**File S1:** Supporting multiple Tables

**Primary microcephaly gene *MCPH1* shows signatures of tumor suppressors and is regulated by miR-27a in oral squamous cell carcinoma**

Thejaswini Venkatesh1, Mathighatta Nagaraj Nagashri1, Shivananda S. Swamy2, S.M. Azeem Mohiyuddin3, Kodaganur S. Gopinath2 and Arun Kumar1

1Department of Molecular Reproduction, Development and Genetics, Indian Institute of Science, Bangalore 560012, Karnataka, India. 2Department of Surgical Oncology, Bangalore Institute of Oncology, Bangalore 560027, Karnataka, India. 3Department of Otolaryngology and Head and Neck Surgery, R.L. Jalappa Hospital and Research Centre, Kolar 563101, Karnataka, India.

**Corresponding Author**

Prof. Arun Kumar

Department of Molecular Reproduction, Development and Genetics

Indian Institute of Science

Bangalore 560012

India

Email: karun@mrdg.iisc.ernet.in

Telephone: 91-80-2293 2998

Fax: 91-80-2360 0999

Table S1: A summary of clinico-pathological features of patients included in the study.

|  |  |
| --- | --- |
| **Characteristics** | **No. of patients (n=93)** |
| **Median age/range**  ≤ 54  ≥ 55  **Gender**  Males  Females  **Site of cancer**  Buccal mucosa  Tongue-anterior 2/3  Othersa  **Tumor classification**  T1  T2  T3  T4  Epithelial Dysplasia  **Lymph node status**  positive  negative  **Differentiation**  Well  Moderate  Poor  **Tobacco usage**  Tobacco positive#  Tobacco negative  **Treatment**  Surgery  Radiotherapy  Chemotherapy | 54 yrs/32-80  47 (50.54%)  46 (49.46%)  36 (38.71%)  57 (61.29%)  57 (61.29%)  15 (16.12%)  19 (20.43%)  8 (8.60%)  25 (26.88%)  22 (23.65%)  36 (38.70%)  2 (2.58%)  72 (79.12%)  19 (20.88%)  43 (47.25%)  35 (38.46%)  13 (14.28%)  85 (91.39%)  8 (8.60%)  93 (100%)  87 (93.54%)  3 (3.22%) |

a The sites of cancer include lower alveolus, floor of the mouth, lip and

retromolar trigone. # indicates addiction to tobacco, bidi or cigarettes for at least

5 years.

