High Resolution Copy Number Analysis of Parkinson's Samples Using Nexus Copy Number and Roche NimbleGen Microarrays

A WHITE PAPER FROM

BioDiscovery, Inc. and Roche NimbleGen, Inc.

September 2008

Introduction

The goal of this study was to utilize high-resolution comparative genomic hybridization (CGH) technologies to identify functional mutations associated with Parkinson's disease. Results from this study identified a disease-causing microdeletion within the PARK2 gene in an affected individual. The data for this study was generously provided by Dr. Christopher E. Mason of Yale Medical School working in Dr. Matthew State's laboratory. The details of the study have been published in Human Mutation (2007 Dec; 28(12):1236-40).

Genomic DNA from an affected patient was prepared and co-hybridized with normal human genomic DNA (pool of 6 individuals) on two different NimbleGen™ 385K arrays. The first array was the Human CGH 385K Whole-Genome Tiling catalog array (6270bp median probe spacing) provided by Roche NimbleGen, Inc. The second array was a focused Human Chromosome 6-specific array consisting of 385K probes (404bp median probe spacing). We processed both arrays using Nexus Copy Number™ Professional version 3.1 and report the findings below.

Identification of a PARK2 Deletion

The raw data were normalized using Roche NimbleGen's NimbleScan™ software and subsequently processed in Nexus Copy Number™ using the built-in Rank Segmentation algorithm. Identical parameters were set for both the "Whole-Genome" and the "Focused" arrays. As shown in figure 1, both samples show a common deletion on chromosome 6. It is also interesting to note that the higher-resolution focused array (Array 104418) identified additional copy number changes on chromosome 6. The whole-genome array (Array 89695) shows copy number changes at a number of different loci throughout the genome, such as on Chromosomes 1, 8, 9, 12, 15, 17, 20 and 22.

Figure 1 – Whole-genome plot of a Parkinson's sample hybridized to an array designed with probes only on chromosome 6 (Array 104418) and to an array with probes spanning the entire human genome (Array 89695).

Identifying a Common Deletion on Chromosome 6

We next looked at the common area of deletion on Chr 6, as shown in Figure 2 below. It is very easy to see that a homozygous deletion is detected at the PARK2 gene on both arrays. Here we used the Nexus' custom track display to show the exon locations of the three different transcript forms of the PARK2 gene. It is very interesting to note that the deletion is taking place on exon 5 of the PARK2 gene, as previously reported, and this exon is present only in one of the three different forms of the gene (PARK2_NM_004562). It is also very interesting to note that without exon 5, this transcript is identical to another form of the PARK2 gene (PARK2_NM_013987).

