Approximately 10% of "*de novo*" *SCN1A* mutations causing Dravet Syndrome are inherited from undetected parental mosaicism

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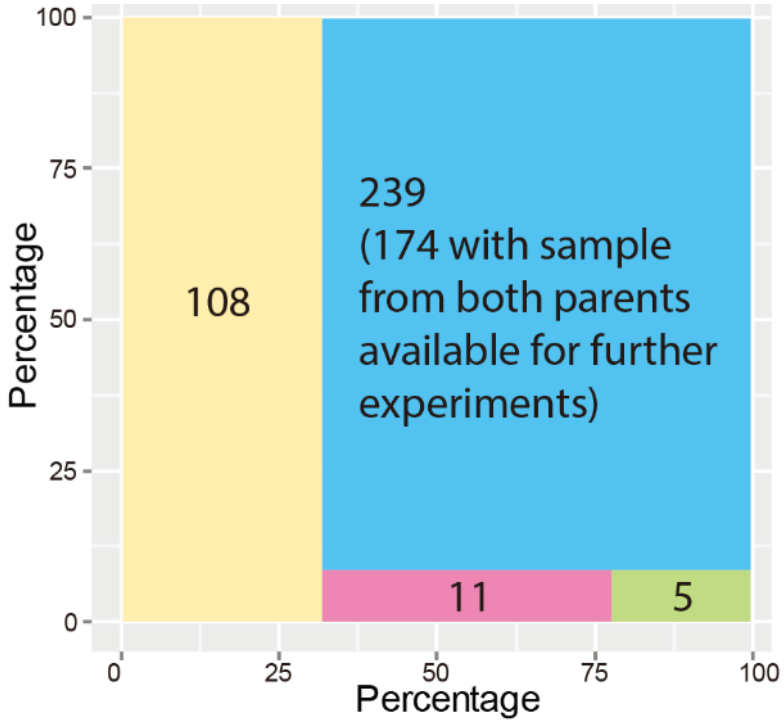
Outline

- Background
  - Dravet syndrome (DS) and cohort description
  - Introduction to postzygotic mosaicism
- Methods
  - Deep sequencing framework we developed for mosaicism detection (PASM)
  - Bayesian model used for calculating the fractions of mutant alleles
- Results
  - Parental mosaicism detected from *SCN1A* "*de novo*" mutation families
  - Fraction of mutant allele and parent phenotype
- Conclusion

Brief introduction to Dravet syndrome and *SCN1A* mutations

- Characteristics
  - Seizure onset within 1 year of age (average age of onset 6 months) with the first event often being seizures induced by fever
  - Normal early development
  - Prolonged generalized or hemiclonic seizures, often triggered by fever
  - Multiple seizure types (myoclonic, focal, atypical absences) in addition to seizures triggered by fever after 1 year of age;
  - Psychomotor slowing after 1 year of age, ataxia and pyramidal signs
  - Normal interictal electroencephalography in the first year of life followed by generalized, focal, or multifocal discharges
  - Seizures that were pharmaco resistant.
- Genetics:
  - 70% DS probands have nonsynonymous mutations on *SCN1A*
  - 90-95% of the mutations are *"de novo"* by Sanger sequencing

Description for the Chinese DS cohort we collected



Mutations not found in *SCN1A* **29.8% (108/363)**

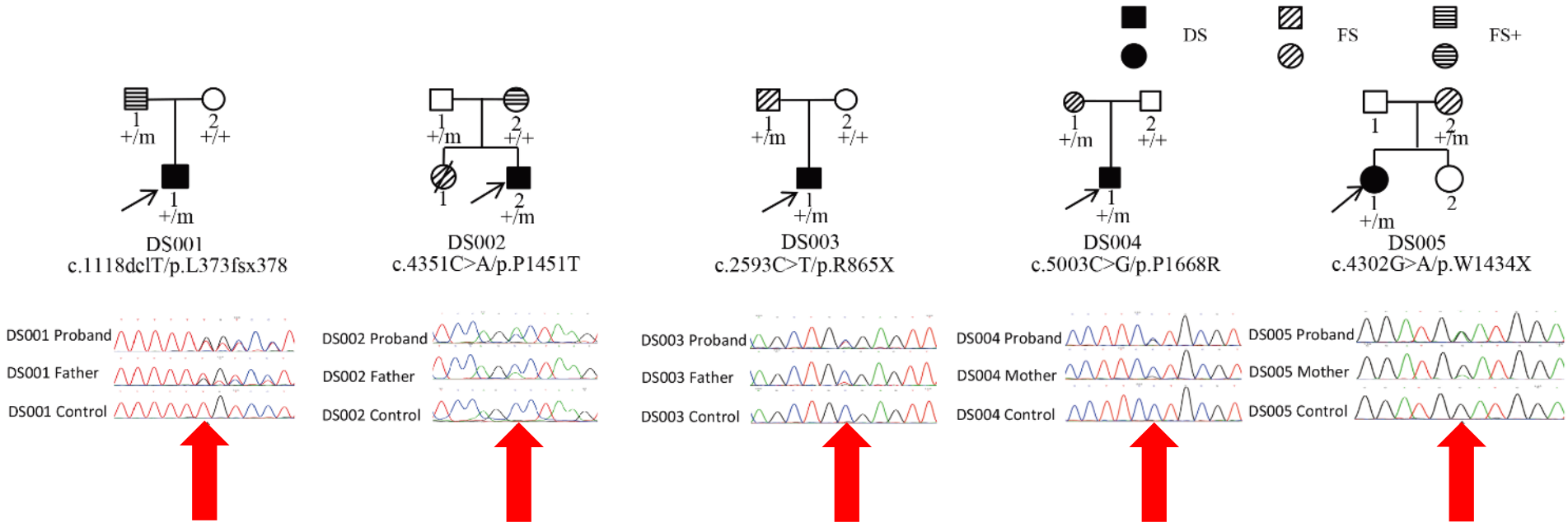
Inherited from parental heterozygous mutations **3.0% (11/363)**

