Combined Effect of CCND1 and COMT Polymorphisms and Increased Breast Cancer Risk

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Abstract

Background
Estrogens are crucial tumorigenic hormones, which impact the cell growth and proliferation during breast cancer development. Estrogens are metabolized by a series of enzymes including COMT, which converts catechol estrogens into biologically non-hazardous methoxyestrogens. Several studies have also shown the relationship between estrogen and cell cycle progression through activation of CCND1 transcription.

Methods
In this study, we have investigated the independent and the combined effects of commonly occurring CCND1 (Pro241Pro, A870G) and COMT (Met108/158Val) polymorphisms to breast cancer risk in two independent Caucasian populations from Ontario (1228 breast cancer cases and 719 population controls) and Finland (728 breast cancer cases and 687 population controls). Both COMT and CCND1 polymorphisms have been previously shown to impact the enzymatic activity of the coded protein.

Results
Here, we have shown that the high enzymatic activity genotype of CCND1\textsuperscript{High} (AA) was associated with increased breast cancer risk in both the Ontario [OR: 1.3, 95\%CI (1.0-1.69)] and the Finland sample [OR: 1.4, 95\%CI (1.01-1.84)]. The heterozygous COMT\textsuperscript{Medium} (MetVal) and the high enzymatic activity of COMT\textsuperscript{High} (ValVal) genotype was also associated with breast cancer risk in Ontario cases, with an OR of 1.3, 95\%CI (1.07-1.68) and 1.4, 95\%CI (1.07-1.81), respectively. However, there was no statistically significant association or increased trend of breast cancer risk with COMT\textsuperscript{High} (ValVal)) genotypes in the Finland cases [OR: 1.0, 95\%CI (0.73-1.39)]. In the combined
analysis, the higher activity alleles of the COMT and CCND1 is associated with increased breast cancer risk in both Ontario [OR: 2.22, 95% CI (1.49-3.28) and Finland [OR: 1.73, 95% CI (1.08-2.78)] populations studied. The trend test was statistically significant in both the Ontario ($\chi^2_{\text{trend}} = 14.62, \text{df} = 1, p=0.00013$) and Finland ($\chi^2_{\text{trend}} = 6.30, \text{df} = 1, p=0.012$) populations across the genotypes associated with increasing enzymatic activity.

Conclusion

Using two independent Caucasian populations, we have shown a stronger combined effect of the two commonly occurring CCND1 and COMT genotypes in the context of breast cancer predisposition.
Introduction

Estrogen demonstrates diverse effects in humans and has a critical role in breast cancer development. Estrogen exerts its effect by simultaneously stimulating the transcription of genes, via the estrogen receptor, necessary for cell proliferation and by causing DNA damage via their catechol estrogen metabolites [1, 2]. The two major estrogens, 17B-estradiol (E2) and estrone (E1), are oxidized to the 2-OH and 4-OH catechol estrogens and 16-a hydroxyestrogen by the enzymes Cyp1A1 and Cyp1B1 [3, 4]. The products of these phase I enzymes are extremely toxic metabolites, which are detoxified through methylation, sulfonation and gluconation. Only catechol estrogens are the substrates for the phase II enzyme catechol-O-methyl transferase (COMT), which catalyzes the conversion of catechol estrogens into biologically non-hazardous methoxyestrogens. COMT is constitutively expressed mainly in brain, liver and kidney, but also in peripheral tissue, including the epithelial cells in the ducti and lobuli of normal mammary tissue. Most detoxification happens in the liver, but it takes place in peripheral tissues as well, including breast [5]. COMT expression is elevated in tumor tissue compared to normal mammary tissue [6]. COMT activity varies among individuals, and lower activity is associated with low thermal stability [7, 8]. A commonly occurring SNP in the 108/158th amino acid of the COMT protein sequence results in two different alleles of COMT (A to G change at position 1947; rs4680), COMT (Met) and COMT (Val). It has been suggested that COMT_Low (Met) may have 3 to 4-fold less enzymatic activity compared to COMT_High (Val) [9, 10].

