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Clinical Findings in Obligate Carriers of Type I Usher Syndrome

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Seventeen obligate carriers from nine families with autosomal recessive Usher syndrome type I underwent otological, audiological, vestibular, and ophthalmological examination in order to identify possible manifestations of heterozygosity. Linkage studies were performed and six families showed linkage to chromosome region 11q13.5 while 3 families have so far failed to show linkage to the candidate regions. Eight obligate carriers had an abnormal pure-tone audiogram. Two different audiometric patterns could be distinguished when hearing loss was corrected for age and sex. Four carriers (24%) had significant sensorineural hearing loss (SNHL) which increased at higher frequencies. The other 13 carriers had SNHL of about 10 dB at 0.25 and 0.5 kHz, but less at higher frequencies. Vestibular findings were generally normal. Electro-oculography demonstrated a significant lower mean light peak/dark trough ratio in Usher type I carriers compared to normal control individuals. The methods used in this study were found not to be specific enough to clinically identify carriers of Usher type I syndrome. Nevertheless it is remarkable that a number of obligate carriers showed significant audiological and ophthalmological abnormalities.

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KEY WORDS: genetic deafness, genetic blindness, autosomal recessive disorder, Usher syndrome, retinitis pigmentosa, obligate carrier

INTRODUCTION

Usher syndrome is an autosomal recessive form of retinitis pigmentosa (RP) and congenital hearing impairment. The association of deafness and RP was first described by Von Graefe [1858]. The syndrome was named after Usher who had emphasized its hereditary nature [Usher, 1914]. The prevalence of Usher syndrome in the general population is estimated at 3-4/100,000 [Hallgren, 1959; Nuutila, 1970; Boughman et al., 1983]; the carrier incidence is approximately 1/100 [Boughman et al., 1983]. Usher syndrome was first classified into two, and later into four, clinical types. The existence of type IV is no longer tenable (Table I). Based on gene linkage analysis, type II has been divided into USH2A, which is linked to chromosome region 1q41, and USH2B which has not shown any linkage so far [Kimberling et al., 1990, 1995; Pieke Dahl et al., 1993]. Type I has been subdivided into USH1A which is linked to 14q32, USH1B linked to 11q13.5, and USH1C linked to 11p13-15 [Kaplan et al., 1992; Kimberling et al., 1992; Smith et al., 1992]. About 75% of the Usher type I patients are typed as 1B [Weil et al., 1995].

Although genetic identification of obligate carriers of different types and subtypes of the Usher syndrome may be possible in the near future, the aim of this study was to detect clinical manifestations of heterozygosity. So far, no such manifestations have been found for any autosomal recessive type of congenital deafness [Wildervanck, 1957]. In a previous study on carriers of Usher type USH2A by our group some typical findings were noted, but they did not prove to be sufficiently specific to enable clinical identification of the carriers [Van Aarem et al., 1995]. In this study we examined obligate carriers of Usher syndrome type I in order to trace sub-clinical characteristics of heterozygosity.

FAMILIES AND METHODS

In this study, the parents of 2 or more affected children were considered to be obligate carriers of Usher syndrome type I. The diagnosis was based on medical history, otoscopic, audiovestibular, and ophthalmological examination, and on gene linkage studies.

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TABLE I. Subtypes of the Usher Syndrome

	Usher syndrome type		
	I	II	III
Hearing loss	Severe to profound congenital	Moderate to profound congenital	Progressive congenital
Vestibular function	Absent	Normal	Variable
Retinitis pigmentosa	Prepubertal	Postpubertal	Variable
Linkage	USH1A: chrom. 14q32 USH1B: chrom. 11q13.5 USH1C: chrom. 11p13-15	USH2A: chrom. 1q14 USH2B: unlinked	USH3: chrom. 3q