Inherited from parental mosaic mutations **1.4% (5/363)**

De novo mutations **65.8% (108/363)**

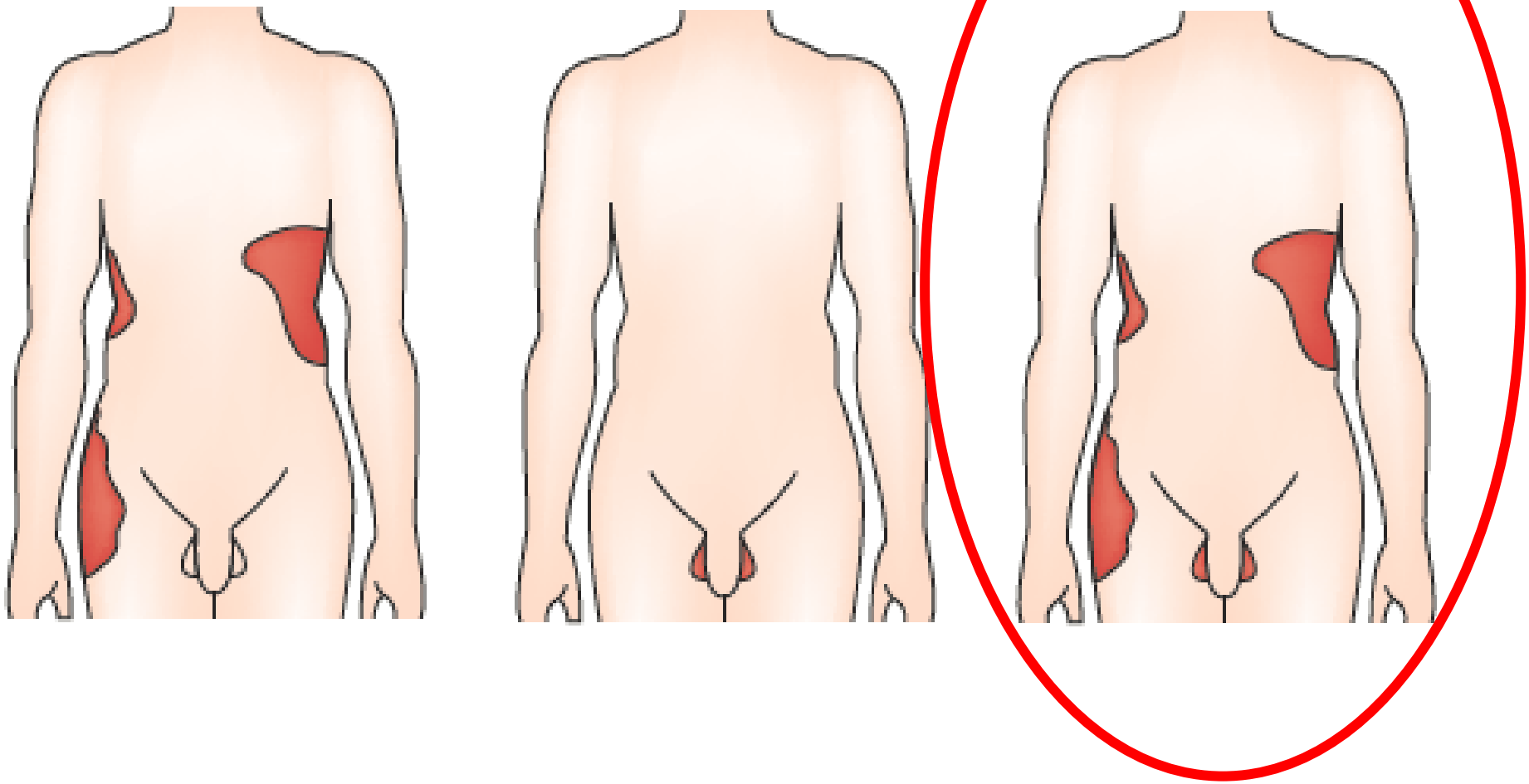
(Xu, *et al*. 2015)

