Genetics

A Founder Mutation in *MYO7A* Underlies a Significant Proportion of Usher Syndrome in Indigenous South Africans: Implications for the African Diaspora

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Purpose. Research over the past 25 years at the University of Cape Town has led to the identification of causative mutations in 17% of the 1416 families in the Retinal Degenerative Diseases (RDD) biorepository in South Africa. A low rate of mutation detection has been observed in patients of indigenous African origin, hinting at novel genes and mutations in this population. Recently, however, data from our translational research program showed two unrelated indigenous African families with Usher syndrome (USH), with the same homozygous *MYO7A* mutation. Therefore, the extent to which this mutation contributes toward the disease burden in South Africa was investigated.

Methods. Cohorts of unrelated indigenous South African probands with different RDD diagnoses were tested for the *MYO7A* c.6377delC mutation. Familial cosegregation analysis was performed for homozygous probands, clinical data were evaluated, and SNP haplotypes were analyzed.

Results. This homozygous *MYO7A* mutation underlies a remarkable 43% of indigenous African USH cases investigated in this study, the majority of which (60%) were diagnosed clinically with Type 2 USH. All homozygotes shared a common haplotype. This mutation does not appear to cause nonsyndromic vision loss.

Conclusions. Of interest is the origin of this common mutation relevant to the Bantu population migration into southern Africa. Further investigation of the phenotype may elucidate the disease biology, and perhaps reveal a larger cohort with the same mutation, with which to assess the impact of environmental and genetic modifiers and evaluate therapeutic trials.

Keywords: Usher syndrome, African, founder mutation, *MYO7A*

Usher syndrome (USH) is characterized by vision and hearing loss, and is the most common cause of deaf-blindness.[1,2] It is an autosomal recessively inherited group of disorders, divided into three major clinical subtypes that are differentiated by the severity of hearing loss and the presence of vestibular dysfunction. Vision loss due to retinitis pigmentosa (RP) is a hallmark of all three USH subtypes. In addition to clinical heterogeneity, Usher syndrome displays genetic heterogeneity, with 12 causative genes identified to date.[3]

Usher syndrome type 1 is the most severe form, exhibiting profound congenital hearing loss and vestibular dysfunction, and prepubertal onset of progressive RP. To date, six genes have been associated with USH type 1, namely *CDH23*, *CIB2*, *MYO7A*, *PCDH15*, *USH1C*, and *USH1G*.[4] Usher syndrome type 2 is less severe, characterized by congenital hearing loss that is moderate to severe, with normal vestibular functioning and a later RP onset. Mutations in three genes, namely *DFNB31*, *GRP98*, and *USH2A*, cause type 2 USH.[1,2] Type 3 USH is characterized by variable onset of RP and hearing loss, as well as varying degree of vestibular dysfunction. Two genes are associated with USH type 3, namely *CLRN1*[2] and *HARS*,[5] with a third gene (*ABHD12*) being associated with a variant of this subtype.[6]

A number of USH protein interactions (or interactomes) have been reported,[4,7] which function in the development and maintenance of stereocilia hair bundles of the inner ear and which also colocalize in the synaptic layer, connecting cilium and the calyceal processes of the photoreceptors of the retina. The exact function of these protein interactomes is not known, as mouse models (of mutated USH genes) have little or no retinal phenotype, but they may have a role in protein trafficking between the inner and outer segments of the photoreceptors, as well as synaptic function of these sensory neurons.[4]

Due to the genetic and clinical heterogeneity, the large size of the genes, and multiple isoforms underlying the syndrome, identifying the molecular basis of USH in affected South African families using traditional candidate gene screening methods has been challenging. Technologic advances, such as the development of microarrays and next generation sequencing, have significantly improved the turnaround time and success rates of genetic mutation screening for inherited retinal degenerative diseases. As part of our translational research program in South Africa,[8,9] families can opt for genetic screening using microarrays (Asper Biotech Ltd., Tartu, Estonia)[10] or for whole exome sequencing of 105 retinal candidate genes (through the

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Manchester Centre for Genomic Medicine, Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK).[11] A review of data obtained from the Asper USH array and whole exome analyses revealed two unrelated indigenous (Black) South African USH probands with a homozygous c.6377delC (p.Pro2126Leufs*5) mutation in *MYO7A*, which subsequently was confirmed by cycle sequencing. This mutation has been reported previously only recently (to the best of our knowledge) in the compound heterozygous state, together with p.Arg1240Trp, in a single Caucasian individual from the United Kingdom with USH type 1.[12] The mutation subsequently was added to the panel of mutations on the Asper Usher Microarray; however, many South African samples had been tested by that time and, therefore, it was deemed necessary to rescreen appropriate samples for this mutation. The p.Pro2126Leufs*5 mutation is predicted to truncate the 2215 amino acid MYO7A protein by 86 amino acids (3.88%).

