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Rebecca Cozad 4/15/12

Mapping and Analyzing Breakpoints of a Recurrent Translocation Between Chromosomes 8 and 12

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Abstract

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The recurrent translocation between chromosomes 8 and 12 is responsible for a novel syndrome and its phenotypic characteristics, which include intellectual disability, seizures, macrocephaly, eczema, and obesity. This translocation, which results in trisomy for part of chromosome 12p and monosomy for part of 8p, is mediated by the presence of segmental duplication regions, or regions of highly repetitive sequence between the two chromosomes. Additionally, one of the genes present in this section of 12p, of which there is an extra copy in this translocation, is *GNB3*, which codes for a G protein subunit and has been found to be an obesity candidate gene, according to previous association studies. In this research, I worked to map the breakpoint junction sites for seven unrelated patients previously diagnosed with this recurrent unbalanced translocation. I also outline the difficulties inherent to the project, as the breakpoint junctions lie within regions of highly repetitive sequence, and are therefore difficult to capture. Furthermore, I discuss future plans to study the mRNA expression of *GNB3* in a mouse model of this translocation as a future project.

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Chapter 1: Background

Translocation Overview

Recombination within the genome is a normal, expected occurrence during the formation of gametes. Homologous recombination maintains genetic variability in the human population by reshuffling genes from parental chromosomes during prophase of meiosis I. Usually, this occurs between homologous chromosomes and does not lead to chromosomal aberrations or alterations in genes. An example of this type of normal chromosomal crossover between homologs during meiosis is given in Figure 1. However, homologous recombination is not completely error-free. Faulty or error-prone DNA-repair mechanisms can result in different chromosomal rearrangements, such as duplications, deletions, translocations, inversions, or a combination of any of these, and can result in cytogenetic imbalance.

Chromosomal translocation is an interesting and noteworthy process to study in the field of genetics because such rearrangements have been reported in a diverse collection of chromosomes and occur at a detectable frequency in humans. When inherited in an unbalanced state, translocations often result in abnormal phenotypes due to an imbalance of genetic material. Approximately one in 600 individuals have a *balanced* chromosome translocation, which is the product of reciprocal exchange between two chromosomes (Van Dyke et al. 1983). At this rate, one in approximately 300 couples may produce offspring with an *unbalanced* translocation, which could result in an abnormal phenotype, making this a rather frequent occurrence.

Chromosome rearrangements can occur between chromosome members of the same homolog pair (also referred to as intrachromosomal rearrangement) or between non-homologous chromosomes (interchromosomal rearrangement). For intrachromosomal rearrangements, there is a further distinction between those that involve only one homolog (where the exchange is only

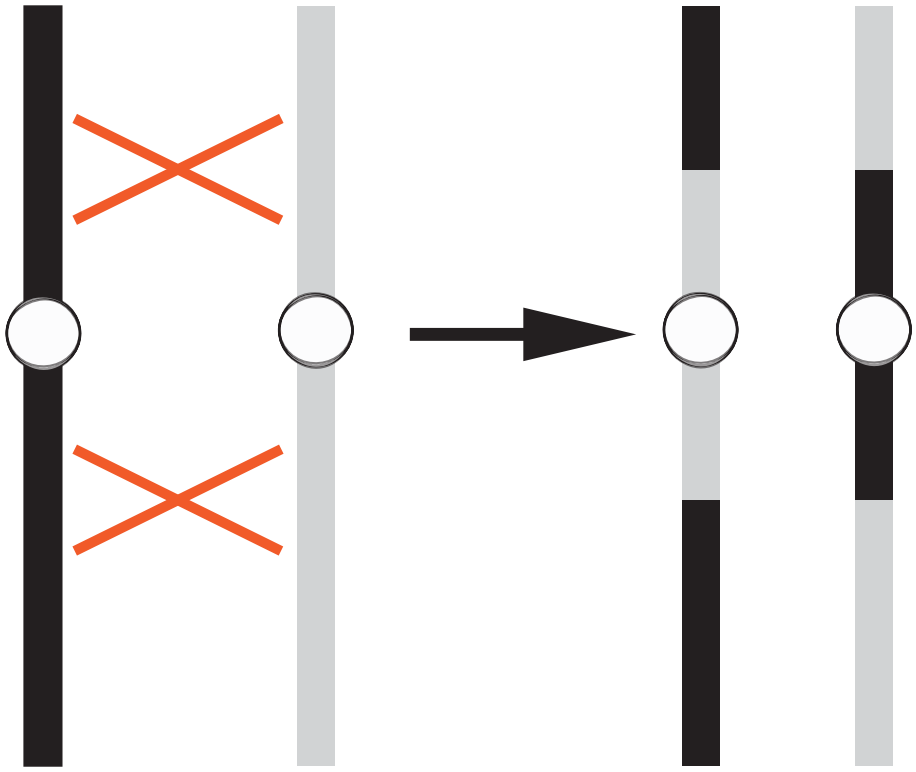


Figure 1. Example of normal recombination event. Homologous chromosomes, represented in black and gray with centromeres represented as circles, recombine to form a new copy with genes from both parental chromosomes.

between sister chromatids of the same homolog) and those that involve both homologs (where the exchange is between the homologous chromosomes, as in normal meiotic recombination) (Shaffer et al. 2000). Even when recombination happens between sister chromatids or homologs, there is still a chance for genomic abnormalities to form and cause abnormal phenotypes. In these instances, intrachromosomal rearrangements can result in inversions (where a portion of the chromosome is reversed), interstitial and terminal deletions, or interstitial duplications. On the other hand, interchromosomal translocations can result in genomic gain or loss for parts of chromosomes when inherited in an unbalanced fashion (Shaffer et al. 2000).

