Supplementary methods

Comparative genomic sequence analysis

MultiPipMaker, http://pipmaker.bx.psu.edu/pipmaker
WebMCS, http://zoo.nhgri.nih.gov/mcs

Sequencing

Primer 3, http://fokker.wi.mit.edu/primer3/input.htm

Electrophoretic mobility shift assay (EMSA)

Infrared dye (IRDye-700) end-labeled and unlabeled oligonucleotide probes were purchased from LI-COR Biosciences (Lincoln, NE) and IDT (Coralville, IA), respectively. We performed EMSAs by using the LI-COR EMSA Kit. Briefly, 6μg of human recombinant AP-2α protein (Promega) was incubated with 2.5nM of labeled probe in 20μl binding reaction for 20 min at 4°C. Each binding reaction contained 2μl of LI-COR 10X binding buffer, 2.5mM DTT, 2.5% Tween-20 and 1%NP-40. To prevent nonspecific binding, 1μg of poly(dI-dC) and 0.1μg of shared salmon sperm DNA were added to each reaction. The samples were then electrophoresed for 2.5 hours at 280V in 0.5XTBE buffer on an 8% polyacrylamide gel at 4°C following pre-run of the gels for 1hr at 200V. The gel was then visualized by Odyssey® Infrared Imaging System (LI-COR). For competition experiments, a 50-, 100-, and 200-fold molar excess of unlabeled competitor oligonucleotide probe was incubated for 20min before addition of the labeled probe. For supershift assay, 3μl of anti-AP-2α antibody (Upstate Biotechnology) was incubated for 20 min at 4°C prior to the addition of the labeled probe.
**Chromatin immunoprecipitation (ChIP) assay**

Protein cross-linked chromatin from ~2x10^7 Ad-AP-2α-infected and uninfected HaCaT keratinocyte cells was isolated as detailed previously. A small amount of the chromatin sample was removed as input control, while the remainder was split into two equal parts and immunoprecipitated with 10µg of anti-AP-2α antibody or control mouse IgG overnight at 4°C with agitation.

Chromatin/antibody complexes were then collected using Protein G agarose followed by washing and elution according to the manufacturer’s instructions. DNA was then purified from input chromatin and immunoprecipitation elutions by reversing crosslinks using 200mM NaCl at 65°C for 4 hr followed by the Qiagen DNeasy Kit according to manufacturer’s protocol. The amount of immunoprecipitated target region was determined by SYBR Green (Applied Biosystems) quantitative real-time PCR with primers for the target sequence in MCS-9.7 and control region (Supplementary Table 4) at a final concentration of 100nM. Specific amplification of the target sequences were tested on agarose gel. Real-time PCR was carried out in triplicate on 5ng of DNA under the following conditions: 10 min denaturation at 95°C, followed by 40 cycles of 95°C for 10 sec and 60°C for 30 sec. Amplification of the target amplicon was monitored as a function of increased SYBR Green fluorescence. An analysis threshold was set and the cycle threshold (Ct) computed for each sample. Fold enrichment of target sequence was calculated using the following formula (Fold enrichment = 2^{(Ct_{AP-2α-Ab IPed})-(Ct_{IgG IPed})}).
**Luciferase reporter assay**

For luciferase reporter assay we generated reporter constructs by inserting 540bp genomic segment (chr1:208055787-208056326, UCSC hg18) containing the entire MCS-9.7Kb region upstream of firefly luciferase open reading frame (ORF) driven by the SV40 promoter. DNA samples from individuals homozygous for -14474A>G, -14523G>A, and rs642961 variants were PCR amplified and cloned into the pGL3-Basic and pGL3-Promoter vectors (Promega) in both orientations. HFK cells were seeded in 12-well plates at ~ 4x10^7 cells/well density in 1mL of K-SFM media supplemented with rEGF (0.16ng/mL) and BPE (25μg/mL) 24 hours prior to transfection. These cells were then cotransfected with 1μg of reporter construct and 20ng hRL-TK renilla luciferase plasmid using 2μg of Lipofectamine™ LTX reagent in 500μl of reduced-serum Opti-MEM® I media (Invitrogen). Dual Luciferase® Assay (Promega) was performed 24 hours after transfection according to manufacturer’s protocol. The pGL3-Basic and pGL3-Promoter vectors were used as controls. Renilla luciferase was used as an internal control to normalize the firefly luciferase activity. Luciferase activity was measured with Wallac Victor^2 luminometer (PerkinElmer).

**References**

1. Provenzano, M.J. et al. AP-2 participates in the transcriptional control of the amyloid precursor protein (APP) gene in oral squamous cell carcinoma. *Exp Mol Pathol* **83**, 277-282 (2007).