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STABLE AND PROGRESSIVE HEARING LOSS IN TYPE 2A USHER'S SYNDROME

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Audiograms were traced or additionally performed on 23 Usher's syndrome patients in 10 Dutch multi-affected families, all linked to chromosome 1q (USH2A locus). Serial audiograms, available in 13 patients, were used for a regression analysis of binaural pure tone average on age (follow-up, 9 to 32 years) to test for "significant progression," ie, a significant regression coefficient, here called the "annual threshold increase" (ATI, expressed in decibels per year). A significant ATI (>1 dB/y) was observed in 3 patients. Analysis of variance of ATI demonstrated significant heterogeneity; hearing loss was either stable or progressive. This implies a significant clinical heterogeneity. A similar analysis performed on our progressive USH2A cases and "type III" cases previously reported by others (ATI of 1 to 5 dB/y), some of which were recently linked to chromosome 3q (USH3 locus), failed to show any significant heterogeneity in the progression of hearing loss.

KEY WORDS — deaf-blindness, gene linkage, genetic deafness, progressive deafness, retinitis pigmentosa, sensorineural hearing loss, type III Usher's syndrome.

INTRODUCTION

Usher's syndrome is an autosomal recessive disorder characterized by congenital sensorineural hearing loss (SNHL) that is combined with progressive pigmentary retinopathy that shows onset in childhood or adolescence and leads to severe constriction of the visual field (tunnel vision). Cataracts usually occur later and cause additional loss of vision, eventually up to the point of (sub)total blindness.

Three clinical subtypes were currently distinguished, before the gene linkage studies started.¹⁻³ Type I involves profound congenital SNHL that causes abnormal speech and language development, combined with vestibular areflexia or severe hyporeflexia, which later in life adds to the problem of spatial disorientation as the visual system further deteriorates. Type II involves moderate to severe SNHL, most pronounced at the higher frequencies, whereas at the speech frequencies there generally is sufficient hearing ability to allow for the normal development of speech and language. Vestibular (caloric) responses can be elicited. The SNHL of this type was defined as stationary throughout life, apart from the develop-

ment of presbycusis at a more advanced age. A clinical type of Usher's syndrome with features similar to type II, in which, however, the SNHL proved to be progressive, was designated as type III.

Gene linkage studies have split type I into three subtypes and type II into two subtypes. Type I has been linked to loci at 14q32 (USH1A),⁴⁻⁶ 11q14 (USH1B),^{5,6} and 11p13-15 (USH1C),⁶ whereas type II has been linked to 1q41 (USH2A).⁷⁻⁹ Usher's cases that fail to show linkage to chromosome 1q41 are classified as Usher's type 2B.^{10,11}

This study shows the long-term results of audiometry in 23 patients from 10 Dutch multi-affected families with Usher's type 2A syndrome. Serial audiograms were collected and analyzed to evaluate whether the hearing loss was stable or showed significant progression.

PATIENTS AND METHODS

Our study population comprised 23 patients from 11 different sibships in 10 kindreds with more than 1 affected person. Blood samples from affected persons and their nonaffected relatives whose medical

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TABLE 1. SUMMARY DIAGNOSTIC AND LINKAGE DATA FOR EACH MULTIPLEX SIBSHIP OR KINDRED ANALYZED

Family	Probability of Linkage to 1q41 Markers	Tentative Audiologic Phenotype
A	.985	II
B	.980	II
C	.972	III
D	.979	II
E	.979	II
F	.997	Mixed
G	.972	II
H and I	.993	Mixed
K	.996	Mixed
M	.955	II

Phenotype II is stable hearing loss. Phenotype III is progressive hearing loss. Mixed phenotype, phenotype III, does occur, but not — with at least some certainty — in all sibs.

history indicated stable hearing loss (type II) were collected and analyzed at Boys Town National Research Hospital for gene linkage. The following chromosome 1 markers had been typed on all family members: D1S245, D1S70, D1S217, D1S237, D1S229, D1S227, PPOL, and D1S81. The Usher's 2A gene is flanked by a 2.4 centimorgan region bounded by D1S237 and D1S229.