The five Sanger sequencing detectable parental mosaicism from DS affected *SCN1A* mutated families



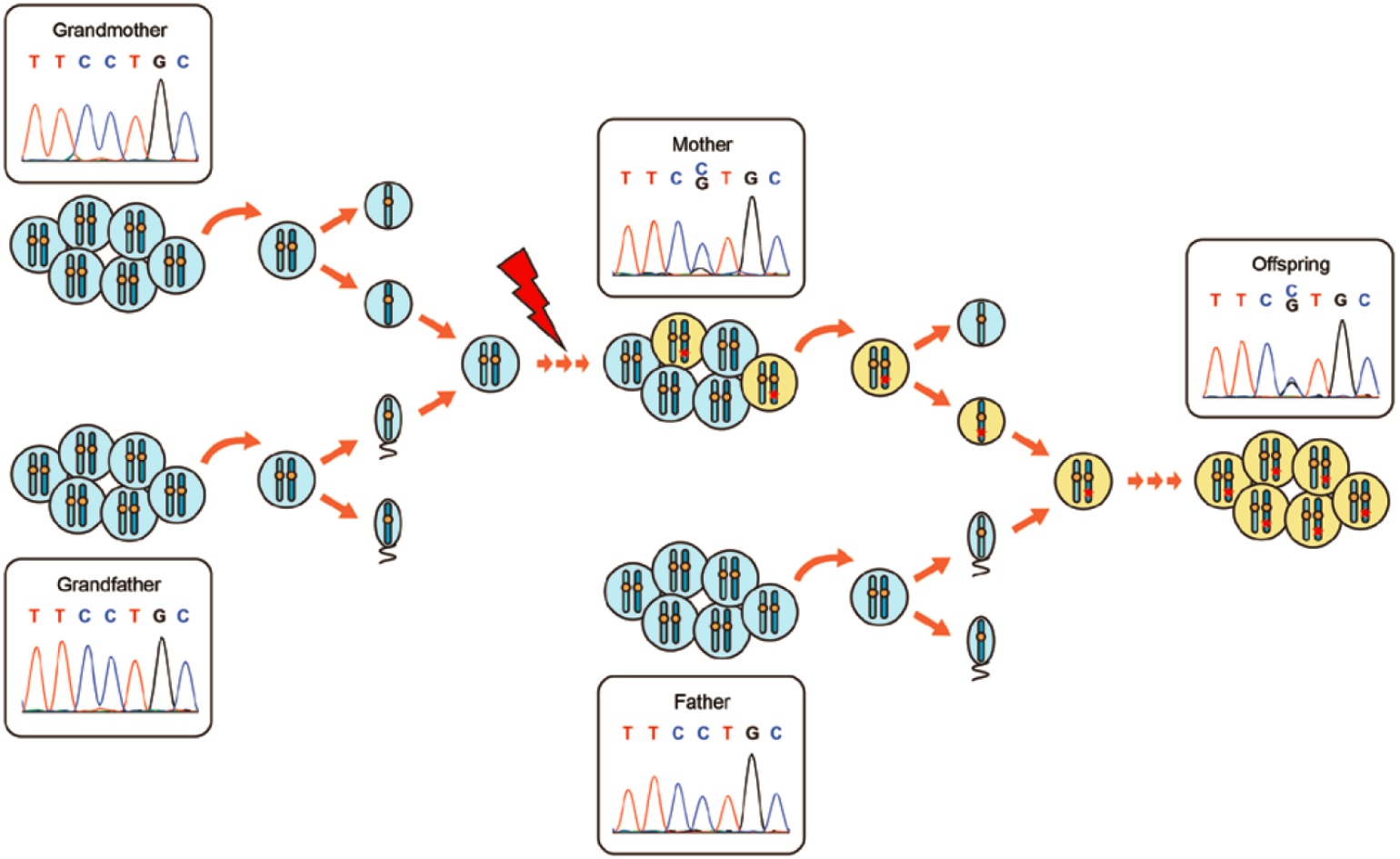
(Xu, *et al*. 2015)