The gene *MYO7A* was the first USH gene identified[13] and has since been recognized as the most frequent cause of USH type 1, which is the most severe form of USH.[14,15] The protein MYO7A is an unconventional myosin expressed in multiple epithelial cell types,[16] including the RPE, where it functions in the light-dependent localization of the visual cycle enzyme, RPE65.[17] It is also expressed in the photoreceptor calyceal processes and cilia, and the stereocilia, together with other USH proteins.

The identification of two unrelated indigenous South African patients (and their respective families) with the same homozygous *MYO7A* mutation warranted further investigation in this population, particularly with respect to a potential founder effect and the clinical manifestation of c.6377delC. Phenotypic variation has been reported previously in USH, with mutations in *USH2A* causing nonsyndromic RP[18,19] and *MYO7A* mutations causing nonsyndromic[20] deafness. Founder mutations have been reported previously in indigenous South Africans,[21,22] and determining whether this USH mutation occurs on the same haplotype in all affected individuals would show a probable founder effect, having diagnostic implications.

Methods

Cohort

Affected individuals and their family members were recruited from throughout South Africa as part of a Retinal Degenerative Disorders (RDD) research project, established in the Division of Human Genetics at the University of Cape Town in 1990. Biological material (genomic DNA extracted from venous blood) is archived in the RDD registry, together with demographic and clinical information. Informed consent is obtained from all RDD research participants according to the tenets of the Declaration of Helsinki (2013), and ethics approval for this specific study was obtained from the institutional Human Research Ethics committee (HREC/REF: 312/2014).

A cohort of 12 unrelated indigenous South African probands with confirmed clinical diagnoses of USH (regardless of the clinical subtype), but no genetic diagnoses, were tested for the c.6377delC mutation. A further six indigenous South African probands with RDD and some hearing loss, but no clinical confirmation of USH, also were tested and considered to be a "query USH cohort." Testing also was performed on samples from three probands of Mixed Ancestry with confirmed clinical diagnoses of USH (who likely share similar ancestry with the indigenous South African individuals, as Bantu-speaking Africans are a major ancestral contributor to this admixed population[23]).

Familial cosegregation analysis was performed where possible for probands carrying the homozygous c.6377delC mutation, to ensure that the mutation cosegregated with disease within the families. A total of 51 indigenous South African population controls, who had not specifically been assessed for the absence of RDD, also was screened for the mutation. No detailed population data were available for these controls, other than that they are of indigenous South African origin. To investigate possible phenotypic variation, a cohort of 10 indigenous South African probands with nonsyndromic autosomal recessive RP (arRP) and 107 indigenous South African simplex RP cases also were tested.

c.6377del C Assay Design

The *MYO7A* Transcript variant 1 NCBI (NM_000260, accessed November 2013) and Ensembl (ENSG00000137474, ENST00000409709, accessed November 2013) sequences were used to compare the region containing exon 47 and to ensure that the entire exon was included in the assay. Transcript variant 1 was selected as it encodes the longest isoform. Primers were designed to span exon 47 and at least 50 bp of intronic sequence flanking the exon. The primers used were Forward 5′ GCAACAGGAGAGGCTGACTTTATC 3′, Reverse 5′ GTGGCTAGGAGGGCTTGTG 3′.

The primers were used to amplify a 287 bp fragment under standard 25-μL PCR conditions: 100 ng genomic DNA, 0.4 μM forward and reverse primer, 200 μM dNTPS, 1X Colourless GoTaq Reaction Buffer, and 0.5U GoTaq DNA Polymerase (Promega, Madison, WI, USA). Cycling conditions were as follows: 95°C – 5 minutes, 30 cycles of (94°C – 30 seconds, 61°C – 30 seconds, 72°C – 40 seconds), 72°C – 7 minutes.

