Assessing Bipolar Disorder through Affymetrix Genotyping of Patient-Derived Fibroblasts and iPS Cells

Irma, Howard Rafal, Mike DaSilva, Jon Madison, Jennifer Moran, Kim Chambert, Pamela Sklar, Andrew Crenshaw, Wendy Winckler, and Stacey Gabriel
Genetic Analysis Platform and Stanley Center, Broad Institute, Cambridge MA, USA

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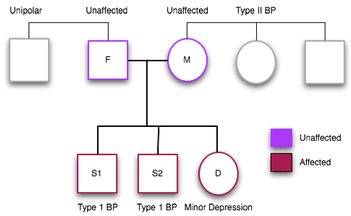
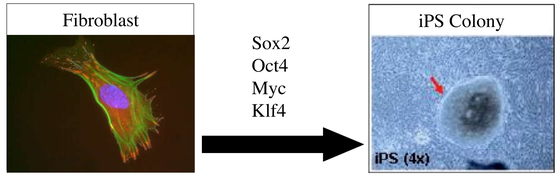
Introduction

Bipolar disorder affects about 1% of the population and although it is believed that bipolar disorder is mainly caused by genetic factors, those factors are still poorly understood. In order to better understand the cause of bipolar disorder, we are attempting to find and observe copy number polymorphisms (CNPs) in bipolar patients to find new genes and confirm known genes that play a role in the development of the disorder. Our study involves the use of fibroblasts taken from a family who has had the disorder through generations.

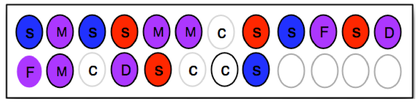


We are also analyzing CNPs from these patients' fibroblasts which were then induced to become induced pluripotent stem (iPS) cells through the viral infection of the Klf4, Sox2, Oct4, and Myc genes into the fibroblast.



DNA samples from iPS cells and fibroblasts taken from this family were genotyped using Affymetrix 6.0 gene chips and analyzed for their copy number polymorphisms and SNPs.

The 20 Samples in Our Plate



Affected Son 1 · Affected Son 2 · Unaffected Daughter · Unaffected Father
Unaffected Mother · Bold = Fibroblast · Not Bold = iPS · Control

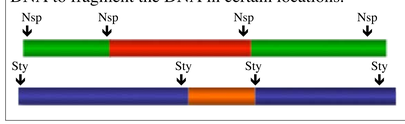
Project Goals:

1. Compare groups of samples to ensure they are from the same individual (i.e no mix-up or contamination occurred).
2. Compare copy number changes in fibroblasts and iPS cells from the same individual.
3. Compare key loci (from previous GWAS studies) between unaffected parents and affected children.

Materials and Methods

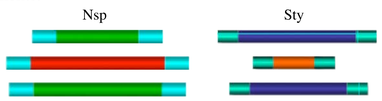
After the DNA samples arrive from the Biological Samples Platform, the DNA undergoes a series of processes to prepare it for the hybridization onto the Affymetrix 6.0 gene chips.

Digestion: Restriction enzymes Nsp and Sty are added to DNA to fragment the DNA in certain locations.

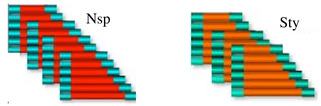


Four-base sticky ends result from the digestion of the DNA, preparing it for Ligation.

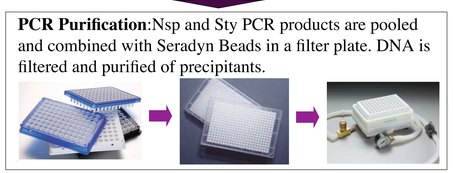
Ligation: Adaptors are ligated to the ends of the DNA fragments.



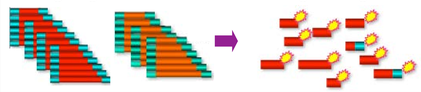
Polymerase Chain reaction (PCR) amplifies the fragmented DNA



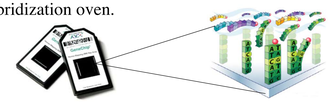
PCR Purification:Nsp and Sty PCR products are pooled and combined with Seradyn Beads in a filter plate. DNA is filtered and purified of precipitants.



Fragmentation and Labeling: Fragmentation breaks the DNA into 50 base pair fragments. Labeling adds biotin labeling reagent to the DNA for use during staining.



Hybridization: Fragmented DNA and hybridization cocktail are added to the gene chips which then rotate in a hybridization oven.