Reciprocal translocations between different chromosomes can also be further broken down into the subcategories of non-recurrent translocations and recurrent translocations. Non-

recurrent translocations are the product of exchanges between chromosomal regions with little or no homology. In this case, double-strand breaks on two different chromosomes occur and there is a mistake in the repair machinery. Instead of repairing these breaks correctly by using genetic material from the homologous chromosome as a template, the damaged chromosomes can swap the genetic information with each other (leading to two aberrant non-homologous chromosomes). These events are described as "non-recurrent" because they involve DNA breaks throughout the genome and therefore have different breakpoints in different individuals.

Recurrent translocations occur via homologous recombination between highly homologous segments of the genome (also referred to as segmental duplications) on different chromosomes. Since segmental duplications, discussed in more depth below, make up approximately 10% of the human genome (Bailey et al. 2002), it is not surprising that unrelated individuals can present with translocations involving the same specific regions, making the name recurrent translocations an apt description. Translocations mediated by segmental duplications can occur between non-allelic regions of homologous chromosomes or between non-homologous chromosomes. By examining the DNA sequences at translocation breakpoints, we can infer the mechanisms of recurrent and non-recurrent translocation formation.

After a balanced translocation occurs, it may be inherited in an unbalanced fashion, resulting in the gain of an entire region of genes from one chromosome and the loss of another set of genes from another chromosome. If any of these genes that have an altered copy number is dose-sensitive, meaning that there needs to be exactly two copies of the gene to maintain normal function, then the individual carrying the unbalanced translocation can present with an abnormal phenotype. Alternatively, translocations can also result in an atypical phenotype by physically disrupting genes at the breakpoints or can alter the regulatory environment of genes, including

non-coding regions of DNA or promoter regions that control the regulation of transcription (Sobreira et al. 2011).

Segmental Duplications

Segmental duplications, or seg dups, are segments of DNA in different parts of the genome that have nearly identical sequences, and play an important role in the evolution of new genes and gene products as well as the production and mediation of genomic rearrangements (Cheung et al. 2003). These sequences, found throughout the genome, can be nearly identical to sequences on the same chromosome, homologous chromosomes, or non-homologous chromosomes. Seg dups are classified as sequences with > 90% identity, and can vary in degree of similarity up to > 99% identity (Bailey et al. 2002). Approximately 298 megabases (Mb) (or roughly one tenth) of the human genome is comprised of repetitive regions such as these (Bailey et al. 2002). The minimum length required for a sequence to be considered a seg dup is 1,000 basepairs, or 1 kilobase (kb), though the most prevalent translocations resulting in abnormal phenotype usually involve segmental duplication regions that have > 95% identity and are greater than 10 kb (Bailey et al. 2002). Segments of high sequence homology shared between different chromosomes can undergo homologous recombination during meiosis and lead to recurrent genomic rearrangements such as those discussed earlier. This process is known as non-allelic homologous recombination (NAHR) and can lead to deletions and duplications when recombination occurs between homologous chromosomes, and translocations when between two different chromosomes (Cheung et al. 2003; Giglio et al. 2002).

Recurrent Translocations

The short arm of chromosome 8 is one such region involved in a number of recurrent translocations mediated by megabases of segmental duplications (Giglio et al. 2002). There are

two main seg dup regions on chromosome 8 known as distal repeating sequences (REPD) and proximal repeating sequences (REPP) that mediate numerous chromosomal rearrangements including deletions, duplications, inversions, and translocations (Giglio et al. 2001; Giglio et al. 2002). REPD and REPP, both located within chromosome band 8p23.1, are each ~ 1 Mb in length and share a 95%-97% percent identity, a level of homology that promotes recombination between these two regions that can result in an inversion polymorphism on 8p (Giglio et al. 2002). The presence of a heterozygous inversion such as this could promote homologous recombination between non-homologous chromosomes and has been shown to participate in other chromosomal rearrangements. For example, similar seg dup regions are also present at 4p16, which is involved in a recurrent translocation between chromosomes 4 and 8 (Giglio et al. 2002).

Ou et al. described two recurrent unbalanced translocations in humans (one between chromosomes 4 and 11 and the other between chromosomes 8 and 12) that are mediated by non-allelic homologous recombination. Though this paper focused mainly on the translocation between chromosomes 4 and 11, it also provided evidence for recombination occurring between other segmental duplication regions on non-homologous chromosomes. The authors also mapped breakpoint junction in five patients with the translocation between chromosomes 4 and 11 and in one patient with the translocation between chromosomes 8 and 12.

