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^{o}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^{o}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^{o}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[1]. 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^{o}C, followed by 40 cycles of 95^{o}C for 10 sec and 60^{o}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 (C_t) computed for each sample. Fold enrichment of target sequence was calculated using the following formula (Fold enrichment = $2^{(Ct\ AP\text{-}2\alpha\text{-}Ab\ IPed)-(Ct\ IgG\ IPed)}$).

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).