

# Structural variant in the RNA Binding Motif Protein, X-Linked 2 (*RBMX2*) gene found to be linked to bipolar disorder



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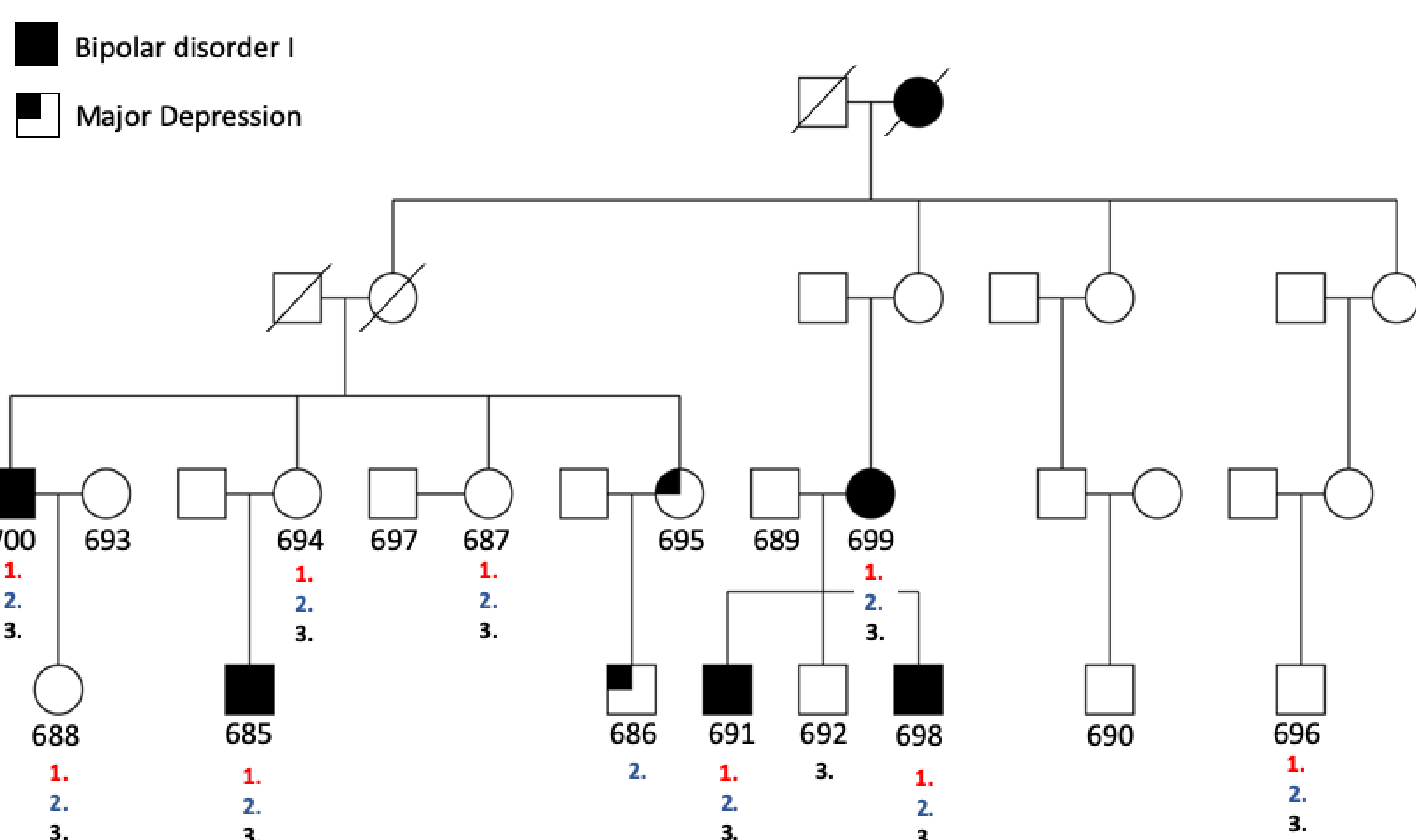
## Introduction

Here, we have used long-read Single Molecule, Real-Time (SMRT) sequencing to characterize a ~12 Mb genomic region on chromosome Xq24-q27 significantly linked (lod score=3.54) to bipolar disorder in an extended family from a genetic sub-isolate.