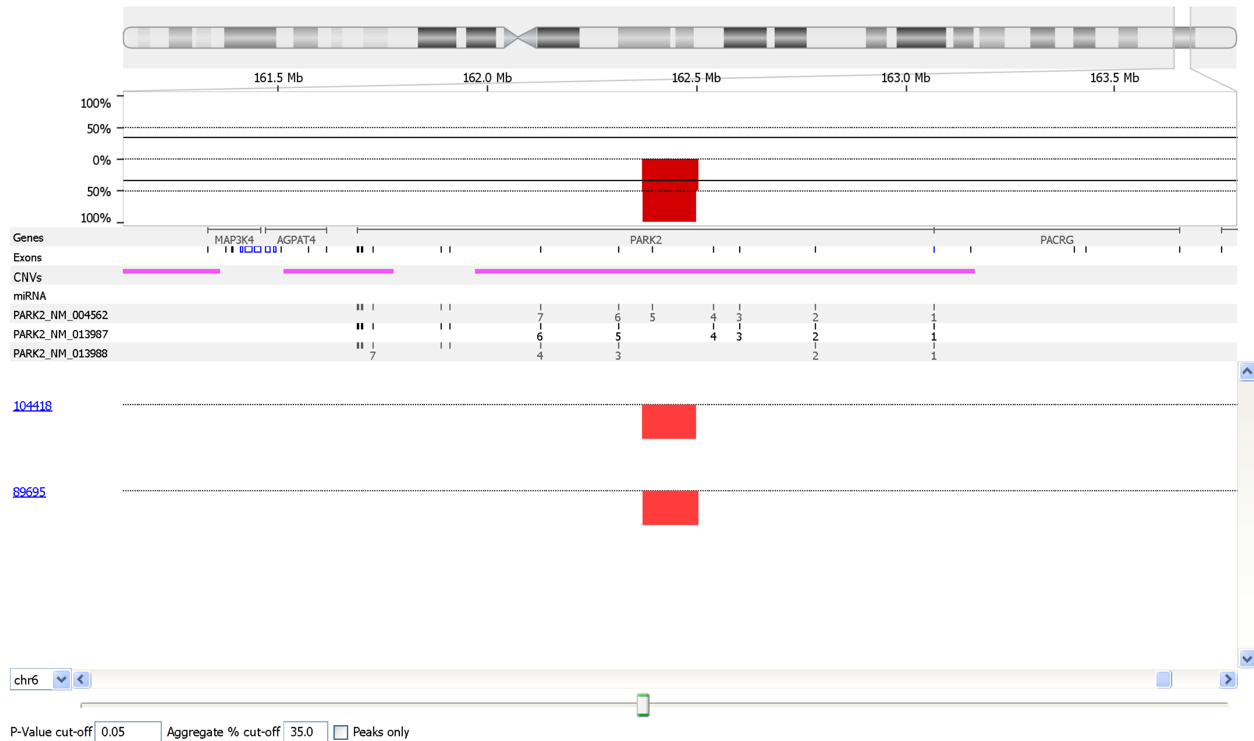

Figure 2 – The Common region of deletion in the two arrays on Chromosome 6

We also noted that there is a slight difference in the boundaries of the deleted area between the two arrays. To understand why, we performed a drill-down operation on both arrays. Figure 3 shows the probe-level view of Array 104418 and Figure 4 shows the probe-level view of Array 89695 both zoomed in on the area of the homozygous deletion. As can be seen from Figures 3 and 4, because the focused array has so many more probes covering the same genomic area, it can offer a better, more precise, estimate of the exact break point boundaries as compared to the whole-genome array.

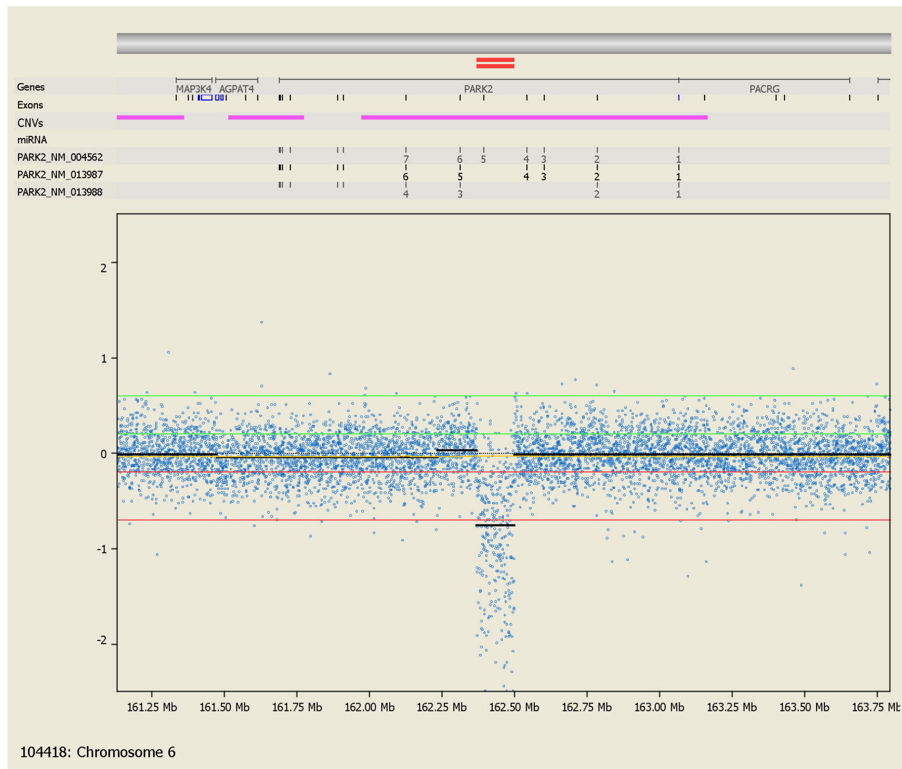

Figure 3 – Probe-level view of the focused Chromosome 6 array indicating the boundaries of the breakpoint in the PARK2 gene

