‘Twin Research and Human Genetics’, *One CNV Discordance in NRXN1 Observed upon Genome-wide Screening in 38 Pairs of Adult Healthy Monozygotic Twins*, Patrik K. E. Magnusson, Donghwan Lee, Xu Chen, Jin Szatkiewicz, Setia Pramana, Shumei Teo, Patrick F. Sullivan, Lars Feuk, Yudi Pawitan

**SUPPLEMENTARY MATERIALS**

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Figure S13. Results from PennCNV estimation and qPCR validation attempts for concordant CNV11

Table S1. Taqman qPCR system for validation

Table S2. Summary of copy numbers estimated from PennCNV and qPCR
Triplicate runs (labeled with red color) and eight internal controls (labeled with dark gray color) for each interesting CNV (CNV1 to CNV11, CNV2 and CNV5 are in the same pair) were performed on three 96-wells plates, each column represents one CNV, each row for one individual. For example, P1-T1-CNVI-Ref means pair1-twin1-CNVI combined with reference assay; P1-T1-Ref (labeled with light blue) means independent reference assay for twin1 in pair1; NTC-CNVI-Ref (labeled with light gray) means no template control (NTC) for CNVI combined with reference assay; NTC-CNVI means no template control for CNVI without the reference assay.

**FIGURE S1**

Plates design for Taqman qPCR validation.

| Plate 1 |
FIGURE S2
Location of the top ranked MZ CNV discordance in relation to the exon/intron structure of NRXN1 gene (GRCh37/hg19 from Ensembl)
The deletion is labeled by a red rectangular frame. The 5bp exon (Chr2: 51125736-51125740) it involves is included in transcript nrxn1-201 and nrxn1-202 shown at the bottom of the figure.
FIGURE S3

Two transcripts (nrxn1-201 and nrxn1-202) including 5bp exon of NRXN1 gene (GRCh37/hg19 from Ensembl and expression pattern from C-Lt-Loci)
Recurrence of CNVs in NRXN1 gene reported from Database of Genomic Variants archive (DGVa)

The region of the validated discordant CNV overlapped with a large proportion of previously reported CNVs in NRXN1 gene.
FIGURE S5

Results from PennCNV estimation and qPCR validation attempts for discordant CNV3

(A). Log R ratio (LRR) and B allele frequency (BAF) of discordant CNV3 (Chr5:140137644-140261235) from PennCNV; (B). Copy number detected from qPCR signal, both of the twin members carry two copies in this region. The color-coding of the bars indicates replicate (on 3 different 96-well plates). The red box shows the triplicate runs for the MZ pair in which the CNV implicated by PennCNV is tested. Bars outside the red box are control samples for the particular CNV in question.
A. PennCNV

B. Taqman qPCR

FIGURE S6
Results from PennCNV estimation and qPCR validation attempts for discordant CNV4
(A). Log R ratio (LRR) and B allele frequency (BAF) of discordant CNV4(Chr1:9321241-9364634) from PennCNV; (B). Copy number detected from qPCR signal, both of the twin members carry two copies in this region. The color-coding of the bars indicates replicate (on 3 different 96-well plates). The red box shows the triplicate runs for the MZ pair in which the CNV implicated by PennCNV is tested. Bars outside the red box are control samples for the particular CNV in question.
FIGURE S7
Results from PennCNV estimation and qPCR validation attempts for discordant CNV5
(A). Log R ratio (LRR) and B allele frequency (BAF) of discordant CNV5(Chr19:49061724-49089795) from PennCNV; (B). Copy number detected from qPCR signal, both of the twin members carry one copy in this region. The color-coding of the bars indicates replicate (on 3 different 96-well plates). The red box shows the triplicate runs for the MZ pair in which the CNV implicated by PennCNV is tested. Bars outside the red box are control samples for the particular CNV in question.
FIGURE S8

Results from PennCNV estimation and qPCR validation attempts for discordant CNV6
(A). Log R ratio (LRR) and B allele frequency (BAF) of discordant CNV6(Chr9:107623626-107626542) from PennCNV; (B). Copy number detected from qPCR signal. The color-coding of the bars indicates replicate (on 3 different 96-well plates). The red box shows the triplicate runs for the MZ pair in which the CNV implicated by PennCNV is tested. Bars outside the red box are control samples for the particular CNV in question.
FIGURE S9
Results from PennCNV estimation and qPCR validation attempts for concordant CNV7
Both the PennCNV and qPCR support the concordant CNV within the seventh MZ Pair. (A). Log R ratio (LRR) and B allele frequency (BAF) of concordant CNV7(Chr21:22790919-23145950) from PennCNV; (B). The color-coding of the bars indicates replicate (on 3 different 96-well plates). The red box shows the triplicate runs for the MZ pair in which the CNV implicated by PennCNV is tested. Bars outside the red box are control samples for the particular CNV in question.
FIGURE S10
Results from PennCNV estimation and qPCR validation attempts for concordant CNV8
Both the PennCNV and qPCR support the concordant CNV within the eighth MZ Pair. (A). Log R ratio (LRR) and B allele frequency (BAF) of concordant CNV8(Chr6:31379109-31453640) from PennCNV; (B). Copy number detected from qPCR signal. The color-coding of the bars indicates replicate (on 3 different 96-well plates). The red box shows the triplicate runs for the MZ pair in which the CNV implicated by PennCNV is tested. Bars outside the red box are control samples for the particular CNV in question.
FIGURE S11
Results from PennCNV estimation and qPCR validation attempts for concordant CNV9
Both the PennCNV and qPCR support the concordant CNV within the ninth MZ Pair. (A). Log R ratio (LRR) and B allele frequency (BAF) of concordant CNV9(Chr11:25150091-25599986) from PennCNV; (B). Copy number detected from qPCR signal. The color-coding of the bars indicates replicate (on 3 different 96-well plates). The missing bars represent the failure to detect the signals from qPCR. The red box shows the triplicate runs for the MZ pair in which the CNV implicated by PennCNV is tested. Bars outside the red box are control samples for the particular CNV in question.
Results from PennCNV estimation and qPCR validation attempts for concordant CNV10