Seventeen carriers entered the study. Pure-tone audiograms were measured according to the ISO 8253 standard at 0.5, 1, 2, 4, and 8 kHz for air and bone conduction [International Organization for Standardization (ISO) 8253-1, 1989]. The individual P50 threshold values for presbycusis, corrected for age and sex, were calculated according to the ISO 7029 method [International Organization for Standardization (ISO) 7029, 1984]. Additional auditory tests included a speech discrimination test, tympanograms, stapedial reflexes, and brainstem auditory evoked potentials. Electrostagnography was performed to record vestibular evoked eye movements, to test smooth pursuit eye movements and gaze positions, and to elicit optokinetic nystagmus responses. Caloric tests were performed according to previously described methods [Van Aarem et al., 1995]. All 17 carriers underwent a complete ophthalmological examination, including corrected visual acuity measurement, slit lamp examination, ophthalmoscopy, visual field examination with Goldmann perimetry, using test targets I-1, I-4, III-4, and V-5, electroretinography (ERG) and electro-oculography (EOG) [Pinckers, 1979; Pinckers et al., 1994]. The carriers were matched for sex and age with normal controls. The EOG light peak/dark trough ratio of the right eye was calculated and compared to that of the controls. Student's *t*-test was used with a one-tailed significance level ($P = 0.05$).

RESULTS

Figure 1 shows the pedigrees of all 9 families who participated in this study. Consanguineous mating was reported in 3 families. Genetic evaluation of 9 families confirmed linkage to 11q13.5 in 6 families (families C, D, E, F, G, H). Three different heterozygous mutations, two missense and one base deletion, in the myosin VIIA gene at 11q were found in three families (C, D, E). Two mutations substituted a cysteine and histidine codon for the same arginine codon in exon three (families C, E) [Weil et al., 1995]. The other mutation was found in exon one and resulted in a deletion of a base with a frame shift in amino acid sequence and premature termination of protein assembly (family D). Three families (A, B, I) have so far failed to show linkage to the candidate regions for Usher type I.

Medical histories of the obligate carriers did not indicate any relevant ear disease, exposure to ototoxic drugs, or hearing impairment. Carriers 1, 5, and 15 reported previous noise trauma. Otoscopy did not show

any relevant abnormalities. Eight carriers had abnormal pure-tone audiograms. The audiograms of carriers 1, 2, 13, and 15 showed a dip at 4 kHz, most likely due to acoustic trauma. Significant sensorineural hearing loss (SNHL) defined as hearing loss in excess of P95 for presbycusis, was found in carriers 4, 5, 6, and 7. Statistics of the air conduction thresholds minus the P50 for presbycusis were calculated for each ear and frequency (Table II). There was no apparent dependence on frequency; the grand average excessive hearing loss was approximately 9 dB. However, it seemed valid to distinguish between the cases with significant SNHL (4, 5, 6, 7) and the other cases. Carriers 4, 5, 6, and 7 showed increasing hearing loss in excess of the P95 at increasing frequencies (mean values of 10, 11, 14, 28, 37, and 41 dB, respectively). The other carriers showed decreasing hearing loss in excess of the P50 at increasing frequencies (mean values 10, 8, 4, 1, 7, and 0 dB). When the cases with noise trauma (average hearing loss in excess of P50 of 16 dB at 4 kHz) were excluded from this analysis, the mean hearing loss in excess of the P50 at 4 kHz is reduced from 7 dB to 3 dB. Other auditory tests results were all within normal range. There was no spontaneous or gaze-evoked nystagmus and ocular motor responses were normal. Caloric responses were also normal in all of the carriers, except for carriers 4 and 14, who both had a mild unilateral canal paresis.

All the carriers had a corrected visual acuity within the normal range. No signs of retinitis pigmentosa were detected during ophthalmoscopy. Carrier 12 was excluded from further ophthalmoscopic examination because of diabetic retinopathy which had been treated with laser therapy. In 4 carriers, capsular and/or nuclear cataracts were detected, but these were normal for age. In 4 carriers (2, 6, 10, and 15) the EOG light peak/dark trough ratio was <1.80 (P95 of normal). Compared to the normal controls, the light peak/dark trough ratio of the EOG was significantly lower in Usher type I carriers ($P < 0.025$) (Table III) [Pinckers et al., 1994]. The ERG was within the normal range in all carriers.

DISCUSSION

The subjects examined in this study were carriers of Usher syndrome type I. When interpreting the results, it is important to bear in mind that different genetic subtypes of Usher type I syndrome were present in this carrier population.

