hearing impairment. The reported prevalence is 34%-50% in autosomal recessive cases, 10% and 37% in sporadic cases. The hearing impairment in these patients has been described as severe or profound. We have studied 76 unrelated subjects, affected by congenital non-syndromic sensorineural hearing impairment, in order to evaluate the prevalence and type of Cx26 mutations and establish better genotype-phenotype correlation. Mutations in the Cx26 gene have been found in 53% of the individuals tested: 35.3% of the autosomal recessive and 60% of the sporadic cases of our series. Three new mutations have been identified.

The hearing deficit varies from mild to profound even in 35delG homozygotes within the same family. Mutations of the gene coding for the Gap-junction protein Connexin 26 are likely to account for at least 60% of all the cases of congenital nonsyndromic neurosensory deafness. It seems appropriate to extend the molecular analysis even to individuals with mild or moderate preverbal hearing impairment of unknown cause.

IS THE PRESBYACUSIS REALLY A SOCIACUSIS - A TWIN STUDY

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Hypothesis: Though a multifactorial pathogenesis of presbycusis is accepted, the sociacusis is considered as main source today. We assume however that genetic factors are underestimated.

Background: By means of a twin study we are able to assess the influence of genetic and environmental effects on presbycusis.

Methods: 60 ears of 31 monozygotic (MZ) twin pairs (average age: 41 years, youngest: 17, oldest: 75) were examined and compared with 68 ears of 34 pairs (average age: 39 years, youngest: 22, oldest: 61) of a control group, which consisted of non-related persons of same age and sex, taken out of the group of MZ twins. The intra-pair-differences of hearing loss (HL) in the pure tone audiogram (PTA) and of DPOAE-amplitudes in the DP-gram for middle (>0.5 to 2 kHz) and high frequencies (>2 to 8 kHz) were calculated.

Results: We could find highly significant differences between the group of MZ twins and control group for middle and high frequencies of the PTA and DP-gram (Whitney-Mann U-test). That means PTA and DP-gram were more similar between the MZ twins than between non-related persons of same age and sex. Furthermore the correlations between intra-pair-differences of PTA and DP-gram and age at the group of MZ twins were established. No correlation for the middle PTA-frequencies, a very small correlation for the high PTA-frequencies and no correlation at all for the middle and high DP-gram-frequencies could be found.

Conclusion: Presbycusis is influenced strongly by genetic effects. The concept of sociacusis as most important reason for presbycusis has to be reconsidered.

Acknowledgement: This work was kindly supported by the *Deutsche Forschungsgemeinschaft* (DFG) (project Be 1676/2-2).

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PREVALENCE OF NON-MENDELIAN MITOCHONDRIAL INHERITANCE IN PEDIATRIC SENSORINEURAL HEARING IMPAIRMENT

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Objective: To assess the relative prevalence of mitochondrial DNA (mtDNA) alterations in a subgroup of pediatric cases of sensorineural hearing impairment.

Background: Hearing impairment affects 1 in 1000-2000 children and is of genetic origin in at least 50% of these cases. Up to 100 independently acting nuclear genes are involved in the disorder, of which around 30 have been mapped, but only a handful identified. Autosomal recessive transmission is frequent but in principle any mode of inheritance have been observed. Maternally-inherited mtDNA mutations play a role in both syndromic and non-syndromic adult-onset sensorineural hearing impairment. However, it is unknown the extent to which alterations in the mitochondrial genome affect the natural history of deafness in childhood.

Methods: Sixty consecutive children with idiopathic sensorineural hearing impairment came to our observation in the past year. Audiological examination included pure tone audiogram, impedance audiometry, and brain-stem evoked responses. Total leukocytes DNA was extracted and the region encompassing the mtDNA A3243G and A1555G mutations were PCR-amplified using *ad hoc* designed oligonucleotides. A semi-quantitative, high resolution restriction fragment length polymorphism analysis was employed to assess levels of mutated genomes.

Results: Out of 60 children, 56 showed clinical and audiological evidence of non-syndromic hearing impairment. Mode of inheritance was compatible with maternal transmission in 16 cases. No patients harbored detectable levels of the investigated mtDNA mutation, ruling out the most prevalent causes of mitochondrial hearing impairment.

Conclusions: In our experience, mtDNA mutations do not play a significant role in childhood onset of sensorineural hearing impairment. We cannot exclude that other, as yet under investigation, mtDNA changes are associated with a limited number of affected individuals. In the absence of means of prevention and treatment for most cases of hereditary hearing impairment, genetic counselling remains the most valuable course.

EPIDEMIOLOGY OF GENETIC HEARING IMPAIRMENT - OUTCOME OF THE EVALUATION PROTOCOL

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In order to reduce the proportion of children with unknown cause of their hearing impairment (HI) and thus obtain an estimate of genetic HI in Europe, an evaluation protocol was outlined as part of the EU-CA:HEAR.

