Neuroscience Campus Amsterdam

CNVs & attention problems in MZ twin pairs

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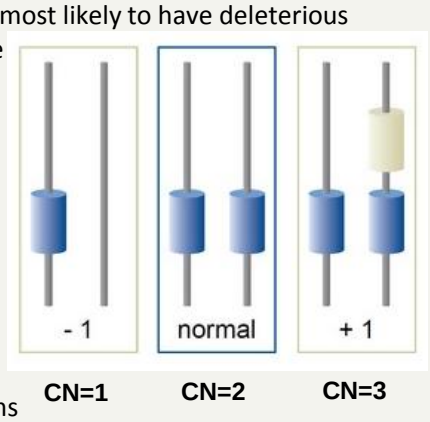
Background

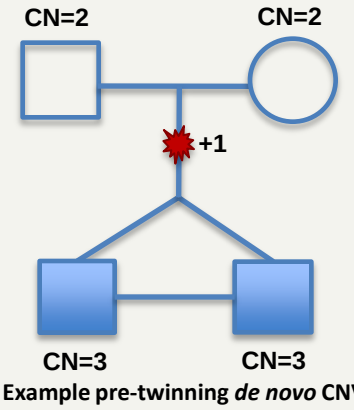
Copy number variants (CNVs) are polymorphisms in the number of copies of chromosomal segments (duplications and deletions) and have recently been recognized as a major contributor to human genetic variability. CNVs collectively encompass a larger part of the genome than single-nucleotide polymorphisms (SNPs), and mutation rates for CNVs are two to four times higher than those of SNPs and affect larger segments of the genome. CNVs have been shown to correlate with changes in gene expression levels. Changes in copy number can also lead to the generation of new combinations of exons between different genes, causing proteins to change their structure and modify their activities. Therefore CNVs are likely to be involved in phenotypic variation. CNVs can be either inherited or *de novo*, where *de novo* CNVs are most likely to have deleterious effects. *De novo* CNV mutations have been linked to several neuropsychiatric disorders, such as schizophrenia and autism. *De novo* CNVs have been demonstrated in monozygotic (MZ) twins and are one mechanism by which discordance in MZ twins may be explained.



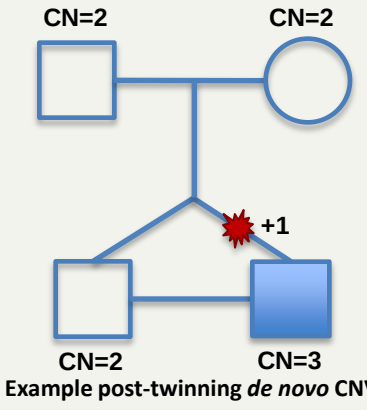
We investigated whether there is an association between CNVs (*de novo* and inherited) and attention problems (AP) measured by the CBCL. The AP scale has been shown to be predictive for ADHD. Heritability estimates for AP and ADHD are about 70% and 75% on average respectively and ~75% of the covariance between the AP scale and ADHD has been estimated to be explained by genetic influences.

Methods

The Attention Problems (AP) scale was collected at ages 7, 10 and 12 years. Twins were identified as affected if they scored high at all time points, and as unaffected if they consistently scored low.
Genotyping was performed on the Affymetrix Human Genome-Wide SNP Array 6. CNV calls of 153 individuals passing quality control, including 45 complete MZ twin pairs (21 concordant unaffected, 10 discordant, 14 concordant affected), of which 25 with both parents.
CNVs were only included if the calling algorithms of Birdsuite and PennCNV both agreed on the copy number (CN) and were > 100 kb.
The presence of two types of *de novo* CNVs was investigated: pre-twinning *de novo* CNVs, where the parental CN calls were compared with those of the MZ twins, and post-twinning *de novo* CNVs, that can be identified by comparing the CN calls between the MZ twins.



Example pre-twinning *de novo* CNV



Example post-twinning *de novo* CNV

Genome-wide CNV burden linked to AP was also tested with permutations in all available twins (corrected for relatedness and gender).

Results

There were 8 possible pre-twinning *de novo* events found. One of those events were in a concordant high twin pair, the rest was in concordant low twin pairs.

There were 21 segments that differed in CN between the MZ twins: 9 in concordant unaffected twins, 6 in discordant twins and 6 in concordant affected twins. The discordant twins have relatively most possible *de novo* CNV events. The *de novo* events in the discordant twins were all gains of 1 copy, were 4 of the six gains were in the affected twin.

After corrections for multiple testing (α = .05 / 14 = .0036), the only significant association of genome-wide CNV burden with AP was found for the average size of the group of CNVs with any deviation from the expected CN (CN = 0, 1, 3 or 4), where the affected individuals had significantly larger CNV events than the unaffected group (p = .0004). Each group of CNVs showed a larger average CNV size in the affected group, except for the CNV group with deletions of 2 copies (CN = 0). This group however was the only group to show a nominally significant result in the amount of CNV events, were the affected group had more events than the unaffected group (p = .036).

CNV event	Average nr of CNVs			Average size of CNVs		
	No AP	AP	*p*	No AP	AP	*p*
Losses (CN = 0, 1)	2.70	2.41	.800	204 kb	211 kb	.399
Deletion: 1 copy (CN = 1)	2.38	1.88	.947	206 kb	238 kb	.150
Deletion: 2 copies (CN = 0)	0.32	0.54	.036	173 kb	171 kb	.547
Gains (CN = 3, 4)	3.83	3.02	.984	564 kb	660 kb	.005
Duplication: 1 copy (CN = 3)	3.68	2.80	.994	585 kb	663 kb	.013
Duplication: 2 copies (CN = 4)	0.15	0.22	.278	180 kb	287 kb	.203
Gains + losses (CN = 0, 1, 3, 4)	6.53	5.44	.978	411 kb	483 kb	**.0004**

Conclusions

There were few *de novo* CNVs found. Of the nine pre-twinning *de novo* CNVs, none were found in the discordant twins, and only one was found in a concordant affected twin pair. The post-twinning *de novo* CNVs seem to be most congruent with AP status, where the discordant twin pairs showed relatively most *de novo* CNVs. Due to the relatively low signal-to-noise ratio of CNVs in the SNP microarray chips, these regions will be re-examined using qPCR methods to confirm these findings.

There also was a significant association with genome-wide CNV burden and AP, where affected children have larger CNV segments than unaffected children. This may be because larger CNV regions affect more genes and/or regulatory regions.

This study suggests that both *de novo* and inherited CNVs may be involved in AP. However, due to the relatively small sample size and the difficulty to measure CNVs accurately with SNP micro-arrays, replication studies are needed.

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