The c.6377delC mutation creates an *Hpy*188III restriction enzyme recognition site 131 bp into the amplicon, resulting in 156 bp and 131 bp fragments in samples with the homozygous mutation, and 287, 156, and 131 bp fragments in samples with the heterozygous mutation. Restriction digests with *Hpy*188III were performed as follows: a standard 20-μL reaction containing 10 μL PCR product, 1X NEB CutSmart Buffer, and 5U NEB *Hpy*188III enzyme (New England Biolabs, Ipswich, MA, USA) was incubated at 37°C for 3 hours. The digest products were subjected to electrophoresis on a 3% agarose gel containing SYBR Safe DNA Gel Stain (Applied Biosystems by Life Technologies, Woolston, Warrington, UK) and visualized under UV light using a UVIpro Gold transilluminator (UVItec, Cambridge, UK). A heterozygous and homozygous sample (confirmed by cycle sequencing) were included as controls.

Haplotype Analysis

To examine the possibility of a founder effect in the indigenous South African population (which would have diagnostic implications), it was necessary to determine whether the mutation exists on a common haplotype in the cases. Additionally, haplotyping of African controls could determine the origin of the mutation in South Africa, and the potential contribution to USH disease burden in other African countries. The mutation of interest is located at position 76924019 on chromosome 11 (human genome build GRCh37.p13/hg19). More than 1 Mb of the sequence flanking the mutation (584 709bp 5′ and 502 265bp 3′) was interrogated for informative SNPs with minor allele frequencies > 20% in the Luhya and Yoruba 1000 Genomes dataset.[24] Three single nucleotide polymorphisms (SNPs) were selected for haplotyping analysis in mutation-positive families: rs6592706, rs948972, and

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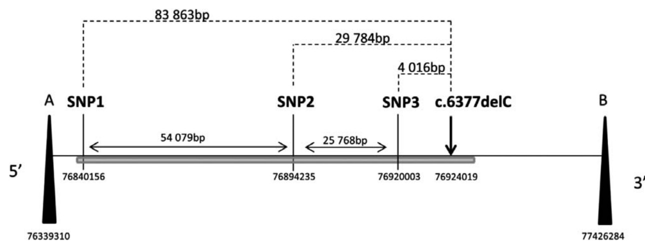

FIGURE 1. Diagrammatic representation of the >1 Mb region of chromosome 11 (between [**A**] and [**B**]) interrogated for informative SNPS, and the three SNPs selected for genotyping (SNP1 = rs6592706, SNP2 = rs948972, and SNP3 = rs11237122). The mutation location is shown as a *bold vertical arrow*. The numbers below the *horizontal solid line* indicate the position (bp) on the chromosome, the numbers above the *horizontal arrows* show the distance between adjacent SNPS, and numbers above the *dashed line* show the distance between each SNP and c.6377delC. The *MYO7A* gene is represented as a *gray box* (located from 76839310–76926284 bp).

rs11237122. All selected SNPS are 5′ of the mutation; no suitable SNPs were identified 3′ of the mutation (Fig. 1).

Primers were designed to amplify a 250, 210, and 247 bp fragment spanning rs6592706, rs948972, and rs11237122, respectively. Polymerase chain reactions (PCR) were performed in a 25-μL volume under standard PCR conditions: 100 ng genomic DNA, 0.4 μM forward and reverse primer, 200 μM dNTPS, 1X Colourless GoTaq Reaction Buffer and 0.5U GoTaq DNA Polymerase (Promega). Cycling conditions were as follows: 95°C – 5 minutes, 30 cycles of (94°C – 30 seconds, annealing temperatures (Ta) – 30 seconds, 72°C – 40 seconds), 72°C – 7 minutes. Primer sequences, annealing temperatures, and assay information are listed in Table 1.

The G allele of rs6592706 creates a *Bsr*BI restriction enzyme recognition site, generating fragments of the following sizes for the various genotypes: A/A, 250 bp; A/G, 250, 159, and 91 bp; G/G, 159 and 91 bp. Restriction digests were performed as follows: a standard 20 μL reaction containing 10 μL PCR product, 1X Tango Buffer, and 2U *Bsr*BI enzyme (ThermoScientific, Waltham, MA, USA) was incubated at 37°C for 3 hours. The digest products were subjected to electrophoresis on a 3% agarose gel containing SYBR Safe DNA Gel Stain (Applied Biosystems by Life Technologies) and visualized under UV light.