Children born 1985-1989 with BEHL 0.5-4 KHz, ≥ 50 dB who had previously been diagnosed, were subjected to the protocol, which resulted in a minor insignificant reduction of unknown cause of the HI. This negative result should further encourage the clinicians to do systematic evaluations preferably in infants and young children. The insufficient collaboration from parents, who have accepted their child's HI, is an obstacle towards the aetiological evaluation. Appropriate information to the parents concerning the importance of an aetiological evaluation is crucial.

MOLECULAR ANALYSES OF THE CONNEXIN 26 GENE IN NON-SYNDROMIC SENSORINEURAL DEAFNESS

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Pre-lingual non-syndromic deafness affects one child in 1000 and 50% of cases have a genetic origin. Autosomal recessive non-syndromic sensorineural deafness NSRD is the commonest genetic form, present in about 80% of cases. Mutations have been identified in the connexin 26 (Cx26) gene at the *DFNB1* locus on chromosome 13 (13q11-12), among which the 35delG mutation has been described as the most common cause of NSRD. This mutation has been seen in 63% of chromosomes from affected patients in the Italian population (1) and in 69% of patients from a mixed population (2). Considering the high frequency of NSRD and the great heterogeneity of clinical forms, it is very important to attempt molecular diagnosis in affected families in order to establish the cause and offer correct counselling.

In the present study we analysed 38 patients from different families with NSRD. Molecular analysis was performed by PCR-amplifying Cx26 sequences from genomic DNA and direct sequencing of the PCR product, using partly manual and partly automatic (ABI 377) sequencing.

We saw Cx26 mutations in 8 patients. 4 were homozygous for the 35delG mutation; 2 were compound heterozygotes for 35delG and a second mutation (a C>A change producing the missense mutation Ala40Glu, and an insertion of 14 bp in position 510 of the cDNA in the other case); 2 subjects were probably compound heterozygotes with 1 35delG and a second mutation not yet identified. As controls we studied 110 normal subjects. The 35delG mutation was seen in 1/220 (0.45%) normal chromosomes and 12/76 (15.8%) chromosomes from subjects with NSRD. We found a lower frequency of the 35delG mutation than previously reported for the population of southern Italy (63% (1)), but similar to an American study (3), which reported a frequency of about 1% (2/192 chromosomes in the general population and 28.4% (33/116 chromosomes) in affected subjects).

Data obtained up to now on mutations in Connexin 26 confirm that the 35delG mutation is the most common in NSRD, but as the number of cases studied increases, other mutations are also seen that cause NSRD. Deeper molecular knowledge will be required in order to be able to use this test for genetic counselling.

MOLECULAR GENETIC ANALYSIS OF A FAMILY WITH MONOZYGOTIC TWINS WITH STAPES GUSHER SYNDROME

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DFN3 is the most frequent non syndromic X-linked hearing loss. Its characteristic clinical feature is a conductive hearing loss resulting from a stapes fixation. Through application of genetic linkage analysis and molecular mapping of extended deletions associated with DFN3 and other abnormalities, it was possible to map the responsible gene to chromosomal band Xq21. In 1995, de Kok et al. showed an association between x-linked mixed deafness and mutations in the POU Domain Gene POU3F4. Since then, point mutations and small deletions have been described in the POU3F4 gene in patients with x-linked hearing loss.

Presently we have identified male monozygotic twins with a phenotype corresponding DFN3 patients. Audiologically both brothers are showing a mixed hearing loss and radiologically a bulbous internal auditory meatus and a deficient bone at the fundus of the Internal auditory meatus. Monozygotic status has been confirmed through genotyping the family with 6 high polymorphic microsatellite markers. We are now testing the twins and their mother for known mutations in POU3F4 as well as DFN3-typical minideletions. POU specific exons have been amplified for SSCP and sequencing. Next southern blot hybridization will be performed with specific POU probes.

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MUTATIONS IN GJB2 IN ITALIAN AND SPANISH FAMILIES WITH DEAFNESS

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Recent progress in the genetics of deafness has shown that mutations in the GJB2 gene, located on human chromosome 13q11 (DFNB1 and DFNA3), are responsible for non-syndromic recessive as well as dominant forms of deafness. Mutations in GJB2 are also detected in 10-40% of sporadic cases of deafness. The most common mutation is the frameshift mutation 35delG, which is present in over 95% of the mutated alleles of the Mediterranean population. The carrier frequency for this mutation is approximately 1 in 35 in most of the Mediterranean countries studied so far, but a lower carrier frequency is detected in central and northern Europe. Several mutations have been detected more than once in patients from the same or different populations, suggesting either recurrence or a common origin. Mutations in the GJB2 gene are a major cause of both inherited and apparently sporadic congenital deafness. Determination of 35delG and other mutations in GJB2 should facilitate diagnosis and counselling for the most common genetic form of deafness.

