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HEARING IN MENOPAUSAL WOMEN AND
IN WOMEN WITH
TURNER SYNDROME, A MODEL FOR
HEARING MATURED IN AN
ESTROGEN-DEFICIENT ENVIRONMENT

Christina Forshell Hederstierna

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ABSTRACT

Epidemiological studies have shown that women have better high-frequency hearing than men in virtually all age groups, regardless of noise-exposure, and that age-related hearing decline starts after age 30 in men but not until after the age of 50 in women. This coincides with the menopausal transition in most women, thus leading us to hypothesize that the menopause triggers auditory deterioration. This may be due to reduced levels of endogenous circulating estrogens, which are known to have protective effects on the auditory system.

Turner syndrome is a chromosomal aberration affecting 1:2000 newborn girls, in which all or part of one X chromosome is absent. This leads to ovarian dysgenesis and little or no endogenous estrogen production. These women have, among many other syndromal features, a high occurrence of ear and hearing problems, and neurocognitive dysfunctions, including reduced visual-spatial abilities. It is assumed that estrogen deficiency is at least partially responsible for these problems.

One objective of this thesis was to describe the prevalence of hearing loss and to classify audiometric configurations in a group of 143 healthy middle-aged women in the general population with respect to menopausal stage and hormone replacement therapy (HRT). A follow-up study including 101 of these women was performed 7.5 years later to describe the rate of hearing decline during the menopausal transition. Another objective was to perform a battery of hearing tests in a group of 30 adult women with Turner syndrome (TS), aimed at localizing the lesion causing the sensorineural hearing impairment and assessing central auditory function, primarily sound localization. Further we carried out a longitudinal study of hearing thresholds in a group of 69 TS women to determine whether the factors initial age, initial hearing level, karyotype, and presence/absence of a mid-frequency dip influences the rate of decline and could serve as prognostic markers.

The main findings in middle-aged women in the general population are that although they have close to normal median hearing thresholds, a large proportion has significant high-frequency losses and dips, which are overlooked if only an average of thresholds at 0.5-4 kHz is used to determine prevalence of hearing impairment. Further, the menopause per se seems to be the starting point for an accelerated period of hearing decline, rather than age alone.

In TS women we showed that cochlear dysfunction is the major cause for the sensorineural impairment. Phase audiometry, a test for sound localization, showed mild disturbances in the TS women compared to the reference group, suggesting that auditory-spatial dysfunction is another facet of the recognized neurocognitive phenotype in TS. Further, the rate of hearing decline in women with TS is comparable to that seen in 70-90-year-old women in the general population, regardless of initial age, hearing level, karyotype, or presence of a mid-frequency dip. The presence of a mid-frequency dip is an especially strong predictor for a future high rate of high-frequency hearing decline with subsequent social consequences.

LIST OF PUBLICATIONS

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LIST OF ABBREVIATIONS

Abbreviations

4FA	The average of thresholds at 0.5, 1, 2 and 4 kHz
ABR	Auditory brainstem response
ABR-V	Latency of ABR wave V
ANOVA	Analysis of variance
ARHI	Age-related hearing impairment
BE	Better ear
BERKO	β estrogen receptor knockout
BLSA	Baltimore Longitudinal Study of Aging
CHD	Coronary heart disease
DPOAE	Distortion product otoacoustic emissions
ER	Estrogen receptor
HFA	The average of thresholds at 3, 4 and 6 kHz
HL	Hearing level
HRT	Hormone replacement therapy
ITD	Interaural time difference
kHz	kilo Hertz
MOC	Medial olivocochlear
PA	Phase audiometry
PTA	Pure tone audiometry
SNHI	Sensorineural hearing impairment
SRS	Speech recognition scores
SRSn	Sound recognition scores in noise; S/N ratio 4 dB; % correct
SRS-Low	Lower limit for SRSn; mean value - 2 SD, calculated considering age and HFA according to Barrenäs and Wikström, 2000
TEOAE	Transient evoked otoacoustic emissions
TS	Turner Syndrome

1 INTRODUCTION

1.1 EPIDEMIOLOGY

1.1.1 Prevalence of hearing loss

The incidence of hearing loss continually rises with increasing age. An important issue concerns the time of onset of age-related hearing impairment. The prevalence of hearing loss, defined as the 4FA (the average of hearing thresholds better ear at 0.5, 1, 2 and 4 kHz) ≥ 20 dB HL was 26 % in men and 20 % in women in a large study of adults in Great Britain: The MRC Study of Hearing Loss (Davis, 1995). The prevalence was low in young adults, 1.8 % in men and 3.1 % in women aged 18-30. The prevalence in the highest age group, 71-80 in this study, was 78.5 % in men and 70.1 % in women.

1.1.2 Gender differences

There are several differences between males and females regarding auditory function. Significantly stronger transient evoked otoacoustic emissions (TEOAE) are seen in newborn girls than in newborn boys (McFadden, 1993, Khalfa et al., 1997, Berninger, 2007). These differences are very apparent in old age, but they are discernible even for young adults. Further studies of these gender differences regarding auditory function may help us understand aging processes involving the auditory system (Helfer, 2001).

Results from several epidemiological studies of middle-aged and elderly people have shown that men have more high-frequency hearing loss than women do (Davis, 1995, Cruickshanks et al., 1998, Gates et al., 1990, Jönsson et al., 1998, Borchgrevink et al., 2005). In very advanced age, 80+, this gender difference tends to diminish or is even non-existent (Parving et al., 1997).

Another finding is the so-called gender-reversal phenomenon, reported in many studies (Jerger et al., 1993). This phenomenon suggests that elderly women have slightly poorer low-frequency hearing than do men of similar age. Reported gender differences are not only restricted to pure tone audiometry.

Women are also known to have shorter auditory brainstem response (ABR) latencies than men do (Wharton and Church, 1990, Rosenhall et al., 1985) and their otoacoustic emissions are also slightly more prominent (Bilger et al., 1990).

1.1.3 Genetic factors

Genetic factors are significant in the development of age-related hearing loss (Van Eyken et al., 2007). There are indications that the genetic influence is more pronounced in women, and the extrinsic influence is more apparent in men (Gates et al., 1999).

1.1.4 Noise exposure

Occupational and leisure-related noise exposure patterns are classically very different in males and females. However, noise exposure can only explain a part of the gender difference; it has been shown that the gender difference was apparent even in non-noise-exposed study populations (Jerger et al., 1993, Rosenhall and Pedersen, 1995).

1.2 TURNER SYNDROME AND HEARING

1.2.1 Background

Turner syndrome (TS) is a chromosomal aberration in which all (monosomy) or part (partial monosomy) of one X chromosome is to a great extent absent. Other relatively frequent karyotypes include structural anomalies, such as isochromosomes and deletions. Cell line mosaicism is common. The incidence of TS is approximately 1:2000 live born females (Saenger, 1996). The features of the syndrome are diverse, and include infertility due to underdeveloped ovaries and low or absent estrogen production, short stature, ear and hearing problems, and certain cognitive defects including reduced visual-spatial abilities. Due to the low endogenous estrogen levels in TS girls and women, the syndrome may be regarded as a human model for the assessment of physiological processes in organs that have matured in a highly estrogen deficient environment.

1.2.2 Turner syndrome and hearing

In early childhood TS girls usually have normal hearing thresholds (Stenberg et al., 1998). Conductive hearing loss commonly develops, due to middle ear effusion or frequent otitis media in childhood, resulting in subsequent problems with chronic infections, tympanic membrane pathology etc. (Hultcrantz and Sylven, 1997, Hultcrantz et al., 1994, Stenberg et al., 1998). A large proportion of adult TS women develop a mid-frequency sensorineural dip, sometimes apparent even before puberty, but usually noticeable in their 3rd to 5th decade of life (Hultcrantz and Sylven, 1997, Stenberg et al., 1998). Further, a majority of TS women develop a moderate to profound high-frequency loss, thus oftentimes leaving only the low frequencies spared (Anderson et al., 1969, Hultcrantz et al., 2006). The prevalence of hearing loss reported in these different studies are, however, not comparable, since different definitions and frequencies in calculating pure tone averages are used. The pathophysiology of the prevailing sensorineural lesions is not yet fully understood, and the nature regarding the progression of hearing impairment and its consequences for the affected women, especially in middle age and older, has not been reported.

The mid-frequency sensorineural dip occurs more frequently in women with a certain karyotype, namely 45,X or 45,X/46,X,i(Xq)(isochromosome). This implies that the locus for the hearing impairment in TS is situated on the p-arm of the X-chromosome, and Barrenäs et al have shown a “dose-response relationship” between the degree of loss of the p-arm and the degree of hearing loss in TS (Barrenäs et al., 1999).

1.2.3 The Turner syndrome associated neurocognitive phenotype

The TS-associated neurocognitive phenotype generally includes normal verbal function with relatively impaired visual-spatial ability, attention, working memory, and spatially dependent executive function (Ross et al., 2006, Ross et al., 2002). It has not been reported whether the spatial difficulties in TS comprise central auditory functions besides the visual.

1.3 ESTROGENS AND HEARING

1.3.1 Estrogens

A contributing cause for the sensorineural hearing loss in TS women has been proposed to be the lack of endogenous estrogens. Estrogens have neuroprotective and neurotrophic effects on the brain (Brann et al., 2007), and can therefore be presumed to have positive effects also on hearing function. Estrogen receptors α and β (ER α and

ER β) have been shown to exist in the inner ear in rats and mice (Stenberg et al., 1999), and also in normal human fetuses and adults and in TS fetuses (Stenberg et al., 2001). A β estrogen receptor knockout (BERKO) mouse develops a neural hypocoellularity in the somatosensory cortex, and early brain degeneration (Wang et al., 2001). The BERKO-mouse also demonstrates a greater threshold shift after acoustic trauma compared to controls, implying a protective effect on hearing function from estrogen- β (Meltser et al., 2008).

Further, studies on CBA mice, where distortion product otoacoustic emissions (DPOAE) were higher in female animals before menopause, compared to after, and compared to male age-matched animals (Guimaraes et al., 2004); have also illustrated that estrogen blockade with tamoxifen administration negatively affected the medial olivocochlear (MOC) efferent feedback system to the cochlea (Thompson et al., 2006). The authors suggest that their results support the hypothesis that estrogen plays an important role in the integrity of the auditory system. Similar conclusions have been drawn from the demonstration of estrogen receptors alpha and beta in the inner ear of rodents and humans; the receptors can be down-regulated with antiestrogens (Stenberg et al., 1999, Stenberg et al., 2001, Stenberg et al., 2003) and vary in expression in fetal and mature rats during the course of development, maturation and pregnancy (Simonoska et al., 2009).

1.3.2 Reproductive hormones

All the human reproductive hormones are synthesized from a common precursor: 5-pregnenolone, formed by a side chain cleavage from cholesterol. *Progesterone* - is produced in the corpus luteum and placenta, but small amounts are also found in the adrenal cortex in men, postmenopausal women and prepubertal children. *Androgens* - 5 α -dihydrotestosterone is produced in the testes and in the ovaries, the adrenal cortex and to a certain extent in the brain. *Estrogens* - are produced in the ovaries of fertile women, the placenta, the testes, the adipose tissue in postmenopausal women and men, and in the brain. Estradiol is the most commonly occurring estrogen, but there are others, e.g. estrone and estriol.

