Supplemental Materials and Methods

Bead-based suspension immunoassay for detection of salivary NID1

The bead-based immunoassay used to measure salivary NID1 level was developed in-house using antibodies and standard protein from R&D systems (Minneapolis, MN). Briefly, the mouse anti-NID1 antibody was first coupled to COOH beads (Millipore, Billerica, MA) using the Bio-Plex Amine Coupling Kit (Bio-Rad Laboratories, Hercules, CA). Then, the coupled beads were added to pre-wetted filter-bottom 96-well microplates (Millipore). After washing with wash buffer (R&D systems), the beads were serially treated with 50 μL of salivary samples or protein standards (recombinant NID1, R&D Systems) for 1 hour, biotin-conjugated goat anti-NID1 antibody (R&D Systems) for 1 hour, and phycoerythrin-conjugated streptavidin (Jackson ImmunoResearch Laboratories, West Grove, PA) for 20 minutes. All procedures were operated at room temperature in the dark. At the end, the beads were suspended in wash buffer and analyzed using the Bio-Plex 200 system (Bio-Rad Laboratories). The concentration of salivary NID1 was calculated based on the standard curve using Bio-Plex Manager software version 4.2 (Bio-Rad Laboratories).

Immunohistochemistry (IHC)

The performance of IHC staining was executed with an automatic IHC device

(BondTM, Vision Biosystems, VIC, Australia) using antibodies against NID1 (1:50 dilution; R&D Systems), IARS (1:50 dilution; Abcam), KARS (1:100 dilution; Abcam, Cambridge, UK), WARS (1:200 dilution; Abcam), and YARS(1:100 dilution; Abcam). The staining procedure was carried out according to manufacturer`s instruction. Briefly, tissue specimens were fixed with 10% formaldehyde, embedded in paraffin, and cut into 4 μm thick sections. Tissue sections were first deparaffinized with xylene, dehydrated with ethanol, and heated in 0.01 M citrate buffer (pH 6.0). After treating with blocking buffer (Dako, Carpinteria, CA) for 5 minutes at room temperature (RT), the sections were serially incubated with primary antibody and substrate, DAB chromogen (Novocastra/Leica Bioosystems, Buffalo Grove, IL). Then, the slides were counterstained with hematoxylin. The expression level of NID1 in tissue was evaluated according to both intensity (3, strong; 2, moderate; 1, weak; 0, no cell staining) and percentage of cell staining. The final score was calculated as the product of score multiplying percentage.