**Table S2**: Details of clinico-pathological features of 93 OSCC and ED patients included in the study.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Pt #** | **Age/Sex** | **Site of tumor** | **TNM** | **Differentiation** | **Tobacco usage** | **Treatment** |
| 5 | 41M | SCC BM | T3N1M0 | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 8 | 50F | SCC BM | T3N1M0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 9 | 45M | Tongue-  anterior 2/3 | T4N2M0 | Well | Chronic Tobacco | Neoadjuvant CT+ surgery |
| 11 | 60F | SCC BM | T4N1MX | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 14 | 60F | SCC BM | T4N1MX | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 13 | 65F | SCC BM | T4N0M0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 12 | 60F | SCC BM | T4N1MX | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 15 | 55F | SCC BM | T3N1M0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 17 | 55F | SCC BM | T4N1MX | Well | Chronic Tobacco | Surgery+ post  operative RT |
| 18 | 60F | SCC BM | T3N2M0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 19 | 48F | SCC BM | T4N2bM0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 20 | 50F | Lower alveolus | T4N1M0 | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 21 | 32F | SCC BM | T4N1M0 | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 22 | 60F | SCC BM | T2N1M0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 26 | 45M | Lower alveolus | T3N1M0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 27 | 40F | SCC BM | T4N1M0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 30 | 65F | SCC BM | T4N1M0 | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 34 | 65F | SCC BM | T3N1M0 | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 38 | 40M | RMT | T4N1M0 | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 39 | 55F | Lower alveolus | T4N1M0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 40 | 50F | SCC BM | T2N1M0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 41 | 57F | RMT | T1N0M0 | Poor | Chronic Tobacco | Surgery+ post operative RT |
| 44 | 60F | SCC BM | T3N1M0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 47 | 45F | SCC BM | T2N1M0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 50 | 50M | SCC BM | T4N2bM0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 52 | 70M | ED | N/A | N/A | No Tobacco | Surgery |
| 53 | 38F | SCC BM | T2N1M0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 54 | 40M | Tongue-anterior 2/3 | T4N1M0 | Well | Chronic Tobacco | Surgery |
| 55 | 40F | SCC BM | T4N1M0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 56 | 50F | ED | N/A | N/A | No Tobacco | Surgery |
| 57 | 62F | SCC BM | T1N0M0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 59 | 70F | RMT | T4N2bM0 | Well | Chronic Tobaco | Surgery+ post operative RT |
| 60 | 40F | SCC BM | T4aN1MX | Poor | Chronic Tobacco | Surgery+ post operative RT |
| 62 | 35F | SCC BM | T3N0M0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 63 | 40F | SCC BM | T4aN1MX | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 65 | 55F | SCC BM | T2N1MX | Well | Chronic Tobacco | Surgery+ post operative RT |
| 66 | 55F | SCC BM | T3N2bM0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 67 | 38F | SCC BM | T2N1M0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 68 | 65M | Lower alveolus | T3N2bM0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 69 | 70F | SCC BM | T2N1M0 | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 70 | 40F | SCC BM | T2N1M0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 71 | 51M | SCC BM | T4N1M0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 72 | 40F | SCC BM | T4aN2bM0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 73 | 50F | SCC BM | T3N1M0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 74 | 54F | SCC BM | T3N0M0 | Well | No Tobacco | Neoadjuvant CT+surgery |
| 75 | 62M | Tongue-anterior 2/3 | T4N2M0 | Poor | Chronic Tobacco | Surgery+ post operative RT |
| 76 | 47M | Tongue-anterior 2/3 | T4N2M0 | Moderate | Chronic Tobacco | Neoadjuvant CT+surgery |