The release hormones are gonadotropin releasing hormone (GnRH) in the hypothalamus and luteinizing hormone (LH) and follicle stimulating hormone (FSH) in the pituitary gland. There are intricate feed-back mechanisms regulating the hormone levels in different portions of the menstrual phase, where for example estradiol has a negative feed-back effect on FSH and a positive on LH. With progesterone the reverse is true. There are many other hormones involved in these regulatory systems, including thyroid stimulating hormone (TSH), prolactin and growth hormone (GH).

1.3.3 Menopause

Menopause is typically defined as one year since the last menstrual period. This usually occurs around the age of fifty. The circulating levels of estradiol and progesterone produced in the ovaries become significantly reduced due to involution of the ovaries, leading to increased levels of FSH. After menopause lower levels of estrogens are still produced from androgens in the adrenal glands and in body fat.

The different phases leading to and occurring after menopause have been staged by the Stages of Reproductive Aging Workshop (Soules et al., 2001). Here the menopausal transition is subdivided into four periods: early and late perimenopause, and early and

late postmenopause. During this transition the menstrual cycles change from being of variable length to being completely terminated.

The period before and during the menopausal transition is a time of many inconveniences for a large proportion of healthy women. Common symptoms include hot flashes, night sweats and mood swings, and are caused by the changes in circulating levels of estrogen and progesterone. There is an increased risk for several diseases in women after menopause, e.g. heart disease, osteoporosis, and other complications.

1.3.4 Hormone Replacement Therapy (HRT)

HRT, especially in a combination of estrogen and progesterone substances, has been extensively used during the second half of the 20th century to relieve the symptoms of menopause. It was earlier believed that the use of these hormones would be beneficial in almost every aspect of menopausal distress, including a reduced risk for heart disease.

1.3.4.1 WHI

In 1991 a large study including more than 160 000 women older than 50 was initiated in the United States, named the Women's Health Initiative (WHI). The clinical trials were designed to test the effects of postmenopausal hormone therapy, diet modification, and calcium and vitamin D supplements on heart disease, fractures, and breast and colorectal cancer.

During the course of this study, there have been many changes in the way hormone therapy is prescribed due to the findings of the study. In 2002, one arm of the WHI-trial (combined estrogen and progestin in women with a uterus) was stopped earlier than planned; based on health risks that exceeded health benefits specifically regarding coronary heart disease, breast cancer, pulmonary embolism and stroke. The WHI-trial continued with the estrogen-only arm until 2004 when this arm was also stopped a year early. Estrogen-alone therapy did not appear to affect (either decrease or increase) the risk of heart disease, the main question of the trial. More importantly, there appeared to be an increased risk of stroke, and the NIH believed that an increased risk of stroke was not acceptable in a prevention trial in healthy women, especially if estrogen did not reduce heart disease risk (WHI, 2008).

1.3.4.2 Current guidelines regarding HRT

Current guidelines in the United States support short-term (up to 5 - 7 years) use of HRT for treatment of hot flashes and other vasomotor symptoms in recently menopausal women who have a low risk for stroke, heart disease, or breast cancer. Beginning estrogen replacement therapy years after menopause has occurred is generally not recommended (WHI, 2008).

In Sweden the corresponding guidelines from the Medical Products Agency (Läkemedelsverket) support the use of HRT for significant menopausal symptoms. It is not the first hand treatment for the prevention of osteoporosis. The lowest effective doses of hormone substances are recommended, during the shortest possible time, usually no more than 3-5 years. In these cases benefits should prevail over risks (Läkemedelsverket, 2008).

1.4 INTERAURAL DIFFERENCES

1.4.1 Left ear disadvantage

For decades a difference in hearing acuity between the left and right ears has been observed. This difference is apparent already in the neonatal period, since newborn girls and boys have slightly, but significantly stronger otoacoustic emissions in the right ear than in the left (Khalifa et al., 1997, Berninger, 2007). The most comprehensive studies of the interaural differences in hearing thresholds have been performed by a Finnish research group (Pirilä et al., 1991a, Pirilä et al., 1991b, Pirilä et al., 1992). They showed that the left ear had slightly better hearing thresholds at all frequencies below 15 years of age, in both boys and girls. After 15 years of age there was noticeable left ear inferiority at 3-8 kHz in all age groups, more pronounced in men. In women, the authors described a slight decrease in the left ear inferiority between 25 and 45 years of age, and after that a slight increase (Pirilä et al., 1992). In the oldest age groups, 70-90 years, the right-left difference is small in males, and virtually non-existent in females at 4 kHz (Jönsson and Rosenhall, 1998).

The etiology of the peripheral left ear disadvantage in the high frequencies has been discussed. Noise induced hearing loss has been proposed as a major cause for the right-left difference that is most pronounced at 4 kHz in both males and females, but several authors have shown that the difference remains regardless of handedness or eyedness in shooting, or hearing level (Job et al., 1998, Nageris et al., 2007, Pirilä et al., 1991a, Rudin et al., 1988). Khalifa et al showed that the MOC-system has a functional right-ear predominance in adults, implying a higher grade of efferent feed-back and as a consequence protection of the right cochlea (Khalifa et al., 1997).

2 AIMS OF THE THESIS

2.1 MIDDLE AGED WOMEN IN THE GENERAL POPULATION

- To describe the hearing function in a population of women around the age of menopause. These women are at the starting point of accelerated hearing-loss, yet the majority has almost normal hearing thresholds.
- To compare the hearing threshold levels with respect to menopausal status.
- To suggest new guidelines for classification of audiometric configurations in age-related hearing loss. More specific and stratified classifications may be useful in planning for the rehabilitation needs of adults and perhaps as a tool for prognostic evaluation.
- To describe the process of hearing decline during the time of the menopausal transition, with respect to time elapsed since menopause, through a longitudinal follow-up. This type of study design will allow for a more detailed description of the transforming configuration at the start of age-related hearing loss.

2.2 WOMEN WITH TURNER SYNDROME

- To investigate several aspects of auditory function in adult women with Turner Syndrome. The women in the selected groups were all born 1966 or earlier, i.e. they were teenagers before implementing the general recommendation to treat all Turner girls with hormonal replacement therapy in puberty. Herein lies an assumption that these older Turner women have had a development of the auditory system in an estrogen deficient environment and therefore constitute a suitable human model for the assessment of a physiological process in an organ that has matured with little or no estrogen effects.
- To determine the location of the lesion that causes sensorineural hearing impairment in Turner syndrome.
- To assess central auditory function, primarily sound localization, in Turner syndrome.
- To study the natural course of sensorineural hearing decline by means of a longitudinal study of hearing thresholds in a group of adult women with Turner syndrome.
- To determine whether the factors initial age, initial hearing level, karyotype, and/or presence/absence of a mid frequency U-shaped audiometric configuration influence the rate of threshold change, and could serve as prognostic markers.

3 MATERIAL AND METHODS

3.1 STUDY GROUPS

3.1.1 Paper I

In this study we assessed 143 healthy women with known menopausal status with pure tone audiometry in both ears. The study was performed as part of the first phase of an ongoing, longitudinal investigation of health and psychosocial work conditions in middle aged women. In all, 2000 women aged 47-53 residing in Stockholm were recruited through the Swedish Population Register. The sample was representative of urban middle-aged women and the participants were randomized from three different socioeconomic groups according to their yearly income level (low, medium, and high) agreeing with criteria established by Statistics Sweden (www.scb.se). The percentage of the participants in each of these groups was 20%, 60%, and 20%, respectively.

During the primary selection procedure the women were mailed a questionnaire regarding socioeconomic background, health, lifestyle, experience of symptoms, and quality of life. In the secondary selection process only women who were active in working life were selected. An exclusion criterion was serious disease, e.g. cardiovascular disease, cancer or diabetes. Of the 940 women who completed and returned the questionnaire - a primary response rate of 47% - a random representative sample of 200 women was drawn. Of these 200 women, 164 agreed to participate in the study. The final percentages in the different income groups were 6.7% (low income), 61.3% (medium income), and 31.9% (high income). The women were invited to take part in a psychological interview, completion of psychological rating scales, blood sampling, and audiometry. In this report we will only discuss the audiometric findings. Twenty women did not participate in all parts of the study.

The mean age at the time of testing was 51.3, (range 48.5-55.0, S.D. 1.5).

3.1.1.1 Menopausal staging

The menopausal staging system as defined according to STRAW (Soules et al., 2001) is used to determine the three main groups of menopausal status:

- Pre-menopause (reproductive) - regular menstrual cycles.
- Peri-menopause (menopausal transition) – variable menstrual cycle length.
- Post-menopause - more than one year since the final menstrual period.

The women who were in the post-menopausal group were further subdivided into two groups, depending on whether or not they were receiving any form of hormone replacement therapy (HRT), thereby yielding the following four groups: 1. Pre-menopause (n=47, mean age 50.7, SD=1.32); 2. Peri-menopause (n=32, mean age 51.2, SD=1.52) 3. Post-menopause (n=21, mean age 52.4, SD=1.52) 4. HRT (n=43, mean age 51.5, SD 1.46).

3.1.2 Paper II

The 30 subjects in this study were chosen from the register of all known females with TS in the Stockholm County. The intention of the study was to recruit a group of TS women in which hearing thresholds were normal or mildly to moderately impaired as several tests in the protocol require a relatively preserved auditory function for optimal

implementation and interpretability. Inclusion criteria were all TS women aged 40 or above. This TS cohort consisted of 70 women, aged 40-76. Exclusion criteria were conductive hearing loss or obvious tympanic membrane pathology, n=12; sensorineural hearing loss of such a degree and extent that it would possibly compromise the interpretation of the test results: asymmetrical hearing loss with a >10 dB difference between the ears in at least two frequencies, n=8; pronounced hearing loss with a 4FA (the average of air conduction hearing level thresholds at 0.5, 1, 2 and 4 kHz, better ear (BE)) exceeding 60 dB HL, n=12; serious disease or cognitive dysfunction impairing the possibility of optimal cooperation and performance during testing, n=4. The remaining 34 women were thus invited to participate, four of these declined due to lack of time or interest.

The included 30 women were examined otoscopically and in most cases further tested with a Grason-Stadler GSI 33 V2 Middle-Ear Analyzer. None was found to have blocking earwax or tympanic membrane pathology at risk of interfering with air conduction thresholds. The mean age of the 30 women in the study group was 52.2 years, range 40.5-67.0 years.

3.1.3 Paper III

The 69 subjects in this study were also recruited through the register of all known females with TS living in Stockholm County. The initial audiograms were performed during the period 1988-2003, except in two women who had undergone audiometry previously. The follow-up audiograms were performed 1998-2008. Only TS women born 1966 and earlier were included (n = 88). In order to include as many TS women as possible in the follow-up, we allowed a minimum of three and a maximum of 16 years interval between the measurements. Exclusion criteria were: 1) women with conductive hearing loss in both ears (n=0); 2) women with initial hearing threshold levels poorer than a 4FA (average of air conduction thresholds at 0.5, 1, 2 and 4 kHz) of 70 dB HL (n=1) as the hearing decline in these women was assumed to have diminished, especially in the high frequency region, and there is not much more additional deterioration to be expected. Eighteen women had only been tested once, or had screening audiograms performed at the first visit, therefore rendering adequate calculation of progression impossible.

A total of 69 women were tested twice, with an average interval of 9.6 years (range 3-16, median 10.2 years). The karyotype of all women was known. The mean initial age was 43.6 years (27.9-61.8).

