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Estrogen-related receptor gamma and hearing function: evidence of a role in humans and mice

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ABSTRACT

Since estrogen is thought to protect pre-menopausal women from age-related hearing loss, we investigated whether variation in estrogen-signalling genes is linked to hearing status in the 1958 British Birth Cohort. This analysis implicated the estrogen-related receptor gamma (*ESRRG*) gene in determining adult hearing function and was investigated further in a total of 6134 individuals in 3 independent cohorts: (i) the 1958 British Birth Cohort; (ii) a London ARHL case-control cohort; and (iii) a cohort from isolated populations of Italy and Silk Road countries. Evidence of an association between the minor allele of single nucleotide polymorphism (SNP) rs2818964 and hearing status was found in females, but not in males in 2 of these cohorts: $p = 0.0058$ (London ARHL) and $p = 0.0065$ (Carlantino, Italy). Furthermore, assessment of hearing in *Esrrg* knock-out mice revealed a mild 25-dB hearing loss at 5 weeks of age. At 12 weeks, average hearing thresholds in female mice$^{(-/-)}$ were 15 dB worse than in males$^{(-/-)}$. Together these data indicate ESRRG plays a role in maintenance of hearing in both humans and mice.

1. Introduction

The progressive loss of auditory function with advancing years, age-related hearing loss (ARHL), is the most common sensory ailment exhibited by the elderly population. Recent estimates suggest that 438 million individuals worldwide experience moderate or severe forms of hearing loss, a large proportion of which is adult onset (Stevens et al., 2011). The etiology of ARHL is complex; the heritability is estimated to be between 35% and 55% (Christensen et al., 2001; Gates et al., 1999; Raynor et al., 2009), and it is exacerbated by environmental factors, particularly noise (Van Eyken et al., 2007a). Histological studies in both humans (Nelson and Hinojosa, 2006) and animals (Fetoni et al., 2011) show that when the cochlea is examined, the predominant pathological feature is loss of the sensory hair cells, with defects in the stria vascularis and spiral ganglion neurons also evident. Relatively few genetic associations with ARHL have been replicated in independent populations. Associations that have been replicated include *GRHL2* (Van Laer et al., 2008), *KCNQ4* (Van Eyken et al., 2006), and *NAT2*6A* (Unal et al., 2005; Van Eyken et al., 2007b) in candidate gene studies, and 2 different metabotropic glutamate receptors, *GRM7* (Friedman et al., 2009; Van Laer et al., 2010) and *GRM8* (Girotto et al., 2011a) have been identified in genome wide association studies (GWAS). The GWAS so far reported for adult hearing status exhibit the phenomenon of "missing heritability" also observed in other common, complex diseases (Manolio et al., 2009). Given that more than 100 genes are known to be involved in congenital deafness then it is likely that a similar number are involved in susceptibility to ARHL. The future challenge in delineating the etiology of ARHL is to discriminate the valid associations that fall below the genome-wide significance threshold, using replication studies and functional genomics.

ARHL is more common (Cruickshanks et al., 1998; Helzner et al., 2005) and more severe (Pearson et al., 1995), with earlier onset (Davis et al., 1995), in men than in women. Historically, this has been attributed to greater occupational noise exposure in men compared to women, but it is clear that sex differences in hearing loss exist in cohorts without a significant history of noise exposure

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(Girotto et al., 2011b; Pearson et al., 1995). It has therefore been suggested that estrogen may act as an auditory protectant, and there is now considerable evidence linking estrogen signaling, the estrogen receptors (ER), and estrogen-related receptors (ESRR) with auditory protection (Hultcrantz et al., 2006; McCullar and Oesterle, 2009). Hence, mice carrying a targeted deletion of *Erβ* display an age-related hearing loss at 12 months, concurrent with a basal to apical degeneration of the organ of Corti in the cochlea (Simonoska et al., 2009). Additional studies with mice deficient for both ERβ and CYP19A1, which encodes the aromatase enzyme responsible for the aromatization of androgens into estrogens, show that these mice exhibit an impaired response of the auditory system to acoustic trauma (Meltser et al., 2008). Furthermore, mutations in the estrogen-related receptor, *ESRRB*, underlie autosomal recessive, non-syndromic hearing loss in humans (DFNB35) (Collin et al., 2008), and *Esrrb* knockout (KO) mice are deaf by 3 months of age (Chen and Nathans, 2007). A decline in hearing sensitivity has been linked to menopause in both humans (Hederstierna et al., 2010) and mice (Guimaraes et al., 2004). In addition, women with Turner's syndrome who are estrogen deficient undergo an early sensorineural hearing loss characteristic of ARHL (Beckman et al., 2004).

Estrogen-related receptor γ (ESRRG; NR3B3; ERR3) is an additional member of the ESRR family, which, together with ESRRB and a third isoform ESRRA, form the NR3B subgroup of the well-characterized, nuclear receptor superfamily. All 3 paralogues are orphan nuclear receptors and share a high structural homology with the classical ERs (Tremblay and Giguere, 2007). *Esrrg* mRNA has been shown to be present in the mouse embryonic inner ear in the cochlear and vestibular ganglion (Hermans-Borgmeyer et al., 2000), which suggests a role in the inner ear. Here, we investigate the relationship between *ESRRG* and adult hearing status in 3 independent cohorts, 2 population-based hearing cohorts and a case-control association study in a London-based ARHL cohort. In addition, we report for the first time that *Esrrg* knock-out mice are hearing impaired, and we characterize the expression of ESRRG in the adult mouse inner ear.