The patient's history was taken and a general otorhinolaryngological examination, including audiometry and collecting previous audiograms, was performed with otoscopy in all instances, and the diagnosis of Usher's syndrome was confirmed by one ophthalmologist with assessment of the extent of retinitis pigmentosa, impairment of the visual fields, visual acuity, dark adaptation testing, and an electroretinogram. Vestibular excitability was confirmed with rotatory tests and caloric tests. This report will be limited to the evaluation of the audiometric data.

Audiometric data could be obtained from the files from the Nijmegen otorhinolaryngology department or elsewhere. In all cases clinical routine or serial audiometry was performed according to common standards and with the usual clinical equipment. Serial audiometry over 9 years up to 32 years was available in 13 patients. Most of the patients were also examined in the University Hospital Nijmegen, where pure tone audiograms were measured with an Interacoustics AC5 audiometer (Interacoustics, Assens, Denmark), calibrated following the ISO 389¹² procedure according to the ISO 8253-1 standard¹³ over the most recent years. The following tentative audiologic phenotypes were distinguished: type II (stable hearing loss), type III (progressive hearing loss), and "mixed" (different types occurring simultaneously in one family). The assignment of each

TABLE 2. MULTIPPOINT LOD SCORES OF DUTCH FAMILIES GROUPED BY AUDIOLOGIC PHENOTYPE

Group	Family	Distance From D1S237 (centimorgans)			
		-10	0	+2	+12
II	A, B, D, E, G, M	2.485	3.535	3.463	2.438
III	C	0.414	0.582	0.683	0.415
Mixed	F, H and I, K	2.800	3.834	3.876	2.838
Total		5.699	7.951	8.022	5.691

sibship was established by inspecting plots of the binaural pure tone average (BPTA, in decibels hearing level [HL]) against age; BPTA is the average of the pure tone averages (PTAs, at the frequencies 0.5, 1, and 2 kHz) of both sides. This tentative phenotype was used to test in first approximation whether it discriminated between the linked and unlinked subtypes of Usher's type II. Statistical tests on audiologic data comprised a regression analysis of BPTA on age to see whether the regression coefficient — here called "annual threshold increase" (ATI) and expressed in decibels per year — differed significantly from zero in each individual with sufficient ($n \geq 3$) serial audiologic data available, and an analysis of variance of the ATI values to test for (non)homogeneity in type of hearing loss.

RESULTS

There were 11 different families and 10 different kindreds (the families H and I were the same kindred) with Usher's type 2A. Two of the families involved multiple sibships of affected persons and the lod score data for these families represent linkage analysis on the whole kindred and are not presented on an individual sibship basis. The heterogeneity analysis (Ott's A-test) was employed to determine the probability that each family was linked to a chromosome 1q marker set. The overall χ^2 was nonsignificant when only the 10 Dutch families were included, and the resulting individual probabilities of each family being type 2A were all estimated at 1.0. Only when the Dutch data were pooled with the total set of 68 Usher II families ascertained worldwide were conservative probabilities obtained (the overall rate of heterogeneity was estimated at 12.5%). The resulting probabilities are shown in Table 1 and indicate that none of the families showed convincing nonlinkage with 1q41 markers. Family M had the lowest probability, .955, because it was the least informative of all the families.