Genomic mosaicism resulted from postzygotic mutations



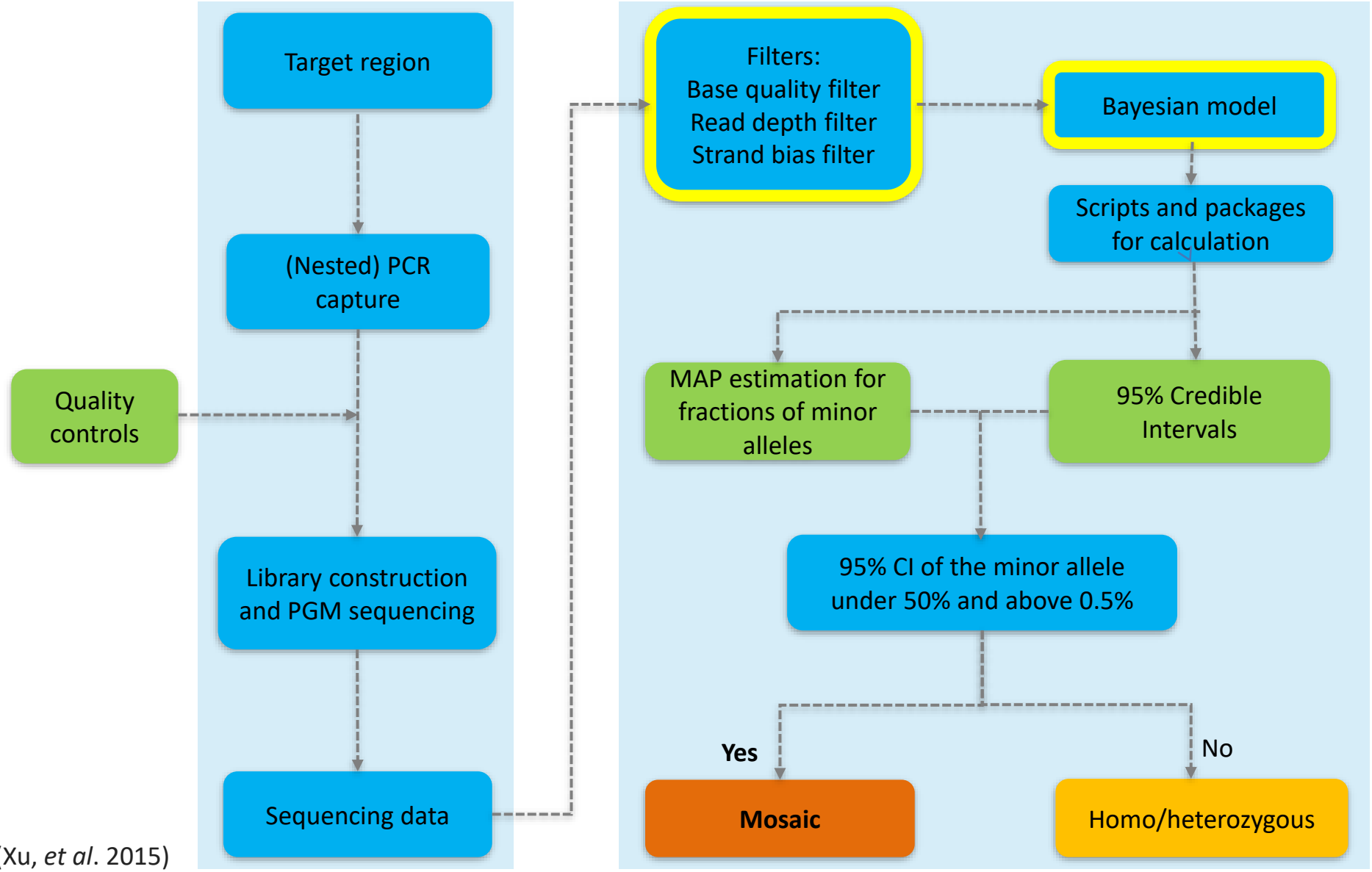
(Biesecker and Spinner,2013)