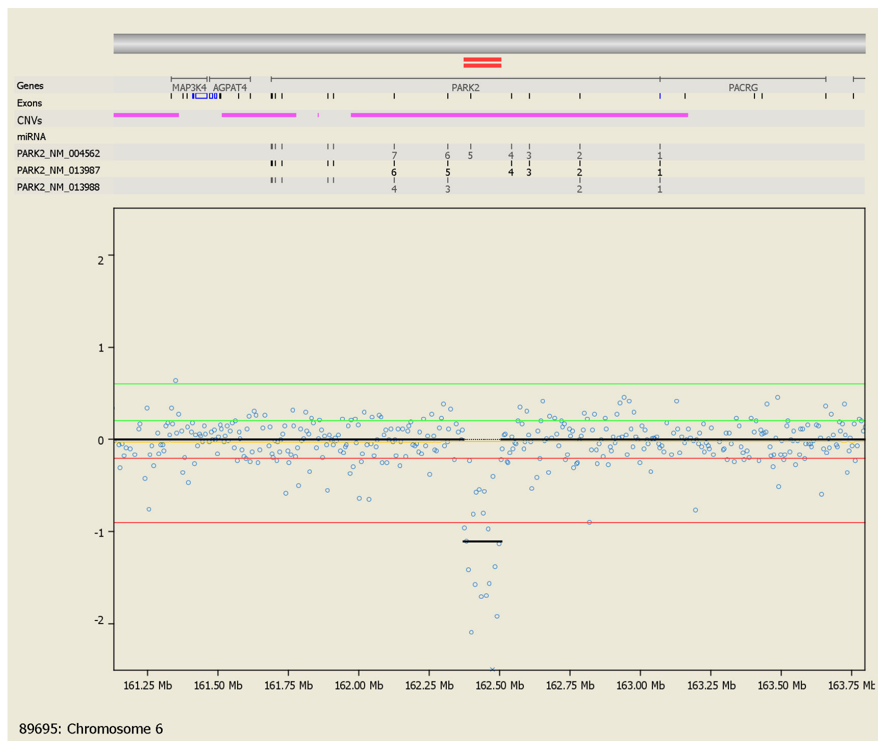

Figure 4 – Probe-level view of the whole-genome array indicating the boundaries of the breakpoint in the PARK2 gene

More Probes Means More CNVs

When we look at all the probes on Chromosome 6 using the whole-genome array, we can only detect the single deletion event at the PARK2 gene. However, looking at the data from the focused Chromosome 6 array, we see a large number of additional copy number variants (CNVs), see Figure 5 below. Taking a closer look at these events, some do correspond to presumably "normal" CNVs from the Database of Genomic Variants. Others may be novel benign CNVs or de novo CNVs related to the phenotype. A good example of this is shown in Figure 6.

104418 - Sample Drill Down

Overview | Whole Genome | Chromosome | Summary | Report

Chromosome Region	Event	Length	Genes	Probes	Cytoband Location
chr6:8,282,177-8,299,298	Loss	17,122	0	42	p24.3
chr6:25,928,007-25,933...	Gain	5,712	1	15	p22.2
chr6:29,959,783-30,007...	Loss	47,631	0	115	p21.33
chr6:33,152,255-33,165...	Loss	13,515	1	34	p21.32
chr6:58,095,437-58,119...	Gain	23,591	0	45	p11.2
chr6:74,646,643-74,658...	Gain	11,716	0	27	q13
chr6:74,764,655-74,775...	High Copy Gain	11,203	0	21	q13
chr6:79,023,844-79,090...	Gain	66,461	0	157	q14.1
chr6:103,838,275-103,8...	Gain	32,088	0	76	q16.3
chr6:162,370,239-162,4...	Homozygous Loss	128,859	1	318	q26
chr6:165,633,429-165,6...	Loss	18,735	1	41	q27

Export TXT

# of regions: 11

Figure 5 – All Copy Number Variations detected on the Focused Chromosome 6Array

The small one copy gain event at approximately 74.65MB corresponds to a previously reported CNV. However, the multiple copy gain event close to 74.8MB does not correspond to a previously reported CNV and may thus be associated with the disease phenotypic. This of course will require further analysis using high resolution arrays and/or additional methods.