As a further test of heterogeneity, the families were divided into three groups, those in which most members fit with the II phenotype with a stable hearing loss (families A, B, D, E, G, and M), those fitting the

TABLE 3. HEARING LOSS, MEASURED BY BINAURAL PURE TONE AVERAGE, AND REGRESSION ON AGE AND ASSIGNED TENTATIVE AUDIOLOGIC PHENOTYPE

Subject	Sex	Age and Binaural Pure Tone Average	Audiologic Phenotype	Regression Parameters		
				r	ATI (dB/y)	SD (dB/y)
A1	F	43 y 0 mo 60 dB HL; 48 y 11 mo 64 dB HL	II			
A2	M	31 y 7 mo 54 dB HL; 44 y 11 mo 65 dB HL	II			
B3	M	55 y 9 mo 34 dB HL	II			
B4	M	24 y 5 mo 32 dB HL; 40 y 6 mo 42 dB HL; 40 y 10 mo 48 dB HL	II	.94	0.805	0.305
C5	F	21 y 3 mo 69 dB HL; 26 y 5 mo 73 dB HL; 31 y 10 mo 82 dB HL; 36 y 10 mo 84 dB HL	III	.97	1.04	0.172
C6	M	11 y 3 mo 52 dB HL; 13 y 5 mo 61 dB HL; 22 y 1 mo 78 dB HL; 28 y 1 mo 82 dB HL; 33 y 7 mo 87 dB HL; 38 y 0 mo 88 dB HL	III	.95	1.31	0.204
D7	M	11 y 4 mo 48 dB HL; 26 y 5 mo 48 dB HL; 27 y 3 mo 53 dB HL	II	.54	0.174	0.271
D8	F	13 y 4 mo 47 dB HL; 22 y 0 mo 48 dB HL; 24 y 2 mo 48 dB HL	II	.98	0.099	0.019
E9	F	6 y 10 mo 49 dB HL; 24 y 3 mo 56 dB HL; 25 y 3 mo 58 dB HL	II	.99	0.450	0.075
E10	M	5 y 7 mo 35 dB HL; 13 y 3 mo 33 dB HL; 20 y 10 mo 38 dB HL	II	.59	0.196	0.266
F11	F	5 y 7 mo 48 dB HL; 10 y 7 mo 48 dB HL; 14 y 4 mo 54 dB HL; 26 y 8 mo 59 dB HL; 37 y 3 mo 87 dB HL	III	.93	1.17	0.272
F12	F	32 y 5 mo 67 dB HL; 34 y 9 mo 62 dB HL	II or III			
F13	F	15 y 0 mo 57 dB HL; 20 y 1 mo 64 dB HL; 25 y 11 mo 64 dB HL	II or III	.85	0.626	0.395
G14	F	39 y 9 mo 61 dB HL; 44 y 6 mo 62 dB HL; 48 y 9 mo 62 dB HL; 48 y 11 mo 61 dB HL	II	.31	0.041	0.090
G15	M	45 y 8 mo 58 dB HL; 50 y 9 mo 66 dB HL	II			
H16	F	45 y 4 mo 88 dB HL; 50 y 3 mo 89 dB HL	III?			
H17	F	42 y 10 mo 81 dB HL; 45 y 10 mo 86 dB HL	III?			
I18	M	15 y 7 mo 48 dB HL; 22 y 8 mo 54 dB HL	II or III?			
I19	F	5 y 4 mo 50 dB HL; 11 y 5 mo 48 dB HL; 12 y 0 mo 46 dB HL; 21 y 3 mo 53 dB HL	II	.52	0.235	0.275
I20	M	6 y 11 mo 50 dB HL; 7 y 3 mo 48 dB HL; 20 y 3 mo 56 dB HL	II	.97	0.529	0.143
K24	F	40 y 2 mo 58 dB HL; 47 y 8 mo 68 dB HL; 49 y 10 mo 61 dB HL; 51 y 9 mo 65 dB HL	II	.80	0.695	0.530
K25	F	41 y 10 mo 95 dB HL	III?			
M29	M	33 y 6 mo 58 dB HL; 38 y 6 mo 62 dB HL	II			

Annual threshold increase (ATI) equals regression coefficient (correlation coefficient r); SD is its standard deviation. Italics indicate significant difference from zero.