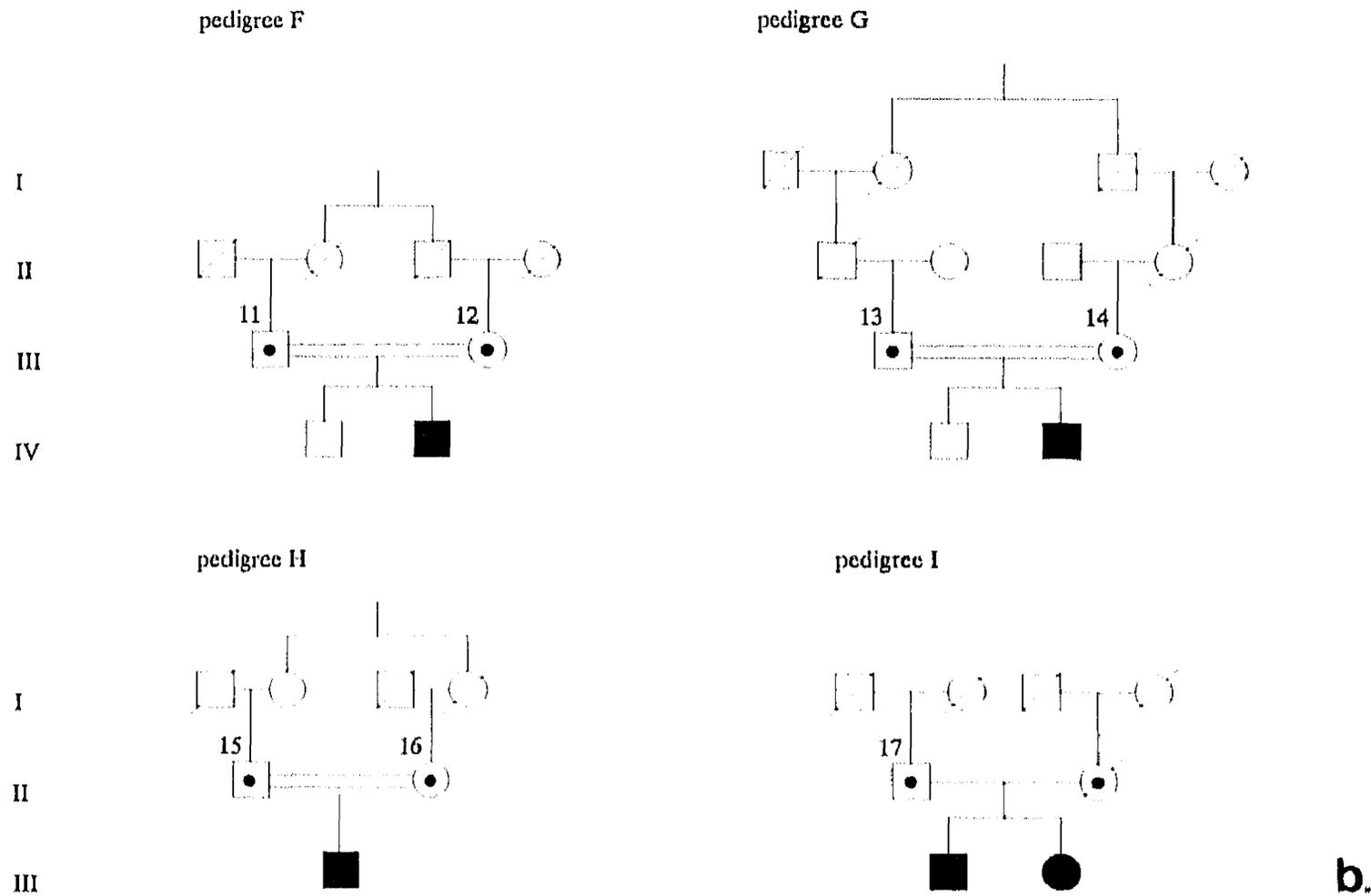
