No Evidence of Measles Virus in Stapes Samples from Patients with Otosclerosis

ALEXIS BOZORG GRAYELI,1* PIERRE PALMER,2 PATRICE TRAN BA HUY,3 JACQUES SOUDANT,4 OLIVIER STERKERS,5 PIERRE LEBON,2 AND EVELYNE FERRARY1

INSERM U.426, Faculté Xavier Bichat, Université Paris 7; Virology Department, Hôpital Saint-Vincent-de-Paul, AP-HP, Université Paris 5; Otolaryngology Department, Hôpital Lariboisière, AP-HP, Paris; ENT Department, Hôpital Pitié-Salpêtrière, AP-HP, Paris; and ENT Department, Hôpital Beaujon, AP-HP, Clichy, France

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Otosclerosis is a localized bone dystrophy of unknown etiology mainly involving the stapes. The hypothesis of a persistent infection by the measles virus was based on the inconstant detection of the virus by various methods, including reverse transcription-PCR (RT-PCR) of patients’ stapes samples. The aim of this work was to investigate the presence of the measles virus in stapedial otosclerosis foci by different sensitive methods. Pathologic stapes samples were obtained from 35 patients suffering from otosclerosis. Measles virus detection was performed by (i) cocultures of Vero cells and primary cell cultures of bone samples (n = 7), (ii) immunofluorescence study of these cocultures (n = 3), and (iii) RT-PCR on RNA directly obtained from fresh frozen samples (n = 28) and on RNA extracted from the primary cell cultures (n = 2). Viral genomic regions coding for N (nucleoprotein) and M (matrix) proteins were separately amplified in 35 samples using the measles virus Edmonston strain. Glyceraldehyde-3-phosphate dehydrogenase mRNA was used as a marker of total RNA recovery. PCR products were tested by Southern blot hybridization technique to improve sensitivity and specificity. PCRs amplifying the M and the N protein genes were able to detect the control measles virus RNA at titers as low as 0.1 and 0.01 50% tissue culture infective dose, respectively. With these highly sensitive methods, we could not evidence the presence of the measles virus in any of our bone samples or primary bone cell cultures. Our results do not confirm the hypothesis of persistent measles virus infection in otosclerosis.

Otosclerosis is a bone dystrophy localized to the otic capsule, an embryonic structure from which develop the inner ear and the stapes footplate (9). This disease is a frequent cause of deafness in adults, affecting over 10% of deaf adult patients seen in outpatient activity by otolaryngologists in the United States (9). Its prevalence is estimated at 0.2 to 0.3% of the population in western Europe and North America (9). About 10% of Caucasian adult temporal bones present histologic otosclerosis foci (12). In the early forms, otosclerosis foci are found only in the stapes and disturb sound transmission, while advanced lesions can involve the cochlea, producing sensorineural hearing loss, or the vestibule, causing vertigo (9, 11). The otosclerosis process in the otic capsule is initiated by an increase in bone resorption with the presence of numerous resorption foci rich in blood vessels, also designated osteonangiogenic foci (11, 27). In response to this increase in bone resorption, a reconstruction phase conducted by numerous osteoblasts present in otosclerotic tissue leads to fibrous bone foci (11, 27). These lesions showing a high bone turnover are similar to those observed in Paget’s disease (27). Although the clinical signs and the histologic aspects of otosclerosis are widely described (9, 12, 27), the pathogenesis of this disease remains unclear, and many hypotheses, including autoimmune and viral origins, have been advanced (1, 2, 4, 15–17, 31).

The hypothesis of persistent measles virus infection in otosclerosis has been advanced by some authors based on electron microscopy observations (15), immunohistologic studies (2, 16, 26), and reverse transcription (RT) followed by PCR results (1, 4, 17–19). However, these studies demonstrated the presence of different viruses in some cases (16, 26), and did not provide reproducible data in order to confirm the implication of the measles virus in otosclerosis foci (1, 4, 17–19). Moreover, the majority of RT-PCR studies were realized on a small number of patients, ranging from 9 to 14 (17–19). Considering the lack of conclusive evidence in favor of this hypothesis, the aim of our study was to detect the presence of the measles virus in fresh otosclerotic samples in a large population (n = 35) using highly sensitive methods.