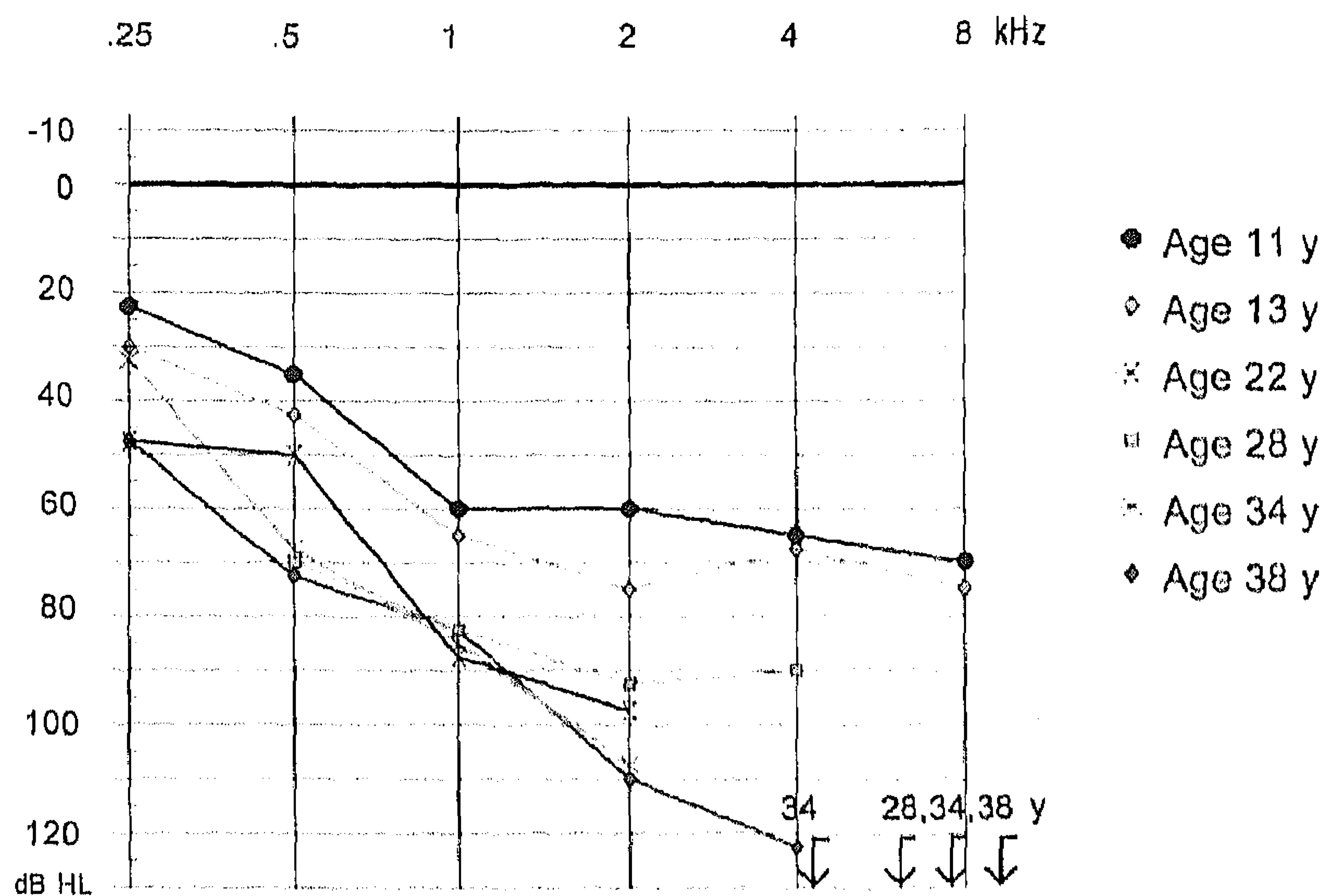
III phenotype with a progressive hearing loss (family C), and those that seemed to be mixtures of the two phenotypes (families F, H and I, and K). The lod scores for these three groups were summed at constant positions -10, 0, +2, +12 centimorgans relative to marker D1S237, and the results are listed in Table 2. An M-test analysis (Morton) was carried out on the grouped data. The result was $\chi^2(2) = 0.20$ (not significant).¹⁴ Thus, it is concluded that dividing the sample into different audiologic phenotypes does not uncover any genetic heterogeneity of Usher's type II. Thus, we would conclude that this clinical variability is not due to the involvement of two (USH) loci.