| 77 | 40F | SCC BM | T4aN1MX | Well | Chronic Tobacco | Surgery+ post operative RT |
| 79 | 67M | SCC BM | T3N1MX | Well | Chronic Tobacco | Surgery+ post operative RT |
| 80 | 67M | FOM | T4N0M0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 83 | 50M | Lower alveolus | T3N2bMX | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 92 | 50F | SCC BM | T4aN2bM0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 95 | 52M | FOM | T3N2bM0 | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 101 | 48F | SCC BM | T2N1MX | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 108 | 56M | Upper alveolus | T4N2bM0 | Poor | Chronic Tobacco | Surgery+ post operative RT |
| 109 | 45M | SCC BM | T3N1M0 | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 110 | 45M | SCC BM | T2N1M0 | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 113 | 80F | SCC BM | T1N0M0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 114 | 68F | SCC BM | T3N1M0 | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 115 | 55F | SCC BM | T4N1M0 | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 116 | 40M | RMT | T4N0M0 | Poor | Chronic Tobacco | Surgery+ post operative RT |
| 121 | 70F | Lower alveolus | T4N1M0 | Poor | Chronic Tobacco | Surgery+ post operative RT |
| 125 | 57M | Tongue-anterior 2/3 | T2N1M0 | Moderate | No Tobacco | Surgery+ post operative RT |
| 127 | 75F | Lip | T1N0M0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 128 | 60F | SCC BM | T2N0M0 | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 133 | 71F | SCC BM | T2N0M0 | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 135 | 41M | SCC BM | T2N0M0 | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 139 | 71F | SCC BM | T4N1M0 | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 140 | 70M | Lip | T4N1M0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 143 | 43M | SCC BM | T3N1M0 | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 144 | 39M | SCC BM | T2N1M0 | Poor | Chronic Tobacco | Surgery+ post operative RT |
| 155 | 60F | SCC BM | T3N0MX | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 156 | 75M | Tongue-anterior 2/3 | T2N0M0 | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 158 | 50F | SCC BM | T2N1M0 | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 157 | 42M | Tongue-anterior 2/3 | T1N0M0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 159 | 40F | Lower alveolus | T4N0M0 | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 165 | 65M | Tongue-anterior 2/3 | T2N1M0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 166 | 50F | SCC BM | T2N1M0 | Poor | Chronic Tobacco | Surgery+ post operative RT |
| 174 | 70F | SCC BM | T2N0M0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 175 | 72M | SCC BM | T4N1M0 | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 177 | 59M | FOM | T2N1M0 | Poor | Chronic Tobacco | Surgery+ post operative RT |
| 180 | 71M | Tongue-anterior 2/3 | T3N1M0 | Poor | Chronic Tobacco | Surgery+ post operative RT |
| 181 | 45M | Tongue-anterior 2/3 | T2N1M0 | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 182 | 65M | Tongue-anterior 2/3 | T1N0M0 | Well | No Tobacco | Surgery+ post operative RT |
| 183 | 50M | Tongue-anterior 2/3 | T1N1M0 | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 191 | 45F | Lower alveolus | T2N0M0 | Moderate | Chronic Tobacco | Surgery+ post operative RT |
| 192 | 56M | Tongue-anterior 2/3 | T1N1M0 | Moderate | No Tobacco | Surgery+ post operative RT |
| 194 | 48F | Lower alveolus | T4N2bM0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 202 | 37F | Tongue-anterior 2/3 | T2N2M0 | Moderate | No Tobacco | Surgery+ post operative RT |
| 206 | 44M | Tongue-anterior 2/3 | T3N2bM0 | Poor | Chronic Tobacco | Surgery+ post operative RT |
| 212 | 65F | SCC BM | T4N1M0 | Poor | Chronic Tobacco | Surgery+ post operative RT |
| 214 | 60F | SCC BM | T2N2M0 | Well | Chronic Tobacco | Surgery+ post operative RT |
| 219 | 68M | SCC BM | T3N1M0 | Poor | No Tobacco | Surgery+ post operative RT |