MATERIALS AND METHODS

Patients. The population was composed of 16 males and 19 females. The mean age was 42 years (range, 27 to 61). All patients presented normal tympanic membrane on otoscopy and progressive conductive hearing loss associated with absent stapedial reflex on preoperative audiometry. Preoperative clinical, audiometric, and imaging data were obtained from medical files. The diagnosis of otosclerosis was confirmed during surgery by the aspect of the stapes and its immobility. The extent of the disease was assessed during surgery and classified in five stages (23): I, stapedial ankylosis with normal aspect; II, stapedial footplate involvement in its anterior or posterior part; III, stapedial footplate bipolar involvement; IV, stapedial footplate entire involvement; and V, total obstruction of the oval window by otosclerosis. During surgery, the involved stapes’ footplate and the superstructure were removed in 24 cases (69%), and only the pathologic superstructure was obtained in 11 cases (31%). The approval of the ethics committee and the patients’ consent were obtained for these samplings.

Cell cultures. Stapedial bone fragments were placed in 10-cm2 culture plates in a culture medium composed of Dulbecco’s minimal essential medium (MEM) with 4.5% glucose (Gibco-BRL Life Technologies, Cergy-Pontoise, France) containing vancomycin (12.5 mg/liter) ( Lilly, Saint-Cloud, France) and 30% fetal calf serum in a humidified atmosphere of 95% air and 5% CO2 at 37°C. Vancomycin was used to prevent the infection of the culture medium by cutaneous saprophytic bacteria. In fact, the middle ear was approached through the external auditory canal for these samplings, and the samples were frequently in contact with the skin. During culture, cells migrated from bone explants, and maximal cellular growth from the explants was obtained in about 21 days, at which time cells were trypsinized, plated homogeneously on the culture surface, and allowed to grow to confluence for 14 to 21 additional days in contact with the explants. At confluence, cells were trypsinized and counted. Half of the cells were used for cocultures with Vero cells (African green monkey renal cells), and the other half were
TABLE 1. Sequences and positions of the oligonucleotides used for measles virus and GAPDH cDNA amplification and Southern blot analysis\(^a\)

<table>
<thead>
<tr>
<th>Gene amplified</th>
<th>Primer</th>
<th>5' position</th>
<th>5'—3' sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measles virus</td>
<td>M protein</td>
<td>MV03</td>
<td>499</td>
</tr>
<tr>
<td>M protein</td>
<td>MV05</td>
<td>913</td>
<td>AAT CGA TTA AGG TCA TTA GTG AT</td>
</tr>
<tr>
<td>M protein</td>
<td>MV04</td>
<td>727</td>
<td>CAT TTT GCA ATA ATC GGC GAC AGT</td>
</tr>
<tr>
<td>N protein</td>
<td>MVNP1</td>
<td>TCA AGA GGA CAC CCG GGA AC</td>
<td></td>
</tr>
<tr>
<td>N protein</td>
<td>MVNP2</td>
<td>1003</td>
<td>GCT CCA GAG CAG AGG GTA T</td>
</tr>
<tr>
<td>N protein</td>
<td>MVNP3</td>
<td>740</td>
<td>AAC CCA GGA TTT CTG AAA TG</td>
</tr>
<tr>
<td>N protein</td>
<td>MVNP4</td>
<td>865</td>
<td>TTC ATG CAC TTT AAC AGC AG</td>
</tr>
<tr>
<td>GAPDH</td>
<td>10</td>
<td>378</td>
<td>AAG GCT GGT GCT CTT TG</td>
</tr>
<tr>
<td>GAPDH</td>
<td>11</td>
<td>1162</td>
<td>GTG TGG TGG GGC ACT GAG</td>
</tr>
</tbody>
</table>

\(^a\) Reverse primers are shown in bold. The pair MV03/MV05 was used to perform the RT-PCR amplification of the matrix protein (M protein) gene. The pairs MVNP1/MVNP2 and MVNP3/MVNP4 were used for the first and the second steps of the nested PCR amplifying the viral nucleoprotein (N protein) gene, respectively. Oligonucleotides MV04 and MVNP3 were used for the hybridization step. The positions on the M protein cDNA sequence for MV03-5 (8) and on the N protein cDNA sequence for MVNP1-4 (22) are shown.

replated in three 10-cm² culture wells and used for RNA extraction at confluence. This stage was defined as the first passage.

Cells obtained from the primary stages cultures at confluence were mixed 1:1 with a suspension of Vero cells and replated in 2.5-cm² wells in the presence of Dulbecco's MEM plus 10% fetal calf serum, 5 mM glutamine, 250 IU of penicillin per ml, and 80 μg of gentamicin per ml. The cocultures were observed for 3 weeks.