The C and G alleles of rs948972 were distinguished by cycle sequencing following purification of PCR products; 8.9 μL PCR products were purified by 1U FastAP Shrimp Alkaline Phosphatase (ThermoScientific) and 2U Exonuclease I (ThermoScientific) in a 10-μL reaction that was incubated as follows: 37°C – 60 minutes, 75°C – 15 minutes, 95°C – 5 minutes. Cycle sequencing was performed in a 20-μL reaction containing the 10 μL purified PCR products, 1 μM reverse primer, 1X Sequencing Buffer, and 1X BigDye Terminator v3.1 Reaction Mix (Applied Biosystems by Life Technologies). Cycling conditions were: 96°C – 5 minutes, 30 cycles of (96°C – 30 seconds, 50°C – 15 seconds, 60°C – 4 minutes). Sequencing products were purified by ethanol precipitation and resuspended in 10 μL Sabax water (Adcock Ingram, Johannesburg, South Africa), after which 5 μL sequencing reaction was loaded, together with 8 μL Hi-Di Formamide (Applied Biosystems by Life Technologies), onto a 3130xl Genetic Analyser (Applied Biosystems by Life Technologies).

The T allele of rs11237122 creates a *Pfl*MI restriction enzyme recognition site, generating fragments of the following sizes for the various genotypes: C/C, 247 bp; C/T, 247, 138, and 109 bp; T/T, 138 and 109 bp. Restriction digest were performed as follows: a standard 20-μL reaction containing 10 μL PCR product, 1X Buffer R and 2U *Pfl*MI enzyme (ThermoScientific) was incubated at 37°C for 3 hours. The digest products were subjected to electrophoresis on a 3% agarose gel containing SYBR Safe DNA Gel Stain (Applied Biosystems by Life Technologies) and visualized under UV light.

Haplotypes of affected individuals with the homozygous c.6377delC mutation were constructed manually based on segregation within the families, and then compared between families, and compared to control data; individual genotypes for the 3 SNPs were obtained from 97 Luhya and 88 Yoruba individuals in the 1000 Genomes dataset.[24] Linkage disequilibrium testing and haplotype analysis was performed subsequently, and χ^2 and Pearson's P values were calculated, using the SHEsis online program (available in the public domain at http://analysis.bio-x.cn/myAnalysis.php).[25,26]

RESULTS

Frequency of c.6377del C

After the initial identification of two unrelated indigenous USH probands homozygous for c.6377delC, another four unrelated homozygotes were identified by screening 12 additional probands. Thus, a total of six homozygotes was identified in the total cohort of 14 confirmed indigenous South African USH cases (42.86%).

No mutation-positive individuals were identified in the additional cohorts screened (Table 2). Furthermore, the mutation was not present in the Luhya or Yoruba individuals in the 1000 Genomes dataset,[24] nor was it present in 200 chromosomes from Zulu individuals sequenced as part of the African Genome Variation Project (AGVP).[27]

USH Mutation-Positive Families: Cosegregation and Haplotyping Analysis

The six identified families came from the following ethnolinguistic groups: one Sotho, two Zulu, two Xhosa, and one unknown indigenous South Africans. The families live in two large, geographically distinct provinces in South Africa, and each family lives in a different town, indicating that consanguinity was unlikely. Familial DNA was available for three of the six probands, and familial cosegregation analysis confirmed that the mutation cosegregated with disease within these families (Fig. 2). Genotyping of 3 SNPs (>83kB from c.6377delC) in all available familial DNA samples also showed the total of 10 homozygotes all shared a common haplotype (Fig. 2). The clinical and demographic information pertaining to the affected individuals is presented in Table 3.

TABLE 1. Primer Sequences, PCR Annealing Temperatures (Ta), and Assay Information for the Genotyping of Three SNPs in Families Carrying the c.6377delC Mutation

SNP	Fwd Primer, 5′→3′	Rev Primer, 5′→3′	Ta, °C	Assay
rs6592706	cttgaaggtggtctagttctca	atgtggattcaacagggcca	60	+ *Bsr*BI
rs948972	agtccaagctcacagaggag	acactcctgtctgcctgatc	60	Cycle sequencing
rs11237122	tgctgtactttggccctgaa	gcagaatctcgaagtcagagg	58	+ *Pfl*MI

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Table 2. Results of Mutation Screening in South African Case and Control Cohorts

Cohort Screened	No. Individuals	No. Chromosomes	No. Chromosomes With c.6377delC
Indigenous South African USH	14	28	12
Indigenous South African query USH	6	12	0
Mixed Ancestry USH	3	6	0
Indigenous South African arRP	10	20	0
Indigenous South African simplex RP	107	214	0
Indigenous South African controls	51	102	0
South African Zulu control AGVP data*	100	200	0
Luhya control 1000 Genomes data*	97	194	0
Yoruba control 1000 Genomes data*	88	176	0

* Control data from publically available datasets.