The available audiometric measurements are presented by age in Table 3; included is the assigned tentative audiologic phenotype. The mean difference between right and left PTA was only about 5 dB (SD about 4 dB). A systematic study of the separate sound frequencies showed that the progression of hearing

loss (when present) could be regarded as being sufficiently represented by the progression at the speech frequencies (0.5, 1, and 2 kHz), especially at 1 kHz. It was thus validated that the PTA (and hence the BPTA) was a suitable measure of the development of hearing loss.

Those patients (n = 13) were selected from whom sufficient serial audiograms were obtained to analyze the regression of BPTA on age. The ATI (expressed in decibels per year) is the regression coefficient. An example of serial audiograms is shown in Fig 1. Histograms and probit plots (not shown) indicated the existence of two separate clusters of ATI values, those pertaining to cases without apparent progression (tentative type II, with a low ATI) and those pertaining to cases with a substantial ATI (tentative type III).

There were 3 patients with an ATI value that differed significantly from zero; ie, they showed



significant correlation and regression (Table 3): C5, C6 (also see Fig 1), and F11; their ATI was in excess of 1 dB/y. The continuous fat lines connecting the separate data points for these 3 patients are shown in Fig 2 to illustrate the progressive character of their hearing loss.

Analysis of variance on all of our cases with an ATI value available ($n = 13$) demonstrated that these values showed significant heterogeneity ($F = 2.44$, df 12 and 22, $p = .033$). A similar analysis showed significant heterogeneity ($F = 2.01$, df 19 and 31, $p = .041$) also for the combination of the present group of patients with known ATI and the cases previously described by others for whom regression analysis could be performed and an ATI value could be obtained (Table 4¹⁵⁻¹⁸). All of the latter cases had a progressive type of hearing loss reported to be type III Usher's syndrome, except for case 1 of Karjalainen et al.¹⁶ Of interest, an analysis of variance performed on the previously reported cases and our own cases (ie, C5, C6, and F11) for whom ATI was in the range of 1 to 5 dB/y, ie, those who showed the most convinc-

ing progression of hearing loss, failed to show any significant heterogeneity in ATI values ($F = 1.20$, df 6 and 15, $p = .36$; after the exclusion of the case of Gorlin et al¹⁵ — because of its large SD — $F = 2.07$, df 5 and 14, $p = .13$).

DISCUSSION

There is little doubt that there is substantial variation in the Usher's 2A audiologic phenotype, and at least two different patterns are apparent: one stable to slowly progressive, ie, in keeping with normal presbycusis,¹⁹ and another more rapidly progressive. Progression was not apparent from the medical history. According to the χ^2 test applied to the data presented in Table 2, the apparent audiologic phenotype did not appear to be associated with linked or unlinked subtypes. No evidence of families showing other than 1q linkage was, in fact, observed. However, the phenotyping used, and thus the grouping of the families, was somewhat arbitrary. We therefore also performed analyses of variance on the ATI variable, ie, for those patients for whom sufficient follow-up data were available. The latter analyses on

