High Resolution Assessment of Copy Number Variation in Seven Pairs of Monozygotic Twins Discordant for Schizophrenia or Bipolar Disorder

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Introduction

- Schizophrenia and bipolar disorder have a concordance rate of about 50% in monozygotic twins
- Copy number variations (CNVs) are important sources of genetic variation and play a clear role in the etiology of many psychiatric diseases, including schizophrenia and bipolar disorder
- aCGH (array Comparative Genomic Hybridization) is a way to detect copy number differences between two individuals

Subjects

- Seven pairs of monozygotic twins were identified using the Swedish Twin Registry
- Upon clinical evaluation, one twin had either schizophrenia or bipolar disorder, the other twin was unaffected and >10 years had passed since disease onset in the affected twin
- There was no known environmental exposure in the affected twin that conceivably might have caused disease
- DNA was collected from peripheral blood and extracted using a salt precipitation method from Qiagen

Pair	Sex	Diagnosis	Age of onset	Year of Birth
1	F	• Schizophrenia • Not Affected	25	1942
2	F	• Bipolar disorder • Not Affected	27	1947
3	F	• Schizoaffective disorder • Not affected	18	1947
4	F	• Bipolar disorder • Not affected	23	1949
5	M	• Schizophrenia • Not affected	30	1950
6	F	• Bipolar disorder • Not Affected	16	1955
7	M	• Bipolar disorder • Not affected	29	1957

Table 1. Diagnoses and age of onset for each twin pair.

Methods

- Conducted an unbiased genome screen for CNVs using Nimblegen's 2.1M CGH tiling arrays. Median probe spacing was 1,169 bp
- Affected twin was compared to unaffected twin using the two-channel array in order to enhance genomic specificity
- Used Nimblegen's software package (NimbleScan and SignalMap) to normalize and make calls, as well as our own normalization algorithm, GenoCN (Sun, PMID 19581427)
- Normalization included
  - Correction for GC content
  - Normalization of signal across the chip

Results

- A varying amount of CNVs were called across the pairs
- Insertions and deletions were called comparatively in each twin pair. A deletion means that the affected twin is missing a copy, and a duplication means that the affected twin has an extra copy (compared to the unaffected twin)

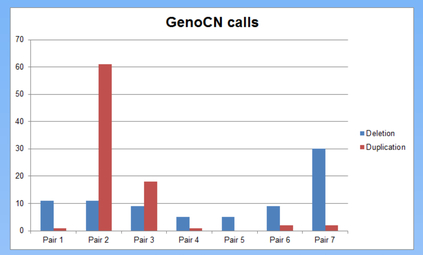


Figure 1. Number of calls (insertions and deletions) for each twin pair

- The number and quality of calls seems to be roughly correlated with both the intensity of probe fluorescence across the chips as well as the overall noisiness of the array
- "Noise" is the first derivative of the median absolute deviation from the norm.
- Average signal intensity refers to each color channel across the chip

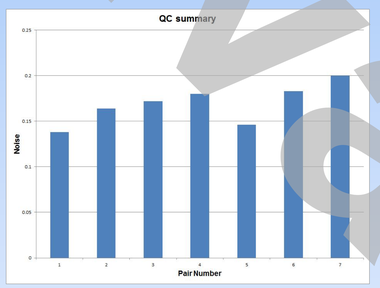


Figure 2. Noise measurements for each twin pair. 0.2 is considered high, but still passes quality control

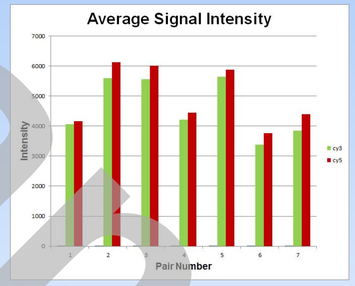


Figure 3. Average signal intensity across the chip for each twin pair. Cy3 labeled the affected twin and Cy5 the unaffected twin

- From the GenoCN calls, we looked for overlaps of gap regions (such as centromeres or telomeres) where CNV calling algorithms can fail, and we did not find any.
- There were a few overlaps with areas of known segmental duplications, which we noted but left in the analysis. Probe design across these areas might be problematic, and interpreting CNVs is hard.
- We compared our CNV calls to known CNVs from other psychiatric genetic findings, and found no overlap.
- We then compiled a list of CNVs that overlapped known genes. We visualized these calls with SignalMap.