We present here the spectrum of mutations in the GJB2 gene in a sample of over 300 unrelated subjects with deafness. Interestingly, the M34T mutation, first reported to be dominant and latter reported as a polymorphism, has been detected in one of the families with recessive deafness, as well as in one individual with a dominant form of deafness.

DISTAL SYMPHALANGISM AND CONDUCTIVE DEAFNESS: A FURTHER ENTITY?

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Conductive hearing loss and symphalangism are associated both in facioaudiosymphalangism (MIM*186500) and in Progressive symphalangism and conductive hearing loss (MIM*185800).

Both the disorders are autosomal dominant and generally involve proximal interphalangeal joints, but the first can usually be differentiated, because of its facial features, frequent hypoplasia of the terminal phalanges and nails, frequent involvement of other skeletal segments.

Here we report a family case that clearly resembles facioaudiosymphalangism, but with some remarkable differences.

The proband showed conductive hearing loss with ossicular dysplasia and stapes ankylosis, and some dysmorphological elements, as short palpebral fissures, hypoplastic alae nasi, pectus excavatum, several nevi and café au lait spots (<0.5cm). He suffered for bilateral distal symphalangism of the V finger and III toe.

Father only suffered for carpal tunnel syndrome and hand X ray showed carpal coalition. A mild carpal coalition was also observed in grandfather X-ray film.

Although the symphalangism was distal, both the facial and the audiological features of the proband were suggestive for facioaudiosymphalangism. The carpal coalition in father and grandfather may be fortuitous finding, but it's rather suggestive of minimal expressivity.

Whether the condition observed is facioaudiosymphalangism, with peculiar expression or a different entity cannot be established at present.

CLONING GENES FOR NON-SYNDROMIC HEARING LOSS

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Over 40 genes for non-syndromic hearing loss have been localized and considerable effort now is being expended to clone the relevant genes. This paper reviews the techniques involved and provides representative examples based on work performed at the Universities of Iowa and Antwerp.

Once fine mapping has been completed, a physical map is usually constructed. This valuable resource serves as a scaffold upon which to place sequenced-tagged sites (STSs), including both microsatellite repeat polymorphisms and expressed sequenced tags (ESTs). Both known genes and ESTs in the candidate interval must be studied and prioritized for further investigation. Particularly useful in prioritization is screening against a cochlear cDNA library. From reported ESTs, full-length cDNAs must be derived, typically using 3' and 5' RACE (rapid amplification of cDNA ends). Open reading frames (ORFs) then can be screened online to establish whether a given EST corresponds to a known sequence or gene family.

Using cDNA as a template, mutation screening can proceed in one of two ways. Directly, by cDNA screening if the gene is illegitimately transcribed in lymphoblastoid cell lines, or after the intronic and exonic boundaries of the genomic structure have been determined. Numerous techniques are used to achieve the latter goal, although simple sequencing with computer analysis is typically the fastest and easiest method.

Although the human genome project has generated enormous data, at this stage the necessity to identify novel ORFs in the candidate interval may still arise. Depending on the properties of the physical region, genes can be identified by cDNA selection, exon trapping, or direct sequencing. Each of these methods has its relative merits.

Candidate genes must be screened for disease-causing mutations. Heteroduplex analysis and/or single strand conformational polymorphism analysis, complemented by direct sequencing, can identify gene variants. Each nucleotide change must be studied to establish whether it represents a benign polymorphism or a disease-causing mutation. Critical factors to consider include the frequency of this change in the general population, its impact at the amino acid level, the importance of the involved amino acid in the protein, especially across species, and, ultimately, the impact of the change on transcription, translation and protein function.

BASIC MECHANISMS IN HEARING AND HEARING IMPAIRMENT

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Sounds enter the ear through the external ear canal, and are transmitted through the middle ear via the ossicles to the inner ear. There, the vibrational energy of sound is transduced into an electrical change in the sensory hair cells, which in turn trigger a neural response via synapses at their base. The neurons form the cochlear nerve, which transmits the action potentials to higher auditory centres in the brain. Abnormalities in any part of this system can lead to hearing impairment, but most cases of permanent hearing impairment are thought to be largely due to defects in the cochlea.