Recurrent Translocation between Chromosomes 8 and 12

The Rudd lab recruited seven unrelated individuals (referred to as Subjects 1-7) previously diagnosed with the same unbalanced translocation between chromosomes 8 and 12, resulting in trisomy for part of chromosome 12p (three copies) and monosomy for part of chromosome 8p (one copy). Subjects exhibit clinical features including intellectual disability,

seizures, macrocephaly, eczema, and obesity. The translocation was identified using fluorescence *in situ* hybridization (FISH), which showed an extra copy of 12p in place of part of 8p. Through the use of DNA microarray, members of the lab found that this particular unbalanced translocation results in a 7.0-Mb loss of sequence on the short arm of chromosome 8 and an 8.5-Mb gain of sequence from the short arm of chromosome 12, which include 23 and 107 RefSeq (National Center for Biotechnology Information Reference Sequence) genes, respectively. The apparent recombination site is located within a 300-kb region of segmental duplications shared between chromosome bands 8p23.1 and 12p13.31, depicted in Figure 2.

One of the genes located in the region of chromosome 12 that is duplicated in this translocation, G protein beta 3 (*GNB3*), is of particular interest. The *GNB3* gene codes for a particular beta subunit of heterotrimeric G proteins, and has been previously implicated as a candidate gene for obesity in genome-wide association studies (Klenke et al. 2011; Siffert et al. 1998).

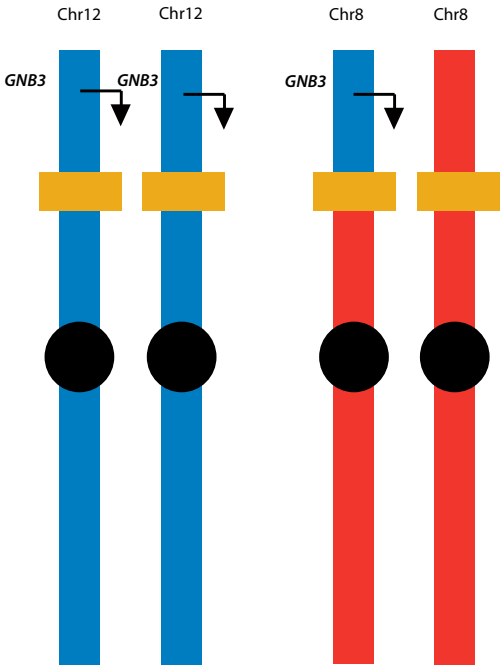


Figure 2. Translocation between chromosomes 8 and 12 resulting in trisomy 12p and monosomy 8p. Blue represents chromosome 12, and red represents chromosome 8. The orange rectangles represents the 300-kb region of segmental duplications shared between these chromosomes and presumed to mediate the translocation. The location of the *GNB3* gene is also denoted with arrows.

Importance of G proteins and their functions

Guanine-nucleotide binding proteins are ubiquitously expressed molecules that are important in regulating transmembrane signals from the cellular surface to secondary intracellular signal cascades (shown in Figure 3). Trimeric G protein complexes consist of three subunits: α, β, and γ. There are 18 distinct α subunits, five β subunits, and 12 γ subunits which, when arranged in different combinations, form distinct G protein complexes that regulate a variety of signaling cascades (Klenke et al. 2011). The inactive state of a G protein exists as a trimeric molecule with the α, β, and γ subunits bound together and guanosine diphosphate (GDP) bound to the α subunit. When a G protein coupled receptor is activated by an extracellular signal (such as a hormone), guanosine triphosphate (GTP) is exchanged for GDP and triggers the dissociation between the α subunit and β,γ complex. The dissociated α subunit with GTP can then activate and regulate a number of other molecules and pathways, like adenylyl cyclase or phospholipase C (Klenke et al. 2011). The β,γ complex is also responsible for the regulation of various other effectors, including ion channels such as K^+, Ca^{2+}, and Na^+, G protein-coupled receptor kinases, and isoforms of adenylyl cyclase and phospholipase C (Klenke et al. 2011). Signaling caused by the activation of these molecules is halted when the GTP is hydrolyzed by the α subunit back to GDP, and the α subunit is then reunited with the β,γ complex to form the trimer and begin the cycle again.

G protein GNB3 (for β 3 subunit) is part of the group of beta subunits of G proteins that are expressed in all tissues (Klenke et al. 2011; Siffert et al. 1998). The *GNB3* gene is comprised of 11 exons and is located on chromosome 12 at band p13.31 near the breakpoint region of the