The SHEsis Linkage Disequilibrium test was used to calculate Lewontin's D' and r^2 between each pair of the 3 SNPs in the 1000 Genomes control data from 97 Luhya and 88 Yoruba individuals. The results indicated that there is no linkage disequilibrium among the three SNPs in the separate control populations or when they are combined, and, therefore, this is not a block of low haplotype diversity in these African population groups.

We performed χ^2 calculations for genotype frequencies (Table 4) of the 3 SNPs in the 185 African controls and 6 unrelated, c.6377delC homozygous probands affected with USH. These statistical tests showed that the cases, separate control groups, and combined controls showed no deviation from Hardy Weinberg Equilibrium. The χ^2 analysis showed a significant difference between the Luyha and Yoruba controls in the frequency of rs6592706 (Pearson's $P = 0.017293$); however, this was no longer significant when the critical value was set to $P < 0.01666$ (Bonferroni correction for testing of 3 SNPs). There was no significant difference in the frequencies of rs948972 and rs11237122 between the Luhya and Yoruba controls (Pearson's $P = 0.122203$ and 0.527811, respectively) and, therefore, these control groups were combined for subsequent comparison with the cases. The results showed that for rs6592706 and rs11237122 there are significant differences in the genotype frequencies between the cases and controls, which remained significant after Bonferonni correction ($P < 0.01666$).

Subsequently, haplotypes were reconstructed and haplotype frequencies compared between the 6 unrelated cases and 185 controls (Table 5). The SHEsis analysis of 370 control and 12 case haplotypes gave a Global χ^2 of 77.993309, while $df = 6$, and a Pearson's P value was 9.27e-015. Haplotypes occurring with a frequency less than 0.05 were excluded. The results indicated a significant difference in the frequency of the ACT haplotype in the African controls compared to the six probands with Usher syndrome.

Discussion

Our experience has shown that testing for known candidate genes and mutations for RDDs (as configured through the Asper Biotech Ltd. Arrays[28]) has a good yield with our subcohort of Caucasian subjects, with 115 of 280 patients (41.1%) having their mutation(s) identified through the use of various Asper arrays, but a rather low return in our indigenous African patients, in whom only 14 of 109 patients (i.e., 12.8%) have a genetic diagnosis after microarray screening (results not shown). This is understandable, since most of the testing arrays are based on mutations generally identified in cohorts of patients of European/Caucasian origin. Our identification of a homozygous *MYO7A* mutation in two USH patients of indigenous African origin was initially surprising, but its emergence as the cause of a large proportion (42.86%) of

Table 3. Clinical and Demographic Data From 10 Affected c.6377delC Homozygous Individuals in 6 Families

DNA Code	Ethnic Group	Age of Onset, y	Clinical Diagnosis and Information
RPU 318.1UNA	Unknown	1	USH Type 2
RPU 340.3JEA*	Xhosa	Congenital	USH Type 2; VA: L = 6/60; R = 6/12 at age 43. Congenital hearing loss.
RPU 340.4PAT*	Xhosa	Congenital	USH Type 2; VA: L = 6/48; R = 6/48 at age 40. Congenital hearing loss.
RPU 340.5PET*	Xhosa	Congenital	USH Type 2; VA: L = 6/24; R = 6/60 at age 37. Congenital hearing loss.
RPU 340.8SIG*	Xhosa	Congenital	Congenital hearing loss, developed RP.
RPU 564.1FRA	Zulu	11	USH Type 2
RPU 954.1ELI	Xhosa	6/7	USH Type 2
RPU 1136.1MAN*	Zulu	Congenital	USH Type 1. Congenital hearing loss, RP developed at 6–8 y, progressive loss of day and night vision.
RPU 1136.2LIN*	Zulu	Congenital	USH Type 1. Congenital hearing loss, RP developed at 6–8 y, progressive loss of particularly night vision.
RPU 1338.1ISI	Sotho	Congenital	USH Type 1. VA: L = 6/18; R = 6/12 at age 30. Night blindness onset at 21 y. Slow disease progression, pigment, attenuation, pale discs.