3.1.4 Paper IV

The 101 women included in this study are part of a prospective longitudinal study of peri-menopausal women in the general population. The initial sample in the hearing part of the study included 143 healthy women, i.e. the women in Paper I. Two years after the first series of tests and interviews, a follow-up was done, including audiometry, psychological interviews and questionnaires (Evolahti et al., 2006, Evolahti et al., 2008). The audiometric results were evaluated, but two years is a short time regarding normal age-related hearing decline, and the findings were not conclusive.

A new follow-up was therefore planned to take place 7-8 years after the initial tests; at the start of this follow-up 120 women from the original group were still living in the Stockholm area, and were invited to participate. 19 declined due to lack of time,

interest, or illness, or have postponed their participation in this study. A final group of 101 women were thus included in this study. They have all been assessed with pure tone audiometry at two occasions, starting in the year 2000. The average interval between tests was 7.5 years, (range 5.7-8.1). The mean age at the first test was 51.3, (range 48.7-56.2), and at the second test 58.8, (range 56.1-61.9).

In all women the menopausal status was known at both occasions; i.e. were they still menstruating, or had they entered menopause, and if so, at what time. At the final audiometry all but two women were in menopause. The number of years since the final menstruation varied between 0 and 24 years, mean time 6.5 years, median 6 years (see figure IV:1). The women were grouped according to years since menopause: 0-5 years (MP 0-5) (n=44, mean age 58.5, range 56.1 – 61.7) or ≥ 6 years since menopause (MP ≥ 6) (n=57, mean age 59.0, range 56.1-61.9). There was no significant age difference between the groups.

Further, the women provided information as to whether they were taking hormone replacement therapy (HRT) or not. A total of 19 women had been using estrogen, progesterone or combination treatment for at least half of the follow-up time. The number of women on estrogen replacement was 8 in MP 0-5 and 3 in MP ≥ 6 ; progesterone replacement was used by 1 woman in MP 0-5 and 2 in MP ≥ 6 and combination treatment was used by 2 women in MP 0-5 and by 3 in MP ≥ 6 . Since the total number of women on any type of HRT was relatively small, and they were relatively evenly distributed between the groups, we concluded that separate statistical analyses on these women would not be reliable.

3.2 REFERENCE MATERIAL

3.2.1 Paper II

For two of the tests included in the study, transient otoacoustic emissions (TEOAEs) and phase audiometry, a reference group was selected, consisting of a group of 33 healthy women (staff at the Karolinska University Hospital) matched for age and with the same inclusion/exclusion criteria as the study group regarding hearing. The mean age in this group was 52.5 years, range 38.2-66.9.

Auditory brainstem response (ABR) results were compared to a clinical reference material of normal hearing ears obtained from age matched female patients (n=34, mean age 53.9, range 40.5-67.4) at the Department of Hearing and Balance, Karolinska University Hospital. These subjects were tested with ABR as part of an investigation for unilateral hearing loss, most often sudden deafness; the non-symptomatic ear used for reference information. Exclusion criteria for this group were middle ear pathology, Ménière's disease, cerebellopontine angle or intracanalicular tumors, or suspicion of central auditory processing disorders.

3.2.2 Papers III and IV

In these papers we compared the results of the included women with several reference materials. These included population based studies of cross-sectional data, i.e. the MRC Study of Hearing Loss, from Great Britain (Davis, 1995), and two longitudinal studies: the BLSA (Baltimore Longitudinal Study of Aging) (Pearson et al., 1995), including women of all age groups 20-80+, and a report from South Carolina (Lee et al., 2005) on women 60-81. The results from these longitudinal studies are shown in figure I:1 where annual decline in dB/year for women of various age groups is plotted against frequency. The BLSA study shows little or negative progression of hearing loss

between 20 and 59 years of age, whereas the oldest age groups (60-89) illustrate an accelerated rate of decline with increasing age. The South Carolina study only includes women 60-81, but the comparison shows that these women have a high and accelerating rate of hearing decline, in effect slightly higher than in the BLSA study.

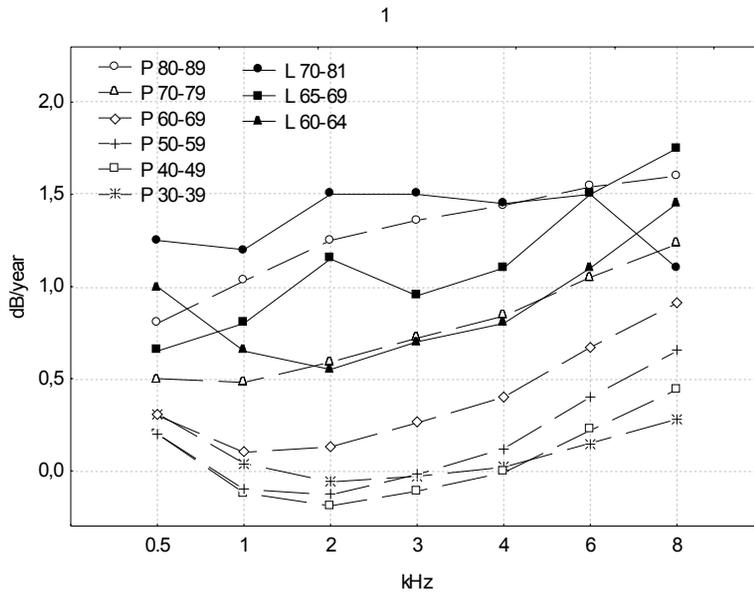


Figure I:1
Reference curves of mean annual decline in dB/year for various age groups in women. P=(Pearson et al., 1995), L=(Lee et al., 2005).

3.3 METHODS

All audiometric testing was performed by licensed clinical audiologists at the Department of Hearing and Balance, Karolinska University Hospital in Solna, according to routine clinical procedure.

3.3.1 Pure tone audiometry

Pure tone audiometry, including air conduction thresholds at 0.125, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6 and 8 kHz and bone conduction thresholds at 0.5, 1, 1.5, 2, 3 and 4 kHz, using Grason-Stadler GSI-16 audiometers with Telephonics TDH-39 ear phones in sound proof booths, was performed by licensed clinical audiologists. The method according to ISO 8253-1, 1989 (ISO, 1989) was applied, and is described in detail by SAME (SAME, 1983)

3.3.2 Transient evoked otoacoustic emissions (TEOAE)

TEOAEs were recorded using a Madsen Capella Cochlear Emissions Analyzer. Results were obtained at band pass widths of approximately 0.5-1 kHz at 1, 1.5, 2, 3 and 4 kHz centered frequency bands.

3.3.3 Auditory brainstem responses (ABR)

ABRs were registered using an Interacoustics EP 25. The stimulus consisted of 80 dB nHL alternating clicks presented monaurally with a frequency of 19.1/s, and band

pass filtered from 0.1 to 3 kHz. Each recording was individually analyzed by the testing audiologist and by one of the authors (CH). The test method has been thoroughly validated as a diagnostic tool for sensorineural hearing loss. Latencies of ABR-waves in cases with sensorineural hearing loss that are within the ranges seen in normal hearing individuals, or with a moderate increase of wave V latency of 0.1 ms or less per 10 dB hearing loss at 4 kHz exceeding 50 dB HL are strongly indicative of cochlear hearing loss (Møller and Møller, 1985). Retrocochlear hearing loss (most often caused by vestibular schwannomas exceeding 1 cm in size) causes significant prolongation of the later ABR-waves. Brainstem lesions cause loss or latency prolongations and configuration disturbances of the later ABR-waves. For a review, see Møller and Møller. Auditory neuropathy causes severe abnormality of the ABR-responses (Berlin et al., 2003).

Assessment of the central auditory function is often compromised by sensorineural hearing loss and age. We therefore chose two tests that have been validated regarding the influence of those two parameters.

3.3.4 Speech recognition scores in noise (SRS in noise)

SRS in noise were measured with the same equipment and under the same conditions as pure tone audiometry. Fifty-word lists, using monosyllabic, phonemically balanced words were presented at a comfortable level chosen by the subject in a fixed speech weighted background noise at a 4 dB S/N-ratio (signal to noise ratio) as described in detail by (Magnusson, 1995). A reference model, accounting for age and high-frequency hearing (average at 3, 4 and 6 kHz) was used for evaluating SRS-scores (Barrenäs and Wikström, 2000).

3.3.5 Phase audiometry

Phase audiometry was performed using an Entomed Phaseaudiometer SA 220. A 0.5 kHz tone was presented binaurally with headphones simultaneously to both ears and adjusted to a comfortable loudness level to give a midline impression. In the TS group the median presentation level was 75 dB HL, range 65-90 dB HL, and in the reference group the corresponding values were 70 dB HL, range 65-85 dB HL. Testing started with a maximum phase lag of 500 μ s (90°) between the ears to give a sensation of lateralization, followed by diminishing decrements of phase lag in order to find the minimum interaural time difference (ITD) at which lateralization was perceived.

3.3.6 The annual rate of hearing decline

The annual rate of hearing decline was calculated for each frequency as the difference between the later and the earlier hearing threshold level, divided by the time difference in years.

3.4 STATISTICAL ANALYSES

All statistical analyses were performed with the computer software program Statistica (Statsoft® Scandinavia AB). The choice of statistical methods was made after consultations with statistician Elisabeth Berg, at the Department of Learning, Informatics, Management and Ethics at the Karolinska Institute.

Student's t-test was used to analyze between-group age differences. Analysis of variance (ANOVA) was applied when comparing ABR results. The Mann-Whitney U

test for non-parametric data was used when analyzing the phase audiometry results in paper II, and in paper IV when comparing differences in decline between groups.

4 RESULTS

4.1 PAPER I

The hearing function of 143 women aged between 47 and 53 is described. The mean hearing threshold levels are significantly poorer in the group of post-menopausal women without HRT, compared to each of the other groups – pre- and perimenopausal, and post-menopausal with HRT, at 2, 3 and 8 kHz. See Figure I:2.

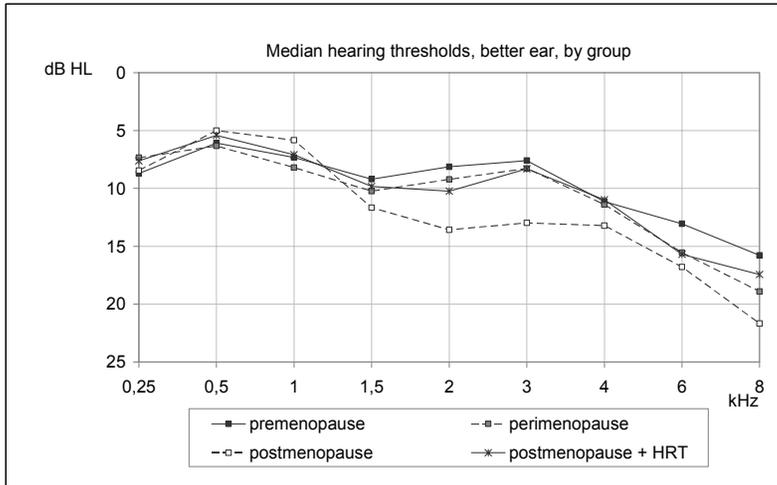


Figure I:2
Audiograms of the women grouped according to menopausal status.

4.1.1 Types and grades of hearing impairment

Fifty-seven women, 40%, (eighty-two ears) had a hearing threshold of ≥ 30 dB HL at any frequency from 0.25 kHz to 8 kHz. Five women, 3.5%, (six ears) with hearing loss exhibited a *conductive hearing impairment* with an air-bone gap of ≥ 15 dB HL at any frequency from 0.5 kHz to 4 kHz. Fifty-two individuals (36.4%) were, accordingly, defined as featuring a *sensorineural hearing impairment*, 28 (19.6%) unilaterally and 24 (16.8%) bilaterally.

