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the REGISTRY

Newsletter of the NIDCD National Temporal Bone, Hearing and Balance Pathology Resource Registry

HEREDITARY HEARING LOSS: FROM HUMANS TO MICE AND BACK

SYMPOSIUM AT THE MEETING OF THE ASSOCIATION FOR RESEARCH IN OTOLARYNGOLOGY (ARO) FEBRUARY 23 , 2005

The goal of this half-day symposium was to bring together investigators dealing with different aspects of genetic deafness, including clinician-scientists and basic researchers, in order to present a state-of-the-art review of this rapidly evolving field. The target audience was broad, and included clinicians, basic scientists and students. The initial part of the symposium concentrated on genotypes and phenotypes of hereditary hearing loss in the human. For a given genetic deafness, an important step in understanding pathophysiology and mechanisms of pathology is to create a mouse model. Subsequent speakers discussed various aspects of mouse models with illustrative examples.

The symposium was moderated by Elizabeth Keithley Ph.D., and was sponsored by the NIDCD National Temporal Bone, Hearing and Balance Pathology Resource Registry. This report summarizes the talks given by each featured speaker.

HEREDITARY HEARING LOSS IN HUMANS: WHERE ARE WE IN 2005?

Richard J.A. Smith, M.D., Ph.D., University of Iowa Hospitals and Clinics

Dr. Smith gave an overview of the genetics of hearing loss from a research and clinical perspective. Non-syndromic deafness is an extremely heterogenetic condition. Table 1 shows the number of loci and genes identified at the present time. Several laboratories in the United States and abroad currently offer genetic testing for some genes involved in hereditary hearing loss. Several issues were covered in this talk:

Why is genetic testing useful for clinicians?

If a hearing loss is established to be genetic, then the diagnosis is no longer one of exclusion and diagnostic tests that are currently used routinely would no longer be necessary (e.g. EKG, urine analysis, blood analysis, etc.). As a consequence, healthcare costs would be decreased. Genetic testing also facilitates genetic counseling and prognostic information can be provided. For example, DFNB1 which is due to mutations in GJB2 has a phenotype that is typically characterized by non-syndromic, prelingual deafness with mild to profound hearing loss, no vestibular abnormalities and no radiographic abnormalities. The genotype does predict the severity of the hearing loss in many instances. The presence of a GJB2 mutation also bodes for a good prognosis with respect to performance after a cochlear implant.

Why is genetic testing useful for families?

Since most deaf children are born to hearing parents, initial parental reactions can include shock, denial, disbelief, pain, guilt and depression. Parents may blame themselves for their child's hearing loss and genetic testing can help parents to understand the basis for the hearing loss. Thus, genetic testing is useful because the diagnosis avoids parents blaming themselves for their child's handicap.

What does a genetic diagnosis tell us?

Currently, genetic testing is available for only 7 of the 45 genes that have been identified as causing non-syndromic deafness. In other words, the majority of non-syndromic deafness does not have routine laboratory testing available at the present. The reason is that non-syndromic deafness is extremely heterogenetic and a very large number of exons have to be analyzed to discover the precise mutation. For example, for autosomal dominant non-syndromic deafness, screening would have to be undertaken for 427 exons involving more than 60 KB of the genome to identify the various reported mutations. This is challenging with current technology. Dr. Smith outlined a hierarchical approach using exon prioritization where the initial screening of mutations might be

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Inside

The REGISTRY is published semiannually by the NIDCD National Temporal Bone, Hearing and Balance Pathology Resource Registry. The Registry was established in 1992 by the National Institute on Deafness and Other Communication Disorders (NIDCD) of the National Institutes of Health to continue and expand upon the former National Temporal Bone Banks (NTBB) Program. The Registry promotes research on hearing and balance disorders and serves as a resource for the public and the scientific community about research on the pathology of the human auditory and vestibular systems.

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done in a way that exons with high reported mutations are sequenced first.

Why should we do better?