Malformations of the inner ear, resulting from very early developmental defects, can lead to hearing impairment, and these can often be detected by imaging techniques such as CT scanning. Abnormal development of sensory hair cells is another obvious cause of deafness, as is progressive loss of hearing due to hair cell degeneration. The recent identification of several dominantly-inherited mutations associated with progressive hearing loss of adult onset has emphasised the importance of the affected genes in the long-term maintenance of the inner ear. The stria vascularis on the lateral wall of the cochlear duct generates the high resting potential in the fluid bathing the tops of the hair cells, and is another site where abnormalities can lead to hearing impairment. However, recent descriptions of the identification of several genes involved in hereditary deafness, together with the localisation of the expression of the normal version of the gene in the cochlea, suggest that several other cell types lining the cochlear duct also may have critical roles in fluid homeostasis. Further genes expressed in tissues previously thought to be mainly responsible for structural support of the organ of Corti (such as fibrocytes of the spiral ligament or spiral limbus) have been found to be involved in hereditary deafness, again suggesting that these tissues may play a more important role than we had thought. The study of the genetics of deafness is really starting to throw light on the molecular basis of cochlear function, with some surprising findings.

EASY DETECTION OF THE CONNEXIN-26 30DELG MUTATION, THE MOST COMMON CAUSE OF NON-SYNDROMIC RECESSIVE DEAFNESS

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The connexin 26 gene has been shown to be a major cause of nonsyndromic recessive deafness. Although many mutations have been found, a single mutation is responsible for the majority of alleles in different populations. This mutation is a deletion of one G from six consecutive Gs between nucleotide positions +30 and +35 (30delG).

Screening for this mutation is usually performed by direct sequencing of PCR products, which is expensive and labour intensive, or by allele-specific PCR analysis. Allele-specific PCR analysis requires two PCR reactions, and has the risk of misdiagnosis due to nonamplification. We developed an easier and more reliable method, based on the creation of an artificial restriction site in the presence of the mutation by PCR-amplification using a modified primer. The resulting PCR fragment is subsequently digested with the restriction enzyme, and the fragments are analysed by gel electrophoresis. As our new screening method is simple and reliable in use, and detects a mutation that is responsible for a significant part of nonsyndromic deafness, it may find a widespread use in DNA diagnostics.

PREVALENCE OF PEDIATRIC HEARING IMPAIRMENT IN FERRARA COUNTY

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In Ferrara and surroundings, the large majority of children at pre-school and school age refer to audiological evaluation. At the Audiology Services of USL 31 of Ferrara, 230 children, 132 males and 98 females, born in the years from 1976 to 1984 and affected by sensorineural and/or conductive hearing loss were identified. Most of these patients were examined during the annual screening of hearing loss and the others were selected by pediatrician.

In the present study, the cases affected by sensorineural and/or conductive hearing loss, excluding those affected by concomitant middle ear acute pathology, were selected.

Age of diagnosis, presence of hearing aid, hearing loss type and etiology, threshold level, threshold profile of each patient were registered and analysed. Data and results are discussed. During this period, the prevalence of the hearing impaired children appeared to be constant. Few variations, especially regarding the percentage of hereditary hearing loss, can be noted over the years.

CONNEXIN 26 MUTATION IN HEREDITARY NON-SINDROMIC SENSORINEURAL DEAFNESS

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Severe hearing loss is the most prevalent inherited sensory disorder, affecting about 1 child every 2,000 births. Hearing losses may result from peripheral auditory defects occurring as a consequence of either middle ear or sensorineural abnormalities. Several numbers of mutant genes have been identified, being responsible both for syndromic and non-syndromic deafness.

The most frequent hereditary deafness (80%) is the non-syndromic sensorineural autosomal recessive deafness (DFNB) in which hearing impairment is not associated with any other abnormalities. Recently a gene of non-syndromic deafness was identified as gene of gap-junction protein or connexin 26 on locus 13q11. The 35delG mutation of connexin 26 is reported as the most common form of recessive non-syndromic deafness on Mediterranean Area (65-80) % .

We report the results in 60 children (40 m-20 f) affected by cryptogenetic pre-lingual hearing loss detected by audiological findings and DNA analysis of patients and their parents.

We observed the absence of 35delG mutation in 48/60 patients (80%) and in 12/60 patients (20%) presence of 35delG mutation. 9/60 patients (15%) resulted homozygous for the 35delG mutation and 3/60 patients (5%) heterozygous for the 35delG mutation .

The identification of 35delG mutation should provide a better understanding of the biology of normal and abnormal hearing, help for the basis for diagnosis and may facilitate development of strategies for treatment of common genetic form of deafness.