Both the PennCNV and qPCR support the concordant CNV within the tenth MZ Pair. (A). Log R ratio (LRR) and B allele frequency (BAF) of concordant CNV10(Chr5:104133164-104688954) from PennCNV; (B). Copy number detected from qPCR signal. The color-coding of the bars indicates replicate (on 3 different 96-well plates). The missing bars represent the failure to detect the signals from qPCR. The red box shows the triplicate runs for the MZ pair in which the CNV implicated by PennCNV is tested. Bars outside the red box are control samples for the particular CNV in question.
A. PennCNV

Both the PennCNV and qPCR support the concordant CNV within the eleventh MZ Pair. (A). Log R ratio (LRR) and B allele frequency (BAF) of concordant CNV11 (Chr8:2346867-2582764) from PennCNV; (B). Copy number detected from qPCR signal. The color-coding of the bars indicates replicate (on 3 different 96-well plates). The missing bars represent the failure to detect the signals from qPCR. The red box shows the triplicate runs for the MZ pair in which the CNV implicated by PennCNV is tested. Bars outside the red box are control samples for the particular CNV in question.

B. Taqman qPCR

FIGURE S13
Results from PennCNV estimation and qPCR validation attempts for concordant CNV11
<table>
<thead>
<tr>
<th>Reaction components</th>
<th>Volume per well(µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2X TaqMan® Genotyping Master Mix</td>
<td>10</td>
</tr>
<tr>
<td>TaqMan® Copy Number Assay, 20X working stock</td>
<td>1</td>
</tr>
<tr>
<td>TaqMan® Copy Number Reference Assay, 20X</td>
<td>1</td>
</tr>
<tr>
<td>Nuclease-free water</td>
<td>4</td>
</tr>
<tr>
<td>Genomic DNA 5ng/ul</td>
<td>4</td>
</tr>
</tbody>
</table>

Note: Amplification procedure: 95°C 10min, 40 cycles of 95°C 15s, and 60°C 60s.
### TABLE S2
Summary of copy numbers estimated from PennCNV and qPCR

<table>
<thead>
<tr>
<th>CNV&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Chr&lt;sup&gt;b&lt;/sup&gt;</th>
<th>N&lt;sub&gt;SNPs&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt;</th>
<th>PennCNV&lt;sup&gt;d&lt;/sup&gt;</th>
<th>qPCR&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Copy number</td>
<td>Confid. score</td>
</tr>
<tr>
<td>CNV1-T1/T2-P1</td>
<td>2</td>
<td>41</td>
<td>2/1</td>
<td>-/124</td>
</tr>
<tr>
<td>CNV2-T1/T2-P2</td>
<td>2</td>
<td>50</td>
<td>1/2</td>
<td>98/-</td>
</tr>
<tr>
<td>CNV3-T1/T2-P3</td>
<td>5</td>
<td>41</td>
<td>2/1</td>
<td>-/74</td>
</tr>
<tr>
<td>CNV4-T1/T2-P4</td>
<td>1</td>
<td>20</td>
<td>2/3</td>
<td>-/66</td>
</tr>
<tr>
<td>CNV5-T1/T2-P5</td>
<td>19</td>
<td>33</td>
<td>2/1</td>
<td>-/52</td>
</tr>
<tr>
<td>CNV6-T1/T2-P6</td>
<td>9</td>
<td>11</td>
<td>2/1</td>
<td>-/50</td>
</tr>
<tr>
<td>CNV7-T1/T2-P7</td>
<td>21</td>
<td>124</td>
<td>3/3</td>
<td>535/380</td>
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<tr>
<td>CNV8-T1/T2-P8</td>
<td>6</td>
<td>137</td>
<td>3/3</td>
<td>466/379</td>
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<tr>
<td>CNV9-T1/T2-P9</td>
<td>11</td>
<td>116</td>
<td>1/1</td>
<td>458/428</td>
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<td>CNV10-T1/T2-P10</td>
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<td>109</td>
<td>1/1</td>
<td>430/249</td>
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<tr>
<td>CNV11-T1/T2-P11</td>
<td>8</td>
<td>99</td>
<td>3/3</td>
<td>387/358</td>
</tr>
</tbody>
</table>

Note: <sup>a</sup>The eleven top ranked CNVs sorted by confidence score from PennCNV; <sup>b</sup> Chromosome number; <sup>c</sup> Number of SNP markers supporting each CNV; <sup>d</sup> Copy numbers in the two members of the twin-pair estimated from PennCNV and the corresponding confidence scores; <sup>e</sup> Copy numbers as judged by the qPCR assay. CNVs 1-6 were selected for discordance and CNVs 7-11 were selected for concordance within MZ twin pairs as estimated from PennCNV.