This family segregates bipolar disorder (BD) in at least 4 generations with 24 affected individuals out of 61 total. Thus, this family portrays a highly elevated recurrence risk compared to the general population. We selected 16 individuals from the family for targeted sequencing. The selected individuals either carried the disease haplotype, were non-carriers of the disease haplotype, or served as married-in controls. We designed a SeqCap EZ probe pool (Roche) enriching for 5-9 kb fragments spanning the entire 12 Mb region that were then sequenced to screen for candidate structural variants (SVs) that could explain the increased risk for BD in this extended family.

Altogether, 201 SVs were detected in the critically linked region. Although most of these represented common variants, three SVs emerged that showed near-perfect segregation among all bipolar disorder type I affected individuals and absence among the married-in controls. Two of these SVs were identified in genes belonging to the RNA Binding Motif Protein, X-Linked (*RBMX*) gene family - a 330 bp *Alu* deletion in intron 3 of the RNA Binding Motif Protein, X-Linked 2 (*RBMX2*) gene and another intergenic variant in the *RBMX* gene. The third SV was a 50 bp tandem repeat insertion in intron 1 of the Coagulation Factor IX (*F9*) gene. These variants are prime candidates for investigating the molecular basis of bipolar disorder in this family.

## Material: Extended family P101



**Figure 1. Family P101.** Sixteen family members were included in the study. Five were affected males (bipolar disorder I: 700, 685, 691, 698, major depression: 686) and two were affected females (bipolar disorder I: 699, major depression: 695). In addition, three married-in unaffected controls were included (693, 697, 689) as well as six unaffected family members (688, 694, 687, 692, 690, 696). See **Table 1.** for explanation on the colored numbers 1-3 seen here.

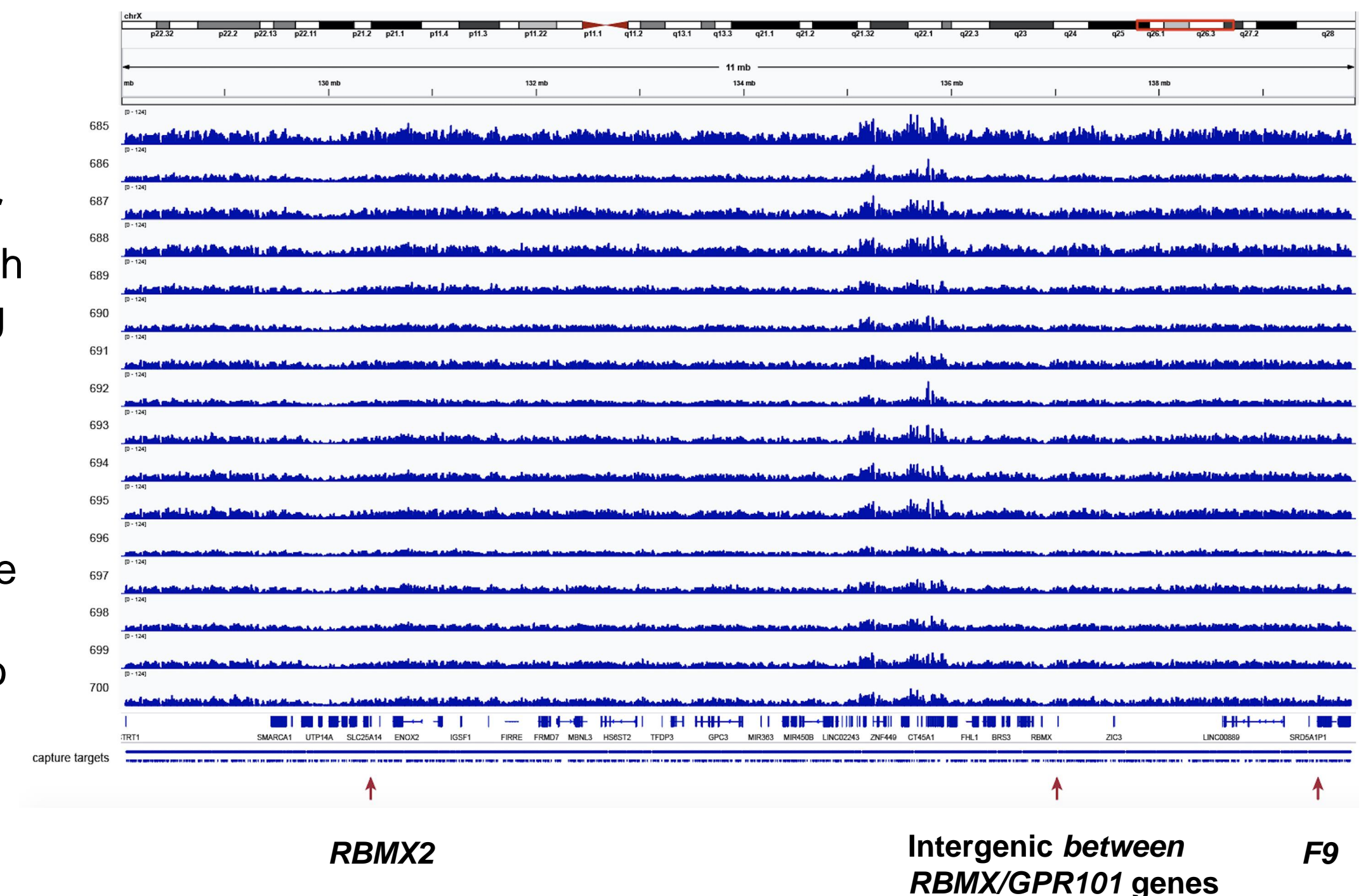
## Methods: SeqCap<sup>®</sup> EZ probe-based capture combined with long-read SMRT Sequencing