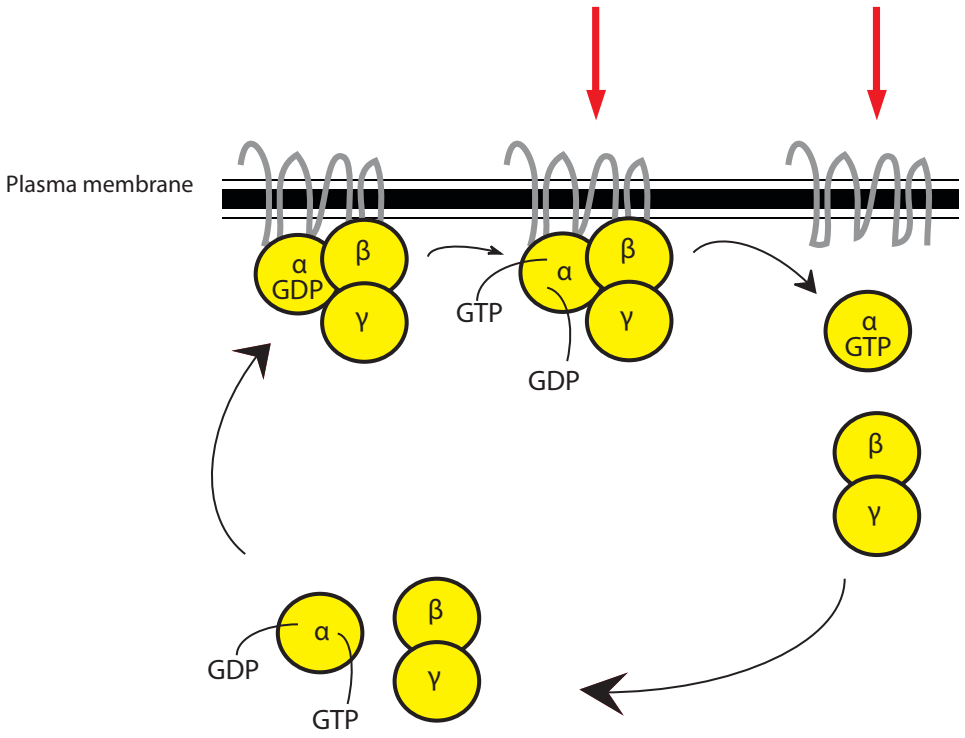


Figure 3. G protein cycle: The inactive state of a G protein is the trimeric molecule with α, β, and γ subunits bound together and GDP bound to the α subunit. When a G protein coupled receptor is activated by an extracellular signal, GTP is exchanged for the GDP, triggering the dissociation between the α subunit and β, γ complex. Both the dissociated α subunit and the β, γ complex can then activate and regulate a number of other pathways. When GTP is hydrolyzed back to GDP by the α subunit, it is reunited with the β, γ complex, halting the signaling from the molecules and reforming the trimer to begin the cycle again.

recurrent translocation between chromosomes 8 and 12. Polymorphisms in this gene have been previously associated with obesity and hypertension (Siffert et al. 1998; Siffert 2005). An extra copy of *GNB3* is present in all seven patients with the recurrent unbalanced translocation.

Chapter 2: Introduction to Experiment

The overarching goal of this project is to study the translocation breakpoint junctions in seven unrelated patients diagnosed with an unbalanced translocation between chromosomes 8 and 12 to learn more about the specific mechanism of recombination that mediates this recurrent translocation. By better understanding the mechanisms of particular chromosome rearrangements, we can learn more about the evolution of the human genome, and identify parts of the genome that are susceptible to recurrent rearrangement.

There are two main aims for this project, the first of which is to successfully amplify and capture the translocation breakpoint junctions in highly repetitive segmental duplication regions on chromosomes 8 and 12. The second aim is to compare and analyze the breakpoint junction data to detect similarities between translocation junctions in different subjects. The recurrence of this translocation with breakpoints in the same region in all seven unrelated patients could suggest that one part of the 8p REPD is prone to genomic instability, and therefore chromosome rearrangements. However, it is also possible that all seven patients have unique breakpoints due to different sites of NAHR, suggesting that there is not a specific 'hotspot' for recombination within the REPD region.

Chapter 3: Experimental Design and Methods

Primer Design

Amplifying DNA sequence in an area with a large amount of segmental duplications is extremely difficult due to the inherent repetitive nature of the region, and typical PCR primer design strategies will fail due to the lack of unique sequence. When attempting to amplify a specific amplicon within this seg dup region, it is common that primers will bind non-specifically to sequences that are repeated throughout the genome. As my target breakpoint junctions are located within highly repetitive regions, I carefully designed my PCR primers to be unique to successfully capture the breakpoint junction region. Using chromosome-specific single nucleotide polymorphisms (SNPs) displayed on the UCSC Genome Browser as a guide, I created unique primers between 18 and 25 bp in length with a minimum of three region-specific SNPs that would only align to one part of either chromosome 8 or 12 using Primer3 software, a PCR primer design tool that allows for the input of specific parameters for the primers (http://frodo.wi.mit.edu/). The forward primers, oriented 5' to 3' on the positive strand of chromosome 12, and the reverse primers, oriented 5' to 3' on the negative strand of chromosome 8, should only amplify chimeric products consistent with this particular translocation, as shown in Figure 4. I also used the two primers described in the Ou et al. (2011) paper for capture of a patient-specific junction site for the same translocation between 8 and 12 in another patient (denoted as primers 12.1 and 8.1 in this project).