Steroid hormones like estrogen are also major regulators of cell cycle progression in breast cancer cells [11]. Several studies have shown the relationship between estrogen and cell cycle progression through activation of CCND1 transcription [12, 13]. CCND1 is the key regulator of transition of the cell from G1 to its proliferative S phase. CCND1 accumulates and activates CDK4/6 in response to mitogenic growth factors in early to mid G1 phase, and initiates the transcription of
transcription factors required in the subsequent S phase. Excess accumulation of CCND1 in a cell due to either amplification of CCND1 gene or over-expression of its protein product has been frequently found in various cancers, including breast cancer [14]. With respect to the genetic variants of CCND1, it is suggested that a commonly occurring G to A substitution at position 6962 (rs603965) (Pro241Pro) in exon 4 produces two alternatively spliced forms of the gene. Splicing form CCND1b produced by the CCND1 (A) allele lacks exon 5 [15]. This last exon contains a rapid protein degradation motif (PEST), and the protein product of the CCND1\textsuperscript{High} (A) allele is hypothesized to be more stable compared to the product of CCND1\textsuperscript{Low} (G) allele [15]. It also has been observed that splicing form lacking exon 5, thus lacking a phosphorylated Thr residue (Thr286), is unable to be transported to cytoplasm and unable to be ubiquitinated [16, 17] and is a nuclear oncogene [18].

In our previous study [19], we examined the breast cancer risk associated with interactions among the SNPs of genes involved in major cancer related pathways. Multivariate analyses revealed several statistically significant SNP-SNP interactions associated with increased breast cancer risk including one between CCND1 Pro241Pro and COMT Met108/158Val substitutions. In this study we have studied the combined effects of CCND1 and COMT polymorphisms in the expanded version of the original study population in Ontario. Additionally, we have also included an independent population from Finland to validate our findings. This study further supports the combined role of CCND1 and COMT genotypes in breast susceptibility.
Materials and Methods

Subject Populations

**Ontario Population: (i) Case-subjects (n=1228)**, were women with pathologically confirmed diagnoses of breast cancer, between 1996 and 1998 were identified through the Ontario Cancer Registry and recruited into the Ontario Familial Breast Cancer Registry (OFBCR), a participating site in the US NIH Breast Cancer Family Registry (BCFR) [20, 21]. Seventy three percent (n=894) of all cases represented women at increased risk of genetically-related breast cancer based on the following criteria: Ashkenazi Jewish background; diagnosed before age 36 years; previous ovarian or breast cancer diagnosis; one or more first- or two or more second-degree relatives with breast or ovarian cancer; one or more second- or third-degree relatives with either breast cancer diagnosed before age 36 years, ovarian cancer diagnosed before age 61 years, multiple breast or breast and ovarian primaries, or male breast cancer; or three or more first-degree relatives with any combination of breast, ovarian, colon, prostate, or pancreatic cancer or sarcoma, with at least one diagnosis before age 51 years were included in the study. Cases without family history defined by the above criteria constituted the remaining Ontario cases (n=330). The age range of all participating women was 25-69 years, with an average of 48.8± 9.26 years. Among the Ontario cases enriched with familial criteria described above, 48% (n=437) had at least one first degree relative with breast or ovarian cancer [20]. Cases were initially identified through the population-based Ontario Cancer Registry. More information regarding the selection and recruitment of cases is given elsewhere [20, 21]. **(ii) Population controls (n=719)** were resourced from the OFBCR. These controls were recruited by calling randomly selected residential telephone numbers from across the province of Ontario and were frequency-matched to all female OFBCR cases by 5-year age group. The reference age range of population control samples from OFBCR is 23-69 with an average of 49.1± 9.55 years.
**Finland Population:** (i) **Case-subjects (n=728)** were unselected for family history, and treated in Helsinki University Central Hospital during 1997-1998 [22], and 2000 [23]. Of these cases 73% (n=534) are sporadic cases without a family history of breast or ovarian cancer, and 27% (n=194) had a family history with at least one or more first degree relatives with breast or ovarian cancer. The reference age range of all Finland cases is 22-69, with an average age of 53.2 ± 9.34. (ii) **Population controls (n=687)** are healthy individuals collected from the same geographical region. The number of the controls was 920 originally, with an age range of 18-65. In order to match the age distribution in Finland and Ontario control samples all of the samples in the age range of 18-20 (n=52) were excluded, and randomly selected 10% of the samples were included in the age range of 21 to 30, thus excluding the 90% (n=181) of the controls in this group. The age range of control samples in the final list (n=687) was 21-65, with an average of 47.1± 10.12 years.