VA, visual acuity; L, left eye; R, right eye.
* Multiple members of Family RPU 340 and Family RPU 1136.

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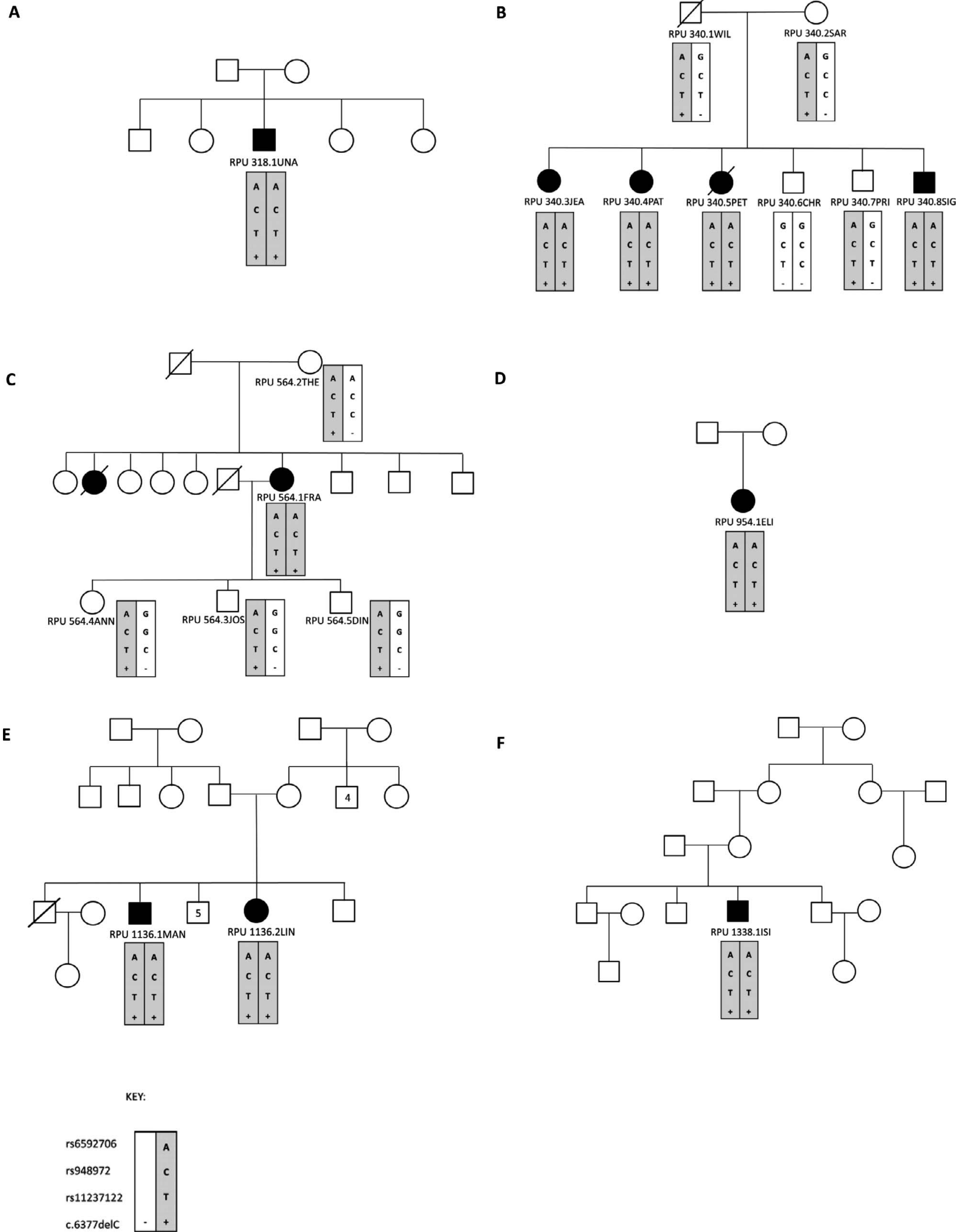

FIGURE 2. (A–F) Pedigrees of six c.6377delC mutation–positive individuals and their family members. *Squares* represent males and *circles* represent females, with *shaded symbols* indicating individuals affected by USH. All individuals from whom DNA was available are indicated by a DNA code. Key: The 3 SNPs and c.6377delC mutation are indicated in the order shown, with the founder haplotype *shaded*.