Amplification of Breakpoint Junction

To amplify breakpoint junction regions, I performed long range PCR using Takara LA (Long and Accurate) Taq polymerase following the manufacturer's protocol. I used LA Taq polymerase because it is designed to amplify longer products (> 5 kilobases (kb)) through the

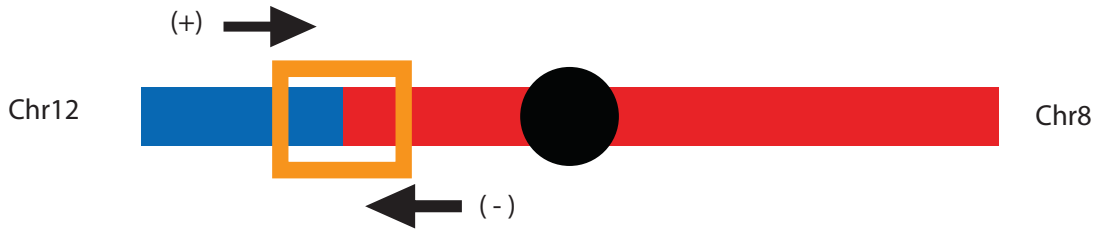


Figure 4. Primer Design. The blue portion of the chromosome represents the chromosome 12 segment of the translocation, and the red represents that of chromosome 8. The orange rectangular outline represents the segmental duplication region. The top arrow represents how the forward primer would correspond to the positive strand of chromosome 12, and the bottom arrow represents the reverse primer that would only correspond with the negative strand of chromosome 8. Figure not drawn to scale.

use of a 3' to 5' exonuclease that detects and removes mismatched base pairs, therefore reducing activity that would hinder elongation. I used this polymerase following the methods described in the Ou et al. paper, whose results showed a patient-specific band approximately 12 kb in length, as a guide. The cycle conditions were 1 minute at 94˚C followed by 30 cycles of 94˚C for 30 seconds and 68˚C for 8 min (increasing by 30 sec every cycle), and 10 min at 72˚C. I started with one patient (Subject 4) and used all of the 169 different primer combinations from the 12 forward chromosome 12 primers and 12 reverse chromosome 8 primers that I designed, along with one chromosome 12 primer and one chromosome 8 primer taken from Ou et al. For the other subjects, I started with a smaller subset of primer combinations, and then focused on the primer pair that was successful in Subject 4 and optimized the PCR conditions. To visualize the PCR results and determine band size, I ran the PCR products through an electrophoresis gel. For primer combinations that resulted in bands, I repeated the PCR under the same cycle conditions with the inclusion of two negative controls: normal male genomic DNA and water; neither should be positive for a translocation junction PCR. After this second PCR, if I visualized multiple or faint bands on the gel, I optimized the PCR conditions with the addition of betaine as a reagent, which

reduces the formation of secondary structures caused by GC-rich regions of DNA to improve amplification, and increased the annealing temperature to increase specificity and reattempted the reaction (Henke et al. 1997).

Product Cloning

After successfully amplifying a patient-specific PCR product and visualizing the band on a gel, I proceeded to isolate the product following a protocol that extracts the DNA product from the gel and purifies the DNA. I performed a tailing reaction that added a poly adenine (A) overhang to the 3' ends of the DNA. I then inserted this purified PCR amplicon into a high copy plasmid vector with complementary 5' thymine overhangs via DNA ligation and transformed the plasmid into Sure2 competent *Escherichia coli* cells. These cells, now containing plasmids with patient DNA inserts, were plated in two volumes (150 uL and 300 uL) onto agar plates with the antibiotic carbenicillin, X-gal, and IPTG (isopropyl β-D-1-thiogalactopyranoside) and incubated overnight at 37°C. After incubation, I performed blue-white colony screening to determine which cells contained inserts. IPTG and X-gal are included on the plates because IPTG is a compound that triggers the transcription of the lac operon, which is located on the plasmid, and X-gal is a substance that can be metabolized by a functional β-galactosidase, resulting in a blue product. If there is no insert present in the plasmid, the mutant *lacZ* gene in the bacterial cell and the mutant *lacZ* gene in the plasmid undergo complementation, resulting in a functional *lacZ* that codes for β-galactosidase. As described earlier, functional β-galactosidase successfully hydrolyzes X-gal to form a blue product, resulting in blue colonies. If an insert is present, the *lacZ* gene on the plasmid is disrupted and does not allow for complementation, so there is no active β-galactosidase to metabolize the X-gal, which results in a white colony.

The number of white colonies present for each sample varied between zero and 25, with the average being five. For each transformation that I plated, I chose between three and five white colonies that would theoretically all include the same DNA insert. These cultures were then incubated overnight in Luria broth (LB), a nutrient rich medium, supplemented with carbenicillin. Afterwards, I mini-prepped the samples using a protocol to separate the plasmid DNA in a purified form from the bacterial genome. To verify that an insert was present in the plasmids, I then performed a restriction enzyme digest with the enzyme *EcoRI*, an endonuclease derived from *E. coli* that preferentially cuts at the sequence GAATTC (and its complement, CTTAAG). If there was no insert, then there should be only one cut, resulting in linear plasmid vector that appears as a single band on an agarose gel. Presence of an insert would lead to multiple restriction sites being cut and result in multiple bands on the gel, that of the plasmid vector and that of the insert. I also used this gel to determine the size of my insert and compare it to the size of the amplicon I cloned.

Sequencing of Amplicon

Plasmids containing inserts were then sent to Beckman Coulter Genomics for Sanger DNA sequencing with primers designed to amplify a portion of the plasmid vector and the insert. Each sample was sequenced from both a forward and a reverse read to cover the entire length of the ~ 1-kb insert, as illustrated in Figure 5. Both the forward and reverse reads were ~ 1 kb in length.