**Molecular Genotyping:**

As described previously [19], the genotyping of Ontario breast cancer and population control DNA specimens for both CCND1 and COMT SNPs were performed by TaqMan 5’ nuclease assay [24] using the ABI PRISM 7900 HT Sequence Detection System (version 2.0). The genotyping of the Finnish DNA samples from the breast cancer cases and population controls was done using Amplifluor fluorescent genotyping (K-Biosciences, Cambridge, United Kingdom), as described previously [25].

**Statistical Analysis**

At the first stage, we calculated crude allele and genotype frequencies for both individual polymorphisms and evaluated Hardy-Weinberg equilibrium using a one-degree of freedom goodness-of-fit test among controls. The association between each the case-control status and each individual SNP was measured by the odds ratio (OR) and its corresponding 95% confidence interval. All analyses
were performed assuming a dominant and a recessive effect for each polymorphism. The alleles of both CCND1 and COMT previously associated with a lower enzymatic activity in the control population were used as reference group both in individual and combined SNP association analyses.

To detect trends from the CCND1 and COMT interactions in breast cancer cases from Ontario and Finland populations, we applied the Trend Analysis Program from the PEPI computer software package [26]. Trend analysis is based on the chi-square test for association in which the data have a natural ordering [27].

Results

Independent Analysis of CCND1 and COMT Polymorphisms

Here we have investigated the independent and combined association of CCND1 Pro241Pro and COMT Met108/158Val polymorphisms using a case control design from two independent populations of Ontario (1228 cases and 719 controls) and Finland (728 cases and 687 controls). The mean age of Ontario cases and controls were 48.8± 9.26 and 49.1± 9.55, and the mean age of Finland cases and controls were 53.2 ± 9.34 and 47.1± 10.12, respectively. We observed that the potentially high enzymatic activity CCND1\textsuperscript{High} (AA) genotype was associated with increased breast cancer risk in both the Ontario [OR: 1.3, 95%CI (1.0-1.69)] and the Finland sample [OR: 1.4, 95%CI (1.01-1.84)] (Table 1). The heterozygous COMT\textsuperscript{Medium} (MetVal) and the high enzymatic activity of COMT\textsuperscript{High} (ValVal) genotype was also associated with breast cancer risk in Ontario cases, with an OR of 1.3, 95%CI (1.07-1.68) and 1.4, 95%CI (1.07-1.81), respectively. However, there was no statistically significant association or increased trend of breast cancer risk with COMT\textsuperscript{High} (ValVal) genotypes in the Finland cases [OR: 1.0, 95%CI (0.73-1.39)].
Combined Analysis of CCND1 and COMT Polymorphisms

The association of the combined CCND1 and COMT genotypes was also tested and the results are presented after grouping the genotypes according to their level of enzymatic activity (Table 2). The low enzymatic activity genotype combinations of CCND1 and COMT (CCND1\textsuperscript{Low} / COMT\textsuperscript{Low}) were taken as a reference compared to the medium (heterozygote combinations) and high activity (CCND1\textsuperscript{High} / COMT\textsuperscript{Medium} and CCND1\textsuperscript{High} / COMT\textsuperscript{High}) combinations. In Ontario, the heterozygote (medium activity) [OR: 1.66, 95%CI (1.18-2.33)] and high activity [OR: 2.22, 95%CI (1.49-3.28)] combinations of CCND1 and COMT genotypes showed statistically significant association with increased breast cancer risk. In Finland, the high activity genotype combinations (CCND1\textsuperscript{High} / COMT\textsuperscript{High} and CCND1\textsuperscript{High} / COMT\textsuperscript{Medium}) were also significantly associated with increased breast cancer risk [OR: 1.73, 95%CI (1.08-2.78)]. The medium activity combinations in Finland sample followed a trend of increased breast cancer risk [OR: 1.21, 95%CI (0.81-1.83)], but did not reach statistical significance. The trend test was statistically significant in both the Ontario ($\chi^2_{\text{trend}} = 14.62, df = 1, p=0.00013$) and Finland ($\chi^2_{\text{trend}} = 6.30, df = 1, p=0.012$) populations across the genotypes associated with increasing enzymatic activity. We have also investigated the COMT and CCND1 genotype interactions by age, familial status and ER subgroups; however we did not observe any differences from the overall analysis (data not shown).