JERWELL AND LANGE NIELSEN SYNDROME IN NORWAY SHOWS HIGH REGIONAL PREVALENCE, CONSIDERABLE CLINICAL VARIATION AND MUTATION HETEROGENEITY IN THE KvLQT GENE

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Jerwell and Lange Nielsen syndrome (JLNS, MIM 220400) is a rare autosomal recessive condition with congenital deafness and long QT interval which was first reported from Norway (Jerwell and Lange-Nielsen, 1957). In an ongoing investigation of all Norwegian patients identified since 1957 we have obtained data on 23 patients from 16 unrelated families and been able to obtain DNA from 12 of them. We reported one non sense mutation in the KvLQT1 gene on 8/18 alleles from 9 un related patients and no mutations in the IsK gene in Norway (Tyson et al., 1997). All patients with that mutation shared the same haplotype and in all cases ancestors originated from one county, which also, historically, was reported to have the highest frequency of deaf-mutism (Uchermann, 1896). A founder effect for that mutation is likely, and the frequency of the mutation among healthy blood donors from that county is under investigation. The estimate based on identified patients indicate a prevalence of approx. 1:55,000 of JLNS in that county, considerably higher than the world prevalence.

Further studies have shown several intriguing findings:

In our present family material we identified one out of three distinct mutations in 9 families (Tyson et al., this meeting). The mutation(s) remain to be identified in three families. So far, we have identified a mutation in 18/24 alleles (75%).

Among the carriers of the mutation we identified, only one suffered from long QT and was independently ascertained with Romano-Ward syndrome. Carriers are probably only rarely affected and other, as yet unidentified, genetic or environmental factors may be critical for developing cardiac symptoms.

Remote relatives suffered from syncopal attacks and congenital deafness in some families but only in one case was it possible to demonstrate mutations and thus to confirm a previously undiagnosed JLNS. Local inbreeding in originally very small villages is assumed to explain this unusual observation in non-consanguineous families.

The patients ranged in ages from 4-83 years illustrating the wide clinical variation since the oldest patient had never been medically treated.

In conclusion: the identification of three different mutations in 18/24 KvLQT1 alleles (75%) now allows specific molecular tests for the Norwegian population in order to establish an early diagnosis in children with congenital deafness. This would also permit an estimate of the frequency of unrecognized JLNS among children with sudden infant death syndrome (SIDS).

(Supported by Medical Research Council of Great Britain, Defeating Deafness, Odd Fellow Medical Research Council and Forskningsfondet til studier af døvhed og tunghørhed)

THE INCIDENCE OF HEREDITARY SENSORINEURAL HEARING LOSS IN GREEK PAEDIATRIC POPULATION AND THE ROLE OF GENETIC COUNSELING

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Hearing loss in childhood is a serious condition, which if not diagnosed early can create problems concerning the child's adjustment to society. Hearing loss often coexists with other medical problems. It can be hereditary or acquired. Hereditary hearing loss involves syndromic and non-syndromic forms. The clinical and genetic evaluation of a child with hearing loss is indicated especially when other family members are also affected. The determination of the etiology is crucial. The genetics department of our hospital in collaboration with the E.N.T Dept. investigated 54 families with one or more affected members over a time period of one year. In 27 of these families (50% of total cases) hearing loss was found to be hereditary and to follow Mendelian inheritance. Of these, 16 (59.3%) followed autosomal dominant inheritance, while 11 (40.7%) followed autosomal recessive inheritance. There were patients in three generations in these families and genetic linkage analysis may be possible in the future. Syndromic hearing loss was diagnosed in 13 families (24 % of total cases) and in some there were more than one affected members. Fourteen patients (26% of total cases) had non-hereditary, non-syndromic hearing loss. In six of them the problem was induced by an ototoxic drug, one was due to a congenital infection, while in the rest seven the cause could not be defined. Clinical evaluation was accompanied by genetic counseling. Genetic counseling included obtaining a thorough family history, as well as medical and pregnancy

DIFFERENT MUTATIONS IN KVLQT1 PREDOMINATE IN THE LONG QT SYNDROMES OF JERVELL AND LANGE-NIELSEN AND ROMANO WARD.

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The autosomal dominant Romano-Ward (RWS) and the autosomal recessive Jervell and Lange-Nielsen (JLNS) syndromes are allelic conditions caused by mutation in either of the genes KVLQT1 and IsK, the two subunits of the slow component of the delayed rectifier current in the heart. Both syndromes are characterised by life-threatening cardiac arrhythmias and prolongation of the QT interval on electrocardiogram (ECG). Persons with JLNS also suffer from profound congenital sensorineural deafness. We report the characterisation of mutations in a group of families with JLNS and compare the type of mutation with those previously found in RWS. We identified novel mutations in KVLQT1, which are predominantly nonsense and frameshift mutations, and are predicted to result in a truncated protein. We now show that mutations in KVLQT1 which underlie JLNS differ from those that cause RWS. This may account for differences in symptoms in heterozygotes for the two conditions, explaining why carriers of missense mutations tend to suffer from RWS, whereas nonsense mutations only rarely give rise to symptoms except in the homozygous state.