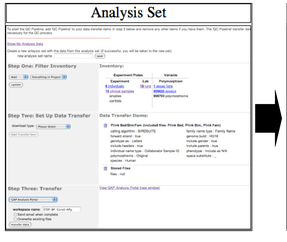


Fluidics Station and Scan: A stain, antibody, and holding buffer are run through the chips during fluidics and then they are scanned.

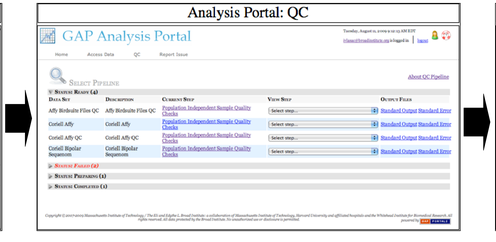
Informatics and Analysis

After the Affymetrix chips have been scanned, a set of algorithms (Bird Suite) is run and data is retrieved from GAP by an analysis set. Bird Suite data is pulled through on the QC Pipeline and downloaded from the Analysis Portal. The Canary files were of most interest because of the Copy Number Polymorphism (CNP) data they held. Areas within the CNP file which showed trends were studied more carefully to investigate whether the changes were due to changes within the cases and controls or if they were a result of genes inserted into the cells during the viral infection that led to iPS cell formation.

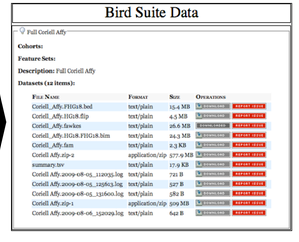
Analysis Set



Analysis Portal: QC



Bird Suite Data



Results and Conclusions

Project Goal 1 Results

We used GAP's Plate Concordance Check to verify the relatedness between samples. When we ran this check, we confirmed the relatedness between siblings and parents. We also received results from Sequenom Fingerprinting where we did not see any discordant mismatches, which indicates that there was no cross contamination between samples. With the fingerprint report we also confirmed the correct gender of the individuals which confirms the samples were plated correctly.

GAP Plate Concordance Check

Project Goal 2 Results

We used GAP's polymorphism search utility (PolyDB) to find SNPs located in the regions of the genes we infected into the fibroblasts to induce iPS cell formation. We then took the SNPs identified and entered them into SNAP, a tool that finds proxy SNP's across commercial arrays, to generate a more complete list of SNPs that are on the Affymetrix 6.0 chip. All this information is then stored on the GAP Analysis Portal to be shared with collaborators.

Gene	PolyDB SNPs	SNAP SNPs	Affy 6 SNAP SNPs
Sox2	4897	22697	3301
Oct4	1118	5253	816
Myc	10610	43612	7927
Klf4	1288	5828	1274

Gene	Total SNPs	# of SNPs with copy # changes	# of changes in fibroblasts
Sox2	51	3	0
Oct4	215	55	17
Myc	678	99	41
Klf4	111	0	0

Project Goal 3 Results

Previous GWAS studies showed SNPs in four regions that associated with bipolar disease and schizophrenia. We compared SNP calls between the affected offspring and the unaffected parents. SNPs rs1938526 from gene CACN and rs2172835 from Chromosome 15q14 are of particular interest because the two affected brothers have the same pairing which is unique from the unaffected parents and sister.

Family	Sample	Father	Mother	Gender	ANK3 rs1938526	DISC1 rs12029109	CACN rs1006737	15q14 rs2172835
CoriellFam1	Mother 1	0	0	F	A A	A A	A G	T T
CoriellFam1	Father 1	0	0	M	A A	A A	G G	C T
CoriellFam1	Aff Brother 1	Father 1	Mother 1	M	A A	A A	G G	T T
CoriellFam1	Aff Brother 2	Father 1	Mother 1	M	A A	A A	G G	T T
CoriellFam1	Sister 1	Father 1	Mother 1	F	A A	A A	A G	C T

Conclusion

In conclusion, we were able to meet all three of our project goals. Through the use of the Concordance report and Sequenom we were able to confirm that our samples belonged to the correct individuals and were correctly plated. Using the Bird Suite files we were able to meet our second project goal of comparing fibroblast and iPS copy number changes to find that copy number changes are affected by the viral genes used to infect the fibroblast and induce it to a pluripotent state. Knowing this will allow us to further study the affects of iPS cells on fibroblasts and to continue searching for ways to induce cells with out affecting their copy number changes. Lastly, we met our third project goal of comparing key loci between the unaffected parents and affected offspring to find a change in pairing in genes which have been shown to influence the development of bipolar disorder. The changes we found in both sons but not in the unaffected parents or sister may show that these regions may in fact be part of the cause of bipolar disorder, and further investigation may help strengthen this theory.

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Acknowledgments

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