**Discussion**

In this study, we have investigated the independent and the combined contribution effects of CCND1 Pro241Pro and COMT Met108/158Val polymorphisms to breast cancer risk in two independent Caucasian populations from Ontario and Finland. Our results suggest a genetic cross-talk between the medium and higher enzymatic activity allele combinations of CCND1 and COMT in breast cancer
development. The biological relevance of the combined effect between CCND1 and COMT polymorphisms can be explained in regard to their common relationship with estrogen, which is considered a critical risk factor for breast cancer. Estrogens have been shown to activate the G1/S transition through both CCND1 dependent and independent mechanisms. The balance is maintained between the estrogen levels in the cell and the functionality of the enzymes that metabolize estrogen, including COMT. Thus in the presence of estrogens the activities of both genes are required for the determination of cell proliferation. Individuals inheriting different combinations will likely have different activity of estrogen metabolism and cell proliferation upon exposure to estrogens.

Both CCND1 and COMT polymorphisms have been shown to have an impact on the function of the protein product, altering their overall enzymatic activity in the cell. The protein product encoded by the COMT Low (Met) allele has been suggested to be 3 to 4-fold less active compared to the COMT High (Val) allele. Also, the protein encoded by the CCND1 High (A) allele has also been hypothesized to produce a more stable protein compared to the CCND1 Low (G) allele. Both COMT and CCND1 polymorphisms occur frequently in the population controls studied. The frequency of CCND1 High (AA) genotype was 21.7% and 21.3%, whereas the COMT High (ValVal) genotype was 22.4% and 20.8 % in Ontario and Finland control populations, respectively.

Analysis of the independent contribution of the CCND1 polymorphism to breast cancer risk has shown a statistically significant association with increased breast cancer risk in both Ontario and Finland. To date a total of three studies with relatively small sample sizes (with a range of ~200-500 cases) have examined the contribution of the CCND1 polymorphism to breast cancer risk; however none of them has shown statistically significant association with breast cancer risk [28-30]. Thus, our study is the first to show the statistically significant association of the CCND1 polymorphism with breast cancer in two independent, relatively large case control studies.

Analysis of the independent contribution of COMT polymorphism has also shown a statistically significant association between the COMT-ValH (GG) genotypes and increased breast
cancer risk in Ontario but not in Finland sample. As summarized in Figure 1, several studies have investigated the association of COMT with breast cancer risk in the Caucasian population; however their findings revealed a continuum of risk ranging from OR 0.78 to 3.29 [31-40]. These findings may explain the variable association of COMT (GG) genotype determined in Finland and Ontario sample of the current study. The heterogeneity in the results may arise from many reasons including differences in study design, sample size, ethnicity, genetic make-up, geographic location, and the environmental factors influencing the populations studied.

Interestingly, in both Ontario and Finland sample, the breast cancer risk was higher in those with high and medium enzymatic activity genotype combinations of CCND1 and COMT polymorphisms. COMT with higher enzymatic activity leads to increased level of estrogen in the cell through negative feedback inhibition of CYP enzymes, and CCND1 with higher activity facilitates estrogen effect to cell cycle progression and proliferation, explaining the background for this combined association. The magnitude of risk was higher in Ontario compared to Finland; however a test for trend supported the association of increased breast cancer risk with increasing activity of both CCND1 and COMT genotypes. The difference in the magnitude of the increased risk may be mainly due to the difference in the independent effects of COMT alleles in the two groups.

**Conclusion**

In this study, we have shown a combined effect between the two commonly occurring polymorphisms associated with higher enzymatic activity of CCND1 and COMT in the context of breast cancer predisposition. The results of this study also supports our initial findings where SNP-SNP interactions between these polymorphisms was observed in the subset of the Ontario sample [19]. Our findings suggest that COMT and CCND1 alleles act in combination and thus contribute to initiate the events
necessary for breast cancer progression. Here we propose that the allelic status of individuals with respect to these two genes alters the relative risk of individuals for breast cancer. This study provides an example of the potential role of combined effect of SNPs with low penetrant alleles, and provides guidance to the understanding of the genetic basis of breast cancer.

**Competing Interest**

The authors declare that they have no competing interests.

**Authors Contribution**

UVO participated in study design, assisted in the production and organization of Ontario samples genotyping data, and participated in manuscript preparation.