An example of the trans-generational impact of *SCN1A* parental mosaicism



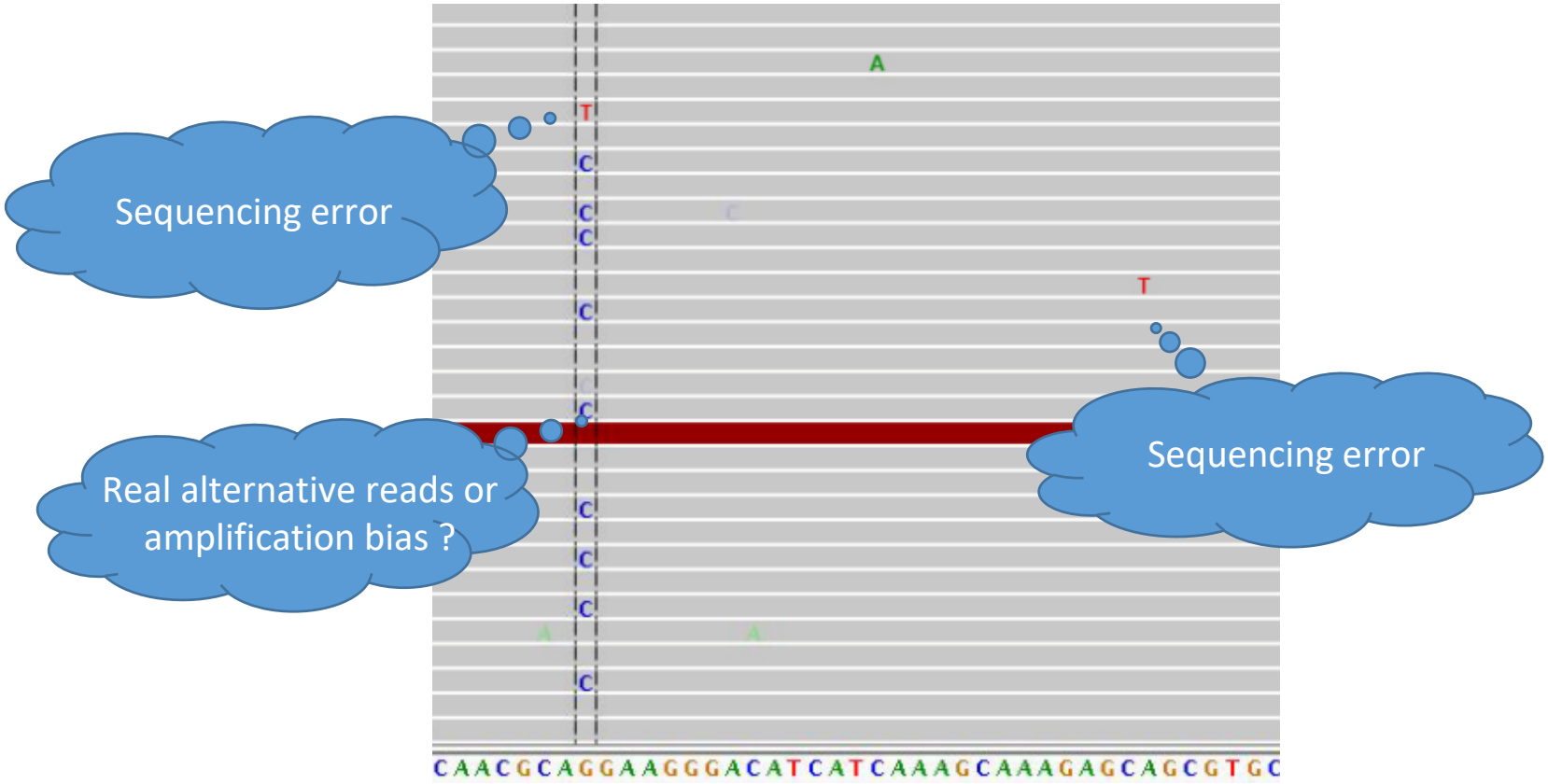
(Huang, *et al*. 2014)

Experimental and computational framework of PGM Amplicon Sequencing for Mosaicism (PASM)



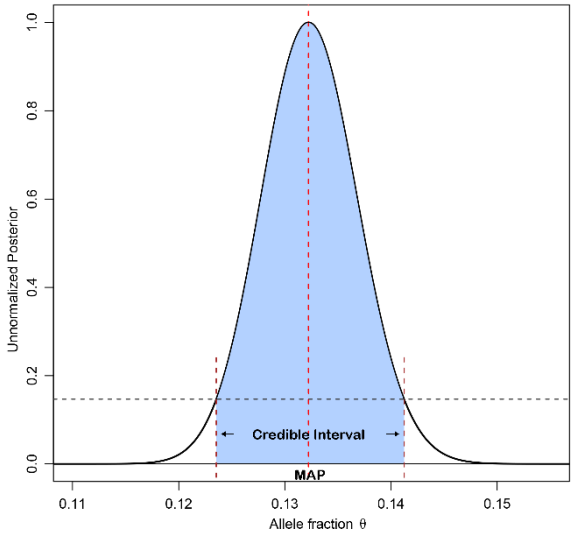
(Xu, *et al*. 2015)

We need data filters to exclude potential sequencing error and keep enough information

