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Sensorineural hearing loss in people with deletions of 18q

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Abstract

Objective—The objective of this study was to characterize hearing loss in individuals with deletions of distal chromosome 18q and to identify the smallest region of overlap of their deletions, thereby identifying potential causative genes.

Study Design—The clinical data were collected via a retrospective case study. Molecular data were obtained via high resolution chromosome microarray analysis.

Setting—The study was conducted as a component of the ongoing research protocols at the Chromosome 18 Clinical Research Center at the University of Texas Health Science Center at San Antonio.

Patients—Thirty-eight participants with a deletion of the distal portion of the long arm of chromosome 18 were recruited to this study.

Interventions—The participants underwent an otologic examination as well as a basic audiometry evaluation. Blood samples were obtained and high resolution chromosome microarray analysis was performed.

Main Outcomes Measures—Pure tone averages and speech discrimination scores were determined for each participant. The region of hemizygosity for each participant was determined to within 2 Kb each of their breakpoints.

Results—Twenty-four participants (63%) had high-frequency hearing loss, similar to the pattern seen in presbycusis. Comparison of microarray results allowed identification of eight genes, including the candidate gene for dysmyelination (*MBP*).

Conclusions—Individuals with a deletion of a 2.8 Mb region of 18q23 have a high probability (83%) of high frequency sensorineural hearing loss.

Introduction

Chromosome abnormalities are a well-known cause of disability. As a result of the Human Genome Project, the relationship between deletions /duplications of genetic material and phenotypic changes is being elucidated. One of the most unexpected discoveries has been the contribution of chromosome deletions and/or duplications to human genetic diversity in normal populations (1, 2). The current estimate is that there are over 57,000 genomic copy number changes between 100 bp and 3 Mb in size, half of which include known genes. Taken together, these changes comprise nearly 30% of the reference genome (3). Clearly, there are genes that are not dosage sensitive, meaning that they can be duplicated or deleted with no ill effect. The current challenge is to determine which genes are dosage sensitive and which are not, and then to link specific abnormalities to specific dosage sensitive genes. We anticipate that only about 5-10% of genes will be dosage sensitive (4).

The focus of the Chromosome 18 Clinical Research Center is the identification of dosage sensitive genes responsible for phenotypic abnormalities in people with chromosome 18 deletions. Deletions of the end of the long arm of chromosome 18 (distal 18q-) occur in approximately 1 in 40,000 births (5). The deletion size varies from 0.5 to 30 Mb of DNA (6). Consequently, the phenotype varies and can include short stature, cognitive impairment, hypotonia, abnormal genitalia, growth hormone deficiency, flattened midface, aural atresia, and renal abnormalities(7). Neurologic manifestations include hearing loss, delayed myelination, and seizures (8).

Hearing loss has long been known to be associated with distal 18q- (9).The aural phenotype was more precisely described in 1971 when Wertelecki and co-workers described individuals with 18q deletions who had conductive hearing loss and aural atresia (10). In 1993, Kline et al. reported that sensorineural hearing was also a part of the aural phenotype. They described 7 people with 18q deletions, three of whom had sensorineural hearing loss (SNHL) and two had conductive loss (11). However, the audiometric pattern and degree of loss were not described.

In 2011, the dosage sensitive gene responsible for aural atresia was identified. In the years prior, several groups had progressively narrowed the aural atresia critical regional of the chromosome and hence narrowed the list of candidate genes in this region of 18q (12, 13). In 2011, Feenstra et al. identified four individuals with aural atresia from 2 families with microdeletions of 18q. The patients' common region of overlapping hemizygosity included a single gene, *TSHZ1*. Subsequently, these investigators sequenced this gene in 11 individuals with isolated aural atresia and found 2 people with mutations in the *TSHZ1* gene, thus identifying it as the gene responsible for isolated aural atresia.

Although the conductive hearing loss component of the distal 18q- has been described, the SNHL phenotype remains uncharacterized. The purpose of this study was to describe the features of SNHL in this population as well as to work towards identification of the gene responsible for these features.

Materials and Methods

All components of the study were approved by the Institutional Review Board of the The University of Texas Health Science Center at San Anotnio. Participants learned of the research through the Chromosome 18 Registry & Research Society as well as the internet. Participants were eligible for the study if they had a distal deletion of 18q identified via karyotype and confirmed by medical records. Individuals with more complex rearrangements involving other chromosomes were excluded. All individuals were and continue to be involved in the informed consent process, which is appropriately documented.