ASSESSMENT OF VESTIBULAR FUNCTION IN CHILDREN WITH AN ESTABLISHED FAMILIAL SENSORINEURAL HEARING LOSS

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Most young people with vestibular impairment compensate for it, thereby not displaying the sensations of dizziness or unbalance. Under certain circumstances, however, decompensation may occur with a return of the symptoms. As prominent subjective features may be minimal, and as identification is important clinically for management, evidence of vestibular impairment should be actively sought by objective measures. Though the correlation between the severity of hearing loss and vestibular hypofunction is not precise, the presence of vestibular impairment in association with hearing loss has been important in classifying different phenotypes in some syndromes eg Ushers syndrome. Therefore, it is possible that identification of vestibular impairment could contribute to research of auditory phenotypes in other conditions and help in early identification of those at risk.

The aims of this study were to assess the presence of vestibular impairment in a cohort of children with familial hearing loss, to correlate subjective and objective evidence of vestibular impairment, and to assess its relationship, if any, with the degree of the hearing loss and the mode of inheritance.

The methodology included a cohort of children born between 1985-1989 with familial sensorineural hearing loss in a London District. The assessments were divided into four broad categories - clinical, etiological, audiometric and vestibular and all subjects had a genetic assessment by a Clinical Geneticist.

The results will be presented and discussed.

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NON-SYNDROMIC HEARING LOSS ASSOCIATED WITH ENLARGED VESTIBULAR AQUEDUCT IS CAUSED BY PDS MUTATIONS

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The enlarged vestibular aqueduct (EVA) is known as the most common anomaly in the inner ear. EVA has recently been of particular interest because this anomaly is associated with characteristic clinical features, including fluctuating and sometimes progressive sensorineural hearing loss as well as dysequilibrium symptoms. Recently, we mapped the locus for non-syndromic sensorineural hearing loss associated with EVA to the same chromosomal region, 7q31, as the Pendred syndrome locus (Abe et al. *Am J Med Genet*, 1999 in press). Pendred syndrome is an autosomal recessive disorder originally described as being associated with deafness and goiter. It has recently been demonstrated that mutations in PDS cause Pendred syndrome (Everett et al., 1997). It is therefore obvious to ask whether PDS mutations are present in non-syndromic hearing loss associated with EVA. We report here the results of PDS mutation screening in EVA families. We obtained DNA samples from 6 EVA families. Mutations in the 21 exons of PDS were analysed using exon by exon genomic sequencing. We also tested 192 unrelated people from the general populations in Japan and the United States as controls.

Seven mutations were found in the EVA families analyzed. One family was homozygous, three families were compound heterozygotes, and two families were heterozygous but with no other mutation detected. The present results indicate that mutations in PDS are also responsible for non-syndromic hearing loss associated with EVA. We therefore propose that PDS mutations can be responsible for a continuum of overlapping clinical conditions including non-syndromic hearing loss, EVA and Pendred syndrome.

THE COCH GENE: A FREQUENT CAUSE OF COCHLEOVESTIBULAR DYSFUNCTION?

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Among the loci for autosomal dominant hearing impairment, the DFNA9 locus on chromosome 14 is the only one involving vestibular problems in addition to non-syndromic deafness. Three mutations in the COCH gene, probably encoding an inner ear specific extracellular matrix protein, have recently been shown to be disease causing.

In this study, we have performed linkage analysis on an extended Belgian pedigree with hereditary sensorineural hearing loss associated with vestibular problems. We found close linkage between the disease and markers surrounding the DFNA9 locus. Mutation analysis of the COCH gene, responsible for DFNA9, revealed a missense mutation changing a Proline into a Serine (P51S). The same mutation was also found in two small Dutch families with similar vestibulocochlear symptoms. Interestingly, one individual from a consanguineous marriage between two patients is homozygous for the P51S mutation. This person differed from the other affected persons in that the age of onset of symptomatology in this individual was lower.

The four COCH mutations that have been found so far are all missense mutations and cluster within a limited portion of the COCH5B2 protein. This region is rich in cysteines and shows homology with the factor C domain in the Japanese horseshoe crab. Possibly, a different type of mutation, or a mutation in another domain, leads to a phenotype that is different from the one observed here.

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FAMILIAL COSEGREGATION OF PROGRESSIVE SENSORINEURAL HEARING LOSS AND OPTIC ATROPHY?

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We examined a Dutch family in which the features of progressive sensorineural hearing loss and optic atrophy seemed to cosegregate. By history, persons were known to be affected in 6 generations. Affected persons in the last 3 generations underwent general, ophthalmological, otorhinolaryngological, audiological, vestibular and ocular motor examinations. The pattern of inheritance is possibly autosomal dominant, but we are still awaiting more definitive diagnoses in some key cases. Possibly related familial disorders are only poorly documented. In our family, the optic atrophy comprised an interesting type of colour vision deficit. Hearing loss

showed progression, which lead to impairment symptoms during the third to fourth decades of life. Vestibular and ocular motor functions were normal.