**Figure 2. Workflow overview.** gDNA<sup>1</sup> was sheared<sup>2</sup> 10 kb fragments which was followed by a ligation<sup>3</sup> of adapters, amplification and size selection<sup>4</sup> using the BluePippin System. Next, the SeqCap<sup>®</sup> EZ capture<sup>5</sup> probes were hybridized, followed by the bead capture step and a washing step to remove any nonspecific and unbound molecules. Finally, the sequencing library was generated<sup>6</sup> which was size selected with BluePippin before sequencing<sup>7</sup> on the PacBio RS II system.

## Results

**Figure 3. Sequencing coverage plot across the targeted 12 Mb region on chromosome Xq24-q27 for all 16 family members.** Each row represents the sequencing coverage for each family member separated by ID number. On the top the chromosomal location is displayed and at the bottom the genes and probe locations are shown. The red arrows point to the three SVs that were located in *RBMX* and *F9* genes.



SV #	SV type	Size	Location	Carriers of SV	Verification methods
1.	<i>Alu</i> deletion	330 bp	chrX:130405824, <i>RBMX2</i> intron 3	685, 687, 688, 691, 694, 696, 698, 699, 700	PBSV workflow, PCR+Sanger, manual data inspection
2.	Tandem repeat deletion	27 bp	chrX:137012126, intergenic between genes <i>RBMX</i> and <i>GPR101</i>	685, 686, 687, 688, 691, 694, 695, 696, 698, 699, 700	PBSV workflow
3.	Tandem repeat insertion	50 bp	chrX: 139536239, <i>F9</i> intron 1	685, 687, 688, 691, 692, 694, 695, 696, 698, 699, 700	PBSV workflow

**Table 1. Three SVs were highlighted in this study.** These included an *Alu* deletion (1, red), a tandem repeat deletion (2, blue) and a tandem repeat insertion (3, black) of various sizes. Two SVs (1 and 2) were located in or in close vicinity of different *RBMX* gene family members, while one was located in the *F9* gene. The pedigree in **Figure 1** shows the segregation by number of each of the SVs.

## Conclusions

### Summary

Long-read SMRT Sequencing was applied to a 12 Mb genomic region on chromosome Xq24-q27 linked to BD in an extended family from an isolated population

- 16 key individuals from the extended family were selected for the study
- Roche's hybridization-based target enrichment of 5-9 kb fragments spanning the region was used in combination with PacBio long-read sequencing
- Altogether, 201 SVs were detected most representing common variants, however 3 SVs emerged that showed near-perfect segregation among all bipolar disorder type I affected individuals

### Conclusions

There is additive evidence that the 330bp *Alu* deletion in intron 3 of the *RBMX2* gene may be involved in the disease development of BD type I in this extended P101 family

- Near-perfect segregation with BD type 1 in family P101
- *RBMX2* play a central role in brain development and function and has previously been associated with mental retardation and epilepsy
- Deleterious *Alu* activity is associated with at least 37 neurological and neurodegenerative disorders

Further studies are warranted to fully understand whether the *RBMX2* gene pathway is involved in the development of BD type I and to confirm the functionality of the identified variant

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