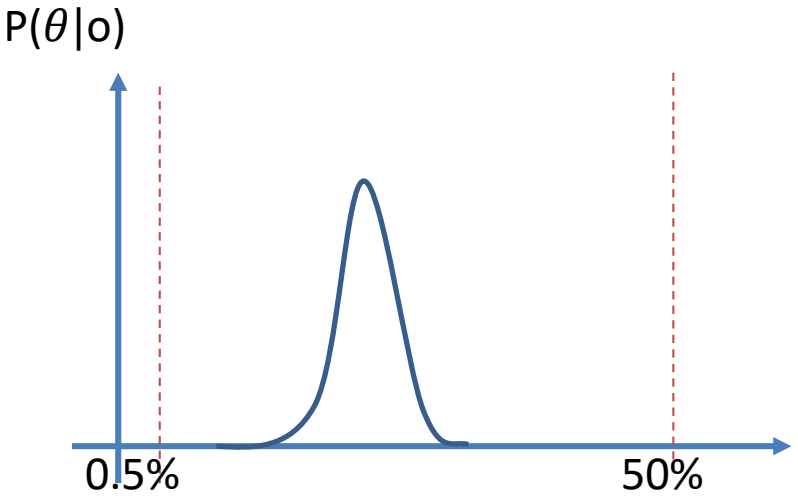


Bayesian model for calculating the fraction of mutant alleles and detecting real mosaicism

ϑ: theoretical fractions of the mutant alleles
o: number of reads support mutant alleles
n: the total number of reads mapped to the position
r: unobserved "real" number of allele count
$\mathrm{P}(\theta)$: prior
$\mathrm{P}(r|\theta;n)$: the likelihood of Bernoulli sampling
$\mathrm{P}(o|r;q)$: the summarized probabilities



$$\mathrm{P}(\theta|o) \propto \mathrm{P}(\theta)\mathrm{P}(o|\theta)$$
$$= \mathrm{P}(\theta)\sum_r \mathrm{P}(o,r|\theta)$$
$$= \mathrm{P}(\theta)\sum_r \mathrm{P}(r|\theta)\,\mathrm{P}(o|r)$$
$$= \mathrm{P}(\theta)\sum_r \mathrm{P}(r|\theta;n)\,\mathrm{P}(o|r;q)$$



List of parental mosaicisms that were missed by Sanger sequence but detected by PASM and validated with pyrosequencing and/or digital PCR

Family	Proband mutation information				Mosaic parent information			Mosaic Related Phenotype	Mosaic site information				
	Chromosome	Position [a]	Nucleotide Variations [b]	Amino Acid Variation [b]	Parent of Origin	Reference Allele	Alternative Allele	Epileptic symptoms in parents	Fractions of mutant alleles by PASM	95% Credible Interval		Validation	
										Lower bound	Upper bound	Pyrosequencing	Digital PCR
DS017	chr2	166848438	c.5347G>A	A1783T	father	C	T	Father, FS before 5	4.0%	3.8%	4.1%	12%	4.41%
DS027	chr2	166915126	c.337C>A	P113T	father	G	T	Father, several FS at the early age	25.3%	22.3%	28.5%	43%	-
DS035	chr2	166894440	c.2792G>A	R931H	father	C	T	Neither	15.0%	14.8%	15.2%	16%	10.24%
DS094	chr2	166848852	c.4933C>T	R1645*	father	G	A	Neither	1.3%	0.8%	1.9%	3%	1.42%
DS101	chr2	166848230	c.5555T>C	M1852T	father	A	G	Neither	6.1%	5.6%	6.7%	26%	6.31%
DS104	chr2	166904137	c.1170+1G>T	-	mother	G	T	Neither	1.1%	0.9%	1.4%	6%	-
DS117	chr2	166895930	c.2589+3A>T	-	mother	T	A	Neither	2.3%	2.0%	2.5%	-	-
DS125	chr2	166868765	c.3733C>T	R1245*	father	G	A	Neither	6.6%	6.2%	6.9%	12%	7.15%
DS128	chr2	166868765	c.3733C>T	R1245*	mother	G	A	Neither	13.2%	12.4%	14.1%	19%	13.02%
DS130	chr2	166868772	c.3726_3727insAT	D1243fsX1270	father	A	T	Neither	3.3%	2.8%	3.9%	-	-
DS136	chr2	166859043	c.4223G>A	W1408*	mother	C	T	Mother, undefined epilepsy	9.2%	8.5%	9.9%	22%	11.71%
DS164	chr2	166915194	c.269T>C	F90S	father	A	G	Father, FS at the early age	8.6%	7.9%	9.4%	15%	9.32%
DS166	chr2	166894396	c.2836C>T	R946C	father	G	A	Neither	3.1%	3.1%	3.2%	6%	3.28%
DS188	chr2	166894554	c.2678T>A	L893*	mother	A	T	Neither	6.3%	1.2%	16.3%	23%	-
DS206	chr2	166901776	c.1439_1442delCAGA	S481fs*488	father	G	A	Neither	10.7%	9.3%	12.3%	-	-

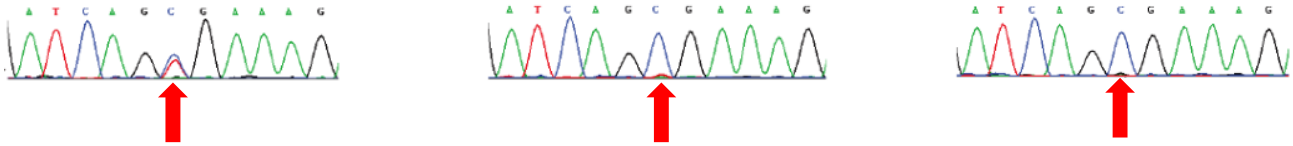
[a] Position coordinates were based on the UCSC human reference genome version hg19.