GENETIC CONTROL OF INNER EAR DEVELOPMENT

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The commitment of the otocyst, i.e. inner ear anlage, into balance (pars superior) and hearing (pars inferior) portions of the inner ear is under the control of patterning genes. Many patterning genes (i.e. >30) expressed in the developing inner ear have been identified and their patterns of expression characterized. Models of cell fate specification in the otocyst have included intersecting patterns of gene expression in an attempt to explain regionalization of the sites for sensory receptor development. New information on the role of patterning genes is being acquired from the analysis of inner ear development of null mutation mice where the expression of single and/or multiple patterning genes have been knocked out. One good example of the consequences of loss of expression of a single patterning gene on inner ear development is the effect of a knockout of the paired box-containing gene, Pax 2, on cochlear development. The Pax 2 null mutant inner ear shows complete agenesis of both its cochlea and spiral ganglion contrasted to normal development of its vestibular receptors and associated Scarpa's ganglion. This is a clear example of the profound effect that a single patterning gene can have on the development of the hearing receptor. However, this is an unusual event, and in general there is usually some redundancy of gene action present during inner ear development as exemplified by the overlapping pattern of expression of two closely related homeobox-containing genes (i.e. Hmx2 and Hmx3) in the developing vestibule. The Hmx2 and Hmx3 genes are both expressed early in otic development. Hmx3 transcripts are present in the developing otic placode and it appears to be one of the earliest expressed patterning genes in the developing inner ear. However, a Hmx3 loss of function mutation did not result in the agenesis of the vestibule. The same was true of the vestibule in Hmx2 knockout mice, but with a more severe vestibular dysmorphogenesis phenotype. The complete lack of a vestibular labyrinth was only achieved once both the Hmx2 and Hmx3 genes were both knocked out. This result shows that there can be redundancy in genes that have similar patterns of regional expression. Because many of the patterning genes have been identified as transcriptional regulators, an area of great interest in the field of genetic control of inner ear development are the downstream targets of patterning genes.

(Supported by Shulsky Hearing Research Fund to TRV)

HEREDITARY OTOVESTIBULAR DYSFUNCTION AND MENIERE'S SYNDROME IN A LARGE BELGIAN FAMILY.

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In nearly all reports of autosomal dominant non-syndromic hereditary hearing impairment, no mention is made of accompanying vestibular problems in the patients. Here, a large Belgian family is presented with a non-syndromic otovestibular dysfunction, inherited in an autosomal dominant way. The impairment is characterized by peripheral degeneration of the inner ear, leading to total deafness and bilateral vestibular areflexia. Genetic analysis confirmed linkage to the DFNA9 locus and mutation identification in the COCH gene in 60 family members.

Clinical data were collected from the anamnesticly affected family members, their parents, siblings and sibs. An otoscopy and a pure tone audiometry was performed and blood samples were taken for DNA extraction. Analysis of the anamnestic, clinical and genetic data revealed a progressive perceptible hereditary hearing impairment starting between the third and sixth decade of life, leading to total deafness. Most of the patients report tinnitus and half of them report pressure in the ears. The vestibular symptoms start around the same age and consist of instability in darkness, tendency to fall sideways, light-headedness, drunken feeling and vertigo attacks. Clinically almost 30% of the 60 patients meet the criteria for Meniere's syndrome. More than 80% of them are between the age of 35 and 55 years. In a smaller number of patients a CT-scan of the petrous bone and an MRI scan of the skull base was performed. All subjects underwent a (clinical) neurological examination. The results of this study will be presented.

PREVALENCE OF 35delG MUTATION IN THE CONNEXIN 26 GENE IN THE PORTUGUESE POPULATION

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Non syndromic neurosensory autosomal recessive deafness (NSRD) is the most common form of genetic hearing loss. Several studies have shown that DFNB1 on chromosome 13 is a major locus for recessive deafness in about 80% of Mediterranean families and that connexin 26 gene (Cx 26) is mutated in DFNB1 families. Mutations in this gene are a major cause of inherited and apparently sporadic congenital deafness, being 35delG one of the most prevalent mutation described. Several lines of evidence indicate that the high prevalence of the 35delG mutation arises from a mutation hot spot present in the Cx26 gene.

The overall carrier frequency of 35delG mutation among Mediterranean populations already analysed is about one in 31, namely Spanish population with one in 43 and Italian population with one in 25. Identification of this mutation should facilitate diagnosis and counselling for NSRD.

In this context, the aim of the present study is the estimation of the frequency of 35delG mutation in the Portuguese population. Preliminary results will be discussed.

SEMICIRCULAR CANAL AND OTOLITH FUNCTION IN A LARGE BELGIAN FAMILY WITH DFNA9.