Dr. Smith discussed novel therapeutic options that are on the horizon as emerging technologies that can be applied in the future if the specific gene defect were known via genetic testing. Examples include those with which might be genotype-specific and those which might be mechanism-specific. RNA interference was discussed, which has great potential as a novel therapy for overcoming the effects of genetic deafness.

Table 1

Non-Syndromic Deafness

–Genes, Loci and Genetic Testing in 2005–

	Loci	Genes	Genetic Testing
DFNA	54	21	1
DFNB	59	21	3
DFN	6	1	1
Mitochondrial	2	2	2*

* 2 of 7 identified mutations

HUMAN OTOPATHOLOGY IN GENETIC DEAFNESS

Saumil N. Merchant, M.D., Massachusetts Eye and Ear Infirmary and Harvard Medical School, Boston, MA

This talk was an overview of human otopathology in genetic deafness and concentrated on five areas.

Why study human temporal bones?

Human otopathology in genetic deafness remains largely unknown. For example, more than 350 genetic conditions have been reported to cause genetic deafness but less than 75 of these have had the otopathology described and this is without genetic confirmation of the mutation. Over 50 genes have been cloned for various syndromic and non-syndromic deafness, but the otopathology has been reported in less than 15 types of deafness where the precise mutation was known. Knowledge of the human otopathology can help in better understanding the mechanism of hearing loss, in developing better diagnostic tests, and in targeting therapy appropriately. The latter will be especially important as therapies develop in the future such as stem cells, gene therapy and other novel therapies. Knowledge of human otopathology can also help in identification of new loci and genes (e.g. DFNA9). It can lead to the development of hypotheses regarding the mechanism of disease which can then be explored more fully in a mouse model. It is important to verify that the pathology in a mouse model is similar to that in the human (this is not always the case even the same gene is altered in the mouse model). Thus, human and mouse otopathology are complementary to each other. Finally, no animal models exist for many types of deafness and so study of the human is the only way to gain insight into the pathology at the present time.

How are temporal bones procured?

Historically, the main source of temporal bones has been from routine autopsies. There has been a steep decline in the number of temporal bones removed at routine autopsy in recent years because of a decrease in the number of autopsies in general and a decrease in the number of active temporal bone laboratories. Fortunately, in the US, the National Temporal Bone Donor Program of the NIDCD National Temporal Bone Hearing and Balance Pathology Resource Registry has provided a source of high quality temporal bones in the past several years. This program of the Registry seeks to solicit pledges from individuals with otologic disease (as well as normal hearing) to donate their inner ears for research after death. The program currently has enrolled over 5,000 subjects across the USA.

How are human temporal bones studied?

The standard method for light microscopy includes fixation in 10% formalin, decalcification using EDTA, embedding in celloidin, serial sectioning at a thickness of 20 microns and staining using hematoxylin and eosin. The processing of human temporal bones is an art which takes many years to acquire. The importance of skilled and experienced temporal bone technicians to process the tissues cannot be overemphasized.

What temporal bone studies have been reported?

The otopathology in known genetic defects include non-syndromic (DFNA9, DFNA17, connexin 26) as well as syndromic defects (Waardenburg I, Alport, Neurofibromatosis type II, Usher I, Usher III, xeroderma pigmentosum, Mohr-Tranebjærg syndrome, Norrie and MELAS syndromes). A pertinent example of the importance of otopathologic study is DFNA9. The disorder was first recognized by its unique otopathology (Fig. 1): namely, atrophy of fibrocytes within the spiral ligament and spiral limbus, degeneration of innervating dendrites, with deposition of an eosinophilic substance. The recognition of this unique abnormality in temporal bones led to investigations in the families of individuals from whom such bones were obtained. These investigations revealed the presence of non-syndromic autosomal dominant sensorineural hearing loss, ultimately leading to the discovery of the DFNA9 locus and mutations in the COCH gene at this locus.

What are the challenges in human temporal bone research and possible solutions?

