DEVELOPING A BIOINFORMATIC PIPELINE FOR HEREDITARY VARIANT ANALYSIS AND IMPRINTING DISORDERS STUDIES

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INTRODUCTION

Genomic imprinting refers to specific allelic transcriptional silencing regulated by differentially DNA methylated regions (DMRs). Imprinting marks elude postzygotic reprogramming and are maintained throughout development on all somatic tissues.

Disruption of expression in parentally imprinted genes, results in imprinting disorders, a group of congenital diseases affecting growth, puberty, behaviour, development and metabolism. Several molecular changes have been described associated with imprinting disorders: cytogenetic rearrangements, point mutations in imprinted genes, uniparental disomy and epimutations.

There are currently two hypotheses on imprinting methylation disruption; environmental exposures causing primary epimutations, and an initial genetic mutation. This mutation should be in a factor involved in the establishment or maintenance of imprinted methylation, resulting in secondary epimutations.

METHODS

Previous studies identified 9 patients carrying a methylation alteration on the GNAS locus, with all four DMRs affected and no deletions in the *NESP55* gene. And 1 patient with partially altered methylation of the GNAS locus.

Pedigree analysis was performed on these 10 patients diagnosed of pseudohypoparathyroidism at the Molecular (Epi)Genetics Laboratory, Araba-Txagorritxu University Hospital. Exome sequencing was performed on 31 individuals (1 quartet and 9 trios) on a HiSeq2000 system (Illumina Inc.) using exome enrichment capture "SureSelect Target Enrichment System Human all exon V5 + UTRs" kit (Agilent©).

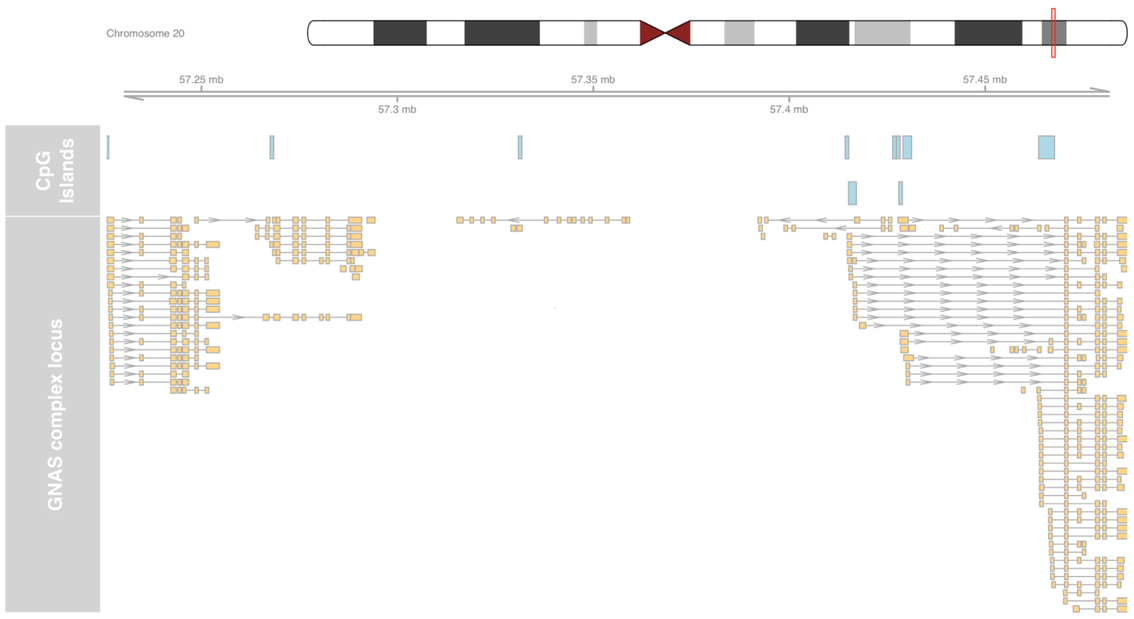


Figure 1. Representation of the GNAS locus; imprinted region affected by aberrant methylation patterns, showing *STX16*, *NESP55* and *GNAS* genes

RESULTS AND CONCLUSIONS

We are developing a tool that performs reproducible and automated genetic inheritance analysis in relation to hereditary diseases and imprinting disorders. We have tested its performance using previous familiar studies (hypobetalipoproteinemia) and have successfully identified the causal variant.

