



Research Article

## ***MTNRIA* Promoter Methylation is Associated with Increased Breast Cancer Risk**

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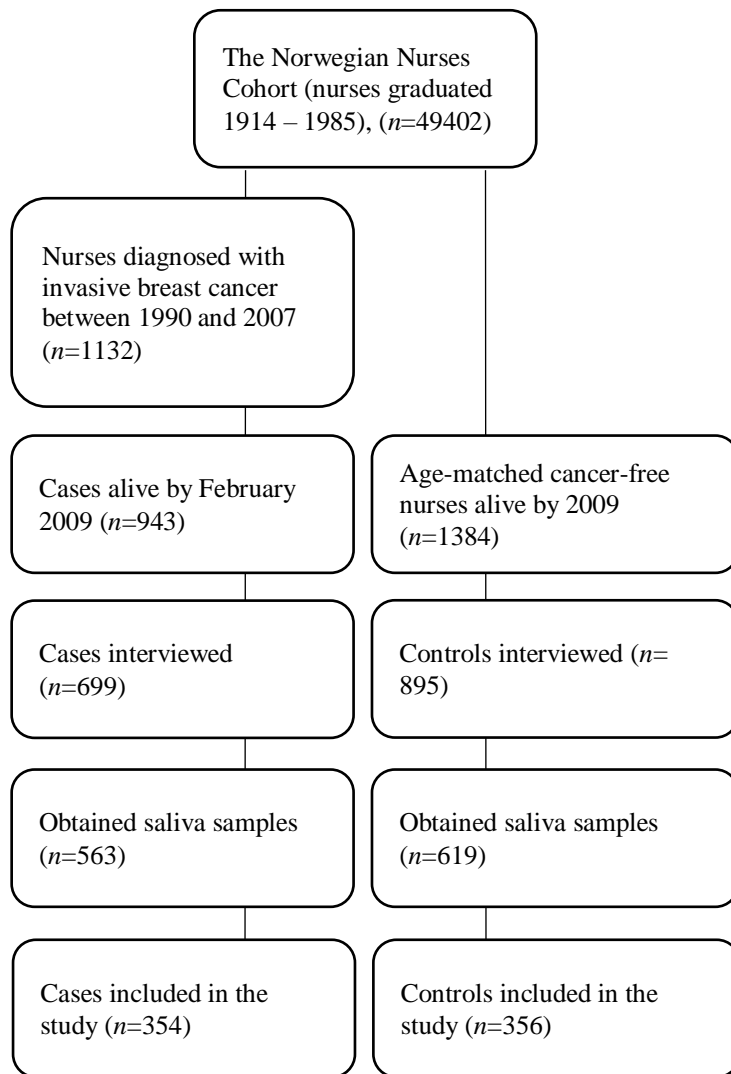
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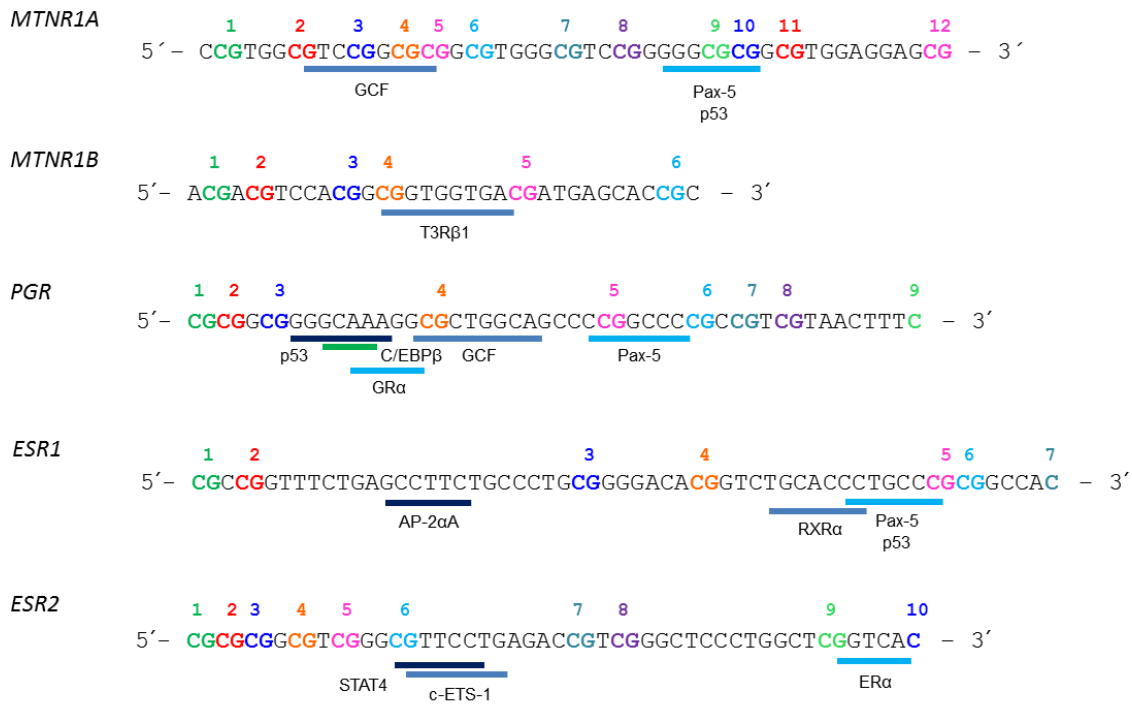
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**Supplementary Figure S1:** Flow chart illustrating study design.