The application of molecular biologic assays (genomics and proteomics) is a challenge as well as an opportunity. The ideal tissue for such assays is that removed at surgery and frozen immediately. This is not practical in the case of human

temporal bones where postmortem times are usually 8 to 12 hours. Temporal bones can be frozen but they need sectioning or decalcification in order to gain access to the delicate membranous labyrinth. There is no easy and practical method to section or decalcify while keeping the bone frozen and preserving the morphology of the delicate membranous labyrinth. Therefore, for practical purposes, bones for genomic and proteomic studies are those that have been fixed, decalcified, embedded and sectioned. Strategies were discussed for optimizing the study of proteins by immunostaining. The use of celloidin embedding gives good morphology but limits immunostaining to selected antibodies. The use of paraffin embedding permits better retrieval of proteins but the morphology of the human labyrinth is suboptimal. The use of a different embedding medium called polyester wax was discussed which has promise in giving good morphology as well as enabling immunostaining more readily. There is also the potential for applying newer methods of study of proteins using mass spectrometry (proteomics) to archival and prospectively acquired human temporal bone sections.

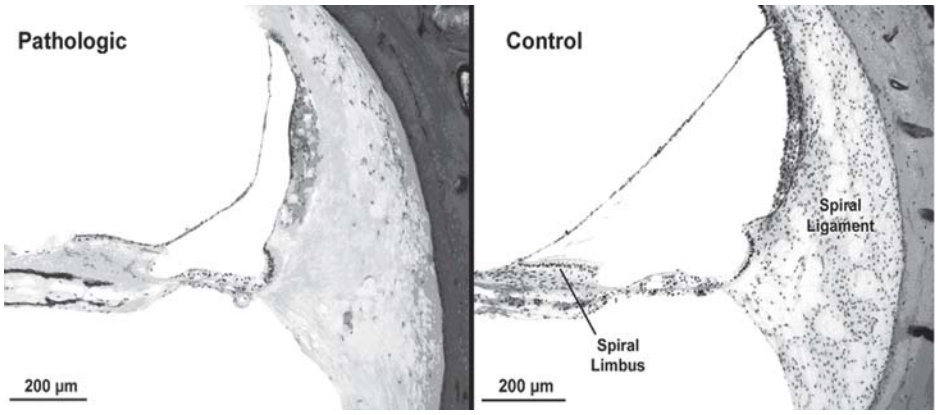


Fig. 1. Photomicrograph of cochlea from a patient with DFNA9 (pathologic) compared to an unaffected individual (control). The DFNA9 ear shows severe atrophy of fibrocytes within the spiral ligament and spiral limbus, degeneration of dendrites, and deposition of an eosinophilic acellular substance within the spiral ligament and limbus.

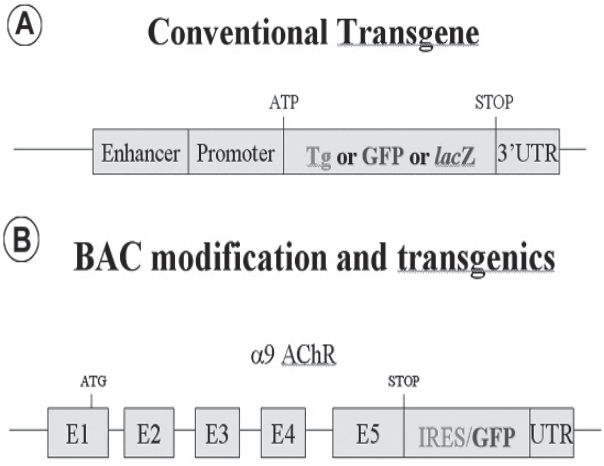
A MOLECULAR GENETICIST'S VIEW OF MOUSE MODELS FOR GENETIC DEAFNESS

Jian Zuo, Ph.D., St. Jude Children's Research Hospital, Memphis, TN

This talk provided an overview of the manipulation of mouse genes to simulate models for human genetic deafness. Various types of manipulations were discussed including transgenic over-expression, knockouts, knockins, tissue or cell-specific expression of various genes and inducible expression of various genes.