Abbreviations: Pt#, patient number; M, male; F, female; ED, epithelial dysplasia; BM, buccal mucosa; SCC BM, squamous cell carcinoma of the buccal mucosa; RMT, retromolar trigone; FOM, floor of the mouth; RT, radiotherapy; and, CT, chemotherapy.

Table S3: Primers and their PCR conditions for the mutation analysis of the *MCPH1* gene*.*

|  |  |  |  |
| --- | --- | --- | --- |
| **Exon #** | **Sequence (5´ to 3´)** | **Amplicon size (bp)** | **Annealing temperature (oC)** |
| 1a | F: CACCTACAGAGAAATCCCGGAAAC  R:AGGGGTGCCGGTCCTCAAACTC | 258 | 60 |
| 2 | F:CTGTGCCGGCCTCGGTTTAC  R:CTCCCACCGCTTACCCATTG | 324 | 64 |
| 3 | F:CTTGTGTGTATGATTCACCGTTG  R:CAAATTACTAGGTGAGGTAAGAG | 442 | 60 |
| 4 | F:GCTAATACATGTGCAGATTTAGTGC  R:TCCCATGGCCAACCACAGGCT | 404 | 64 |
| 5 | F:CCTGCCTTAAGCAGTTGCAGTAC  R:ATCAGCTCTCTCATGCTGAACCC | 265 | 64 |
| 6 | F:TGAAGTATGAAGGCACTTTTTGGTC  R:GAAAGCTTTCCACCATAATTGAATCG | 262 | 64 |
| 7 | F:TAGAATCACCTATGATTAATAGGAGGAC  R:CACATCAGAAGTTGCTACATGAAATTC | 281 | 64 |
| 8 | F:GAGAAGAACTCAAGTGTGGTTAATAG  R:CAAACGATACTTCTCTTCAAACGTC | 464 | 64 |
| 8a | F:TGCAGGTAAAGTAGTCACCCCTG  R:CTTCTTAGAAAAGACTTCTGCAGCTC | 433 | 55 |
| 8 | F:CTGGAGGCTCTTAGCTGTGG  R:CATCATGTCCTTTTGGAAGAGC | 415 | 64 |
| 8 | F:GAAGCCCTAAGGTGTTGTAGAC  R:AGGTGACTTGGAAAAGGAGATTC | 342 | 64 |
| 9 | F:GACGGAGTTGATGCTGTAGAAC  R:GGTTTATGTTTCATTGACCCACAG | 435 | 64 |
| 10 | F:GCTGGCTAACTGGTGGAACAG  R:CCTAAAGGCACCCAGAATTAGAG | 400 | 64 |
| 11 | F:GTGTAACTGCTTTGATGGGCATG  R:TGCAGATCCACAAGGGTGCACG | 345 | 64 |
| 12 | F:CTTGGTTTATTGCCTGCTAAGG  R:CTGCATTTACCATCGTAAAACAAC | 177 | 64 |
| 13 | F:ACGCTATGGACTGGAGTGGTCC  R:CAGATCTGGACCACACCACAGCG | 340 | 64 |
| 14 | F:AGGTATGTGTGCTCTATGGACGTGG  R:GTCACCACAGGCCAGTGAGGTCAC | 238 | 59 |

Abbreviations: F, forward primer; R, reverse primer; and, bp, base pair. awork with 5%

DMSO. A typical PCR consists of 1X buffer, 1.5 mM of MgCl2, 0.2 nM of each dNTP, 50 ng of each

primer, 1 U of *Taq* DNA polymerase (Sigma-Aldrich, St Louis, MO) and 50 ng of genomic DNA.

Table S4: Primers and their PCR conditions used in RT-PCR and COBRA.

|  |  |  |  |
| --- | --- | --- | --- |
| **Name of amplicon** | **Sequence (5´ to 3´)** | **Amplicon size (bp)** | **Annealing temperature**  **(o C)** |
| *GAPDH* | F:GAAGGGTGAAGGTCGGAGTC  R:GAAGATGGTGATGGGATTTC | 226 | 60 |
| *MCPH1* | F:TCACCACAGCGCAATGGAGAAGAGA  R:ATCACGTGAAATGTTCAAAGGTGCTTC | 145 | 62 |
| *ß-actin* | F:GAGCCTCGCCTTTGCCGATCC  R:GACCCATGCCCACCATCACGC | 196 | 70 |
| miR-27a | RT6-miR- 27a:TGTCAGGCAACCGTATTCACCGTGAGTGGTGCGGAA  MP-fw  Short-miR-27a: CGTCAGATGTCCGAGTAGAGGGGGAACGGCGTTCACAGTGGCTAAG  MP-rev  MP-fw: TGTCAGGCAACCGTATTCACC  MP-rev: CGTCAGATGTCCGAGTAGAGG | 81 | 58 |
| *5S rRNA* | F:GCCCGATCTCGTCTGATCT  R:AGCCTACAGCACCCGGTATT | 94 | 60 |
| *BRCA1* | F:ATCAGATTCAGGGTCATCAGAGAAG  R:CACAGTTGCTCTGGGAGTCTTCAG | 178 | 64 |
| First PCR of CpGIa | F:TTTGAGGTTTGGAGGTATTTTTG R:TAAATTTTTCTCTTCTCCTAAAAAT | 453 | 54 |
| Second PCR of CpGIa | F:GGGTTATTTTGTGGGGGTTTGAAG R:CCCCTAACCCCTAAATTAACCTTC | 373 | 60 |
| First PCR of CpGIIa | F:TTTTAGTTTAGGTGAGTTTAGATGAGG R:AACAAAACTCCTCCCACAAAATCCC | 465 | 54 |
| Second PCR of CpGIIa | F:AAATAGAAGGTTAATTTAGGGGTTAGG R:AAAACAAACAACAAAAAATACCTCACC | 337 | 54 |
| *ASPM* (exon 1)b | F:AGGAGATCCAGGAGGGGTCTCG  R:GCCTGGAGCACGCTCCTCCTG | 356 | 60 |