Clinical Assessment

Subjects were invited to the Chromosome 18 Clinical Research Center for a series of evaluations, including an otologic examination. This study included thirty eight patients, twelve of which were examined /tested serially. Basic audiometry, including air conduction, bone conduction, and speech testing, was performed on all participants. The testing was performed in a sound proof booth using several different audiometers (GSI 16, GSI 61, Avant, Madsen Astera, Maico), between 1997 and 2012. Results were always within 12 months of the most recent audiometer calibration. The bone conduction scores were used to determine the pure tone average (PTA) and thresholds when air conduction values could not be obtained, as in cases of external auditory canal stenosis or atresia. Masking was used except when the dB difference between ears at each frequency did not exceed the intra-aural attenuation that would necessitate its' use. Responses from the right and left ears were recorded separately. Live voice was used for spondees and recorded speech was used for the NU-6 word list. Normal responses for pure tones were considered to be less than or equal to 25 dB HL. Hearing loss was defined as a pure tone response greater than 25 dB HL at any given frequency.

Molecular Assessment

In order to determine precise breakpoints and thus determine the genes involved in the deletion, we performed high resolution high resolution microarray comparative genomic hybridization as previously described (6). If a gain or loss of non-18q material was identified, the subject was excluded from the study.

Results

The current study population included 14 males and 24 females between the ages of 5 months and 40 years of age with an average age of 16.7 years. The youngest age for audiometric data used in this report was five years based on reliability and completeness of testing. In this cohort of 38 participants, fourteen had normal hearing thresholds at all frequencies either by air conduction or bone conduction. Twenty-four participants were identified with high frequency sensorineural hearing loss.

In the normal hearing group the mean PTA was 14dB bilaterally, with a range from 0 dB to 20 dB Figure 1. At 4kHz the average threshold was 10 dB for the right and 11 dB for the left ear with a range from 0-25 dB. At 8 kHz, the average threshold was 9 dB for the right ear

and 10 dB for the left ear with a range from 0-25 dB. The speech discriminations scores (SDS) were greater than 98% for both ears in all participants with normal hearing. Of this group, two participants had aural atresia and an additional two had canal stenosis. Bone conduction was used to determine hearing thresholds in those with aural atresia.

Twenty four subjects demonstrated hearing loss beginning at or above 4 kHz. This group also demonstrated a normal PTA and speech discrimination score (Figure 2). The average PTA for the right ear was 16 dB, and for the left ear it was 17 dB. The average SDS was 92% for the right ear and 94% for the left ear. The average thresholds at 4kHz and 8 kHz were markedly different from the normal group. At 4kHz the average right ear threshold was 27 dB, and left ear threshold average was 33 dB. At 8kHz the average threshold was 66 dB for the right ear and 67dB for the left ear. The pattern of loss was down sloping for 23/ 24 individuals, while in one subject it was a flat loss between 4-8 kHz at 45 dB. Aural atresia was present in seven participants and canal stenosis in four others. Bone conduction was used to determine thresholds in six of these participants.

Since high frequency sensorineural hearing loss (HFSNHL) is most often associated with age related hearing changes, we compared the ages of both groups of individuals. If the HFSNHL were age-related, we would expect the patients with hearing loss to be older than those without. Thus, we compared the youngest age at which a diagnosis of HFSNHL was made with the oldest age of evaluation for those with normal sensorineural hearing. The 14 individuals with normal sensorineural hearing had an average age of 12 years at the time of evaluation with an age range between 4.2 years and 24.6 years. The 24 individuals with HFSNHL had an average age of 14.6 years with a range between 5 years and 32.7 years. The ages of the two groups were not significantly different.

Molecular Analysis

The molecular data illustrating the chromosome 18 content for each of the study participants is shown in Figure 3. The findings are presented in two groups. Those with normal high frequency hearing are grouped at the top of panel A. Those found to have a high frequency sensorineural hearing loss are illustrated below them in panel A. The common region of overlapping hemizygosity in the group with hearing loss is shown between the two vertical red lines. Only those individuals with hearing loss are considered when defining the critical region for any phenotype. Specifically in this case, high frequency hearing loss may be later onset so participants with normal hearing are not considered when defining a critical region for a phenotype. The individuals whose breakpoints define this region are individuals 18q-31C and 18q-197C. The region between their breakpoints, which is the common region of hemizygosity is shown expanded in panel B in relation to the known genes in that region. This defines the critical region for high frequency sensorineural hearing loss and identifies 8 candidate genes (dark blue) as well as several hypothetical genes (light blue).