Immunofluorescence assays were performed on cocultures obtained from three patients (patients 1 to 3) using anti-measles virus monoclonal antibody (Biosys, Compiegne, France) and a mixture of monoclonal antibodies against parainfluenza viruses 1, 2, and 3, adenovirus, and respiratory syncytial virus (Sanofi-Pasteur, Paris, France) on separate slides for each sample. Mouse monoclonal antibodies were used as the primary antibodies, and polyclonal goat anti-mouse immunoglobulin antibody conjugated with fluorescein isothiocyanate was used as the conjugated antibody (Sanofi-Pasteur). Cocultures were prepared in duplicate on slides and allowed to grow to confluence for 7 days. At this stage, cells were fixed with 90% acetone for 10 min at 4°C and dried. Slides were subsequently incubated with primary antibodies diluted 1:10 to 1:20 in phosphate-buffered saline (PBS) solution for 30 min at 37°C, washed three times with PBS, incubated with the conjugated antibody for 30 min at 37°C. Finally, slides were washed three times with PBS and observed under a fluorescence microscope.

RNA extraction, RT-PCR, and Southern blot assays. To extract total RNA from bone fragments obtained from patients 8 to 35, the bone fragments were crushed in 1 ml of lysis buffer (guanidium thiocyanate, 4 M; sodium citrate, 25 mM [pH 7.0]; N-laurylsarcosine, 0.5%; β-mercaptoethanol, 0.1 M) using a Polytron. RNA was then extracted using phenol-water and isoamyl alcohol-chloroform (1:1, vol/vol) and precipitated with isopropranol (7). The pellet was re-suspended in 20 μl of nuclease-free water and used for RT. For RNA extraction from the first-passage primary cell cultures (patients 1 and 2), cells were lysed in a similar lysis buffer using a cell lifter, samples were processed as described above, and the pellet was suspended in 50 μl of water. RNA solution was heated at 95°C for 3 min and cooled to 4°C before RT.

Synthesis of cDNA was performed in reverse transcriptase buffer (50 mM Tris·HCl, [pH 8.4], 40 mM NaCl, 10 mM dithiothreitol, 6 mM MgCl₂ containing each dNTP 200 μM, U of RNasin (Promega, Charbonnières, France), random hexamer at 2.5 μM, 10 μl of avian myeloblastosis virus reverse transcriptase (Promega), and 10 μl of the RNA solution in a total volume of 20 μl). The reaction mix was incubated for 10 min at room temperature, followed by 45 min at 42°C, and finally heated at 95°C for 5 min.

cDNA amplification of the measles virus genome was performed in two separate regions, one coding for the matrix protein (M protein) and the other coding for the nucleoprotein (N protein) (Optimal PCR conditions, including MgCl₂ concentration, annealing temperatures, and the effect of different additives (formamide and glycerol), were determined in preliminary experiments.

Sequence alignments between several reported strains responsible for acute and persistent infections permitted verification of the conservation of the target sequences. The M protein gene was amplified by a single-step PCR, using the oligonucleotide pair designated MV03/MV05 already described (28). This PCR yielded a DNA fragment of 414 bp (Table 1). The N protein gene was amplified by a nested PCR (2) (two oligonucleotide pairs designated MVNP1/MVNP2 and MVNP3/MVNP4. The pair MVNP1/MVNP2 amplified a 284-bp DNA fragment, and the internal pair MVNP3/MVNP4 gave a final product of 125 bp (Table 1). A 784-bp fragment of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was amplified using a pair of oligonucleotides designated 10/11 (Table 1). For PCR, 6 μl of cDNA was added to 44 μl of an amplification mix containing 10 mM Tris·HCl ([pH 8.4], 50 mM KCl, 1.5 mM MgCl₂ ([M protein gene and GAPDH]) or 2 mM MgCl₂ ([for N protein gene]), 50 μM each of the four dNTPs, each primer at 0.5 mM (MV03/MV05 and 10/11) or 1 mM (MVNP1 to 4) final concentrations, and 1.5 U of Taq DNA polymerase (Boehringer Mannheim, Meylan, France). After an initial denaturation step at 94°C for 3 min, the cDNA was amplified by 40 cycles of heating at 94°C for 15 s, annealing at 55°C (M protein gene), 58°C (N protein gene), or 60°C (GAPDH cDNA) for 15 s, and polymerization at 72°C for 1.5 s. The reaction was ended by an elongation step at 72°C for 5 min. The specific DNA band was detected by 2% agarose gel electrophoresis containing ethidium bromide.