Abbreviations: F, forward primer; R, reverse primer; bp, base pair. aindicates PCR works with 2 mM of MgCl2; the remaining PCRs work with 1.5 mM MgCl2. b indicates PCR works with 5% DMSO.

Table S5: Details of constructs used in the present study.

|  |  |  |  |
| --- | --- | --- | --- |
| **Construct** | **Primer sequence (5’ to 3’)** | **Amplicon size (bp)** | **Annealing temperature (o C)** |
| pcDNA3/  pre-miR-27a/*EGFP* | F:CCGAAAGCTTCCTGGGGATGGGATTTGCTTC  *Hind* III  R: TCGACTCGAGACCCCTGTTCCTGCTGAACTG  *Xho I* | 366 | 64 |
| pMIR-Report-3’-UTR-S | F:GATAGAGCTCGCGGAGGCCGGGTCAGCCAAG  *Sac* I  R:GACAAAGCTTGAAATGAGACAGTTAGTTTTTATTTATTCC  *Hind* III | 5456 | 55 |
| pMIR-Report-3’-UTR-AS | F: GACAAAGCTTGCGGAGGCCGGGTCAGCCAAG  *Hind* III  R: GATAGAGCTCGAAATGAGACAGTTAGTTTTTATTTATTCC  *Sac* I | 5456 | 55 |
| pMIR-Report-3’-UTR-M1F | F:CAAATGAGAAACAAAAC**CACA**AAGAGAAGGAACTGG  R:CCAGTTCCTTCTCTT**TGTG**GTTTTGTTTCTCATTTG | 5456 | 55 |
| pMIR-Report-3’-UTR-M2F | F:GAATCTGGGCAGCAC**CACA**AAACCAACATTTCTTCAG  R:CTGAAGAAATGTTGGTTT**TGTG**GTGCTGCCCAGATTC | 5456 | 55 |
| pMIR-Report-3’-UTR-MF | F:CAAATGAGAAACAAAAC**CACA**AAGAGAAGGAACTGG  R:CCAGTTCCTTCTCTT**TGTG**GTTTTGTTTCTCATTTG  F:GAATCTGGGCAGCAC**CACA**AAACCAACATTTCTTCAG  R:CTGAAGAAATGTTGGTTT**TGTG**GTGCTGCCCAGATTC | 5456 | 55 |
| pMIR-Report-3’-UTR-S1 | F:GATAGAGCTCGCGGAGGCCGGGTCAGCCAAG  *Sac* I  R:GACAAAGCTTTCAGTCATCAGAAAGTCACCTATGAG  *Hind* III | 340 | 64 |
| pMIR-Report-3’-UTR-S2 | F:GATAGAGCTCGTCTTTCTCTGCAGTCCCCAGCC  *Sac* I  R:GACAAAGCTTGAAATGAGACAGTTAGTTTTTATTTATTCC  *Hind* III | 402 | 56 |
| pMIR-Report-3’-UTR-AS1 | F: GACAAAGCTTGCGGAGGCCGGGTCAGCCAAG  *Hind* III  R: GATAGAGCTCTCAGTCATCAGAAAGTCACCTATGAG  *Sac* I | 340 | 64 |
| pMIR-Report-3’-UTR-AS2 | F: GACAAAGCTTGTCTTTCTCTGCAGTCCCCAGCC  *Hind* III  R: GATAGAGCTCGAAATGAGACAGTTAGTTTTTATTTATTCC  *Sac* I | 402 | 56 |
| pMIR-Report-3’-UTR-M1 | F:CAAATGAGAAACAAAAC**CACA**AAGAGAAGGAACTGG  R:CCAGTTCCTTCTCTT**TGTG**GTTTTGTTTCTCATTTG | 340 | 55 |
| pMIR-Report-3’-UTR-M2 | F:GAATCTGGGCAGCAC**CACA**AAACCAACATTTCTTCAG  R:CTGAAGAAATGTTGGTTT**TGTG**GTGCTGCCCAGATTC | 402 | 55 |