Figure 2A shows a schematic of a conventional transgene. While this is relatively simple to construct and can be done in a relatively short time, it does require well characterized promoters, a large numbers of founders and the effects of position cannot always be predicted. Figure 2B shows the use of BAC modification to create a transgene. Bacterial artificial chromosome (BAC) with more than 100 kb of genomic DNA normally contains an endogenous promoter and enhancer for a gene with known pattern of expression. BAC transgenics yields a small number of founders. The number of copies may be low and efficiency to obtain founders may also be low. For gene knockouts or knockins by using embryonic stem (ES) cells, the time line from a gene construct to creation of a model can take approximately 10 to 12 months with costs ranging from $15,000 to 45,000. Examples of knockout and knockin models for the Prestin gene were discussed.

Fig. 2. Transgenic constructs for over-expression of disease genes or markers. BAC: bacterial artificial chromosome; Tg: transgene; GFP: green fluorescent protein; lacZ: lacZ reporter gene; IRES: internal ribosomal entry site; UTR: un-translated region; E1: exon 1.



The Cre-LoxP system was also discussed which is useful to create mouse models with tissue specific or cell specific effects. An example of this was using the system to create hair cell specific Cre expressing lines. The Cre systems can also be coupled with an estrogen receptor so that gene expression in hair cells can be induced by an agent such as Tamoxifen and the expression can be controlled at a particular time in development.

Various resources for mouse models were discussed including the Jackson Laboratory (Bar Harbor, ME), the Mutant Mouse Regional Resource Centers (Missouri, California, N. Carolina, New York) and various web-based resources for available ES cells for gene targeting (www.lexgen.com, www.baygenomics.ucsf.edu, www.genetrap.de, www.sanger.ac.uk). A key reference on this topic was provided: Gao et al., Molecular Brain Research 132 (2004): 192-207.

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PHENOTYPE ASSESSMENT IN MOUSE MODELS OF GENETIC DEAFNESS

M. Charles Liberman, Ph.D. and Stephane Maison, Ph.D.
Massachusetts Eye and Ear Infirmary and Harvard Medical School, Boston, MA

The mouse is a widely used mammalian model to study the peripheral auditory system. The availability of various strains of transgenic mice with specific gene alterations along with the sequencing of the mouse genome makes the mouse a powerful model of the study of physiology and pathology resulting from genetic defects. Various studies investigating the mouse auditory periphery have shown that the mouse inner ear behaves physiologically in a manner similar to that of other mammals including man.

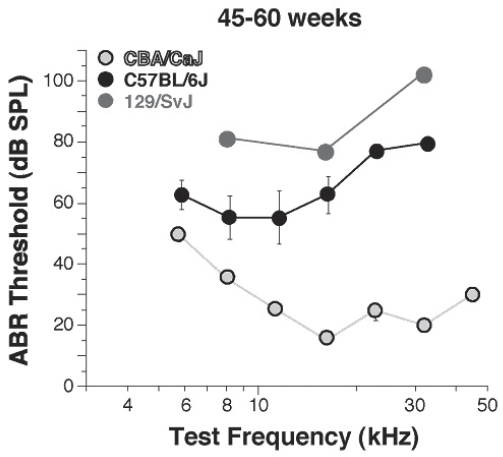

Fig. 3. Hearing thresholds determined by auditory brainstem responses (ABR) for three strains of mice aged 45 to 60 weeks. The thresholds are markedly elevated for the C57BL/6 and the 129/SvJ strains. As a result, it is possible to have confounding hearing losses due to the influence of these background stains when a knockout mouse model is created by hybridizing these two strains (as is often the case).