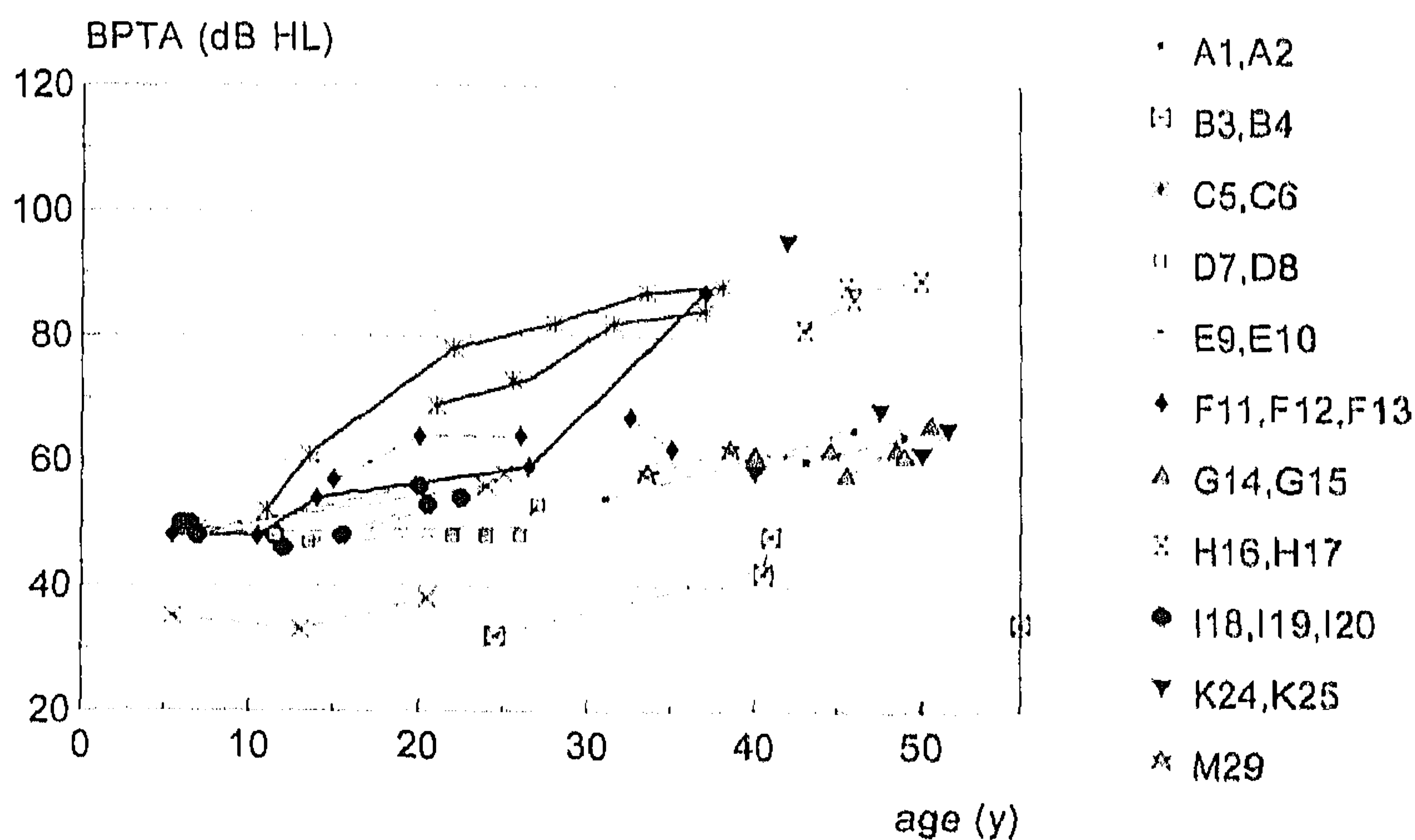


Fig 2. Plot of BPTA as measure of hearing loss against age for all of available data (Table 3). All separate data points are shown. Continuous fat lines connect individual longitudinal data in 3 patients with significant progression as apparent from their ATI (C5, C6, and F11). Dashed thin lines connect individual longitudinal data in other cases.