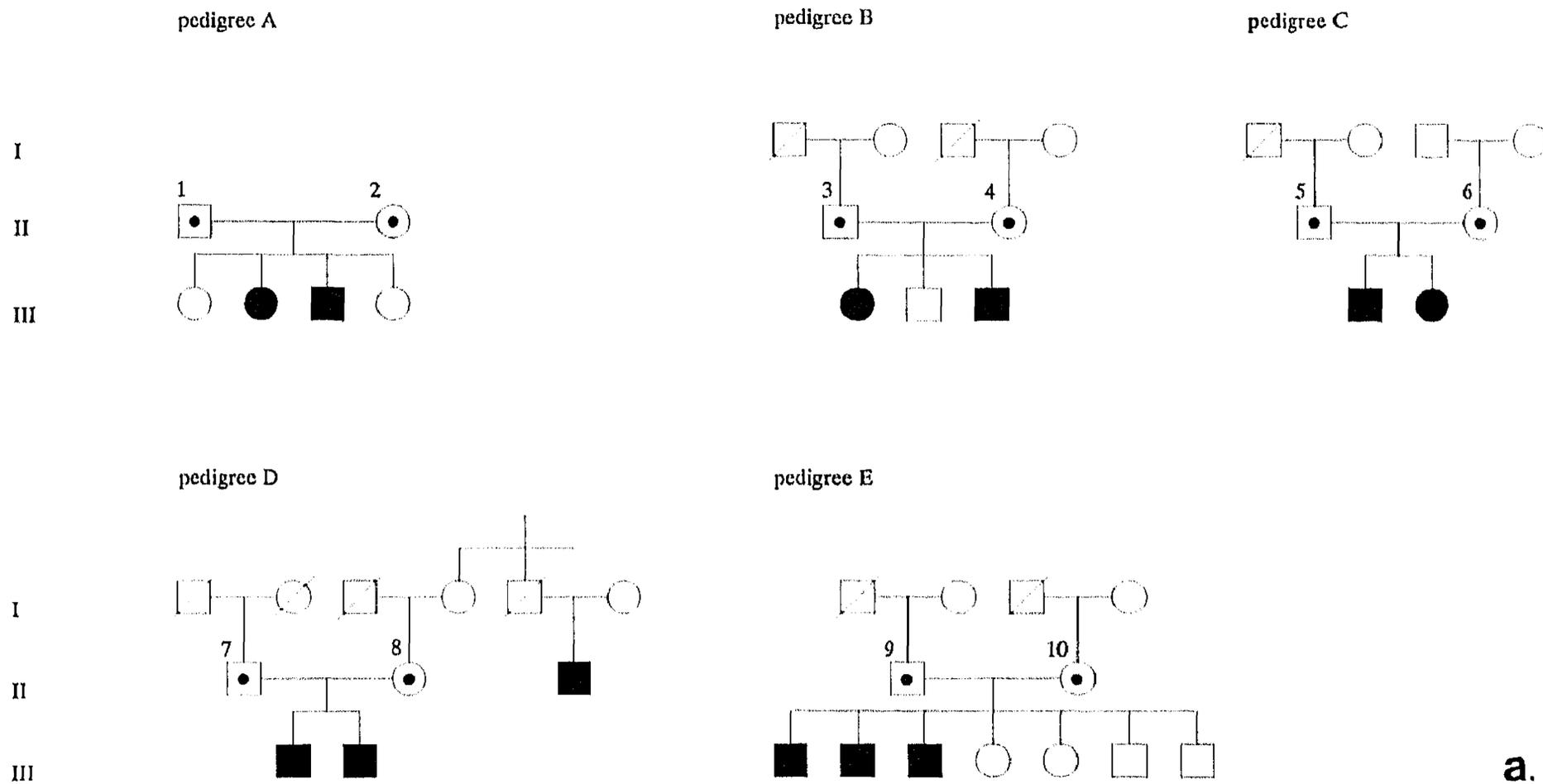