Abbreviations: F, forward primer; R, reverse primer; and, bp, base pair. The mutated residues are in bold in primers for the generation of pMIR-Report-3’-UTR-M1F, pMIR-Report-3’-UTR-M2F, pMIR-Report-3’-UTR-MF, pMIR-Report-3’-UTR-M1 and pMIR-Report-3’-UTR-M2 constructs.

Table S6: *In silico* identification of microRNAs targeting the 3’-UTR of *MCPH1.*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **MicroCosm** | **miRTAR** | **microRNA** | **miRDB** | **TargetScan** |
| hsa-miR-101  hsa-miR-872  **hsa-miR-27a**  **hsa-miR-27b**  hsa-miR-367  hsa-miR-872  hsa-miR-643  hsa-miR-19b  hsa-let-7a  hsa-miR-181a  hsa-miR-593  hsa-miR-548 | hsa-miR-1278  hsa-miR-30a  hsa-miR-143  **hsa-miR-27a**  **hsa-miR-27b**  hsa-let-7a  hsa-let-7b  hsa-let-7c  hsa-miR-146a  hsa-miR-192  hsa-miR-139-5p | **hsa-miR-27a**  **hsa-miR-27b**  hsa-miR-145  hsa-miR-494  hsa-miR-33a  hsa-miR-33b  hsa-miR-599  hsa-miR-653  hsa-miR-381  hsa-miR-300  hsa-miR-599  hsa-miR-129-5p  hsa-let-7a  hsa-let-7b  hsa-let-7c | hsa-miR-532-5p  hsa-miR-616  hsa-miR-409-3p  hsa-miR-579  hsa-miR-1284  hsa-miR-607  **hsa-miR-27a**  **hsa-miR-27b**  hsa-miR-145  hsa-miR-139-5p  hsa-miR-942  hsa-miR-588  hsa-miR-361  hsa-miR-33b  hsa-miR-129-5p | hsa-miR-197  hsa-miR-450  hsa-miR-513  hsa-miR-128a  hsa-miR-128b  **hsa-miR-27a**  **hsa-miR-27b**  hsa-miR-412  hsa-miR-361  hsa-miR-224  hsa-miR-181a  hsa-miR-181b  hsa-miR-181c  hsa-miR-494  hsa-miR-340 |