Five microliters of the PCR product obtained with the MVNP3/MVNP2 pair was subjected to 35 cycles of further amplification with the MVNP3/MVNP4 internal pair in the conditions described above.

The specificity of the reaction was confirmed by Southern blot hybridization using digoxigenin-labeled oligonucleotide MVO4 for the M protein gene PCR products and MVNP3 for the N protein gene first-step PCR products. After denaturation (0.4 N NaOH, 30 min), the gel was blotted by capillarity on a positively charged membrane (Hybond N; Amersham, Les Ulis, France). The membrane was hybridized overnight with the amplified fragment in hybridization buffer containing 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.02% sodium dodecyl sulfate (SDS), 0.1% N-laurylsarcosine, 2% blocking reagent, and 10% formamide. The filters were washed twice with 0.1% SDS in 2× SSC at 42°C max. followed by two 15-min washes at 0.1% SDS at 42°C for 15 min. Antidigoxigenin Fab fragments conjugated with alkaline phosphatase were incubated with the membrane for 30 min before washing and revelation by an enzyme-catalyzed color reaction with nitroblue tetrazolium (NTB)-5-bromo-4-chloro-3-indolylphosphate (BCIP) as the substrate. The entire procedure (labeling and detection) was performed according to the manufacturer's instructions (Boehringer-Mannheim).

Positive and negative controls were inserted in every PCR run, and their results were as expected. The sensitivity of the PCR detection method was evaluated on dilution series of the Edmonston virus strain. Titration of the viral infectivity of this strain was performed by culture in Vero cells and was expressed as 50% tissue culture-infective dose (TCID₅₀). In order to decrease the number of noninfectious particles, the viral stock was prepared from Vero cells with a low-multiplicity infection (≈ 0.1 TCID₅₀/50 cells) and was exposed to an additional 30°C, 5% CO₂ for 30 min before washing and revelation by an enzyme-catalyzed color reaction with nitroblue tetrazolium (NTB)-5-bromo-4-chloro-3-indolylphosphate (BCIP) as the sub-

RESULTS

Clinical, audiometric, and imaging data. Clinical and audiometric data were obtained for all 35 patients. Otosclerosis involved both ears in 21 patients (61%) and was unilateral in 14 cases (39%). The disease appeared as sporadic in 30 cases (87%), while other family members were reported to be suffering from otosclerosis in 5 cases (13%). The mean interval between the onset of symptoms and surgery was 65 months (range, 6 to 204 months). Vestibular signs such as imbalance and episodic vertigo were reported in three cases (9%). Twenty patients (57%) presented with pure conductive hearing loss, and the remaining 15 patients (43%) suffered from mixed conductive and sensorineural hearing loss. The mean air conduction hearing loss measured on pure-tone audiometry on frequencies ranging from 125 to 8,000 Hz was 50 dB (range, 40 to 70 dB), and the mean bone conduction hearing loss on frequencies ranging from 250 to 4,000 Hz was 16 dB (range, 5 to 58 dB). Preoperative temporal bone computed tomography scans were obtained in 11 cases (31%). Among these patients, lytic foci in the anterior part of the oval window (fissula ante fenestram) were observed in four cases (36%), bilateral lytic foci extending to the perilabyrinthine bone were evidenced in three cases (27%), and a normal bone aspect was noted in four cases (36%).

The extent of the otosclerotic foci was evaluated during surgery as stage II (anterior or posterior footplate involvement) in 11 patients (31%), stage III (bipolar footplate involvement) in 10 patients (29%), stage IV (bipolar footplate involvement) in 4 patients (11%), stage V (bilateral footplate involvement) in 3 patients (9%), and a normal bone aspect was noted in four cases (11%).
volvement) in 13 patients (37%), and stage IV (entire footplate involvement) in 11 patients (31%). Stages I and V of otosclerosis extension were not observed in this series.

**Primary cell cultures.** Primary cell cultures and cocultures with Vero cells were performed for patients 1 to 7. Pathologic stapes samples in these cases comprised both the superstructure and the footplate.

In the primary cell cultures (Fig. 1), cells grew in a centrifugal manner from the explants. In bone cell cultures at confluence, numerous mineralization foci surrounded by polygonal plump cells resembling osteoblasts were observed. No morphologic signs of measles virus infection, such as syncytium formation, fuzzy cytoplasmic inclusions, or stellate and dendritic cells already described in vitro (5) could be seen in these first-passage primary cell cultures.