Fig. 1. **a, b:** Pedigrees of the families. □ = male obligate carrier; ○ = female obligate carrier; ■ = affected man; ● = affected woman; □ = unaffected man; ○ = unaffected woman; |/ = deceased man; /| = deceased woman.

TABLE II. Statistics of Hearing Loss (Air Conduction Threshold Minus P50 for Presbycusis in dB HL)

n = 17	KHz					
	0.25	0.5	1	2	4	8
AD						
Mean	9.1	11.4	5.5	6.9	11.8	8.6
SD	6.8	8.5	8.5	11.9	17.8	20.7
AS						
Mean	10.5	5.8	6.6	8.7	17.1	11.0
SD	5.5	5.5	10.6	17.4	17.8	21.5

Four carriers (carriers 4, 5, 6, and 7) had significant SNHL and 13 carriers did not. After correction for the cases with noise trauma, the remaining 13 cases had a hearing loss in excess of the P50 of some 10 dB at 0.25 and 0.5 kHz but lower values at higher frequencies. We did not measure bone conduction thresholds systematically in this study; however, if we assume that there was an average conductive loss of 5 dB as the audiograms seemed to indicate, then there would only be a threshold increase of approximately 5 dB at the lower frequencies. This was found to be valid for all the carriers except for those with significant SNHL. Similar results have been described in carriers of Usher type 2A [Van Aarem et al., 1995]. In 4 obligate carriers (4, 5, 6, and 7) with significant SNHL, we found increasing thresholds at consecutive frequencies (of these carriers, carrier 4 failed to show linkage to chromosome 11q). An increasing threshold at higher frequencies has also been found in patients with the Usher syndrome.

Ophthalmological manifestations of heterozygosity of the Usher syndrome have been reported previously. Kloepfer et al. [1966] reported RP in 13% of heterozygotes of the Usher syndrome. Holland et al. [1972] re-examined these patients and mentioned fundoscopic changes (gyrate atrophy) in 3 of 10 carriers. In contrast with the studies referred to above, no fundus abnormalities were found in the study by Sondheimer et al. [1979] on non-classified carriers of the Usher syndrome or in a study by Van Aarem et al. [1995] on type 2A carriers. We were also unable to demonstrate retinitis pigmentosa-specific changes in the carriers of Usher type I. Subjective dark adaptation (DA) measurements have frequently been used in the past for carrier detection, but the results were contradictory [Goodman and Gunkels, 1958; De Haas et al., 1970]. For this reason we chose more objective methods: EOG and ERG. De Haas et al. [1970] noticed that the incidence of abnor-

mal EOG in his series was too high and too constant to be accounted for by normal variability. In other studies, abnormal EOGs were also found in a high proportion of carriers [Holland et al., 1972; Davenport et al., 1978; Pinckers et al., 1994; Van Aarem et al., 1995]. The mean EOG light peak/dark trough ratio in carriers of Usher syndrome type I was significantly lower compared to that in normal controls. In carriers of Usher syndrome type II, the difference in this ratio was even more significant ($P < 0.0005$) compared to normal controls [Pinckers et al., 1994].

Although the audiological and ophthalmological findings demonstrated abnormalities in Usher syndrome heterozygotes, these tests were not specific enough to be used for individual carrier identification. Therefore, gene linkage studies will be required to identify heterozygotes.

It is nevertheless remarkable that significant abnormalities were found in the carriers. This once again raises the question as to whether Usher carriers are more susceptible to noise trauma, viral infections, otological intoxication, or degenerative processes [McLeod et al., 1971; Karjalainen et al., 1985]. Gene linkage and gene isolation may in due course provide more insight into the pathophysiology of these dual sensory impairments and may help to explain the phenomena found in carriers of the Usher syndrome.

A detection method for heterozygotes of autosomal recessive genes might significantly increase the efficiency of genetic mapping efforts, because the small size of individual families severely limits the amount of data that can be obtained in genetic studies. Our data suggest that it might be possible to obtain a modified estimate of the risk that a clinically unaffected person in an Usher family is a carrier. Using Bayes theorem, this information may then be incorporated into a lod score calculation. However, it should be emphasized that general applicability of this approach requires information on a larger sample of obligate carriers in order to assess the proportion of Usher heterozygotes that fall outside the normal range.

So far, information on heterozygotes in linkage calculations has improved the genetic mapping of several forms of X-linked deafness, but it has not yet proved to be useful in studies on autosomal recessive forms of deafness [Brunner et al., 1988a, b; Shiloh et al., 1990]. The current results are nevertheless encouraging and express the need for further evaluation of this approach in genetic studies on the Usher syndrome and non-syndromal types of autosomal recessive childhood deafness.

TABLE III. Statistics of the EOG Light Peak/Dark Trough Ratio

Group	N	Mean age	EOG Lp/Dt ratio				% Abnormal
			Mean	SD	Min	Max	
USH I	16	55.6	1.98	0.33	1.33	2.66	25
Normal	16	54.5	2.27	0.40	1.63	3.05	12.5

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