Most knockout mice are created by hybridizing two strains: the 129/SvJ (the source of the embryonic stem cells used in gene targeting) and the C57BL/6 strain (used as the source of the blastocyst for implantation and the parental strain for line propagation). It is often overlooked, but very important to understand, that both of these strains have genes that cause age-related hearing loss (Figure 3). The C57BL/6 strain shows a progressive age-related hearing loss due to a locus on chromosome 10. Similarly, there is an age-related hearing loss (which is even more severe) in the 129/SvJ strain due to a locus whose chromosome location has yet to be determined. As a result, when studying targeted deletion of the gene of interest, it is possible to have confounding hearing losses due to the distribution of hearing loss alleles from the two background strains. This confound can cause both false positive as well as false negative phenotypes.

There are two different solutions to the problems seen with strain hybridization. One solution is to back cross the knockout mouse strain to a CBA/CaJ strain in order to remove the genes responsible for age-related hearing loss. Another solution is to create a knockout without using strain hybridization. In the latter case, the embryonic stem cell and breeders are derived from only one strain, such as the 129/SvEv strain which shows less age-related hearing loss.

MOUSE MODELS OF USHER'S SYNDROME

Karen P. Steel, Ph.D., The Wellcome Trust Sanger Institute, UK

Usher's syndrome type I consists of severe to profound congenital sensorineural hearing loss with vestibular dysfunction and progressive retinitis pigmentosa. Several subtypes exist (USH1A through USH1G) caused by mutations in different genes. Dr. Steel's talk focused on USH1B caused by mutations in the Myosin 7A gene (Myo7A), the mouse model of which is the Shaker 1 (sh1). She also described the Waltzer (v) mouse model of the USH1D syndrome due to mutations in Cadherin 23 (Cdh23).

The sh1 mutant mice have mutations in Myo7A which encodes an unconventional myosin molecule that is present in the stereociliary hair bundles. The hair bundles appear disorganized (but not splayed) in the sh1 mice. Cross links and tip links appear to be present. Electrophysiological studies using recordings of transducer currents showed that the tip links can function, but not at normal physiologic levels.

The critical defect in the Waltzer (v) mice is disorganization of the stereociliary hair bundles of the hair cells as shown in Figure 4. These stereocilia show near normal orientation at birth but then show progressive disorganization which is evident by postnatal day 4.

Additional studies of heterozygotes of these mutants (carriers) were done to detect possible susceptibility to noise-induced hearing loss (NIHL). Mice carrying the Myo7A mutation showed normal auditory function without susceptibility to NIHL, whereas those carrying the Cdh23 mutation showed increased sensitivity to noise-induced damage.

Thus, the critical defect in Usher's syndrome due to defects in the Myo7A and Cdh23 genes appears to be disorganization of the stereociliary hair bundles. Myo7A is required for normal gating in sensory hair cells and may serve to anchor the cell membrane to the stereociliary core. These findings in the mouse can help to explain the sensorineural hearing loss that is seen in patients with Usher's syndrome.

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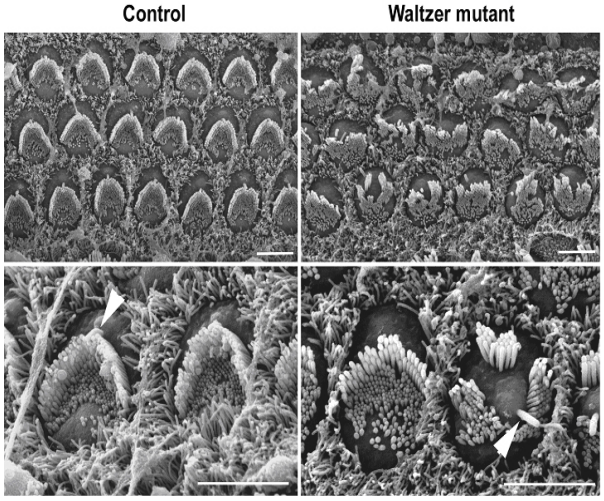

Fig. 4. Scanning electron micrographs of stereociliary hair bundles from the cochlea from Waltzer mutant mice compared to normal controls. Both sets are from postnatal day 4. The Waltzer mutants show disorganization of the stereociliary hair bundles. The mutation is in cadherin 23 which in humans causes Usher syndrome type ID.