Cocultures with Vero cells performed for patients 1 to 7 followed by an observation period of 3 weeks did not evidence any cytopathic effect such as syncytium formation (data not shown). Immunofluorescence assays on cocultures performed on patients 1 to 3 did not reveal the presence of measles virus, parainfluenza viruses 1, 2, and 3, adenovirus, and respiratory syncytial virus antigens in any of the cocultures tested (data not shown).

**Viral genomic material detection.** The single-step PCR amplifying the M protein cDNA from a measles virus RNA extract containing 1 TCID₅₀ showed a signal of the expected size (414 bp). The nested PCR amplifying the N protein cDNA from a measles virus RNA extract containing 0.01 TCID₅₀ also showed a signal of the expected size (125 bp) (Fig. 2A). Southern blot on M protein PCR and the N protein first-step PCR products both yielded a sensitivity of 0.1 TCID₅₀ on titrated control virus samples (Fig. 2B). No signal could be detected with respiratory syncytial virus, parainfluenza viruses, Epstein-Barr virus, or cytomegalovirus.

RT-PCR assays were performed on RNA directly extracted from the bone samples in 28 cases (patients 8 to 35) and on RNA extracted from first-passage primary cell cultures in two samples (patients 1 and 2). The RT-PCR amplifying GAPDH was performed in all 30 cases (Fig. 3). A GAPDH signal of the expected size (784 bp) could be evidenced on the agarose gel in 22 of 30 samples (73%) (patients 1, 2, and 8 to 27). None of the 30 otosclerotic samples tested in parallel for the M and N proteins showed a signal of the expected size on the agarose gel (Fig. 3). The Southern blot assays did not detect any specific PCR product in the 30 otosclerotic samples tested (Fig. 3).

**DISCUSSION**

The hypothesis of persistent measles virus infection in otosclerosis is mainly based on RT-PCR studies (1, 4, 16, 19). Our observations in stapedial otosclerotic samples using Vero cell cocultures, immunofluorescence, and sensitive RT-PCR methods did not detect the measles virus in the otosclerotic foci. Clinical, audiometric, and imaging data for our population were consistent with ongoing otosclerosis in all cases, and the disease was extensive (stage IV lesions and associated sensorineural hearing loss) and highly active (lytic foci on computed tomographic scan) in more than one third of the patients.

Vero cell cocultures did not show any cytopathic effect during the 3-week observation period in our study. Although this method represents a sensitive routine method for the diagnosis of acute measles virus infections (20) and is a preferred means of isolating defective virus in subacute sclerosing panencephalitis brain samples (20), the absence of cytopathic effect alone could not eliminate the possibility of an infection by either a defective virus or a virus present at low titers (20). The immunofluorescence was used to enhance the sensitivity and specificity of viral detection in the cocultures. This technique did not reveal any viral antigen in our cocultures, but immunofluorescence may also yield negative results for a defective virus (20).

Consequently, in addition to these methods, samples were assessed by RT-PCR. Two different genomic regions coding...
for the M and the N proteins, which are highly conserved among characterized strains responsible for acute and persistent measles virus infection, were chosen for the RT-PCR assays (6, 28). The RT-PCR amplifying the M protein gene had already demonstrated its high sensitivity in clinical samples (28). However, since N protein mRNA is reported to be more abundant and less prone to mutations in persistent infection (6), samples were also subjected to RT-PCR to amplify this region. This amplification yielded high sensitivity levels in terms of TCID50. The recovery of total RNA from the bone samples or the cell cultures was verified by GAPDH mRNA detection. Although maximal precaution was employed in the handling of the samples for RNA conservation, GAPDH mRNA could not be detected in some samples (8 of 30). This is probably related to the low cellular content of the samples, since their volumes did not exceed a few tenths of a cubic millimeter. In a different series, including more than 50 samples of similar size obtained in similar technical conditions, we obtained primary cell cultures in all cases (unpublished data). This observation indicates that all the samples contain viable cellular material. Although maximal precaution was employed in this study to avoid RNA degradation, another possible factor explaining negative GAPDH detection is spontaneous RNA degradation. Finally, insufficient sensitivity of GAPDH detection may also explain this negative result. By amplifying a relatively long cDNA fragment (784 bp), we aimed to verify the presence of nonfragmented RNA in our samples, and the GAPDH RT-PCR sensitivity could probably be enhanced by choosing a smaller cDNA fragment to amplify. Consequently, the negative GAPDH mRNA detection in some samples does not completely invalidate the measles virus detection results, and in the majority of our samples the presence of a GAPDH signal associated with a negative RT-PCR for the measles virus N and M proteins constitutes a solid argument in favor of the absence of the virus in otosclerotic foci.