According to the classification of grades of hearing impairment by HEAR (Martini, 2001), ten women had a hearing impairment: eight women had a bilateral mild impairment, one woman had a mild impairment in one ear and a moderate in the other, and one woman had a bilateral moderate impairment (7.0%). According to the classification of grades of hearing impairment by WHO (WHO, 2008), eight women had a hearing impairment: seven women had a bilateral slight impairment, and one woman had a bilateral moderate impairment (5.6%). None had an asymmetric sensorineural hearing loss exceeding 11 dB HL difference in 4FA between the ears.

4.1.2 Audiometric configurations

Audiometric configuration is classified as suggested in Table I:1.

Suggested classification of audiometric configurations in age-related hearing loss

Frequencies involved	Type	Description
Low-frequency hearing loss ≤ 0.5 kHz	R, Rising	The average of the thresholds at 0.25 and 0.5 kHz is ≥ 15 dB poorer than the average of the thresholds at 4, 6 and 8 kHz.
Mid-frequency hearing loss between 0.75 and 2 kHz	MU, U-shaped (dip)	One or more adjacent thresholds between 0.75 and 2 kHz are ≥ 20 dB poorer relative to any threshold at lower frequencies and ≥ 15 dB poorer relative to any threshold at higher frequencies.*
High-frequency hearing loss ≥ 3 kHz	GS, Gently sloping	kHz is ≥ 15 dB better than the average of the thresholds at 4, 6 and 8 kHz.
	SS, Steeply sloping	The average of the thresholds at 0.5 and 1 kHz is ≥ 30 dB better than the average of the thresholds at 4, 6 and 8 kHz.
	HU, U-shaped (dip)	One or more adjacent thresholds between 3 and 6 kHz are ≥ 20 dB poorer relative to any threshold at lower frequencies and ≥ 15 dB poorer relative to any threshold at higher frequencies.#
Miscellaneous	F, Flat	Thresholds across frequencies 0.25-8 kHz do not vary more than 15 dB from each other.
	U, Unspecified	Configuration that does not meet the criteria for inclusion in any of the above categories.

Table I:1

* Mainly describes configurations which in audiological practice are commonly referred to as trough-shaped or cookie-bite.

Mainly describes sharp dips, or notches at 3, 4 and 6 kHz.

The six ears with conductive hearing impairment (6/82; 7.3%) consisted of four *rising* (4.9%), one *flat* (1.2%), and one *unspecified* (1.2%) configuration. When audiograms with conductive hearing impairment were omitted, 76 ears with a sensorineural hearing impairment resulted, and the breakdown of the distribution of configurations (Figure I:3) was the following: High frequency impairment configurations (*Gently* and *steeply sloping* and *high frequency-U-shaped*) accounted for approximately three fourths of all audiograms (76%). *Mid-frequency U-shaped* configurations accounted for 9% of all audiograms. *Flat* configurations were rare in this group (0.7%, one ear). *Rising* configurations were not identified among the ears with sensorineural hearing impairment. Ten audiograms (13%) did not fit into any of the first six categories described in Table I:1, and were therefore categorized as *unspecified* configuration.

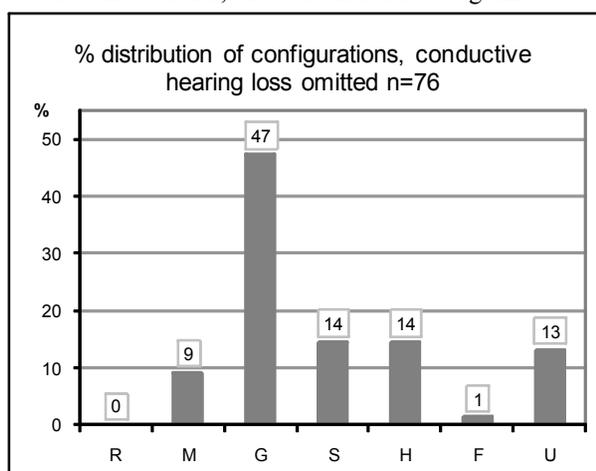


Figure I:3.

R=Rising; M=Mid-frequency U-shaped; G=Gently sloping; S=Steeply sloping; H=High frequency U-shaped; F=Flat configuration; U=Unspecified configuration

4.2 PAPER II

4.2.1 Audiometry

In this group of 30 Turner women eleven had 4FA-thresholds of the better ear within the normal limit (<20 dB HL). Small mid-frequency dips and/or solitary high frequency impairment, exceeding what is normal for the age, were seen in 2 cases. Mild hearing loss, with a 4FA within 20-39 dB HL was seen in ten cases, and moderate loss, below 70 dB HL 4FA in nine cases, see Table 2. The TEOAE responses were in accordance with the hearing threshold levels and did not differ significantly from the reference material.

4.2.2 ABR

ABR wave I was reliably identified in 12 cases, wave III in 26 and wave V in 28. The mean latencies were normal in the Turner group. There was no significant difference between the study group and references regarding the latencies of waves I and III, but wave V was significantly shorter in the Turner group (ANOVA, $p < 0.05$). See Table II:1.

4.2.3 Speech recognition scores in noise (SRS in noise)

SRS in noise were within the calculated normal range (control mean - 2SD) corrected for age and high frequency hearing BE according to the information supplied by Barrenäs and Wikström in the reference model (Barrenäs and Wikström, 2000) in 26 women. Two women had scores below the calculated lower limits (cases 26 and 27) and two women could not recognize the words at all during the test procedure (cases 18 and 30). These four women had 4FA of 47.5, 55.0, 51.3 and 52.5 dB HL BE respectively, see Table 2, and they all also had moderate mid frequency loss.

4.2.4 Phase audiometry

The Turner women performed significantly poorer than the reference group. The median interaural time difference (ITD) was 50 μ s (range 20-500 μ s) in the TS group, significantly higher than in the references, 30 μ s (range 10-80 μ s) (Mann-Whitney U statistics for non-parametric data $p < 0.05$). See table II:2.

Case no. ¹	Age	4FA ²	HFA ³	ABR-V ⁴	SRSn% ⁵	SRS-Low ⁶	500 Hz ⁷	PA- μ s ⁸
8	44.8	2.5	0.0	5.6	82	62	10	30
11	45.6	2.5	8.3	5.4	78	54	5	30
14	51.4	2.5	6.7	5.5	76	44	5	20
5	42.1	3.8	10.0	5.5	82	62	5	50
24	59.4	3.8	8.3	5.4	82	60	0	30
6	43.4	6.3	1.7	5.3	78	62	10	30
1	40.5	7.5	16.7	5.3	78	62	5	40
7	43.7	12.5	10.0	5.5	86	62	15	30
29	63.5	13.8	31.7	5.8	64	47	20	50
4	41.6	15.0	16.7	6.3	82	54	15	40
23	58.1	16.3	15.0	5.6	82	54	25	60
10	45.8	21.3	31.7	5.2	56	35	15	500
15	54.2	26.3	35.0	5.6	66	50	20	50
28	64.3	26.3	46.7	5.8	60	48	20	50
3	41.4	27.5	56.7	5.4	65	27	30	40
16	54.2	28.8	48.3	5.2	50	37	15	20
19	56.1	28.8	28.3	5.6	70	45	30	100
12	48.6	35.0	53.3	5.5	58	21	10	50
21	56.5	37.5	48.3	5.9	58	47	30	70
22	57.6	37.5	36.7	5.5	44	38	40 ⁹	-
2	40.8	38.8	33.3	5.4	54	37	35	100
13	50.1	40.0	24.3	5.2	58	49	30	70
25	60.5	40.0	38.3	5.2	64	45	30	50
17	54.4	42.5	46.7	5.8	62	53	30	80
27	61.7	47.5	60.0	5.4	14	22	30	40
30	67.0	51.3	60.0	n.r. ¹⁰	0	4	45	-
18	55.5	52.5	58.3	5.2	0	28	35	500
9	45.6	53.8	38.3	5.2	54	41	45	-
26	60.8	55.0	56.7	5.4	30	40	55	-
20	56.4	58.8	58.3	n.r.	26	8	55	-

Table II.1.

Results of ABR, speech recognition scores in noise and phase audiometry in 30 TS women. The cases are ordered according to hearing level (the value of 4FA). Subdivisions (horizontal lines) mark normal hearing (4FA<20 dB HL), mild (20-39 dB HL) and moderate (40-69 dB HL) hearing loss.

¹ Case numbers assigned according to age at testing.

² Average of thresholds at 0.5, 1, 2 and 4 kHz, better ear.

³ Average of thresholds at 3, 4 and 6 kHz, better ear.

⁴ Latency of ABR wave V, better ear.

⁵ Sound recognition scores in noise; S/N ratio 4 dB; % correct, better ear.

⁶ Lower limit for SRSn; mean value - 2 SD, calculated considering age and HFA according to Barrenäs and Wikström, 2000.

⁷ Threshold at 500 Hz, worse ear.

⁸ Phase audiometry. Interaural time difference in μ s.

⁹ Numbers in bold figures indicate thresholds at 500 Hz worse ear > 35 dB HL. Phase audiometric results are not given.

¹⁰Not reproducible.

	0.5 kHz > 35 dB HL excluded	Median ITD (*)	Mean ITD, μ s	Range ITD, μ s	Mean threshold at 0.5 kHz, dB HL	Range at 0.5 kHz, dB HL
TS	25	50	85.2	20-500 μ s	19	0-35
References	33	30	40	10-80 μ s	4	0-20

Table II.2.

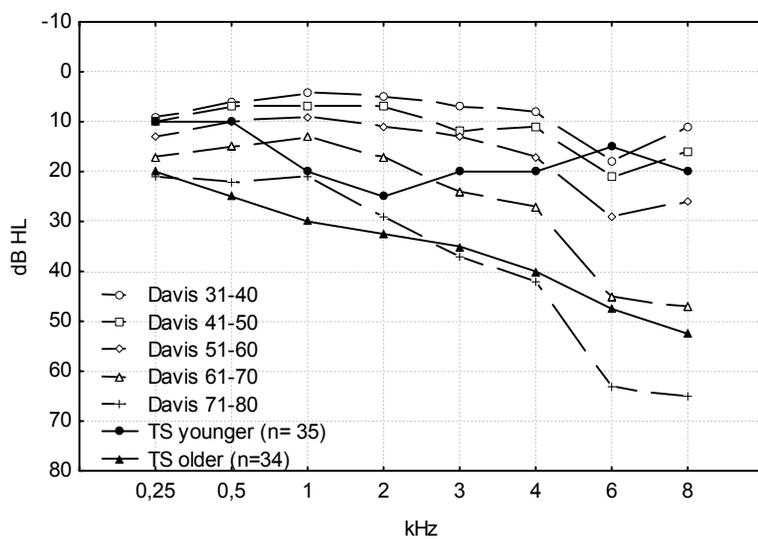
Results of phase audiometry expressed in median and mean interaural time differences (ITD) in μ s. *) significant difference between TS and reference group, $p < 0.05$ (Mann Whitney U statistics for non-parametric data). ITD= interaural time difference. TS=Turner Syndrome.