BRANCHIO-OTO-RENAL SYNDROME-FROM HUMANS TO MICE AND BACK AGAIN

Rick A. Friedman, M.D., Ph.D., House Ear Clinic, Los Angeles, CA

Branchio-oto-renal (BOR) syndrome is known to be due to mutations in the EYA1 gene. The syndrome is characterized by a) hearing loss which may be mixed, conductive or sensorineural, b) branchial fistulae and c) renal defects. A spontaneous waltzing mouse mutant (3H/HeJ) was found to display absent ABR responses and on histology was shown to have a rudimentary cochlea with abnormalities of the semicircular canals (see Figure 5). This mouse also displayed renal defects. Thus, this mutant mouse has a phenotype similar to that of the BOR syndrome.

Sequencing of the mouse EYA1 gene did indeed show that this mouse mutant arose as a result of a mutation in the EYA1 gene, and thus is a representative mouse model to study the human EYA1 mutation resulting in BOR syndrome. The EYA1 gene produces a transcription factor that has nonspecific phosphatase activity. Dr. Friedman and his colleagues investigated the hypothesis that EYA1 is part of a complex genetic network involving the SIX1 gene and that these two factors plus other unidentified factors are necessary for cochlear and vestibular ganglion cell survival as well as epithelial survival and differentiation.

Studies of development of the mouse inner ear have shown downstream effects of the EYA1 mutation which are consistent with the hypothesis. Additional studies are underway to identify the modifier genes that might be involved in determining variable penetrance and variable expressivity of the phenotype in BOR syndrome. Once these modifier candidate genes are discovered in mouse models, one can then go back to patients and individuals with BOR syndrome to investigate these same candidate genes in BOR families. Thus, hypotheses regarding pathophysiology of BOR defects can be explored in the mice model and then correlated back to the human condition.

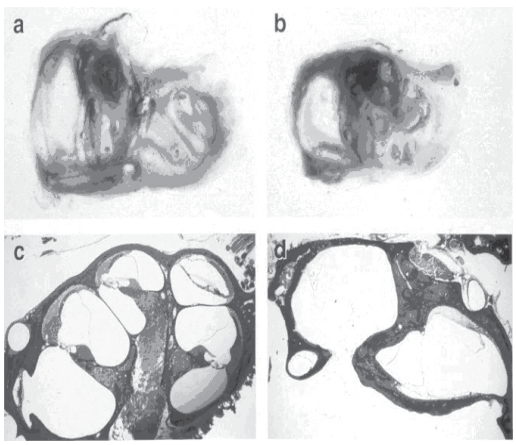

Fig. 5. Photomicrographs showing abnormalities of the inner ear in mouse mutants with defects in the EYA1 gene. The mutant mice are shown in b and d with controls in a and c. The mutants have a rudimentary cochlea with abnormalities of the semicircular canals. This otopathology is similar to that seen in human cases with BOR syndrome.

The synopsis of the workshop was prepared by Saumil N. Merchant, M.D.

Digital Photography of the Temporal Bone and Ear on a Limited Budget

L. Michaels, M.D.[1], R. Chapman[2], T. Upile, F. R.C.S.[1]

1. Royal National Thoat, Nose & Ear Hospital and University College, London
2. Histopathology Dept., Charing Cross Hospital, London

Photography of temporal bone and ear is an essential part of the study of the pathology of this region. Clear images of gross and microscopic findings of this region are necessary not only for recording research observations, but also for teaching. Digital photography has now largely replaced film in all forms of medical work and, at the same time, the acceptance by the public of digital cameras and scanners for recreational purposes has led to the availability of good quality digital cameras and scanners at low cost. Nevertheless, the price of equipment produced specifically for digital photomicroscopy is still high. The purpose of this article is to suggest how digital photographic images of ear and temporal bone pathology can be prepared at low cost using consumer-type equipment. Satisfactory digital images can be obtained with such equipment to record pathological changes of the temporal bone and ear in the following categories: a) low power photomicrography; b) medium and high power photomicrography; c) gross photography of pathologic specimens; d) radiologic images on film; e) otoscopic photography of tympanic membrane and external auditory canal.