2. Methods

2.1. Ethics considerations

In regard to human participants, all studies had appropriate ethical consent, and consent forms for clinical and genetic studies were signed by each participant in the study. Ethical approval for the London ARHL cohort was granted from the Royal Free Local Research Ethics Committee (ref 6202). For the Isolated Populations Cohort, approval was granted by the relevant local ethical committee. Details of the ethical permission and consent for the 1958 British Birth Cohort (B58C) can be found at http://www.b58cgene.sgul.ac.uk/consent.php. In regard to animal use and care, Sprague-Dawley rats and C57BL/6J mice used in this study were sacrificed according to the UK Scientific Procedures Act, 1986. Generation and care of the animals and experimental procedures were in accordance with institutional guidelines and national laws for protection of experimental animals, and were approved by the local animal ethics committee (Hamburg 69/01).

2.2. Subjects

2.2.1. B58C cohort

The B58C and the collection of hearing data have been described previously (http://www.b58cgene.sgul.ac.uk/; Ecob et al., 2008; Strachan et al., 2007). In brief, participants were drawn up from 17,638 individuals born in England, Scotland, and Wales in 1 week of March 1958. Of the original cohort, 9377 members were revisited by a research nurse for a biomedical follow-up in 2002–2004. Hearing measure consisted of pure tone audiometry at 1 kHz and 4 kHz at age 44–45 years and were adjusted for sex, nuisance variables (noise at test, nurse performing test, audiometer used in test), conductive loss, and hearing loss in childhood. DNA was collected from 3900 of these individuals and genotyped for 555,164 single nucleotide polymorphisms (SNPs) on the Illumina Infinium Human Hap550 array (data deposited by Dr Panos Deloukas, Wellcome Trust, Sanger Institute, Cambridge, UK). These genetic data have been used extensively as part of the Wellcome Trust Case Control Consortium (WTCCC), (https://www.wtccc.org.uk/) (Barrett et al., 2009; WTCCC, 2007). No associations from the analysis of B58C genetic data and hearing thresholds at age 44-45 reached genome-wide significance (a version of this analysis can be accessed at: http://www.b58cgene.sgul.ac.uk/).

2.2.2. London ARHL cohort

A total of 260 patients with sensorineural hearing loss (SNHL) consistent with an age-related decline were recruited from the adult hearing aid clinic at the Royal National Throat Nose and Ear Hospital, London; this formed our initial patient group (ARHL_1). All were interviewed by an audiological physician and underwent an audiometric examination. Air conduction and bone conduction thresholds at 0.25, 0.5, 1, 2, 4, and 8 kHz and 0.5, 1, 2, and 4 kHz, respectively were measured with masking according to BSA Recommended Procedures (http://www.thebsa.org.uk/docs/RecPro/PTA.pdf). At interview, a questionnaire was completed that recorded relevant medical history, family history of hearing loss, and history of noise exposure. This questionnaire was then amended based on answers to stage 1 questions to become self-directional, and patient recruitment was extended to the Royal Free Hospital, London. An additional 323 patients were recruited across both hospitals, forming our replication group (ARHL_2) and bringing the total number of patients to 583 (ARHL_COM). Patients were subsequently categorized for family history and for noise exposure. Noise exposure was graded using the occupations listed by Lynch and Kil, 2005 and Tak and Calvert, 2008 as a guide: Grade 0 = no noise exposure documented; Grade 1 = low to medium noise exposure; and Grade 2 = medium-to-high noise exposure. (For questionnaires, see Supplementary information S1). Patients were not excluded from the study if there was an asymmetric hearing loss as long as the better hearing ear was consistent with the criteria for late-onset SNHL. (Full details of the exclusion criteria are available upon request). With regard to controls, the control sample group comprised ECACC Human Random Control (HRC) DNA panels. In association analysis, all samples were of white European origin.

2.2.3. Isolated populations cohort

Isolated populations were recruited from Italy and Silk Road countries (for an overall number of 1651 subjects) belong to the International Consortium G-EAR, described previously (Girotto et al., 2011a). In brief, several quantitative measures of hearing function were undertaken: air conduction thresholds were determined at 0.25 kHz, 0.5 kHz, 1 kHz, 2 kHz, 4 kHz, and 8 kHz and pure tone averages (PTAs) of air conduction thresholds were determined for: PTA_{low} (0.25, 0.5 and 1 kHz), PTA_{medium} (0.5, 1 and 2 kHz) and PTA_{high} (4 and 8 kHz).

2.3. Genotyping

2.3.1. London ARHL cohort

Genomic DNA was extracted from blood, using a standard phenol-chloroform extraction procedure, or from saliva, using Oragene DNA extraction kits (DNAgenoTek, Kanata, Ontario, Canada). The *ESRRG* rs2818964 SNP genotyping was performed using ABI TaqMan SNP genotyping assay (C_222941_10) on a SDS7500 Real Time PCR System (Life Technologies, Paisley, UK). Each plate