4.3 PAPER III

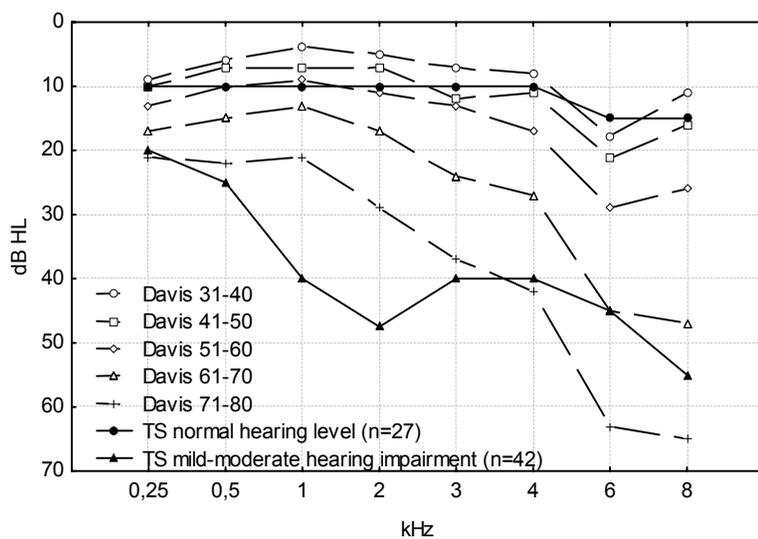
4.3.1 Hearing at baseline

In this population of Turner women, aged 28-62 at baseline, hearing deteriorates at a rapid pace. Figures III:1a-d show the baseline median hearing threshold levels in the better ear, in each figure with two subdivisions according to III:1a) initial age; younger (mean age 36.1) and older (mean age 51.3); III:1b) initial hearing level; normal hearing (4FA < 20 dB HL) and hearing impairment (4FA \geq 20 dB HL); III:1c) karyotype: "mosaics" and "45,X / XiXq"; and III:1d), subdivided by presence or absence of a dip. The cross-sectional reference data according to Davis' corresponding age groups (median, better ear, sample "ALL", occupational group "Overall") are shown (Davis, 1995).

III:1a



III:1b



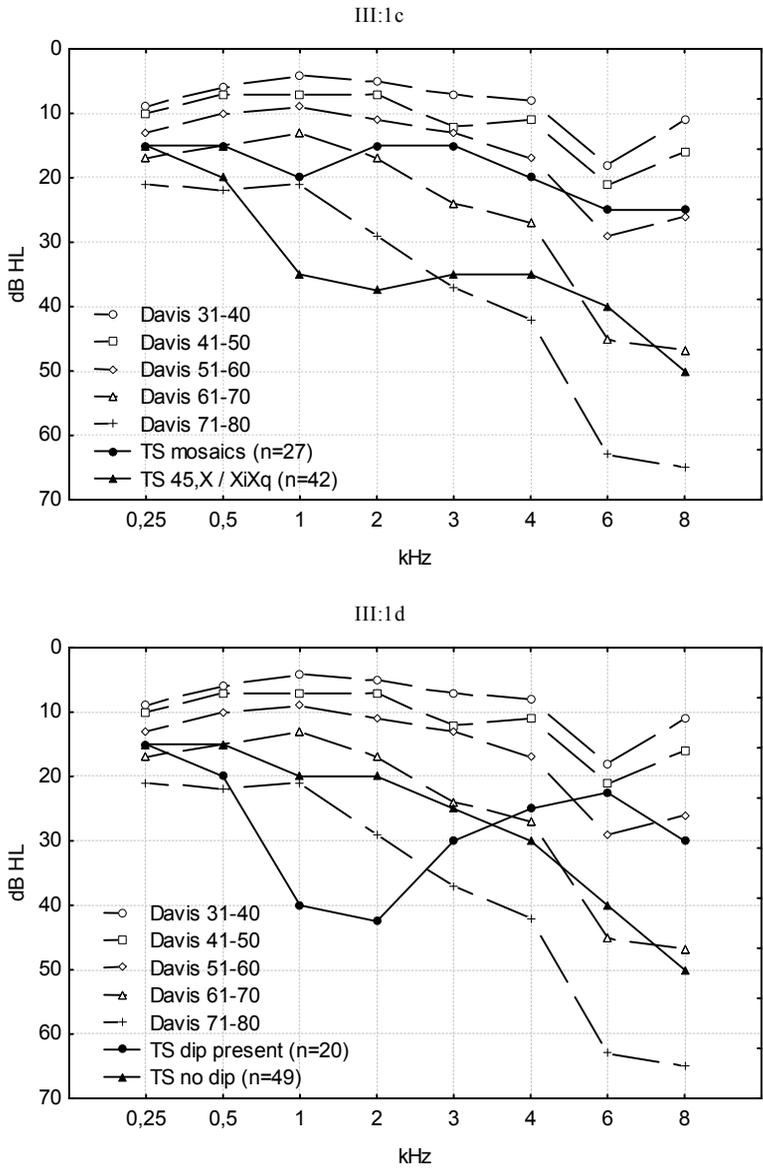


Figure III:1a-d. Baseline median hearing thresholds in dB HL in 69 TS women: III:1a. Divided into two age groups: younger (n = 35, mean age = 36.1 years) and older (n = 34, mean age = 51.3 years). III:1b. Divided into normal (4FA \leq 20 dB HL) hearing (n = 27, mean age 40.5 years) and mild-moderate (4FA > 20 dB HL) hearing impairment (n = 42, mean age 45.6 years). III:1c. Divided into two groups according to karyotype: mosaics (n = 27, mean age 44.7 years) and 45,X / XiXq (n = 42, mean age 42.9 years). III:1d. Divided according to presence (n = 20, mean age 40.0 years) or absence (n = 49, mean age 45.1 years) of a mid-frequency dip. In all four figures comparisons to normative median data from Davis, 5 age groups are supplied (Davis, 1995).

The initial median hearing threshold levels in the mid-frequencies were comparable to those in normal women 20-40 years older than the actual age of the TS women. In low

and high frequencies the discrepancy was not as pronounced in the younger women, but quite apparent in the older TS women.

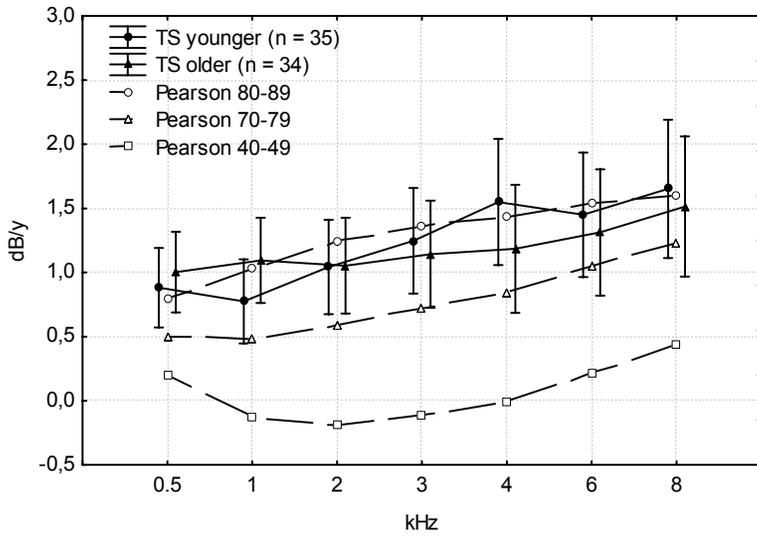
4.3.2 Hearing decline

The mean annual rate of hearing decline is shown in Figures II:2a-d, subdivided according to initial age, initial hearing threshold levels, karyotype group and presence/absence of a dip. For reference purposes, mean curves for the annual decline of the two oldest age groups (70-79 and 80-89) and the age group matching the TS mean age (40-49) are included in each figure (Pearson et al., 1995).

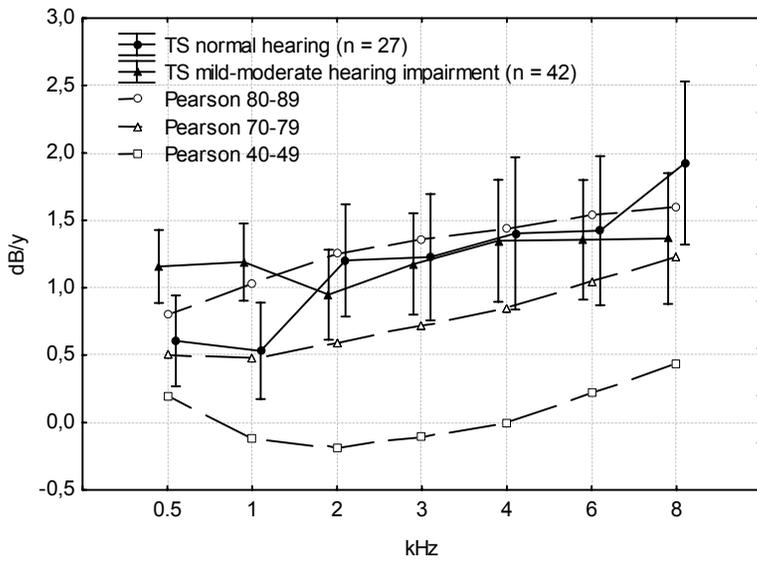
The average rate of decline in all groups is at a level of 0.5-2.2 dB/year for all frequencies, in general higher in the high frequencies. The women with an initial dip have the highest rate of decline of all subgroups, as high as 0.9-2.2 dB per year, especially high (1.6-2.2 dB per year) in the high frequencies (3-8 kHz). This means that on average the TS women will lose 5-22 dB hearing threshold levels every ten years regardless of age, initial hearing level, karyotype or presence/absence of a dip. The rate of decline in all sub-groups is thus comparable to that of 70-89 year old women in the population. The corresponding Pearson age group matching the mean age of our TS cohort (40-49) has an annual rate of decline between -0.2 and 0.4 dB per year.

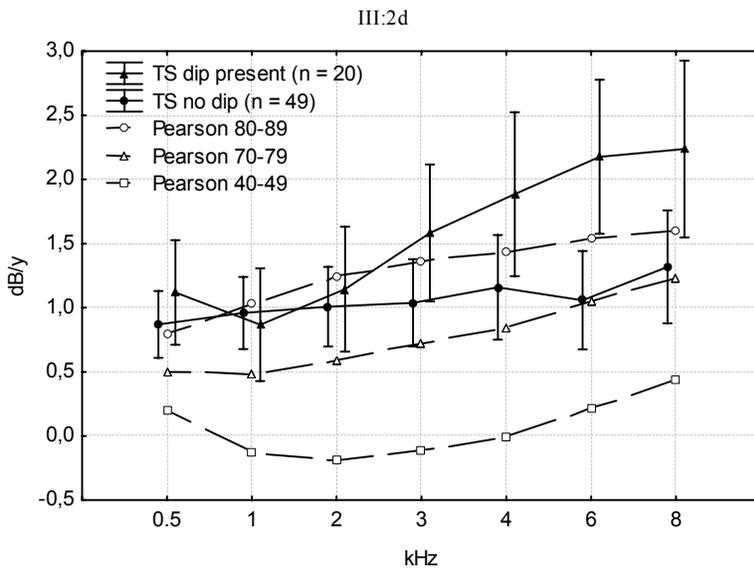
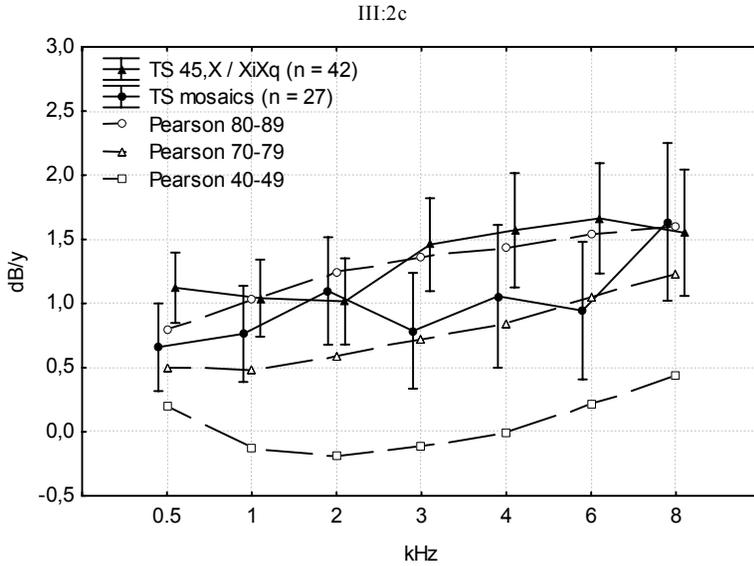
Further subdivisions of the groups were applied, e.g. to see whether the younger TS women with dips had a different rate of acceleration than the older women with dips, however this rendered too small groups to be able to draw any statistically significant conclusions.