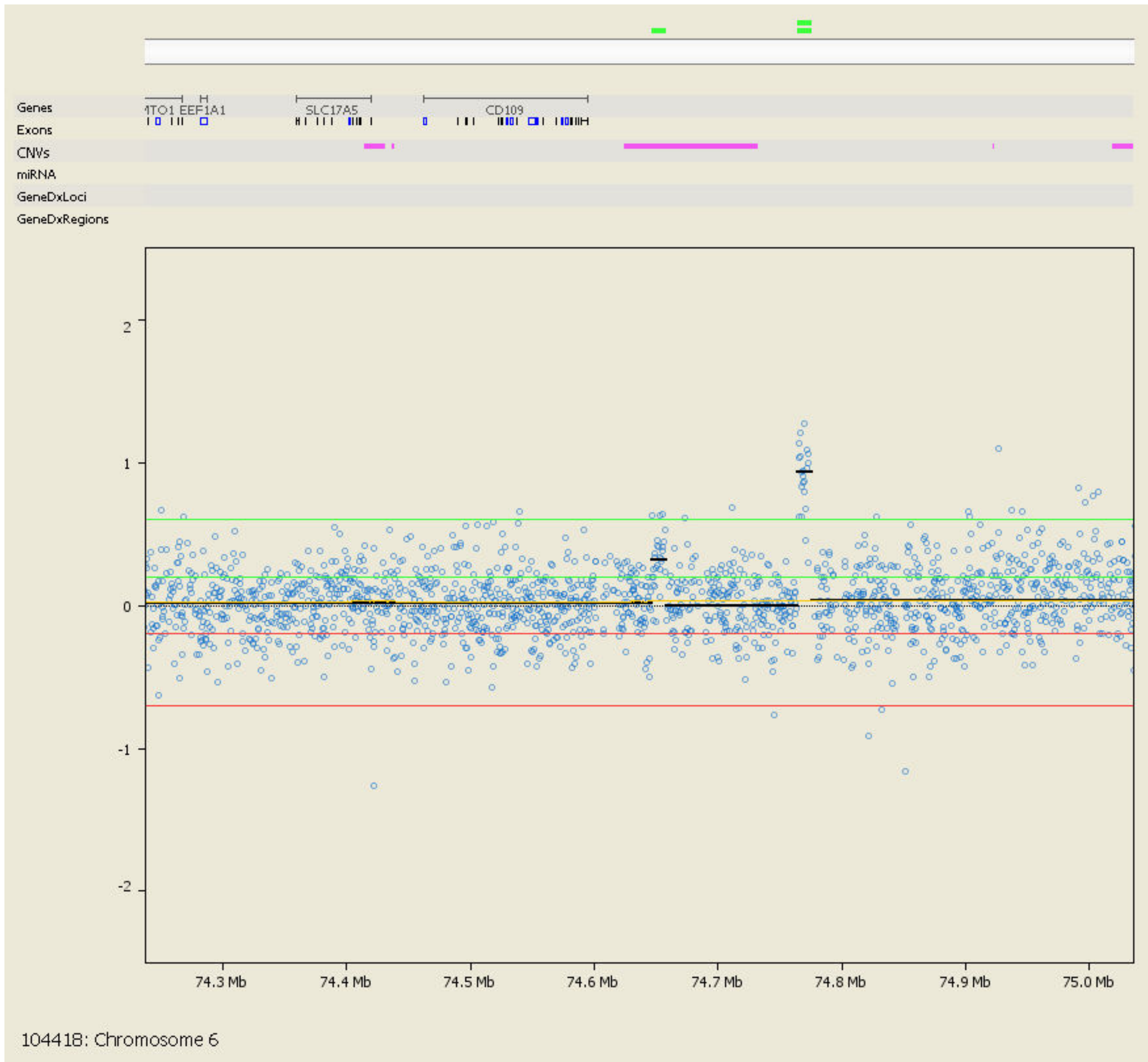

Figure 6 – Small CNVs detected on the focused array

Acknowledgments

We would like to thank Dr. Mason for his assistance in preparation of this white paper.

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