Equipment

Cameras

A wide variety of consumer-type digital cameras is available. These machines are capable of a high degree of resolution and are reasonably priced; indeed, prices are steadily diminishing. We have found cameras of the Nikon Coolpix series to be suitable for ear and temporal bone surgery, in particular the Nikon Coolpix 995, which has a resolution of 3.3 megapixels. The Nikon Company replaced the 995 in 2003 by the similar Coolpix 4500, which has a resolution of 4.0 megapixels. Both machines can produce fine close-up images with the macro setup and can easily be adjusted to the delicate requirements of photomicrography. A unique feature of these cameras is the ability to rotate the lens separately from the main camera body.

Monitor for Focussing

The monitor of the 995 is larger than that of the 4500, but still presents too small an area for comfortable focussing of temporal bone histology. A small home model television set, connected to the camera by the cable provided with the camera, mirrors changes of focus in "real time" and is a cheap and efficient monitor for this purpose.

Adapters

It is our experience that the previously described method of simply placing the camera lens onto the microscope eyepiece[1], while useful as a rough record is unsatisfactory for producing publishable photographs, because it produces a severe degree of "vignetting" – the dark shadow that appears around the periphery of the image in photography using the separate lens systems of a scope and a camera. To reduce or even completely exclude vignetting an adaptor system is necessary so that microscope or endoscope eyepiece can be closely applied to the camera lens in a stable manner. Such a system may be obtained from, or even built specially, at reasonable prices, from such firms as SRB Film Service, UK. Eyepieces from some older Leitz microscopes can thread onto the Coolpix camera lens so that when such an eyepiece is available an adaptor system is not necessary.

Microscope

The microscopes commonly used in pathology laboratories are suitable for digital microphotography of temporal bones. A central tube with eyepiece is preferable for installation of the camera so that the binocular eyepieces are free for selection of the field before photography.

The group of instruments required for digital microphotography of temporal bones is shown in Figure 1.

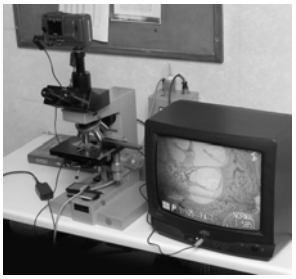

Figure 1. Microscope with digital camera mounted onto central tube through an adapter. The TV set is connected to the camera and, by showing the image and data depicted on the camera monitor in real time, becomes a useful large monitor for focussing.

Scanner

It is necessary to display much of temporal bone pathology by low power images, but clear, sharp, low-power microscopic images are difficult to obtain with digital cameras except in some purpose built digital photomicroscopic systems, which are expensive. Satisfactory results with magnifications of up to about 20 x in temporal bone histology sections can be obtained, however, by producing digital scans at high resolution. Two types of scanners can be used for this purpose.

The slide/film scanner will take a glass-mounted, temporal bone section for scanning in the slot provided for the transparency. With the flatbed scanner, the section is scanned near the center of the larger scanning field. We have used the Epson Perfection 4870 flatbed scanner and obtained good, clear images of temporal bone changes at low power.

Software

Images are downloaded to the computer hard disc from digital camera or scanner using a card reader. In the case of images produced by the digital camera some editing is always required. Scanned images may require little or no editing. The Adobe Photoshop software is an excellent tool for this purpose.

Methods

a) Low Power Photomicrography

Two budget methods are available for low power photomicrography: scanning and macro photography of the slide.

The scanning method is the simplest. With the Epson flatbed 4870 Perfection scanner the temporal bone section can be scanned at a resolution of 4,800 d.p.i. Scanning is carried out after placing the section in the middle of the scanning field and removing the reflective document mat. In the digitized image, the required portion of the section is magnified to the requisite degree and the image cropped down to this area. A small degree of sharpening of the scanned image may be required.