KA and HN provided the genetic and clinical data for Finland samples, and critically revised the manuscript.

LB and JAK provided statistical assistance and critically revised the manuscript.

OK and CB provided specimens for Finland data, and participated in the critical revision of the manuscript.

NP provided statistical assistance and participated in critical revision of the manuscript.

ILA provided specimens for Ontario data, and participated in the critical revision of the manuscript.

HO participated in study and manuscript preparation.

All authors read and approved the final manuscript.
Acknowledgements

We would like to thank Priscilla Chan, Nayana Weerasooriya and the members of the OFBCR biospecimen repository for their expert assistance, Sean Wells and Hong Li for genotyping the Ontario samples, and Nina Puolakka for help with the Finnish patient data. The Finnish study was supported by Helsinki University Central Hospital Research Fund, Academy of Finland (110663), Finnish Cancer Society, Sigrid Juselius Foundation and the Finnish Medical Foundation (Finska Läkaresällskapet). The Ontario study was supported by DOD-BCRP grant (DAMD17-00-1-0353) by U.S. Army Medical Research and Materiel Command, and by the National Cancer Institute, National Institutes of Health RFA # CA-95-011 and through cooperative agreements with members of the Breast Cancer Family Registry (BCFR) and P.I.s. The content of this manuscript does not necessarily reflect the views or policies of the National Cancer Institute or any of the collaborating centers in the CFR, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government or CFR.
References


Figure Legends

**Figure 1**: The risk estimates (OR) of COMT<sup>Low</sup> (Met) allele in various studies of breast cancer cases and population controls.
Table 1: Characterization of the main effects of CCND1 and COMT polymorphisms in Ontario and Finland cases

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls N (%)</th>
<th>Cases N (%)</th>
<th>OR</th>
<th>95% CI</th>
<th>Controls N (%)</th>
<th>Cases N (%)</th>
<th>OR</th>
<th>95% CI</th>
</tr>
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<tbody>
<tr>
<td><strong>ONTARIO</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CCND1 Low</td>
<td>GG</td>
<td>217 (30.2)</td>
<td>335 (27.4)</td>
<td>1</td>
<td>-</td>
<td>195 (29)</td>
<td>179 (25.1)</td>
<td>1</td>
</tr>
<tr>
<td>CCND1 Medium</td>
<td>AG</td>
<td>346 (48.1)</td>
<td>573 (46.9)</td>
<td>1.1</td>
<td>0.86-1.33</td>
<td>334 (49.7)</td>
<td>355 (49.8)</td>
<td>1.2</td>
</tr>
<tr>
<td>CCND1 High</td>
<td>AA</td>
<td>156 (21.7)</td>
<td>314 (25.7)</td>
<td>1.3</td>
<td>1.01-1.69</td>
<td>143 (21.3)</td>
<td>179 (25.1)</td>
<td>1.4</td>
</tr>
<tr>
<td>CCND1 Medium / CCND1 High</td>
<td>AG / AA</td>
<td>502 (69.8)</td>
<td>887 (72.6)</td>
<td>1.15</td>
<td>0.93-1.4</td>
<td>477 (71)</td>
<td>534 (74.9)</td>
<td>1.22</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>719</td>
<td>1222</td>
<td></td>
<td></td>
<td>672</td>
<td>713</td>
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</table>

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls N (%)</th>
<th>Cases N (%)</th>
<th>OR</th>
<th>95% CI</th>
<th>Controls N (%)</th>
<th>Cases N (%)</th>
<th>OR</th>
<th>95% CI</th>
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<tr>
<td><strong>FINLAND</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>COMT Low</td>
<td>AA (Met)</td>
<td>201 (28.2)</td>
<td>273 (22.4)</td>
<td>1</td>
<td>-</td>
<td>168 (30.60)</td>
<td>206 (29.10)</td>
<td>1</td>
</tr>
<tr>
<td>COMT Medium</td>
<td>AG (Met) (Val)</td>
<td>353 (49.5)</td>
<td>642 (52.8)</td>
<td>1.3</td>
<td>1.07-1.68</td>
<td>267 (48.63)</td>
<td>361 (50.99)</td>
<td>1.1</td>
</tr>
<tr>
<td>COMT High</td>
<td>GG (Val)</td>
<td>160 (22.4)</td>
<td>302 (24.8)</td>
<td>1.4</td>
<td>1.07-1.81</td>
<td>114 (20.77)</td>
<td>141 (19.92)</td>
<td>1</td>
</tr>
<tr>
<td>COMT Medium / COMT High</td>
<td>AG (Met)(Val) / GG (Val)</td>
<td>513 (71.9)</td>
<td>944 (77.6)</td>
<td>1.3</td>
<td>1.1-1.67</td>
<td>381 (69.4)</td>
<td>502 (70.91)</td>
<td>1.1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>714</td>
<td>1217</td>
<td></td>
<td></td>
<td>549</td>
<td>708</td>
<td></td>
</tr>
</tbody>
</table>
Table 2: CCND1.COMT interaction in breast cancer cases from Ontario and Finland population