III:2a



III:2b





Figures III:2a-d.

Mean annual rate of hearing decline in dB per year in 69 TS women:

III:2a. Divided into two age groups: younger (n = 35, mean age = 36.1 years) and older (n = 34, mean age = 51.3 years). III:2b. Divided into normal (4FA ≤ 20 dB HL) hearing (n = 27, mean age 40.5 years) and mild-moderate (4FA > 20 dB HL) hearing impairment (n = 42, mean age 45.6 years). III:2c. Divided into two groups according to karyotype: mosaics (n = 27, mean age 44.7 years) and 45,X / XiXq (n = 42, mean age 42.9 years). III:2d. Divided according to presence (n = 20, mean age 40.0 years) or absence (n = 49, mean age 45.1 years) of a mid-frequency dip. Vertical bars denote 95 % confidence intervals (two-sided). Reference values, according to Pearson et al. age groups 40-49, 70-79 and 80-89 are supplied in each figure (Pearson et al., 1995)

4.4 PAPER IV

4.4.1 Pure tone thresholds

The mean hearing thresholds for the left and the right ears at baseline, mean age 51.3, and at follow-up, mean age 58.7, are presented in figure IV:1. At baseline, the mean thresholds at all frequencies were better than 20 dB HL. At follow-up, the mean thresholds at 6 and 8 kHz were poorer than 20 dB HL. In the low frequencies the thresholds were better in the left ear, and in the high frequencies the reverse was seen.

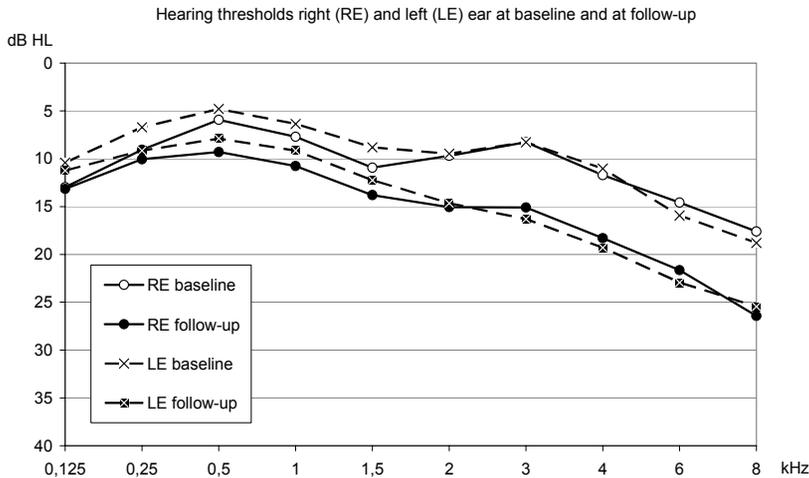


Figure IV:1. Mean hearing thresholds in dB HL of the left and the right ears at baseline and at follow-up.

4.4.2 Hearing decline

The mean annual rate of hearing decline in dB/year, between baseline and follow-up, for the right and the left ears, is shown in figure IV:2, with thresholds from the Baltimore Longitudinal Study of Aging (BLSA), six different aged cohorts, supplied in the figure for reference (Pearson et al., 1995). The rate of decline in the studied group of women most closely resembles that of the age group in the BLSA study referred to as 70-79 years. Further subdivisions for each ear into two groups according to time since menopause, 0-5 years since menopause (MP 0-5) or at least six years since menopause (MP ≥ 6), are shown in figures IV:3a-b.

The mean rate of decline in frequencies from 0.125 to 1.5 kHz does not exceed 0.5 dB/year in either the left or the right ear. In the high frequencies, 3-8 kHz, the mean rate of decline is approximately 1 dB/year in both ears.

The mean rate of decline in the right ear is significantly higher at 1.5, 2 and 4 kHz ($p < 0.01$, $p < 0.01$, $p < 0.05$) in the subgroup of women where at least six years have passed since the final menstrual period (MP ≥ 6) as compared to the subgroup where the menopause was more recent (MP 0-5). In the left ear the corresponding difference is significant only at 1 kHz ($p < 0.05$). There exists a correlation between age and years since menopause (correlation coefficient 0.25, $p < 0.05$), but there is no significant age difference between the women with a recent menopause (MP 0-5) and the women in which menopause occurred at least six years earlier (MP ≥ 6).

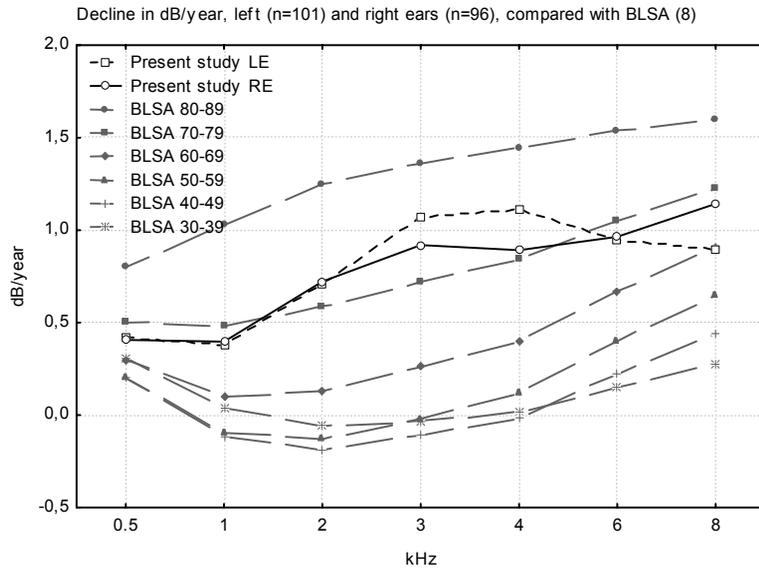
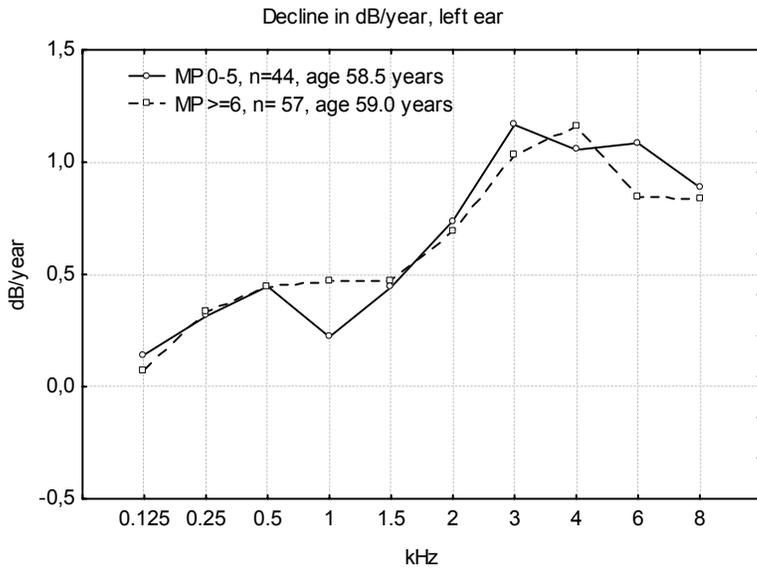
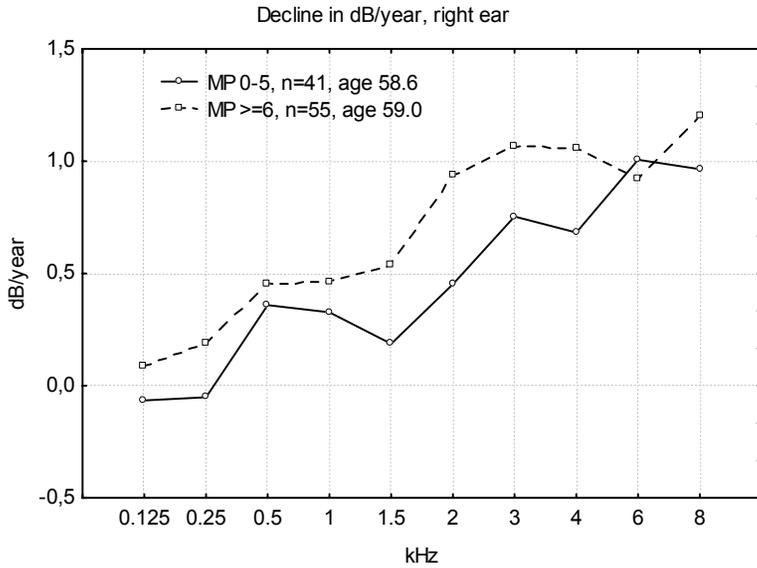


Figure IV:2.
 Mean annual rate of hearing decline in dB/year for the left and the right ears between baseline (mean age 51.3) and after an average of 7.5 years of follow-up (mean age 58.7). Total n=101. Five right ears with conductive hearing loss are excluded. Hearing decline curves from the BLSA for six different aged cohorts (30-39, 40-49, 50-59, 60-69, 70-79 and 80-89 years) are supplied in the figure for reference (8).



Figures IV:3a and b.
 Mean annual rate of hearing decline in dB/year for a) the right ears and b) the left ears. In each figure the total group of women is subdivided according to time in years since the final menstrual period: not more than five years since the final menstrual period (MP 0-5), at least six years since the final menstrual period (MP >=6). Total n=101. Five right ears with conductive hearing loss are excluded.

5 DISCUSSION

The aim of this thesis was to study various aspects of hearing function in middle aged women in the general population and in women with Turner syndrome. These studies are parts of a broader investigation of both women and animals, focused on elucidating if, and to what extent, estrogen protects hearing function.

5.1 MIDDLE AGED WOMEN IN THE GENERAL POPULATION

5.1.1 Hearing thresholds

At baseline our study group of 143 women in peri-menopausal age (47-53 years) had median hearing threshold levels closely resembling a comparable group of Swedish women in an epidemiological study by Johansson and Arlinger (Johansson and Arlinger, 2002). A small proportion of the 143 women had hearing levels meeting the requirements for at least a mild or a slight hearing impairment in the better ear by the respective classifications of grades of hearing impairment by HEAR (7 %) (Martini, 2001) and the WHO (5.6 %). However, averaging the hearing thresholds at the frequencies 0.5 – 4 kHz, in the better ear, does not provide any information about very minute hearing loss, affecting only occasional frequencies, causing either dip-shaped threshold elevations, or a very restricted high frequency hearing loss. In order to identify hearing losses at a very early stage, before any functional deficiency is apparent, such minute hearing impairments must be observed. If only the better ear is considered, all cases with unilateral hearing loss with rehabilitative needs are missed.