TABLE 4. BINAURAL PURE TONE AVERAGE AND REGRESSION ON AGE FOR PREVIOUSLY REPORTED PATIENTS FOR WHOM SUFFICIENT FOLLOW-UP DATA WERE AVAILABLE

Reference	Case No.	Age and Binaural Pure Tone Average	Audiologic Phenotype	Genotype	Regression Parameters		
					<i>r</i>	ATI (dB/y)	SD (dB/y)
Gorlin et al ¹⁵	(One case)	4 y 57 dB HL; 5 y 85 dB HL; 9 y 90 dB HL	III		.75	5.07	4.41
Karjalainen et al ¹⁶	Case 1	36 y 64 dB HL; 44 y 63 dB HL; 51 y 72 dB HL	II	USH3*	.79	0.518	0.405
	Case 3	9 y 52 dB HL; 18 y 59 dB HL; 32 y 72 dB HL	III	USH3*	1.00	0.875	0.041
	Case 4	10 y 86 dB HL; 24 y 102 dB HL; 31 y 105 dB HL	III	USH3*	.98	0.939	0.177
	Case 5	9 y 28 dB HL; 20 y 62 dB HL; 27 y 71 dB HL	III	?*	.98	2.45	0.487
	Case 7	5 y 63 dB HL; 13 y 77 dB HL; 17 y 113 dB HL	III	?*	.91	3.82	1.794
Sankila et al ¹⁷	Case 8	7 y 59 dB HL; 8 y 75 dB HL; 15 y 92 dB HL	III	?*	.92	3.50	1.443
	Case II-1, family 15	7 y 55 dB HL; 8 y 80 dB HL; 10 y 97 dB HL; 18 y 98 dB HL; 18 y 108 dB HL	III	USH3	.80	3.08	1.338
Pakarinen et al ¹⁸	Case II-5, family 7	35 y 73 dB HL; 42 y >100† dB HL; 51 y 115 dB HL	III	USH2A*		-2.6	

Italics indicate significant values.

*Dr L. Pakarinen, personal communication 1995.

†Not measured at 2 and 4 kHz (pure tone average right ear).

the one hand clearly demonstrated heterogeneity in the progression of hearing loss in our patients, whereas on the other hand there was no significant difference in progression between our 3 cases with the most convincing progression and the similar cases previously reported for which sufficient data were available. Perhaps there is still lack of sufficient data, but it should be emphasized that 3 of our cases and 1 case previously reported (case 3 of Karjalainen et al¹⁶) showed significant progression.

The Dutch families are homogeneous with regard to linkage, and all are type 2A. Thus, the source of variation in audiologic phenotype seen here is not due

to different mutations at different USH loci (insofar as presently known and therefore screened in our patients).

These results argue only that phenotypic variation in terms of the progression of hearing loss occurs within the Usher's 2A sample of families. There is only a single previous report that noted no difference between the hearing loss seen in type 2A versus 2B.¹⁰ Thus, an investigation into possible audiologic differences between the type 2A and 2B subtypes is in order.

There are no linkage data published from families with the tentative clinical "type III Usher's," ie,