<table>
<thead>
<tr>
<th>Combined Enzymatic Activity</th>
<th>Genotype CCND1&amp;COMT</th>
<th>Controls N (%)</th>
<th>Cases N (%)</th>
<th>OR</th>
<th>CI</th>
<th>Controls N (%)</th>
<th>Cases N (%)</th>
<th>OR</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCND1 Low / COMT Low</td>
<td>GGAA</td>
<td>73 (10.2)</td>
<td>74 (6.1)</td>
<td>1.0</td>
<td>-</td>
<td>51 (9.4)</td>
<td>52 (7.4)</td>
<td>1.0</td>
<td>-</td>
</tr>
<tr>
<td>CCND1 Low / COMT Medium</td>
<td>GGAG, GGGG, AGAA, AGAG, AGGG, AAAA</td>
<td>534 (74.4)</td>
<td>897 (73.6)</td>
<td>1.66</td>
<td>1.18-2.33</td>
<td>416 (76.6)</td>
<td>515 (73.4)</td>
<td>1.21</td>
<td>0.81-1.83</td>
</tr>
<tr>
<td>CCND1 Medium / COMT Medium</td>
<td>AAAG, AAGG</td>
<td>107 (14.9)</td>
<td>240 (19.8)</td>
<td>2.22</td>
<td>1.49-3.28</td>
<td>76 (14)</td>
<td>134 (19.1)</td>
<td>1.73</td>
<td>1.08-2.78</td>
</tr>
<tr>
<td>CCND1 High / COMT High</td>
<td>714</td>
<td>1211</td>
<td>543</td>
<td>701</td>
<td></td>
<td></td>
<td>p=0.00013</td>
<td>p=0.012</td>
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Trend Test
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<tr>
<th>Study</th>
<th>OR</th>
<th>95% CI</th>
<th>Weight (%)</th>
<th>Case</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitrune et al [34], FIN</td>
<td>1.19</td>
<td>0.88 - 1.62</td>
<td>7.36</td>
<td>115 / 481</td>
<td>100 / 480</td>
</tr>
<tr>
<td>Millikan et al [32], USA</td>
<td>1.23</td>
<td>0.88 - 1.71</td>
<td>6.19</td>
<td>103 / 389</td>
<td>86 / 379</td>
</tr>
<tr>
<td>Thompson et al [33], USA</td>
<td>0.88</td>
<td>0.60 - 1.28</td>
<td>5.60</td>
<td>69 / 281</td>
<td>78 / 289</td>
</tr>
<tr>
<td>Gaudet et al [39], USA</td>
<td>1.11</td>
<td>0.92 - 1.35</td>
<td>19.03</td>
<td>287 / 1048</td>
<td>277 / 1092</td>
</tr>
<tr>
<td>Ahsan et al [37], USA</td>
<td>1.29</td>
<td>0.88 - 1.89</td>
<td>4.46</td>
<td>84 / 313</td>
<td>58 / 262</td>
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<td>Wedren et al [36], SWE</td>
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<td>Onay et al [this study], FIN</td>
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<td>114 / 549</td>
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<td>Kocabas et al [35], TUR</td>
<td>0.97</td>
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<td>35 / 103</td>
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<td>160 / 714</td>
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<td>Lavigne et al [31], USA</td>
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<td>27 / 114</td>
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<td>Sazci et al [38], TUR</td>
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<td>0.54 - 1.45</td>
<td>3.28</td>
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<td>62 / 224</td>
</tr>
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more in controls  | more in cases