Analytical Methods

The results from the sequencing were two reads (a forward and a reverse read) for each sample that I sent in that covered the plasmid insert in its entirety along with a small portion of the plasmid itself. To analyze these sequences, I first created a contiguous sequence (contig) by

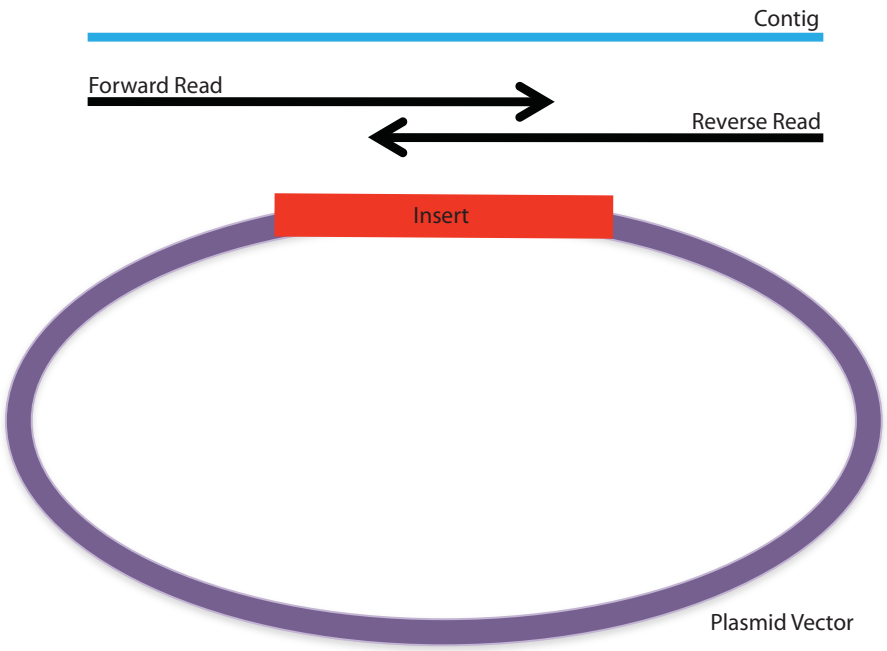


Figure 5. Contiguous sequence assembled from sequencing and aligning plasmid reads. Plasmid is not drawn to scale.

aligning the overlapping forward and reverse reads for each sample using the BioEdit Sequence Editing software. This singular contiguous sequence, derived from my patient sequence, included all of the bases sequenced for the forward read, the reverse read, and their overlapping portions in one clean sequence ~1.2 kb in length for analysis. With this new contig sequence, I used the program BLAT (BLAST-like Alignment Tool, where BLAST stands for Basic Local Alignment Search Tool) on the UCSC Genome Browser to align my patient sequence with the reference human genome to verify if it aligned with both chromosome 12 and chromosome 8 in the expected regions (12p13.31 and 8p23.1, respectively) and that it represented the true breakpoint junction. I also used the multiple sequence alignment program MultAlin to align my patient sequence with the reference genome sequence for chromosome 12 and chromosome 8 at nucleotide resolution and determine the percent identity between the three sequences

(http://multalin.toulouse.inra.fr/multalin/). With the MultAlin results, I also looked for where the nucleotides of my patient-specific sequence matched identically to the chromosome-specific single nucleotide polymorphisms on chromosomes 8 and 12, and used these SNPs as a guide to map the breakpoint junction site.

Chapter 4: Results

PCR amplification of genomic DNA from Subject 4 with forward primer 5' GAA CCC GAT GTC AAC AAC AC 3' (12.4) and reverse primer 5' GTG TAA GAC GTC GAT ACG ATA CGG CAC TTC 3' (8.1) successfully resulted in a ~1-kb patient-specific amplicon (Figure 6A). As anticipated, the sequencing data from this amplicon was consistent with the predicted junction overlap between the reference sequences (including chromosome-specific SNPs) for chromosomes 12 and 8 on the UCSC Genome Browser. The contig I created from this amplicon aligned to chromosome 12p13.31 at position 8,377,047-8,378,103 on the UCSC Genome Browser with a percent identity of 97.9% and to chromosome 8p23.1 at position 7,432,148-7,433,257 with 96.7% identity. Results from the MultAlin program also demonstrated a clear delineation of the region that contained the breakpoint junction to within a region 40 nucleotides in length. This region included sequence with which the contig for Subject 4 aligned with chromosome 12 and its chromosome-specific SNPs and then switched to align with chromosome 8 reference sequence and its chromosome-specific SNPs (Figure 6B).

For the other six patients, the breakpoint junctions were not successfully captured and amplified. When I began the initial primer combination testing with the other six patients one at a time (in the same manner as Subject 4), I did not have success with amplifying clear, patient-specific bands. Due to time and reagent amount restrictions, I narrowed my focus from testing each individual separately and instead tested the primer combination of 12.4 and 8.1 (successful for Subject 4) with all patient DNA templates (including Subject 4 as a positive control, and the two negative controls of normal male DNA and water) in the same set of reactions. For three of the six additional patients, the product amplified by this primer combination was a non-unique