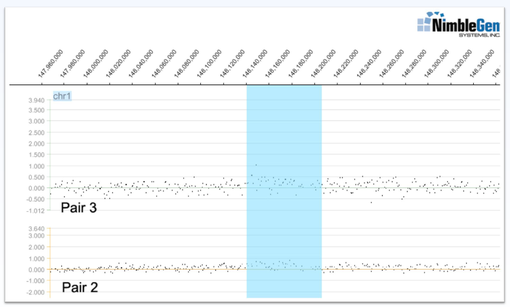


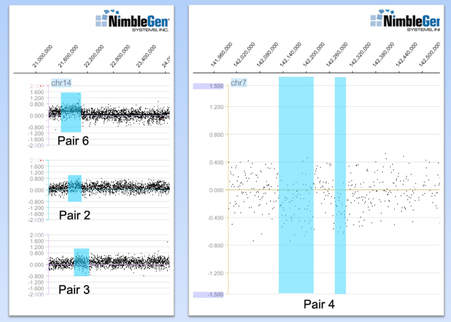
Figure 4. Probes slightly shifted over *SV2A* for two pairs

- Upon visual inspection, most calls did not appear to be real, however, two pairs had the same CNV called, overlapping the gene *SV2A* (synaptic vesical glycoprotein 2A
- We assessed these calls with Taqman Copy Number Assays, and none verified (i.e. all subjects were copy number two).

- There were subtle copy number differences detected in areas of known CNVs

hg18chr	Start	End	Association	Pair
2	88,937,989	89,411,302	lymphocytes, Ig light/lambda chain, V(D)J recomb.	2, 4
2	88,966,183	89,377,035	lymphocytes, Ig light/lambda chain, V(D)J recomb.	
2	89,589,457	89,897,555	lymphocytes, Ig light/lambda chain, V(D)J recomb.	
7	38,245,705	38,323,462	lymphocytes, T cell receptor recomb.	
7	141,647,263	142,221,209	lymphocytes, T cell receptor recomb.	4
11	72,794,675	72,986,876	lymphocytes, T cell receptor recomb.	7
14	21,159,850	22,042,614	lymphocytes, T cell receptor recomb.	2,3,6
14	105,065,301	106,352,275	lymphocytes, Ig heavy chain, V(D)J recomb.	
22	20,715,572	21,595,082	lymphocytes, Ig light/kappa chain, V(D)J recomb.	

Table 2. Chromosomal locations of common CNVs, their association, and which twin pair they presented in.



Figures 5 and 6. Subtle shifts in probes might indicate CNVs in these areas of known CNVs

Conclusions

- CNVs between monozygotic twins implies a genomic change after the zygote has split, leading to genetic mosaicism. For instance, a mutation might occur at such a time point that a CNV only occurs in 1/3 of white blood cells. Or, it might have occurred only in cells of neuronal lineage, and not in white blood cells.
- aCGH is limited both by the size of the CNV as well as the proportion of the cells that have that variation. For instance, a complete deletion of a large amount (>50kb) of DNA will be readily detected, while a smaller one that is not present in all cells might be missed.
- The study of discordant MZ twins has intuitive appeal. We were unable to detect CNVs that correlated with disease status. Future studies of these unusual but informative twins with higher resolution technologies would be of use.

Acknowledgements

Funded by 5R01MH052857 (US PI Cannon, Swedish PI Hultman) and a Distinguished Investigator Award from NARSAD (PFS).

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