5.1.2 Audiometric configuration

In our study of presumably very early age-related hearing impairment, the gently sloping audiometric profile was by far the most common one (47%). Further, steeply sloping audiograms and high frequency troughs and dips were common, seen in 29% of the cases. We do not know in detail to what extent these women have been occupationally or otherwise exposed to noise, but it is likely that this exposure is low as the median hearing thresholds of the total group of women closely correspond to the by Johansson and Arlinger suggested levels for otologically unscreened and non-occupationally noise exposed women of the same age (Johansson and Arlinger, 2002). None of the women was working in an environment with high levels of occupational noise at the time of the study. Further the mean hearing threshold levels are markedly better in the 3-8 kHz range as compared to the corresponding curve in Davis' study from Great Britain, indicating that there nevertheless is a difference in noise-exposure, although the comparison is made with the non-occupationally noise exposed sub-group (Davis, 1995).

Existing definitions of audiometric configurations were defined to classify genetic hearing impairment in children or noise-induced hearing loss in adults (Martini, 2001, Perez et al., 2000). Other criteria appear to be more relevant in classifying configurations of adults in the early process of age-related hearing impairment. With the arrival of a new generation of hearing aids, intended for persons with mild to moderate high-frequency hearing losses and possibly normal hearing thresholds in low and mid frequencies, a four frequency average including 0.5, 1, 2 and 4 kHz will not suffice to identify these potential hearing aid users. We therefore propose a more detailed structure for classification (Table I:1). We experienced a need for having separate categories for mid- and high frequency U-shaped configurations (dips or notches), as these different types of configuration have different implications

concerning speech perception. 13% of audiograms did not meet the criteria for the proposed configurations. With more liberal implementation of the definitions this percentage may be further lowered. Thus, the combination of a generous definition of hearing impairment and a detailed description of the audiometric configuration gives us more adequate information in the process of identification of persons with rehabilitative possibilities.

5.1.3 Thresholds and decline

At base-line the hearing thresholds were very accurate in the majority of the women. We regarded this as a favorable situation, as it reduced the risk of confounding by other types of inner ear disorders. The specific selection criteria provided us with a group of healthy women, at the brink of entering the period in life where we know from epidemiological studies that ARHI in women starts. Accordingly, the deterioration of hearing thresholds of this cohort had been very minute up until this age.

A follow-up after two years was performed, but did not yield any significant information, although our findings showed a trend that the group of women on HRT had a higher rate of hearing decline than other women. This information initiated a prolonged longitudinal study with yet another follow-up after an additional 5-6 years. During this period, a large proportion of the women stopped taking combination-HRT, most certainly at least partly due to information provided from the WHI-study, implying increased risk for CHD, breast cancer, pulmonary embolism and stroke. This meant that we did not have sufficiently large groups on HRT for statistical analysis of the possible effect of HRT on hearing function leading to a focus on the actual time for menopause as a possible initiator of hearing decline.

At follow-up, on average 7.5 years after the initial hearing test was performed, the hearing decline in the 101 participating women was very apparent (see figure IV:2), and higher than expected when comparing with age-matched reference data (Lee et al., 2005, Pearson et al., 1995).

5.1.4 Protection from estrogen

There was a clear tendency towards a higher rate of hearing decline in post-menopausal women, the more years that had passed since the final menstrual period, regardless of age. Further, it appeared that women who had not yet, or only recently, entered menopause, still had a protection against hearing decline in the right ear, whereas the protection had already been reduced in the left ear. When more than five years had passed since the final menstrual period, even the right ear had a surge of decline, catching up with the left ear. We interpreted this as indirect evidence that the diminishing levels of endogenous estrogen during and after menopause were responsible, at least in part, to the accelerated hearing decline in these women. The findings are in accordance with the previous findings in animals of Guimaraes et al., (2004) where distortion product otoacoustic emissions (DPOAE) were higher in female animals before menopause, compared to after, and compared to male age-matched animals, indicating that DPOAEs are better where endogenous estrogen levels are higher; and of Thompson et al., (2006), who illustrated that estrogen blockade with tamoxifen administration negatively affected the protecting medial olivocochlear (MOC) efferent feedback system to the cochlea, i.e. estrogen appears to boost the protection of the efferent system.

5.1.5 Interaural difference - Left ear disadvantage

We found the right-left differences intriguing and to some extent surprising in this rather small study group. The existence of differences in hearing acuity between the left and right ears is well established, and is apparent already in the neonatal period, since newborn girls and boys have slightly, but significantly stronger otoacoustic emissions in the right ear than in the left (Khalifa et al., 1997, Berninger, 2007). The most comprehensive studies of interaural differences in hearing thresholds have been performed by a Finnish research group (Pirilä et al., 1991a, Pirilä et al., 1991b, Pirilä et al., 1992). They showed that the left ear had slightly better hearing thresholds at all frequencies below 15 years of age, in both boys and girls. After 15 years of age there was noticeable left ear inferiority at 3-8 kHz in all age groups, more pronounced in men. In women, the authors described a slight decrease in the left ear inferiority between 25 and 45 years of age, and after that a slight increase (Pirilä et al., 1992). In the oldest age groups, 70-90 years, the right-left difference is small in males, and virtually non-existent in females at 4 kHz (Jönsson and Rosenhall, 1998). The etiology of the peripheral left ear disadvantage in the high frequencies has been discussed. Noise induced hearing loss has been proposed as a major cause for the right-left difference that is most pronounced at 4 kHz in both males and females, but several authors have shown that the difference remains regardless of handedness or eyedness in shooting, or hearing level (Job et al., 1998, Nageris et al., 2007, Pirilä et al., 1991a, Rudin et al., 1988). Khalifa et al showed that the MOC-system has a functional right-ear predominance in adults, implying a higher grade of efferent feed-back and as a consequence protection of the right cochlea (Khalifa et al., 1997).

Estrogen, as described under 1.1.4, appears to protect hearing through the MOC efferent system, and if this is functionally predominant in the right ear this may be the reason for the right ear maintaining hearing thresholds longer than the left ear during the course of the menopausal transition. When the circulating endogenous estrogen levels decline, during the course of the menopausal transition, the left ear loses its protection through the MOC-system first, and the right ear follows, but several years later, congruent with the findings of Pirilä et al (1992) in women aged 25-45, and those of Jönsson and Rosenhall (1998) in older women.

5.2 WOMEN WITH TURNER SYNDROME

In both studies in TS women the intention was to study the nature of the sensorineural and central auditory function, unaffected by confounding factors such as conductive or severe to profound hearing impairment and otological disease, as these factors are quite likely to have an influence on the interpretability of test results. Further we chose not to study the younger women who received HRT in adolescence; as such a treatment in itself, according to our hypothesis of hearing protection from estrogen, may be confounding. These deliberately selected groups are thus not representative of the whole TS-population for the purpose of obtaining “pure” materials for studying sensorineural and central aspects of hearing function.

5.2.1 Auditory function

We found that the studied TS women had normal TEOAEs, ABR recordings and speech recognition scores in noise, with few exceptions, thus implying a cochlear origin for the hearing loss according to Møller and Møller (1985), and concordant with previous reports in TS women (Anderson et al., 1969). The mean ABR wave V-latencies in TS women were slightly but significantly shorter compared to the control

group. This intriguing finding may be explained by anatomical differences with a shorter length of the cochlear nerve. However, there are contradictory reports in the literature regarding the concept that differences in size of anatomical structures may be the basis for gender differences in ABR-latencies (Dempsey et al., 1986, Durrant et al., 1990). There was no evidence of auditory neuropathy, retrocochlear pathology or brainstem lesions.

5.2.2 Sound localization

Phase audiometry is based on the ability of the auditory system to detect minute phase shifts (Δp) between the ears of a dichotically presented 0.5 kHz tone. Young and middle aged individuals with normal peripheral and central auditory function can identify interaural time differences (ITD) of 10–60 μs . Normal sound localization is dependent on fairly intact peripheral hearing and on normal processing in the central auditory system. One great advantage with the method is that it is relatively insensitive to sensorineural hearing impairment of cochlear origin. High-frequency hearing loss can be tolerated, provided that the low-frequency hearing is normal or only mildly impaired. Patients with retrocochlear or central lesions have ITD varying from 160 to more than 400 μs (Almqvist et al., 1989, Rosenhall, 1992, Rosenhall et al., 1998).

We found that women with TS had median phase audiometric results (50 μs) significantly higher than the reference group (30 μs). The results show resemblance with findings in an elderly population where 75-year old women had slightly abnormal sound localization: a mean ITD of 104 μs compared to 59 μs in controls (Jönsson and Rosenhall, 1994). The results may not be directly comparable in these two different studies, owing to the fact that the test settings may have been different, and the level of presentation of the 0.5 kHz tone is not supplied by Jönsson and Rosenhall, and, but the ratio between TS women and our controls resembles the ratio between older and younger subjects in the Jönsson and Rosenhall study.

5.2.3 The neurocognitive phenotype in Turner syndrome

The characteristic neurocognitive phenotype in TS, described in detail by Ross et al., consists of selective deficiencies in certain domains (Ross et al., 2006, Ross et al., 2002). Verbal abilities are usually normal; TS girls, on average, have a variety of neurocognitive impairments, among them impaired visual-spatial and visual-perceptual abilities. In adult age many of these disabilities are not as apparent, possibly due to the hormonal treatment in puberty. The visual-spatial and visual-perceptual abilities seem to remain decreased in adulthood despite hormonal treatment, therefore giving rise to speculations that the decline in these functions has a genetic etiology. A small interval of distal chromosome Xp has been identified where a deletion showed a statistically significant association with the hormone independent defined TS neurocognitive phenotype, concluding that it is genetic in etiology and is due at least in part to haploinsufficiency for gene(s) in distal Xp22.3 (Zinn et al., 2007).

Holzappel et al. performed neuroimaging on females with Turner syndrome in search of altered areas causing the visual-spatial and sensorimotor dysfunction in these women (Holzappel et al., 2006). They report changes in the left parieto-occipital region extending into the frontal lobe and bilaterally in the internal capsule extending into the globus pallidus and in the right prefrontal region. Studies on visual-spatial working memory in Turner females showed reduced sustained activation in fronto-parietal regions on functional MRI (fMRI) compared to controls (Hart et al., 2006).

The neural basis of temporal and spatial auditory discrimination has been studied through fMRI, (Pastor et al., 2006) revealing among other findings, that both types of auditory discrimination activated a network of brain areas including regions of the prefrontal cortex and basal ganglia. Patients with Parkinson's disease, with impairment of function in basal ganglia, have significantly reduced acuity of sound lateralization compared to age-matched healthy controls (Lewald et al., 2004). At present there is no evidence that the visual-spatial functions and sound localization ability activate the same neural mechanisms.

Our findings suggest that auditory-spatial dysfunction is another facet of the neurocognitive problems experienced by the TS women. Since it resembles the sound localization performance of 75-year old women it could possibly indicate early aging of the central auditory system.