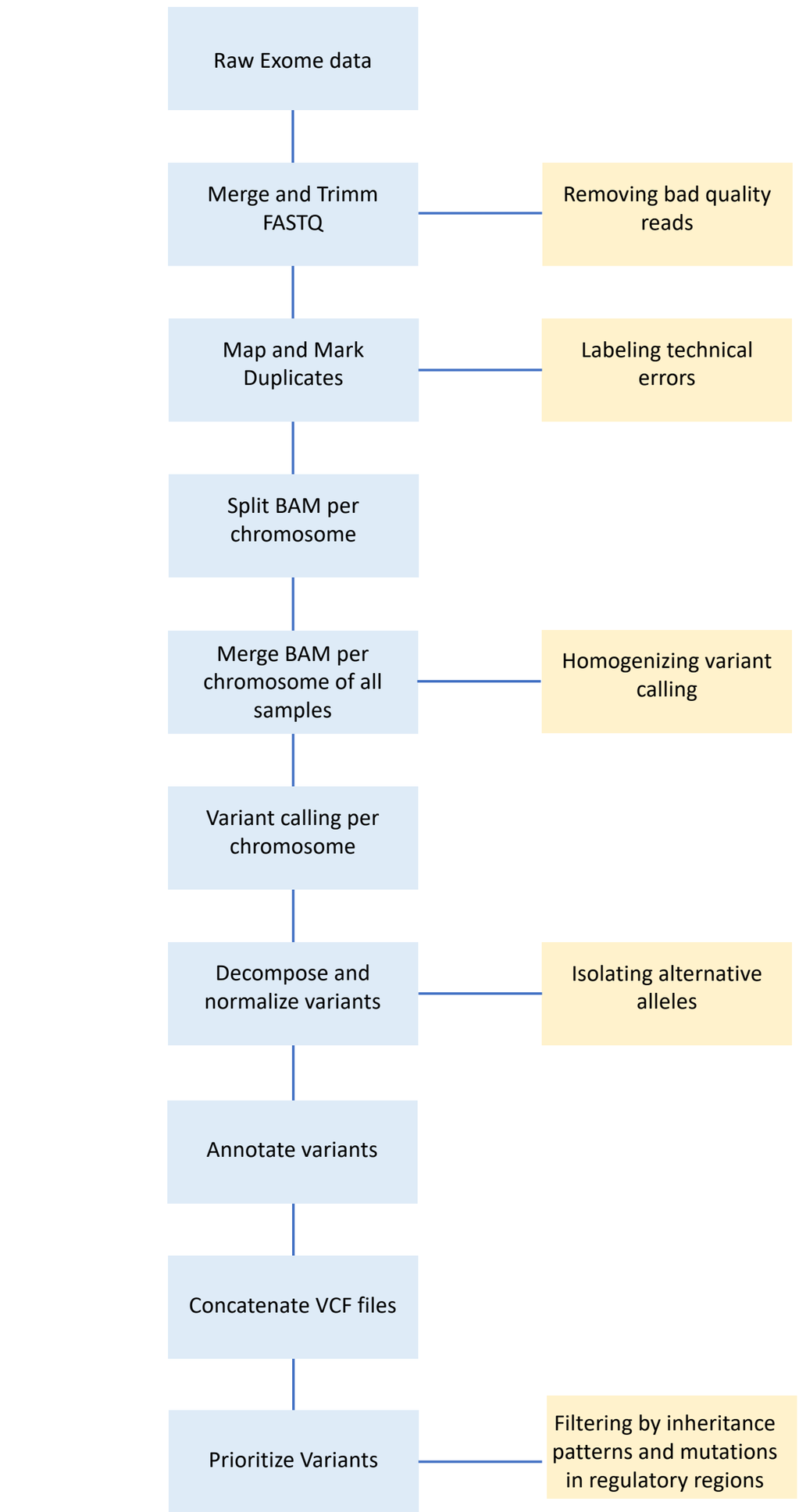
Joint analysis of all individuals in the study permits identification of parent-of-origin variants and segregation analysis, as well as subgroup comparison using known phenotypic or genomic characteristics.

OBJECTIVES

So far mutations in four genes have been associated with imprinting disorders caused by multilocus imprinting disturbances: *NLRP7* (MIM #609661), *KHDC3L* (MIM #611687), *NLRP2* (MIM #609364) and *ZFP57* (MIM #612192). Whereas microdeletions and point mutations have been detected at the ICRs regulating locus-specific DMRs

- Short term objective: developing a specific bioinformatic pipeline for pedigree analysis.
- Long-term objective: identifying unknown genes associated with disruption of imprinting patterns (secondary epimutations).

PIPELINE



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