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Table 4. Genotype Frequency Comparison for the 3 SNPs Between 185 African Controls and 6 Homozygous c.6377delC Probands

SNP	rs6592706			rs948972			rs11237122		
Genotype Count, Frequency	A/A (freq)	A/G (freq)	G/G (freq)	C/C (freq)	C/G (freq)	G/G (freq)	C/C (freq)	C/T (freq)	T/T (freq)
Case, $n = 6$	6 (1.000)	0 (0.000)	0 (0.000)	6 (1.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	6 (1.000)
Luyha control, $n = 97$	14 (0.144)	45 (0.464)	38 (0.392)	46 (0.474)	44 (0.454)	7 (0.072)	28 (0.289)	49 (0.505)	20 (0.206)
Yoruba control, $n = 88$	23 (0.261)	46 (0.523)	19 (0.216)	53 (0.602)	27 (0.307)	8 (0.091)	20 (0.227)	45 (0.511)	23 (0.261)
Total control, $n = 185$	37 (0.200)	91 (0.492)	57 (0.308)	99 (0.535)	71 (0.384)	15 (0.081)	48 (0.259)	94 (0.508)	43 (0.232)
χ^2/df		21.320929/2			5.073668/2			17.951681/ $df = 2$	
Pearson's P		2.35e-005*			0.079116			0.000126*	

* $P \leq 0.001$.

unselected USH cases from this population group was remarkable. Several possibilities could explain the existence of this relatively frequent mutation underlying USH in this part of Africa, including a mutational hotspot at this nucleotide position of the *MYO7A* gene, evolutionary advantage conferred, or genetic drift. The c.6377delC mutation has been reported only once previously (in the compound heterozygous state, in a Caucasian individual from the United Kingdom with type I USH),[12] and the mutation has not been detected in the 1000 Genomes dataset, indicating that this codon is not particularly susceptible to mutagenesis. It is unlikely that the mutation confers an advantage to carriers, as heterozygous mutations in *MYO7A* can cause autosomal dominant hearing loss.[29,30] Furthermore, in the present study, USH patients from different ethnolinguistic subgroups, namely Xhosa, Zulu, and Sotho, were identified with this homozygous mutation, negating genetic drift as a cause of the mutation.

The majority of sub-Saharan Africans speak "Bantu" languages, which are believed to originate from a core region in the north west of the African continent, specifically Nigeria and western Cameroon.[31] The "Bantu expansion" refers to the movement of people approximately 5600 years ago, across and down Africa. Bantu speakers arrived in South Africa approximately 1500 years ago, where they diverged further. Today, there are two main Bantu-speaking groups in South Africa, the Southeastern (subgroup S) and Southwestern (subgroups R and K) groups. The S subgroup of languages comprises the following ethnolinguistic groups: Sotho-Tswana, Venda and Nguni (which includes Xhosa and Zulu).[32] The different ethnolinguistic groups described in this affected cohort, therefore, represent a derivation of the original Bantu expansion. All mutation-positive patients in the present study, whether Xhosa, Zulu, or Sotho, shared a common haplotype spanning >83kB of sequence which is 5′ of the mutation. This denotes that c.6377delC is a founder mutation that arose in speakers of the S-subgroup of Bantu languages before their divergence.

Table 5. Haplotype Reconstruction and Frequencies for rs6592706, rs948972, rs11237122 in 6 USH Cases and 185 African Controls

Haplotype	Case (freq)	Control (freq)	χ^2	Pearson's P
A C C	0.00 (0.000)	70.59 (0.191)	2.947	0.086060
A C T	12.00 (1.000)	38.47 (0.104)	77.993	1.09e-018*†
A G C	0.00 (0.000)	35.20 (0.095)	1.313	0.251864
A G T	0.00 (0.000)	20.74 (0.056)	0.742	0.389170
G C C	0.00 (0.000)	53.38 (0.144)	2.106	0.146678
G C T	0.00 (0.000)	106.56 (0.288)	5.060	0.024500
G G C	0.00 (0.000)	30.83 (0.083)	1.135	0.286700

* The haplotype associated with c.6377delC.
† $P \leq 0.001$.