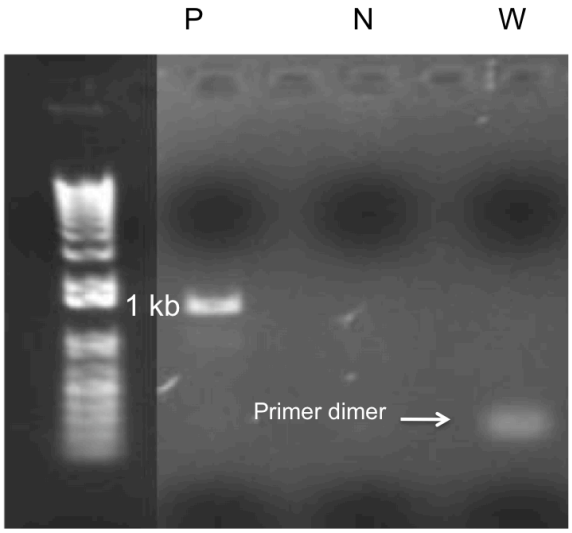
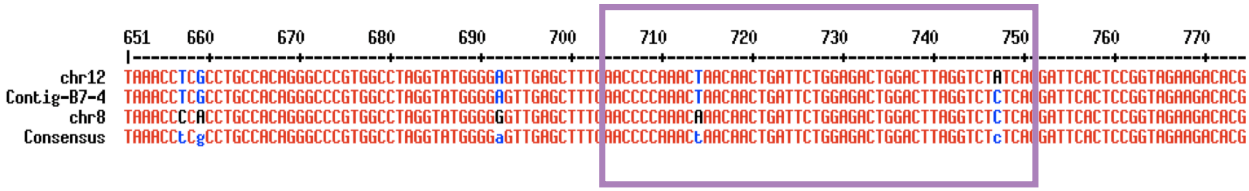


Figure 6.
6A) Electrophoresis gel image of patient specific band amplified by primers 5' GAA CCC GAT GTC AAC AAC AC 3' (12.4) and 5' GTG TAA GAC GTC GAT ACG ATA CGG CAC TTC 3' (8.1). First lane shows the Invitrogen 1 kb Plus ladder used to size the bands; lane 2 (P) is the ~ 1-kb product from Subject 4 DNA; lane 3 (N) is normal male control DNA; lane 4 (W) is water. Note: part of gel was removed to show band sizes relative to the ladder used.
6B) Portion of alignment of chromosome 12 reference genome sequence, patient specific contig, and chromosome 8 reference genome sequence. Numbers along top of image refer to nucleotide number of the contig (not location on reference genome). Nucleotides in red indicate identical bases on all three sequences, blue and black nucleotides indicate single nucleotide polymorphisms (SNPs) present in the sequences. The area within the purple box signifies the breakpoint junction region, where the patient DNA aligned with chromosome 12 sequence and then switched to align with chromosome 8 sequence within a 34-bp region.



sequence that aligned to multiple locations in the genome and with lower identity than previously found with Subject 4.

The failure of this PCR in amplifying patient specific bands in multiple individuals could be due to a number of factors. As mentioned earlier, attempting to amplify products in segmental duplications is a very difficult process, as there are very few unique portions within the region that can be used to design specific primers. The PCR conditions were optimized for a larger product, and thus required a longer annealing time, which is not an ideal condition when using primers from repetitive regions as it allows for less stringent binding to DNA (as in non-specific binding of primers to multiple locations on the genome). There also exists the possibility that

these patients also have an inversion on one of the chromosomes involved in this translocation. If, for example, there was also an inversion of genetic material on chromosome 8 within the region of interest for amplification, the orientation of the bases will have switched from positive to negative and from negative to positive, making the primers I designed (with their specific orientation) ineffective and therefore resulting in no patient-specific band.

Chapter 5: Discussion

Though not the ideal result of this experiment, the successful capture and amplification of only one patient breakpoint supports the hypothesis that the breakpoint junction region for the recurrent translocation between 8 and 12 is located within the 300-kb segmental duplication region shared between the two chromosomes. Failure to capture the other breakpoints in this same region also demonstrates the complexity of this rearrangement and the difficulty of trying to successfully amplify sequences within a highly repetitive region of the genome. This is also supported by the fact that the authors from the Ou et al. paper were also only able to successfully capture the breakpoint junction site in one patient with this particular translocation, but were able to capture breakpoints in five patients for a different translocation.

Ou et al. successfully amplified an ~ 12-kb patient-specific band with forward primer 5' TTCTTAATATCACTTTTCCCCACTCTAGTTC 3' (primer 12.1) and reverse primer 5' GTGTAAGACGTCGATACGATACGGCACTTC 3' (primer 8.1), and narrowed the junction site down to a 55-bp region that aligned to chromosome 12 at position 8,374,216-8,374,332 and chromosome 8 at position 7,884,979-7,885,033. The breakpoint on chromosome 12 lies within ~ 2.5 kb of the breakpoint I found for Subject 4, while the breakpoint sites on chromosome 8 are much harder to define. As the chromosome 8 portion of my insert mapped to a different places on the reference genome, I cannot be confident about mapping the breakpoint at a nucleotide resolution. Since the Ou et al. paper used the same chromosome 8 primer as I did, I also cannot be sure that they mapped the chromosome 8 breakpoint at a nucleotide resolution either. Though I cannot say where exactly on chromosome 8 Subject 4's breakpoint lies, I am still confident that I did capture the junction. I was able to narrow down the junction region to within a 34-bp region shared in common between chromosomes 8 and 12. The difference between breakpoint sites

suggests that, though the REPD region itself may indeed be more prone to chromosomal rearrangement as a result of its repetitive nature, there does not seem to be a specific recombination 'hotspot' within the region. The breakpoints for this translocation are variable, and depend on the individual patient genome.

