**Chapter 4**

%% PRDM9 gene, meiotic recombination, hotspots

%% PRDM9 allele is determined through zinc finger repeat expansion and the mutations on the zinc finger repeat expansion

%% PRDM9 binding site per allele

%% PRDM9 genotype per sample

%% PRDM9 genotype per DNA molecule per sperm

%% relationship between PRDM9 allele and gene evolution rate

%% relationship between LD and meiotic recombniation hotspots

%% Trio-sequencing has been used to determine or the aftermath of meiotic recombniation per chromosome from a single meiotic event

%% long-range PCR has been used to genotype meiotic recombination from thousands of sperm samples in a target region

%% gene conversion tract length

%% meiotic mutagenesis: small snvs, indels and copy number variations or structural variations

%% linkage disequilibrium

%% gene conversion as a violation of mendelian ratio

\section{Introduction}

Gene conversions and crossovers resulting from meiotic recombination is one of the ways Nature generates genetic diversity and provide the solutions for natural selection to act upon and select the solutions that is best adapted towards the environment and is responsible for speciation and evolution. The study of meiotic recombination products has been abled by trio-sequencing, sperm-typing and population-level haplotype map generation. Trio-sequencing enables the study of 1 meiotic recombination product per chromosome per child while sperm-typing allows for the analyses of thousands of meiotic recombination products at the target site.

The detection of meiotic recombination products at scale has been difficult as their detection requires haplotype phasing of the target region and Illumina short reads with the limited insert size cannot traverse multiple heterozygous SNPs (hetSNPs) to determine the haplotype of the target region.

Meiosis is a critical part of gamete generation and genetic diversity generation. In the beginning stages of meiosis, DNA is replicated, sister chromatid formation, double strand break is introduced by PRDM9 and SPO11, [………………………………] and double strand break repair results in the formation of gene conversions and crossovers. Gene conversion is a product of mismatch repair (MMR) and leads to a DNA molecule where the maternal haplotype is flanked by paternal haplotype both upstream and downstream while crossover leads to a DNA molecule where maternal haplotype sequence is continued with a paternal haplotype sequence.

Meiotic recombination is required to hold the non-sister chromatids in place before their segregation in …

We hypothesized that CCS reads, however, with their longer read length and high base accuracy, should 1) enable the haplotype phasing of large blocks of the genome if hetSNPs are present and if there is sufficient coverage to phase these hetSNPs, 2) the high base accuracy should allow the algorithm to distinguish erroneous haplotype switches resulting from sequencing errors and that arising from biological processes. We sequenced sperm samples of different ages at 20 to 30 sequence coverage to call meiotic recombination products across the genome. In addition, we also CCS sequenced granulocytes from Bloom syndrome patients to examine mitotic recombination products [Table X]. Bloom syndrome patients have defective BLM protein that is involved in the double strand break repair process and suppresses crossover as an outcome of the homology-directed repair process. This study allowed for not only the detection of gene conversion and crossover across the genome, allowing the determination of both meiotic and mitotic recombination hotspots and determinants of these hotspots, but also the comparison of characteristic differences between meiotic and mitotic recombination for the first time and the riddles in these processes that could not be answered with existing sequencing technologies and the resolution of the studies.

In addition, we also sequenced granulocytes from normal individuals of different ages to determine the normal gene conversion rate and crossover rate during clonal hematopoiesis.

%% what are the questions? Meiotic recombination hotspots, changes in meiotic recombination rate with age?

%% double strand break repair

%% double strand break repair during meiosis

%% double strand break repair in mitotic cells

%% differences in double strand break repair mechanism in meiotic and mitotic cells

%% diagram

%% meiotic recombination

%% mitotic recombination can happen during different stages of the cell cycle

%% gene conversion and both crossovers can lead to loss of heterozygosity events that can be a potential oncogenic mechanism

%% transfer of epigenetic status from one haplotype to other haplotype during mismatch repair process

%% the real-time detection of MMR through the analysis of single-strand consensus sequences

%% when was meiotic recombination first used?

%% is it only used in eukaryotic cells?

%% how does prokaryotic cells evolve?

To date, gene conversions and crossovers resulting from meiotic recombination and mitotic recombination have not been compared or contrasted.

\section{Material \& Methods}

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\subsection{Haplotype phasing}

\subsection{Gene conversion and crossover candidate calling}

\section{Results}

\section{Discussion}

We sequenced multiple sperm samples of different ages with similar PRDM9 genotype. PRDM9 is known to be the critical factor for determining the double strand break sites and determining the locations where double strand break repair happens.

We do not know how ethnicity, environment, carcinogens impact meiotic and mitotic recombination rates.