**Table S7**: Mutations reported so far in the *MCPH1* gene.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **SI.#** | **Mutation** | **Location** | **Nature of mutation** | **State of zygosity** | **Predicted effect on protein** | **Disease condition/**  **cell lines** | **Reference** |
| 1 | del exon 1-6 | Exon1-6 | Deletion | Homozygous | Results in truncated protein | MCPH | Darvish et al. (2010) |
| 2 | del exon 2-3 | Exon 2 & 3 | Deletion | Homozygous | Results in truncated protein | MCPH | Darvish et al. (2010) |
| 3 | c.74G>C(p.Ser25X) | Exon 2 | Nonsense | Homozygous | Results in truncated protein | MCPH | Jackson et al. (2002) |
| 4 | c.80C>G(p.Thr27Arg) | Exon 2 | Missense | Homozygous | Results in impaired function of N- terminal BRCT domain | MCPH | Trimborn et al. (2005) |
| 5 | c.147C>G (p.His49Gln) | Exon 3 | Missense | Homozygous | Results in impaired function of N- terminal BRCT domain | MCPH | Darvish et al. (2010) |
| 6 | del exon 3 | Exon 3 | Deletion | Homozygous | Results in truncated protein | MCPH | Darvish et al. (2010) |
| 7 | c.215C>T(p.Ser72Leu) | Exon 3 | Missense | Homozygous | Results in impaired function of N- terminal BRCT domain | MCPH | Darvish et al. (2010) |
| 8 | p.Trp75Arg | Exon 3 | Missense | Homozygous | Results in impaired function of N- terminal BRCT domain | MCPH | Ghani-Kakhki et al. (2012) |
| 9 | del exon 4 | Exon 4 | Deletion | Homozygous | Results in truncated protein | MCPH | Darvish et al. (2010) |
| 10 | c. 302C>G(p.S101X) | Exon 4 | Nonsense | Homozygous | Results in truncated protein | MCPH | Farooq et al. (2010) |
| 11 | c.321delA(p.Lys107fsX39) | Exon 4 | Frame-shift | Heterozygous | Results in truncated protein | Endometrial cancer; SCC084 & SCC131 cell lines | Bilbao et al. (2010); present study |
| 12 | c.436+1G>T | Intron 5 | Intronic | Homozygous | Results in truncated protein | MCPH | Darvish et al. (2010) |
| 13 | c.427\_428insA(p.Thr143fsX5) | Exon 5 | Frame-shift | Homozygous | Results in truncated protein | PCC | Trimborn et al. (2004) |
| 14 | c.427delA(p.Thr143fsX3) | Exon 5 | Frame-shift | Heterozygous | Results in truncated protein | Endometrial cancer | Bilbao et al. (2010) |
| 15 | c.566\_567insA (p.Asn189fsX15) | Exon 6 | Frame-shift | Homozygous | Results in truncated protein | MCPH | Darvish et al. (2010) |
| 16 | c.1179delG(p.Arg393fsX50) | Exon 8 | Frame-shift | Homozygous | Results in truncated protein | MCPH | Hussain et al. (2012) |
| 17 | c.1402delA(p.Thr468fsX32) | Exon 8 | Frame-shift | Heterozygous | Results in truncated protein | Endometrial cancer; SCC084 & SCC131 cell lines | Bilbao et al. (2010); present study |
| 18 | c.1561G>T(p.Glu521X) | Exon 8 | Nonsense | Homozygous | Results in truncated protein | OSCC | Present study |
| 19 | del exon 10 | Exon 10 | Deletion | Homozygous | Results in truncated protein | Breast cancer | Rai et al. (2006) |

Abbreviations: MCPH, autosomal recessive primary microcephaly; OSCC, oral squamous cell carcinoma; SCC084 and SCC131, oral cancer cell lines; and, PCC, premature chromosome condensation syndrome.