5.2.4 Hearing decline and audiometric configuration

In this study we have shown that women with TS have a progressive type of hearing impairment, deteriorating at a very rapid pace in adult age. Regardless of initial age or hearing level, karyotype, or presence/absence of a mid frequency U-shaped audiometric configuration, the rate of hearing decline is much higher in TS women than in age matched women from the general population. The rate of progression in young and middle aged TS women is on a level comparable to that seen in 70-90-year old women in the general population. The highest progression rates are seen in high frequencies (3-8 kHz).

Cross-sectional studies on hearing thresholds in different age groups in the general population have shown that the threshold levels are fairly constant in women up until the age of 50 or 60, whereas the threshold decline in men starts in the high frequencies as early as in the third or fourth decade (Borchgrevink et al., 2005, Davis, 1995). Longitudinal studies of hearing threshold change are generally considered to be more accurate, as each subject acts as her own control, thus minimizing effects of confounding factors, such as other health issues and other cohort related factors. Age related hearing impairment progresses with increasing age, but the extent of the progression has been debated (Jönsson et al., 1998, Cruickshanks et al., 1998, Lee et al., 2005, Pearson et al., 1995).

In TS women several studies have shown that there is an early onset mid-frequency hearing loss, sometimes seen as early as before puberty although late teens and early adulthood seem to be a more common age for the start of this hearing loss (Hultcrantz and Sylven, 1997, Stenberg et al., 1998, Beckman et al., 2004, Sculerati et al., 1996). Twenty of 69 women in this study fulfilled the specific requirements for a mid-frequency U-shaped configuration according to the chosen definition (one or more adjacent thresholds between 0.75 and 2 kHz are at least 20 dB poorer relative to any threshold at lower frequencies and at least 15 dB poorer relative to any threshold at higher frequencies). It is further likely that a large proportion of the women have had this type of configuration earlier, before a high frequency decline appeared. Fifteen of the remaining 49 women fulfilled the requirements for the mid-frequency dip, with a clear demarcation towards the lower frequencies, but they had no rise towards the higher frequencies since they had marked high-frequency impairment. An additional ten to fifteen women had audiograms that demonstrated the typical configuration but did not fulfill the requirements, i.e. the dip was not as pronounced. This mid-frequency dip shows resemblance to other genetic patterns of hearing impairment, and progresses

in severity with age. This dip has been attributed to Turner karyotype, where genotypes with loss of all or part of the p-arm appear to run a greater risk of acquiring this loss. The presence of a dip has therefore been suggested as a poor prognostic sign regarding hearing (Barrenäs et al., 1999, Hultcrantz and Sylven, 1997, Hultcrantz et al., 1994).

In general, it is considered that mid-frequency hearing impairment implicates a genetic influence. Different loci on various chromosomes, e.g. the TECTA-gene on chromosome 11, have been identified in persons with this type of audiometric configuration (Iwasaki et al., 2002). Histopathological findings of temporal bone studies in two cases with clinically well-documented, non-syndromic, autosomal dominant, mid-frequency SNHI include loss of hair cells, stria vascularis, and cochlear neurons in one case and loss of hair cells and stria vascularis in the second case (Shah et al., 2005).

5.2.5 High-frequency decline and ARHI

A high-frequency decline appears in adulthood, and seems to engage a large proportion of TS women. It has been speculated that also the degree of high-frequency decline might be attributable to karyotype, yet this has not been qualitatively shown. The high frequency decline resembles the most common type of ARHI, starting in the high frequencies, and gradually engaging mid and low frequency regions.

The pathophysiological features corresponding to the hearing loss in aged TS women is not fully known, but cochlear morphology in a Turner mouse, described by Hultcrantz et al, is associated with outer hair cell (OHC) loss, most pronounced in the basal turn of the cochlea (Hultcrantz et al., 2000). There is also pathology of the inner hair cells (IHC), except in the apical turn, swelling and degeneration of the nerve endings and disintegration of the stria vascularis and the spiral ligament. This pathology is more pronounced in older animals. These findings have striking similarities to ARHI in research animals (Ohlemiller, 2006).

ARHI in humans was characterized through temporal bone studies by Schuknecht and Gacek as sensory, neural, metabolic or cochlear conductive. The classification was based on audiometry and on the morphologic features on light microscopy (Schuknecht and Gacek, 1993).

The general trend of the progression pattern of the SNHI in TS women thus appears to have two components: a mid frequency decline similar to assumed genetic hearing loss patterns, and a high frequency decline resembling ARHI, or sensory or mixed presbycusis as termed by Schuknecht and Gacek (Schuknecht and Gacek, 1993). The etiology of ARHI is certainly multifactorial, but the influence of genetic factors is assumed to be high, in the order of 50% (Karlsson et al., 1997).

5.2.6 Rate of hearing decline

Hearing decline in women in general is, as stated earlier, fairly slow up until the age around fifty, but accelerates after menopause. At this stage in a woman's life, the circulating levels of endogenous estrogens drop rapidly and markedly, and the menopausal transition appears to be the starting point for a more rapid age-related hearing deterioration as seen in papers I and IV. In analogy, the low estrogen levels in TS may be a contributing cause for their rapidly progressive hearing decline.

As mentioned, the rate of hearing decline is high in all adult TS women; 0.5-2.2 dB per year at 0.25-8 kHz. The karyotypes 45,X and 46,XiXq suggest a high predictive value for rapid decline at 3-8 kHz (1.5-1.7 dB per year), as has also been implied by Barrenäs et al (Barrenäs et al., 1999). However, an even higher predictive value lies in the presence of a mid-frequency dip: these women have the highest average rate of decline in the high frequency region (3-8 kHz); 1.6-2.2 dB per year. It is important to note that a large proportion (80%), but not all of the TS women with a dip configuration have the karyotype 45,X or 46,XiXq. Four women (20%) with prominently deep dips had a mosaic karyotype (all four had 45,X/46,XX). More details regarding the degree of mosaicism in these four women are presently not available. In addition, the hearing impairment most likely becomes more socially handicapping when the mid-frequency loss is accompanied by the high frequency loss, as opposed to those affected by only high-frequency loss.

Some caution must be applied when interpreting the mean rate of hearing decline in these TS women, as the sample size is relatively small, and the follow-up times vary considerably in length. There is also a certain variability in the median hearing threshold levels at baseline, and the group sizes differ somewhat, but the main focus of this study was the rate of hearing decline, which seems to be fairly independent of the initial hearing level, see figures III:1b and III:2b.

5.2.7 Hearing loss and social consequences

It is apparent that the high degree of hearing loss seen in such a large proportion of the TS women implicates similarities with the problems of ARHI: when left without rehabilitation there is a great risk of isolation, depression and possibly dementia. We therefore recommend that follow-up strategies for TS women include repeated audiometry, at least every five years, but preferably every two to three years, for all TS women, regardless of initial age, initial hearing threshold levels, karyotype and/or presence of a dip. This ought to facilitate the possibility for these women to access early and adequate technical and other rehabilitative measures. The presence of a mid-frequency U-shaped audiometric configuration in TS may serve as the single most important prognostic factor implying risk for rapid hearing decline with social consequences in adult age.

Women with TS have increased mortality and a reduced life expectancy (Stochholm et al., 2006). As many of their various syndrome-related conditions become both treatable and curable, it is likely that we will see a larger proportion of older TS women with such a high degree of hearing impairment that they will benefit from cochlear implants, and regain hearing function. It is a particularly important task for health providers to identify these TS women with severe to profound hearing impairment, and refer them to appropriate centers. TS women rarely have children, and as they grow older and their needs become greater; it is more likely that they will not have significant others looking after their best interests concerning social and health matters.

5.3 FUTURE STUDIES

5.3.1 Women in the general population

Cross-sectional material on hearing threshold levels in women and men at 70, 75, 85 and 90 will be compiled, to determine the prevalence of various audiometric configurations at various ages, and how they differ between men and women.

These studies are parts in a greater research project where the main goal is to elicit to what extent reproductive hormones may influence the hearing decline in adults. Other goals that are achieved along the way are e.g. the descriptions of audiometric configuration, in TS and in women in general, and the new guidelines for classification. Hopefully this new classification will be a helpful tool in planning for rehabilitative needs in age-related hearing impairment and other adult onset hearing losses. The classification guidelines have already been utilized in a study of hearing problems at working age in 60 patients (Ternevall, 2008).

5.3.2 TS women

Audiograms of all adult TS women in the Stockholm area (close to 200 women) are currently being collected, for epidemiological and descriptive studies of the whole adult TS population, aiming at correlating phenotypes and karyotypes. We will also specifically compare hearing thresholds in older TS women with those in younger women when the groups are large enough to compare non-HRT-substituted TS women with substituted, of the same age.

6 CONCLUSIONS

6.1 MIDDLE AGED WOMEN IN THE GENERAL POPULATION

- In this group of women aged 47-53 at baseline, a large proportion, 40%, have a minimal to moderate hearing impairment, whereas only 7% of the total group meet the requirements for a mild or a moderate hearing impairment according to the suggestions of the European work group HEAR (Martini, 2001) or 5.6% according to the definitions of WHO (WHO, 2008) (for definitions, see Table 1:1). If only an average hearing threshold level at the “speech-frequencies” 0.5-4 kHz is used to identify persons in need of hearing rehabilitation a large number of people who might benefit from hearing aids may be missed. It is therefore important to analyze the audiometric configuration as well – many considerable high frequency hearing impairments are thus identified. Modern technological advances in hearing aid function allow for broader rehabilitative possibilities.
- The most commonly occurring audiometric configurations in the group with identified sensorineural hearing impairment were: gently sloping 47%, steeply sloping 14% and high-frequency U-shaped 14% (for definitions, see Table 1:2).
- 3.5% of the total group had conductive hearing impairment.
- High frequency hearing decline in peri-menopausal women is relatively rapid; at 3-8 kHz the rate of decline is close to 1 dB/year, whereas in the low frequencies, a more subtle decline is seen, no more than 0.5 dB/year between 0.125 and 1.5 kHz.
- The menopausal transition, rather than age alone, sets off a period of accelerated hearing decline in healthy middle-aged women.
- The mid and high frequency hearing decline in peri-menopausal women commences earlier in the left than in the right ear, possibly due to estrogen protection of the more prominent MOC efferent system on the right ear. When at least six years have passed since the final menstrual period, there is rather a catch-up effect where an increased decline is seen in the right ear.

6.2 WOMEN WITH TURNER SYNDROME

- The sensorineural hearing impairment that commonly affects women with TS seems mostly to be of a cochlear origin.
- Mild disturbances of sound localization are seen in TS women who have not been substituted with estrogens during puberty, and can be added to the neurocognitive deficits previously reported in TS. Our study provides no evidence of other forms of central auditory processing disorders in Turner syndrome.
- Young and middle aged women with TS have a progressive type of hearing impairment, deteriorating at a very rapid pace in adult age. The rate of hearing decline is much higher in TS women than in age matched women from the general population, and on a level comparable to that seen in 70-90-year old women, regardless of initial age, initial hearing level, karyotype, and presence/absence of a mid frequency U-shaped audiometric configuration. The rate of decline is high at all frequencies, but most prominent in the high frequency region.

- The presence of a mid-frequency dip is an especially strong predictor for a future high rate of hearing deterioration at 3-8 kHz with following social consequences.
- The hearing decline seems to consist of two patterns, one a genetic mid-frequency dip and the other a high-frequency loss, possibly estrogen-associated, resembling age related hearing impairment.

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