Figure 2. Film and transparency copying apparatus screwed on to lens of digital camera. The section, secured by Blue Tack, has been placed in a compartment between the camera lens and a white diffuser. Low power photomicrographs of high quality can be prepared by this method.

If a high resolution scanner is not available, a high quality microphotograph of the temporal bone section may be made using a digital camera and a slide (transparency) copier setup. The section is put into the copier in place of the transparency and secured at the edges by Blue-Tack. There is a diffuser behind the section for even illumination and daylight may be used satisfactorily to illuminate the section through the diffuser (Fig. 2). The image is magnified and then cropped as with the scanned image described above.

Figure 3. Digital camera attached externally to microscope eyepiece of central tube by adaptor which is screwed to thread on camera lens.

b) Medium and High Power Photomicrography

This is carried out with the camera lens closely apposed to the microscope eyepiece by an adaptor, as described above (Fig. 3).

When appropriate settings are made in the Nikon Coolpix 995 or 4500, the results are rewarding. We have obtained good results following the recommendations of Messing[2], setting the exposure program to "Fixed Aperture" and the aperture to the lowest (greatest size) setting manually. The camera focus should be set to infinity which incapacitates the autofocus facility of the digital camera.

The specimen is brought into focus with the microscope controls. The dark circular edge of the image of the microscope tube is first removed from the field at low power using the optical zoom controls. For subsequent higher power use, these controls are not required and the specimen can be delicately focussed on the television set.

Some degree of editing of these digitally acquired images is always necessary and this is done on the computer with the software, as described above.

c) Gross Photography of Pathologic Specimens

Slices, such as microslices, and gross specimens derived from the temporal bone and even lesions of the external ear in the living patient may be conveniently photographed with the Nikon Coolpix using its macro setup. Satisfactory illumination of the specimen should be carefully prepared.

d) Radiologic Images on Film

Radiographs on film, including CT and MRI scans can be digitally recorded in the same way as gross specimens described above, except that light is transmitted through the film from a light box on which the film is placed.

e) Otoscopic Photography of Tympanic Membrane and External Auditory Canal

Clinical photo-otoscopy can be carried out with an illuminated otoscope passed into the external auditory meatus which is attached via an adapter to a digital camera . Most office based otolaryngology practices have 4mm Storz Hopkin's rod otoscopes and high quality Halogen/ Xenon light-sources for illumination as standard. The Coolpix 995 or 4500 cameras are both suitable. Commercially available endoscope camera adapters are expensive. An adapter can, however, be fashioned by a local engineering facility such as that referenced at a above in such a manner that it is threaded into the eye piece of the digital camera. We have found that three spring load bearings fitted into adjustable screws produce a firm fit of the adapter onto the eyepiece of the Hopkins rod ensuring that the latter is closely applied to the Nikon objective lens.

Fig. 4. Digital camera mounted to Hopkins rod with light source for digital otoscopic photography.

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The endoscope is attached to an external bright cold light source and connected via the adapter to the digital camera (Fig. 4). The Nikon Coolpix camera is placed on the following menu driven settings:- Macro (this allows a short image to objective distance whilst suppressing the flash function in shooting mode); a matrix weighted exposure time; image quality set on “high”; picture size set on “large”; the other settings are placed at default values. The camera is placed in shooting mode with LCD display on. The image of the external auditory canal and the tympanic membrane are viewed on the monitor and final adjustments made to the optical magnification and fine focusing. Several images can be rapidly captured in quick succession by a “Continuous” setting on the camera’s menu. The images can be further processed with standard software, to allow enhancement or digital measurement.

Conclusion

Clinical, gross and low, medium and high power microscopic digital images may be obtained of the ear and temporal bone using equipment purchased at relatively low cost. Such images may be used not only for teaching, but also can attain the high quality of resolution required for publication.

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