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Twenty-six patients with hearing impairment and vestibular problems from a large Belgian family with a non-syndromic otovestibular dysfunction, inherited in an autosomal dominant way, were investigated by means of an extensive otovestibular examination. They were all members of a family for which genetic analysis confirmed linkage to the DFNA9 locus and mutation identification in the COCH gene.

Since the impairment is characterized by peripheral degeneration of the inner ear, and most family members report unsteadiness, especially in the dark, we investigated the integrity of the vestibulo-ocular reflex as well as the utricular function.

Using electronystagmography (ENG) we tested, next to the oculomotor function, the horizontal semi circular canal function by means of rotation tests and bi-thermal caloric tests. Also positional tests were performed. Often a caloric asymmetry was found that corresponded to the degree of asymmetry in the hearing.

The otolith function was investigated by means of on-line three-dimensional video-oculography (VOG). Hereto, we measured the ocular counter roll during lateroflexion first with the head (bending in the roll-plane to the left and right shoulder) and then with the body (bending of the torso to left and right). In some cases there was still a utricular response present in absence of a caloric response. The results were compared with the data obtained in a group of age-matched controls (N=25) using the same test protocol.

Detailed results of this otovestibular study will be presented.

SURGICAL TREATMENT OF OTOLOGIC MANIFESTATIONS OF APERT'S SYNDROME: A CASE REPORT.

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Apert's syndrome is a congenital disease transmitted by autosomal dominant inheritance. The syndrome is referred acrocephalo-syndactily because its main features are the cranio-facial dysostosis and the fusion of fingers. Associated lesions are seizures (related to the craniosynostosis), spina bifida, and ankylosis of major joints.

It affects 1/100.000 - 1/160.000 neonates, and the middle and inner ear are involved in 3/4 of the cases, with malformations of the ossicles and tympanic cavity, enlarged cochlear aqueduct and internal auditory canal.

CASE REPORT

T.D., female, 13 yrs., came to observation for a recent worsening of her previously diagnosed bilateral conductive deafness. Her clinical history included multiple orthopedic and maxillo-facial surgical procedures for the cranial and facial dysostosis. From 1993 she had suffered of seizures, controlled with barbiturates.

Clinical examination revealed brachicephaly with prominent frontal and malar bones, hypertelorism, asymmetric bilateral ocular proptosis, arcuate hard palate, dental malocclusion.

The external ear was normal in appearance and the external ear canal narrow in its medial end. At micro-otoscopy a glue ear was evident, with a bluish prominence in the hypotympanum, consistent with the CT finding of high jugular bulb. No other anomalies of the middle or inner ear were detected by CT scan of the temporal bones.

Pure tone audiometry assessed a conductive loss of PTA=60 dB (fig.1); a flat tympanogram was obtained bilaterally.

A significant improvement of hearing was achieved with insertion of ventilation tubes (VTs) through a myringotomy, but a residual air-bone gap was noted at low frequencies in one ear (fig. 2). The gap persisted at six months after removal of the VTs without evidence of effusion behind an intact eardrum, suggesting congenital partial fixation of the stapes. Due to the limited amount of residual hearing loss and the anatomical difficulties, stapes surgery was considered not indicated.

Fig. 1: pure tone audiogram at admission

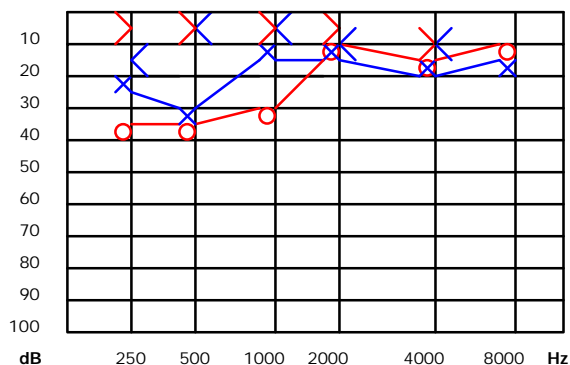
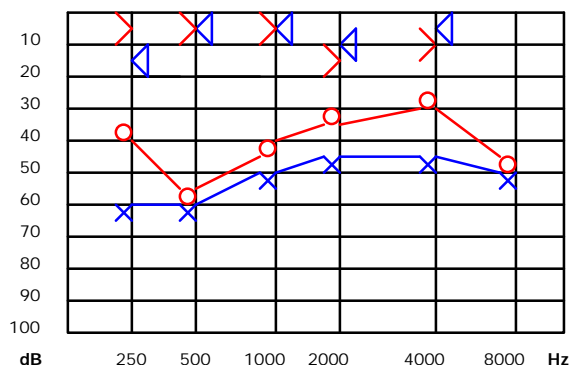


Fig. 2: pure tone audiogram 6 months after ventilation tube extrusion; normal tympanic membrane