[b] Nucleotide and amino acid variations were based on RefSeq sequence NM_001165963.1.

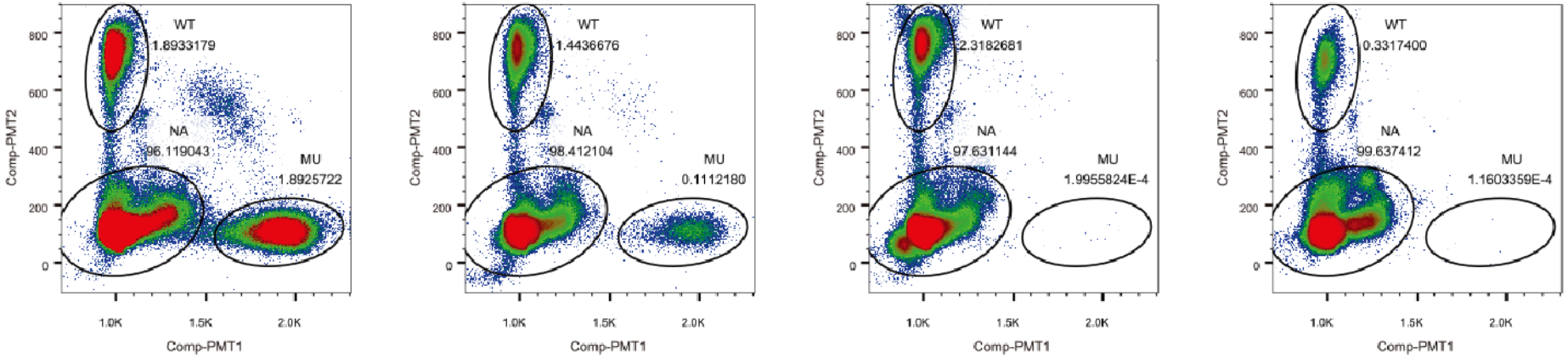
(Xu, *et al*. 2015)