Discussion

We evaluated a cohort of 38 individuals with distal chromosome 18q deletions for hearing acuity. We found 24 individuals with a sensorineural hearing loss at or above 4 kHz. The

loss ranged from mild to profound. Frequencies below 4 kHz were unaffected, and resulted in a normal PTA and SDS.

We also performed high resolution microarray comparative genomic hybridization to determine the exact gene copy number for each gene on chromosome 18 for each participant. Comparison of the chromosome regions of hemizyosity in those with HFSNHL revealed a shared hemizygous region of 2.8 Mb containing eight known genes. Three of those genes' roles in human biology is not yet known (*ZADH2*, *Corf62* and *ZNF236*). Two other of the genes (*ZNF516* and *GALNR1*) have been found to be hemizygous in control populations, implying that they do not play a role in producing a phenotype in distal 18q- (14, 15). However, it should be noted that the individuals in those studies are self-declared to be "normal" and were not tested for hearing acuity. Two other genes in this region have a known role in the distal 18q phenotype. Single gene deletions or loss of functions mutations in *ZNF407* have recently been found to cause intellectual disability, communication delay, and autistic features (16). As discussed in the Introduction, single gene deletions and loss of function mutations in *TSHZ1* have been linked to the aural atresia phenotype (17).

Recent work suggests that presbycusis may be due to myelin degeneration of the auditory nerve that results in a loss of myelin basic protein (18). We would hypothesize that a congenital deficiency of myelin basic protein caused by hemizyosity of the *MBP* gene could also result in HFSNHL. Indeed, the region identified in this paper includes the entire critical region for dysmyelination (8, 19). In fact, in the cohort described here all of the individuals with HFSNHL on whom we have MRI data (22/24) also have delayed myelination of the brain as do 9 of the 13 with normal HFSN hearing. No one with HFSNHL has normal myelination. It is therefore logical to hypothesize that *MBP* hemizyosity results in dysmyelination as well as high frequency sensorineural hearing loss. This is an area for further investigation.

It should be noted that several study participants with normal high frequency hearing also had deletions that included all or part of the critical region portrayed in Figure 1. In fact, there are a total of 29 study participants who have a deletion of the entire critical region, yet only 24 of them have HFSNHL at this point in time. These five individuals are considered to be non-penetrant, suggesting that the deletion is necessary but not always sufficient to cause hearing loss in the distal 18q- population. As 24 of 29 individuals are hemizygous for this region, the condition is 83% penetrant. However, should any of these individuals develop HFSNHL in the future the penetrance will increase, making the current estimate of penetrance the lower limit.

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Figure 1. Normal hearing group

PTA is 14 dB for both the right and left ears. Hearing level at 4kHz and 8 kHz is less than 25 dB for all participants. SDS is greater than 98% for all subjects.

Figure 2. Abnormal hearing group

PTA is less than 25 dB for both the right and left ears. At 4kHz the average threshold for the right ear is 27 dB, and 33 dB for the left. At 8 kHz the average threshold for the right ear is 66 dB, and 67 dB for the left. The speech discrimination score is above 92% for all subjects.

Figure 3. Molecular analysis of individuals with 18q deletions

At the top of panel A is an ideogram showing the banding pattern of chromosome 18. Below it is a representation of the chromosome microarray data displayed using the UCSC Genome Browser custom track feature. The data are in two groups, those with normal hearing in the top group and those with HFSNHL in the lower group. The individual participant's study number is shown down the left next to a bar showing the extent of their abnormal chromosome 18. At the end of each, the breakpoint region is shown by a darker line. Small duplications at the breakpoint (a common feature) are indicated by the dark grey (red) regions. Panel B shows a magnified representation of the smallest region of common hemizygosity in everyone with HFSNHL. This is the critical region of the chromosome for this phenotype. This region is bracketed by the breakpoints of individuals 18q-8C and 18q-197C. The locations of the known genes are shown below the participant data thereby indicating the genes in the HFSNHL region.