Table S8: Summary of the observed results across all the experiments.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Pt #** | **Real-time quantitative RT-PCR** | **IHC** | **LOH** | **Mutation** | **Promoter methylation** | **Western blotting** |
| 5 |  |  | - |  |  |  |
| 8 |  |  | + |  |  |  |
| 9 |  |  | - |  |  |  |
| 11 |  |  | - |  |  |  |
| 14 |  |  | - |  |  |  |
| 13 |  |  | - |  |  |  |
| 12 |  |  | - |  |  |  |
| 15 |  |  | - |  |  |  |
| 17 |  |  | - |  |  |  |
| 18 | N |  | + |  | - |  |
| 19 |  |  | - |  |  |  |
| 20 |  |  | - |  |  |  |
| 21 | N |  | - |  | - |  |
| 22 | L |  |  |  | - |  |
| 26 |  |  | - |  |  |  |
| 27 |  |  | - |  |  |  |
| 30 |  |  | - |  |  |  |
| 34 |  |  | - |  |  |  |
| 38 | L |  | - |  | - |  |
| 39 |  |  | - |  |  |  |
| 40 |  |  | - |  | - |  |
| 47 | L |  | - |  | - |  |
| 44 |  |  | - |  |  |  |
| 49 | N |  | - |  | - |  |
| 50 |  |  | + |  | - |  |
| 52 |  |  | - |  | - |  |
| 53 |  |  |  |  | - |  |
| 54 |  |  | - |  |  |  |
| 55 |  |  | - |  |  |  |
| 56 | L |  | - |  | - |  |
| 57 | L |  |  |  | - |  |
| 59 |  |  | - |  | - |  |
| 60 |  |  | - |  | - |  |
| 62 | L |  | - |  | - | N |
| 63 | L |  | - | - | - | L |
| 65 |  |  | - |  |  |  |
| 66 | H |  | - |  | - |  |
| 67 | N |  | - |  | - |  |
| 68 | L |  | + | - | - | L |
| 69 | H |  | - |  |  |  |
| 70 |  |  | - |  |  |  |
| 71 |  |  | - |  |  |  |
| 72 |  |  | - |  |  |  |
| 73 |  |  | - |  |  |  |
| 74 |  |  | - |  |  |  |
| 75 |  |  | - |  |  |  |
| 76 | L |  | + | - | - |  |
| 77 |  |  | - |  |  |  |
| 79 | N |  | - |  | - |  |
| 80 | N |  | + | - | + |  |
| 83 |  |  | - |  |  |  |
| 92 | L |  |  |  | - | N |
| 95 | N |  | - |  | - |  |
| 101 | L |  | - | - |  |  |
| 108 | H |  | - | - |  |  |
| 109 | H |  | - |  |  | L |
| 110 | L | L | + | + | - |  |
| 113 | N |  | + |  | - |  |
| 114 |  |  | - |  |  |  |
| 115 | H | L | + |  |  |  |
| 116 | L | L | - |  | + |  |
| 121 | L |  |  |  | - |  |
| 125 |  | L | - |  |  |  |
| 127 |  | L |  |  |  |  |
| 128 | H | L |  |  |  | N |
| 133 | L | L | - |  | - |  |
| 135 | N | L |  |  | - |  |
| 139 | H | H | - |  |  |  |
| 140 | N | H | - |  |  | N |
| 143 |  | H | - | - |  |  |
| 144 | L | L | + |  | - |  |
| 155 | N |  | - |  | - | L |
| 156 |  | L | + |  | - |  |
| 158 |  | L |  |  |  |  |
| 157 |  | L | - |  |  |  |
| 159 |  | L | - | - |  |  |
| 165 |  |  | - |  |  |  |
| 166 |  |  | - |  |  |  |
| 174 |  |  | - |  |  |  |
| 175 |  | L | - |  | - |  |
| 177 | L | L | - |  | + |  |
| 180 |  |  | + |  | - |  |
| 181 | L | L | + | - | - |  |
| 182 |  |  | + |  |  |  |
| 183 |  |  | - |  |  | L |
| 191 |  |  | - |  |  | L |
| 192 |  |  | - |  |  |  |
| 194 |  | H |  |  |  |  |
| 202 | L | L | - | - | + |  |
| 206 | N | L |  | - |  |  |
| 212 | N | L | - | - |  |  |
| 214 | L | H |  | - | - |  |
| 219 | L | H | - | - | - |  |

Note that the data of the real-time quantitative RT-PCR is indicated according to 50%-150% cut-offs. Empty boxes indicate experiments not performed. Abbreviations:

-, not seen; +, seen; H, upregulated; L, downregulated; and, N, no change.