**Supplementary Figure S2:** Analyses of transcription factor binding in the target CpG site for each of the genes were performed using PROMO database. Transcription factors with DM ≤ 5% were included in the illustration.

| Gene          | CpG target sites   | Forward Primer                  | Reverse Primer                         | Sequencing primer          |
|---------------|--|---------------------------------|--|----------------------------|
| <i>MTNR1A</i> | <u>CCGTGGCGTCCGGCGCGGCGTGG</u><br><u>GCGTCCGGGGCGCGGCGTGGAG</u><br><u>GAGCG</u>        | TGGTTTTGTTG<br>GGTAAAAG         | CTACCCCTACCCTA<br>CATAATCCCTATAC       | GGATATTAGGT<br>GTTATTTG    |
| <i>MTNR1B</i> | <u>ACGACGTCCACGGCGGTGGTGA</u><br><u>GATGAGCACCGC</u>                                   | AGTTGGGTAG<br>GGAAGAGA          | AACCCCATACCAA<br>CACCCAACAT            | ATCACCAAAAA<br>ATTACCC     |
| <i>PGR</i>    | <u>CGCGGCGGGGCAAAGGCGCTGGC</u><br><u>AGCCCCGGCCCCCGCCGTCTAAGT</u><br><u>TTC</u>        | GGTGAAGAG<br>GTATAGGGTG<br>AAT  | TCATCCACCTACCT<br>ATCCTACCTCTC         | GAGGTATAGGG<br>TGAATT      |
| <i>ESR1</i>   | <u>CGCGGTTTCTGAGCCTTCTGCCCT</u><br><u>GCGGGACACGGTCTGCACCCTGC</u><br><u>CCGCGGCCAC</u> | GGGTTGTGTTT<br>TTTTTTTAGGT<br>G | ACAATAAAACCAT<br>CCCAAATACTTTAA<br>TAT | TGTTTTTTTTTTT<br>AGGTGGT   |
| <i>ESR2</i>   | <u>CGCGCGGCGTTCCTGAG</u><br><u>ACCGTCGGGCTCCCTGGCTCGGT</u><br><u>ACG</u>               | TTGTGGGTGG<br>ATTAGGAGT         | ACTTAACCATAAAC<br>CCCTTCTCCTTT         | GGTTAGAGGGT<br>GTTTTTATTAG |