The haplotype is imputed to be present in the 1000 Genomes data at a frequency of 10%, although the mutation is not present on this haplotype in the African populations in east Africa, that is, the Luhya of Kenya and in west Africa, that is, the Yoruba of Nigeria. Furthermore, there is no linkage disequilibrium of the three SNPs in these two populations, implying that the c.6377delC mutation arose on the haplotype after the Bantu speakers expanded southwards in Africa. A limitation of this study is the sole use of the Yoruba and Luhya data as proxy control populations, with which to investigate the haplotype frequency. It has been shown that these populations are genetically diverse from the Bantu-speaking South Africans,[32] and that proxy populations may not be applicable due to the vast genetic diversity of African populations.[33] This study highlights the paucity of genetic data from indigenous South Africans, as no local population frequency data were available for the SNPs of interest in this study in the SNP dataset recently made publically available by Ramsay et al.[32] This underscores the importance of the Southern African Human Genome Programme (SAHGP)[34] and the Africa Genome Variation Project.[27] Nevertheless, the use of the Yoruba and Luyha datasets was valuable in showing that this founder mutation arose as a more recent event: post-Bantu expansion but predivergence into the different ethnolinguistic groups of South Africa.

The 51 indigenous South African population controls used in this study to establish the frequency of the mutation comprises individuals speaking the S-group of Bantu languages (including Xhosa, Zulu, and Sotho), although a complete and defined ethnolinguistic breakdown of these samples is unavailable. Nonetheless, a recent study on the genomic structure of indigenous southern African populations shows the relatively recent divergence of the Sotho-Tswana, Zulu, and Xhosa populations, suggesting that these may serve as proxies for one another, to a greater extent than the Luhya and/or Yoruba.[35] Thus, this cohort was appropriate to compare the frequency of the mutation between cases and controls, especially when supplemented by the 200 Zulu chromosomes of the AGVP data.

Interestingly, 6 of the 10 homozygous mutation–positive patients had been clinically diagnosed with type 2 USH, whereas *MYO7A* mutations previously generally have been associated with the more severe type 1 USH. This is not the first report of *MYO7A* mutations causing type 2 USH,[14] but the observation is rare. The milder phenotype diagnosed could be due to the fact that the mutation affects the C-terminal FERM domain, and less than 4% of the protein is predicted to be truncated. A mouse study of a different *MYO7A* mutation (albeit a splice variant), affecting the same C-terminal FERM domain, showed tissue-dependent mRNA instability[36]; truncated mRNA in the ear appears to be degraded by nonsense-mediated decay, whereas mRNA expressed in the retina is not. The majority of our

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homozygous cohort reported congenital onset of USH, yet the clinical diagnosis of USH type 2 indicated no vestibular dysfunction is present and RP onset is later.

Mutations in *MYO7A* are associated with nonsyndromic hearing loss[20] and it would be interesting to evaluate whether this particular founder mutation contributes to the burden of hearing loss in indigenous South Africans and other African populations. Our screening indicates that this mutation is not associated with nonsyndromic RP, which is not surprising given the lack of prior reports correlating *MYO7A* mutations with RP, and the tissue-specific protein effects reported.[36]

Providing a genetic diagnosis to a family means that individuals within that family can elect to have diagnostic, carrier, or predictive testing. Genetic testing, therefore, provides patients and their relatives with more accurate risks of developing disease, upon which they can base their informed life decisions and reproductive choices. The identification of this founder mutation will allow targeted genetic testing, based on clinical diagnosis and patient ethnicity, which will reduce the costs of genetic testing and facilitate a rapid test turnaround time. Although the c.6377delC mutation was not present in 51 indigenous South African controls, screening larger numbers of unaffected controls from Xhosa, Zulu, and Sotho populations, and combining these results with data from the AGVP[27] and SAHGP,[34] will provide mutation carrier frequency information that could be useful for genetic counseling purposes and risk calculations. Further screening in larger cohorts of Mixed Ancestry USH patients and nonsyndromic deafness patients is warranted as these patients likely share some ancestry with indigenous South Africans. Furthermore, there is potential for identifying a large number of individuals with the same pathogenic mutation, which could facilitate detailed genotype-phenotype investigations[37] and studies of phenotypic modifiers. Finally, identification of these indigenous South African patients with a *MYO7A* mutation is important given the development of UshStat, the *MYO7A* gene replacement therapy[38] currently in trials (https://clinicaltrials.gov/ identifiers NCT01505062 and NCT02065011).

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