Measles virus genomic material was previously detected in otosclerotic stapes samples by RT-PCR methods, but methodological limitations prevented definitive conclusions in these studies (1, 4, 17–19). Indeed, these positive results have been exclusively reported by two groups which have amplified the N protein gene in otosclerotic stapedial samples using a high number of amplification cycles (two steps of 35 and 40 cycles) (4, 17–19). McKenna et al. (17) evaluated 12 otosclerotic and 10 normal stapedial postmortem archival samples. They employed a nested PCR with two amplification sets of 35 and 40 cycles. The tests were repeated three times for each sample. At least one positive test out of three was observed in 30% of control samples versus 92% of pathologic samples. Additionally, the reproducibility of the tests seemed low, since only 25% of pathologic samples had three positive tests. Arnold and Niedermeyer et al. (4, 18, 19) reported a similar proportion of positive tests by performing a similar RT-PCR method in fresh frozen samples. They amplified the N protein gene by RT-PCR including two amplification steps of 35 cycles each. Primers used for the cDNA synthesis and the PCR amplification of the viral mRNA sequences were the same in their three reports (4, 18, 19). These authors observed 44, 93, and 83% of positive tests in series of 9, 14, and 29 otosclerotic patients, respectively. Each series comprised only two control samples for which the tests were negative. The specificity of the final PCR product was tested by Southern blot assays. In these studies, the small number of control cases does not permit any conclusion concerning the relationship between the presence of the measles virus and otosclerosis in the studied population.

Data concerning anti-measles virus immune status were not available for our population, but considering the high inci-
ence of this infection in France, which is estimated at 300,000 to 500,000 annual cases (21), the majority of our patients have probably encountered the virus during childhood.

In any case, data on the anti-measles virus immune status of our patients could not support the hypothesis of a local persistent measles virus infection. On one hand, if otosclerosis occurred in patients who have never encountered the virus, this would work against the hypothesis of persistent measles virus infection in otosclerosis. On the other hand, similar conclusions can be reached from the fact that we did not detect the virus in stapes samples from patients having a history of measles virus infection.

A persistent infection by measles virus has been advanced in many chronic diseases with an inflammatory component, such as multiple sclerosis, Paget’s disease, and Crohn’s disease (24). However, in these cases, the detection of the virus has been inconstant and nonreproducible by different authors (24). The high incidence of acute infections by viruses such as herpes simplex virus and measles virus in the general population and the persistence of these viruses in different organs, including the inner ear (3, 13, 14), in normal individuals hamper the establishment of a causal relationship between the presence of the virus and the chronic disease. Consequently, the responsibility of measles virus for these pathologic processes remains controversial (24).

To explain the implication of a virus in such pathologies in spite of its inconstant detection, the hypothesis of a “hit and run” mechanism has been advanced (29). According to this hypothesis, some viruses, such as enterovirus in diabetes melitut, have the capacity to trigger pathologic processes which can develop in a chronic course after elimination of the virus with the participation of an autoimmune process (29). This hypothesis may be speculated to occur in otosclerosis, which develops in patients between 30 and 50 years old (10) who have probably encountered the virus during childhood, as in the general population (5, 21). In addition, autoimmunity has been suspected to play a possible role in triggering otosclerosis lesions (31).

Another common trait of the above-mentioned diseases is the presence of predisposing genetic factors (22). Otosclerosis has a hereditary character in about 50% of cases (9). Recently, a locus designated Otosclerosis 1 has been identified on chromosome 15q25-q26 by linkage disequilibrium mapping (30). The most important candidate gene reported in this locus codes for “aggrecan,” an important component of the cartilage matrix, from which develops the stapes footplate during the embryonic phase (30). Although the nature of the triggering event in otosclerosis remains unknown, it appears to act on a complex genetic background.

In conclusion, with highly sensitive methods, measles virus could not be detected in a large number of otosclerotic samples. This observation does not confirm the hypothesis of persistent measles virus infection in the pathogenesis of otosclerosis.

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