Future Aim of Project

The continuation and optimization of PCR conditions is necessary to exhaust the possibility of amplifying patient breakpoints with my available primers. In utilizing more stringent PCR conditions and tailoring the *Taq* and other parameters used, I will be able to test the remaining primer combinations on patient DNA to map their specific breakpoint junctions.

Another future aim of this project that has already begun in the Rudd lab is to successfully recapitulate the obesity phenotype associated with gene *GNB3* in a mouse model and study the transcriptome levels (mRNA expression) of *GNB3*. Through the transcriptome study, we will determine how the extra copy of this gene leads to upregulation or downregulation of other genes downstream, and how that is related to the obesity phenotype demonstrated in all of the patients with this particular rearrangement. We have already succeeded in creating a mouse model for the extra copy of *GNB3*, and are currently designing experiments to measure the expression of the GNB3 transcript levels in mouse tissues.

References:

Abeysinghe SS, Chuzhanova N, Krawczak M, Ball EV, Cooper DN. Translocation and gross deletion breakpoints in human inherited disease and cancer I: Nucleotide composition and recombination-associated motifs. Hum Mutat. 2003 Sep;22(3):229-44.

Bailey JA, Gu Z, Clark RA, Reinert K, Samonte RV, Schwartz S, Adams MD, Myers EW, Li PW, Eichler EE. Recent segmental duplications in the human genome. Science. 2002 Aug 9;297(5583):1003-7.

Cheung J, Estivill X, Khaja R, MacDonald JR, Lau K, Tsui LC, Scherer SW. Genome-wide detection of segmental duplications and potential assembly errors in the human genome sequence. Genome Biol. 2003;4(4):R25. Epub 2003 Mar 17.

Giglio S, Broman KW, Matsumoto N, Calvari V, Gimelli G, Neumann T, Ohashi H, Voullaire L, Larizza D,Giorda R, Weber JL, Ledbetter DH, Zuffardi O. Olfactory receptor-gene clusters, genomic-inversion polymorphisms, and common chromosome rearrangements. Am J Hum Genet. 2001 Apr;68(4):874-83. Epub 2001 Feb 26.

Giglio S, Calvari V, Gregato G, Gimelli G, Camanini S, Giorda R, Ragusa A, Guerneri S, Selicorni A, Stumm M,Tonnies H, Ventura M, Zollino M, Neri G, Barber J, Wieczorek D, Rocchi M, Zuffardi O. Heterozygous submicroscopic inversions involving olfactory receptor-gene clusters mediate the recurrent t(4;8)(p16;p23) translocation. Am J Hum Genet. 2002 Aug;71(2):276-85. Epub 2002 Jun 10.

Gilman AG. G proteins: transducers of receptor-generated signals. Annu Rev Biochem. 1987;56:615-49.

Klenke S, Kussmann M, Siffert W. The GNB3 C825T polymorphism as a pharmacogenetic marker in the treatment of hypertension, obesity, and depression. Pharmacogenet

Genomics. 2011 Sep;21(9):594-606.

Ou Z, Stankiewicz P, Xia Z, Breman AM, Dawson B, Wiszniewska J, Szafranski P, Cooper ML, Rao M, Shao L, South ST, Coleman K, Fernhoff PM, Deray MJ, Rosengren S, Roeder ER, Enciso VB, Chinault AC, Patel A,Kang SH, Shaw CA, Lupski JR, Cheung SW. Observation and prediction of recurrent human translocationsmediated by NAHR between nonhomologous chromosomes. Genome Res. 2011 Jan;21(1):33-46.

Segel R, Peter I, Demmer LA, Cowan JM, Hoffman JD, Bianchi DW. The natural history of trisomy 12p. Am J Med Genet A. 2006 Apr 1;140(7):695-703.

Shaffer LG, Lupski JR. Molecular mechanisms for constitutional chromosomalrearrangements in humans. Annu Rev Genet. 2000;34:297-329.

Siffert W. G protein polymorphisms in hypertension, atherosclerosis, anddiabetes. Annu Rev Med. 2005;56:17-28.

Siffert W, Rosskopf D, Siffert G, Busch S, Moritz A, Erbel R, Sharma AM, Ritz E, Wichmann HE, Jakobs KH, Horsthemke B. Association of a human G-protein beta3 subunit variant with hypertension. Nat Genet. 1998 Jan;18(1):45-8.

Sobreira NL, Gnanakkan V, Walsh M, Marosy B, Wohler E, Thomas G, Hoover-Fong JE, Hamosh A, Wheelan SJ, Valle D. Characterization of complex chromosomal rearrangements by targeted capture and next-generation sequencing. Genome Res. 2011 Oct;21(10):1720-7. Epub 2011 Sep 2.

Websites:

MultAlin. http://multalin.toulouse.inra.fr/multalin/

Primer3 Input. http://frodo.wi.mit.edu/

UCSC Genome Bioinformatics. http://genome.ucsc.edu/