**Supplementary Table S1:** Selected core circadian genes and settings used for analyses.

| Transcription Factor | Gene Symbol | Gene Name  | MTNR1A | MTNR1B | PGR | ESR1 | ESR2 |
|----------------------|-------------|--|--------|--------|-----|------|------|
| AP-2alphaA           | TFAP2A      | transcription factor AP-2 alpha  |        |        |     | X    |      |
| Bach1                | BACH1       | BTB domain and CNC homolog 1   | x      |        |     |      |      |
| C/EBPbeta            | CEBPB       | CCAAT/enhancer binding protein beta                                    |        |        | x   |      |      |
| c-Ets-1              | ETS1        | ETS proto-oncogene 1   |        |        |     |      | X    |
| CTCF                 | CTCF        | CCCTC-binding factor   | x      |        | x   |      | X    |
| E2F-1                | E2F1        | E2F transcription factor 1   |        |        | x   |      |      |
| E2F-6                | E2F6        | E2F transcription factor 6   |        |        | x   |      |      |
| Egr-1                | EGR1        | early growth response 1  | x      |        |     |      |      |
| ER-alpha             | ESR1        | estrogen receptor 1  |        |        |     |      | X    |
| EZH2                 | EZH2        | enhancer of zeste 2 polycomb repressive complex 2 subunit              | x      | x      | x   | X    |      |
| GCF                  | GCFC2       | GC-rich sequence DNA-binding factor 2                                  | x      |        | x   |      |      |
| GR-alpha             | NR3C1       | nuclear receptor subfamily 3 group C member 1                          |        |        | x   |      |      |
| Max                  | MAX         | MYC associated factor X  |        |        | x   |      |      |
| p53                  | TP53        | tumor protein p53  | x      |        | x   | X    |      |
| Pax-5                | PAX5        | paired box 5   | x      |        | x   | X    |      |
| POLR2A               | POLR2A      | RNA polymerase II subunit A  | x      |        | x   | X    |      |
| RAD21                | RAD21       | RAD21 cohesin complex component  | x      |        |     |      |      |
| RBBP5                | RBBP5       | RB binding protein 5, histone lysine methyltransferase complex subunit |        | x      |     |      |      |
| REST                 | REST        | RE1 silencing transcription factor                                     |        | x      |     |      |      |
| RXR-alpha            | RXRA        | retinoid X receptor alpha  |        |        |     | X    |      |
| STAT4                | STAT4       | signal transducer and activator of transcription 4                     |        |        |     |      | X    |
| SUZ12                | SUZ12       | SUZ12 polycomb repressive complex 2 subunit                            |        |        | x   |      |      |
| T3R-beta1            | THRB        | thyroid hormone receptor beta  |        | x      |     |      |      |
| TAF(II)55            | TAF7        | TATA-box binding protein associated factor 7                           | x      |        |     |      |      |
| USF1                 | USF1        | upstream stimulatory factor 1  |        |        |     |      | X    |
| ZNF265               | ZRANB2      | zinc finger RANBP2-type containing 2                                   |        |        |     |      | X    |

**Supplementary Table S2:** Analyses of transcription factor binding in the target CpG site for each of the genes were performed using PROMO and ENCODE databases. From PROMO database analysis, only transcription factors with  $DM \leq 5\%$  were included.

| Characteristics   | Controls (n=356)     | Cases (n=354)        | P                  |
|---|----------------------|----------------------|--------------------|
| <sup>a</sup> Age (years), mean ± SD (min-max)           | 54.48 ± 7.62 (36-74) | 54.79 ± 7.47 (36-74) | 0.720 <sup>b</sup> |
| No. of children, mean ± SD (min-max)                    | 2.24 ± 1.28 (0-9)    | 2.03 ± 1.15 (0-5)    | 0.033 <sup>b</sup> |
| Age at first birth (years), mean ± SD (min-max)         | 26.75 ± 4.13 (19-41) | 26.76 ± 4.22 (18-43) | 0.932 <sup>b</sup> |
| <sup>d</sup> Breast cancer in first-degree family (Y/N) | 34/321               | 65/286               | 0.001 <sup>c</sup> |
| Alcohol consumption ≥twice/week (Y/N)                   | 18/338               | 31/323               | 0.052 <sup>c</sup> |
| Daily exposure to x-rays (Y/N)                          | 63/293               | 69/285               | 0.565 <sup>c</sup> |
| <sup>e</sup> Hormone therapy in the past 2 years (Y/N)  | 71/279               | 87/261               | 0.159 <sup>c</sup> |
| <b>Night work exposure</b>                              |                      |                      |                    |
| Never night work  | 90                   | 73                   |                    |
| Low (never ≥ three consecutive night shifts)            | 27                   | 33                   |                    |
| Medium (three consecutive night shifts < five years)    | 58                   | 51                   |                    |
| High (three consecutive night shifts ≥ five years)      | 181                  | 197                  |                    |

**Supplementary Table S3:** Characteristics of the study subjects and night work exposure parameters.



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