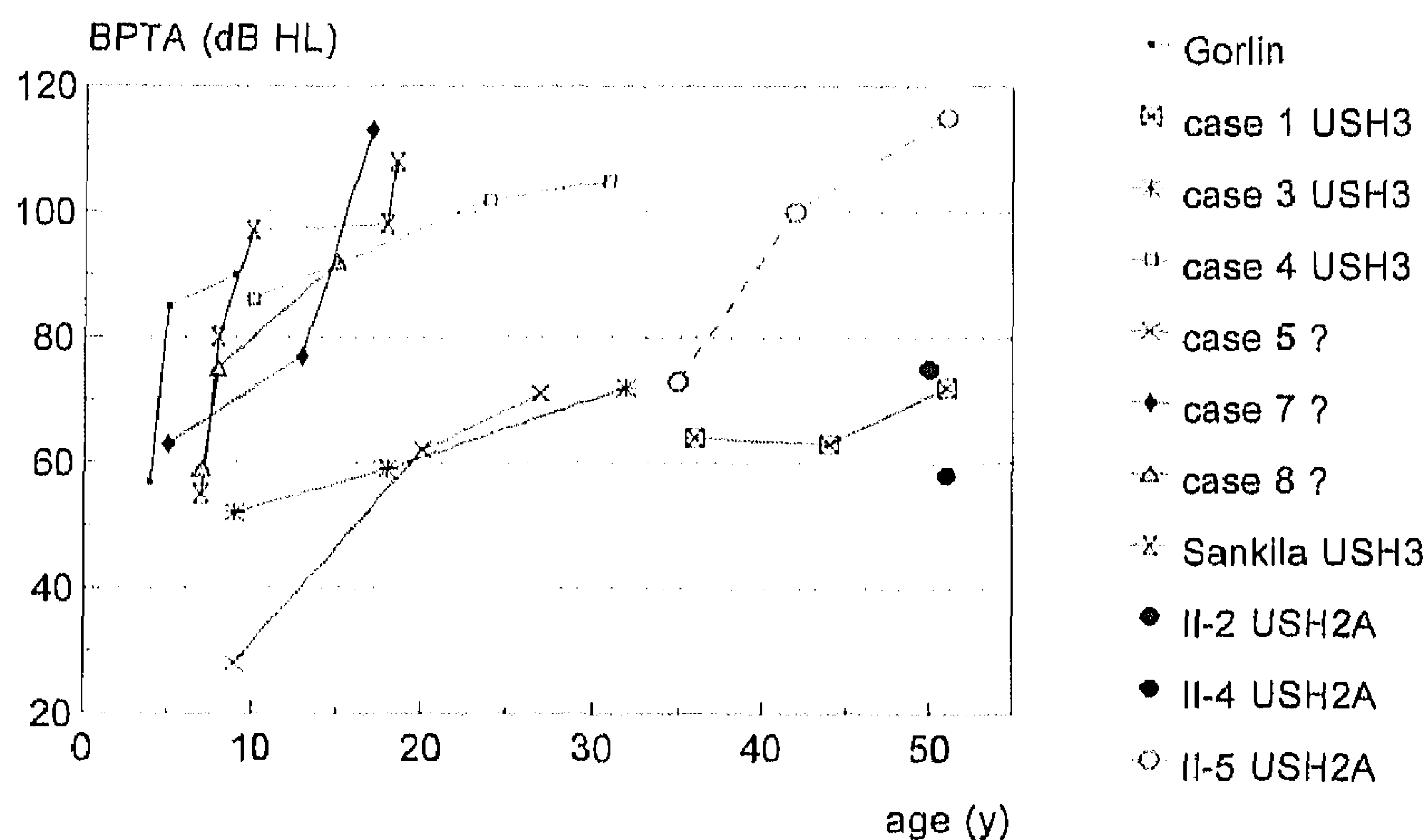


Fig 3. Plot of BPTA against age for previously reported patients, most of whom have disease designated as Usher's type III, with sufficient follow-up data to allow regression (solid lines) or trend (dashed lines) analysis (Table 4). Data for patients II-2, II-4, and II-5 were obtained from Dr L. Pakarinen (family 7, personal communication 1995).

progressive hearing loss, such as the patients described by Gorlin et al¹⁵ and Karjalainen et al¹⁶ whose hearing data were included in the present analysis (Fig 3 and Table 4), with one exception. Most recently, in the Finnish population Usher's syndrome type III has been linked to chromosome 3q (USH3 locus),¹⁷ and some of the cases included in our analysis (Table 4 and Fig 3) recently turned out to be

USH3. It seems that we have to face the situation that not only Usher's III patients but also Usher's 2A patients show, or may show, progression of hearing loss. Nevertheless, more detailed and more numerous data on the audiologic features of Usher's III patients, also based on gene linkage results, will be needed to conclude more precisely whether those types can be divided clinically only on audiometric data.

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