Validation of mosaicism by micro-droplet digital PCR and pyrosequencing

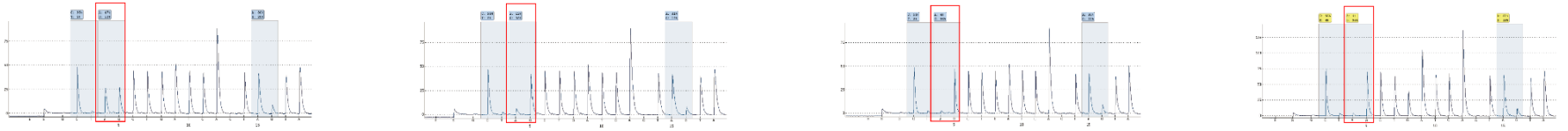
Sanger sequencing



Micro-droplet digital PCR



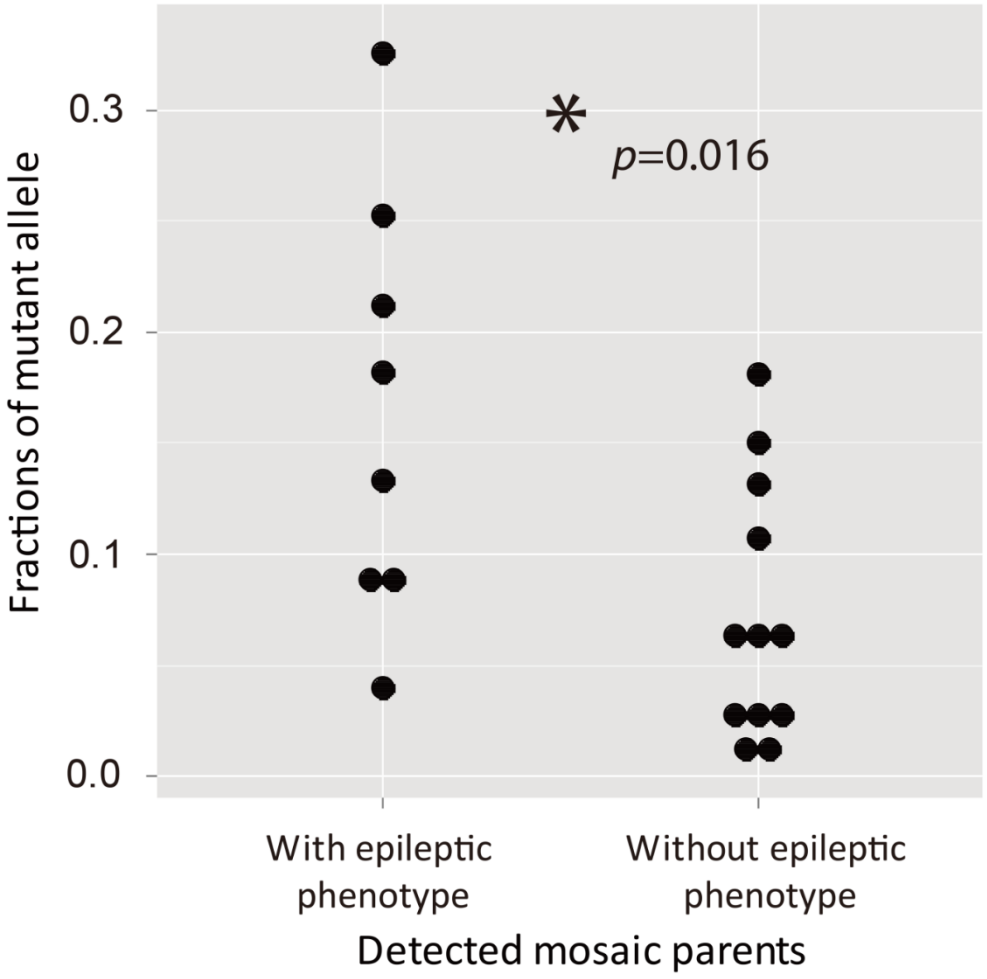
Pyrosquencing



Positive control (Proband) | Father | Mother | Negative control

(Xu, *et al*. 2015)

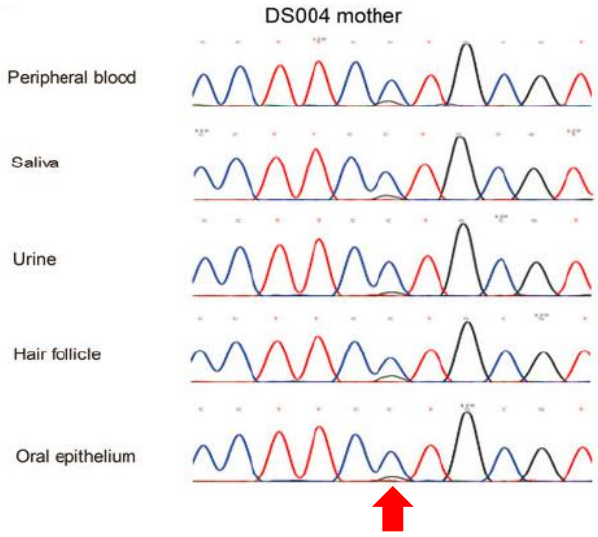
Parents with epileptic phenotypes have significant higher fractions of mutant allele

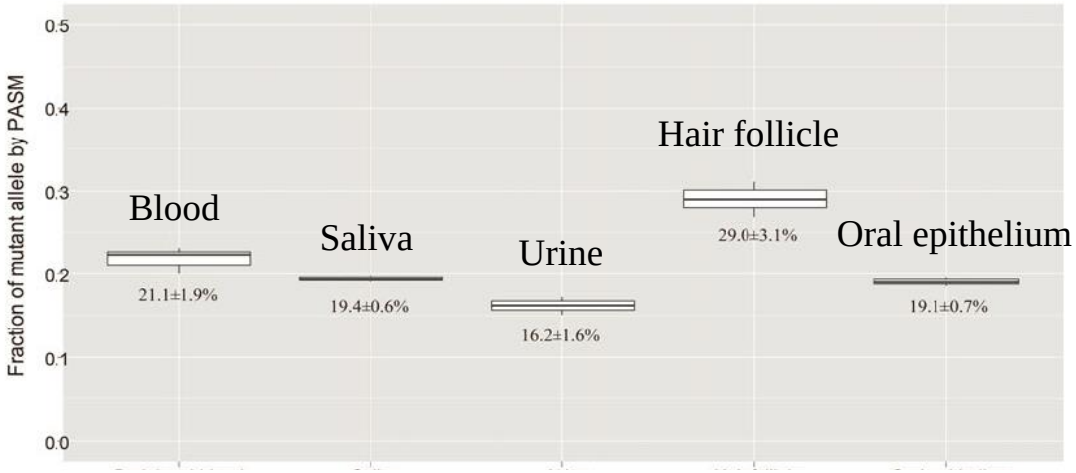


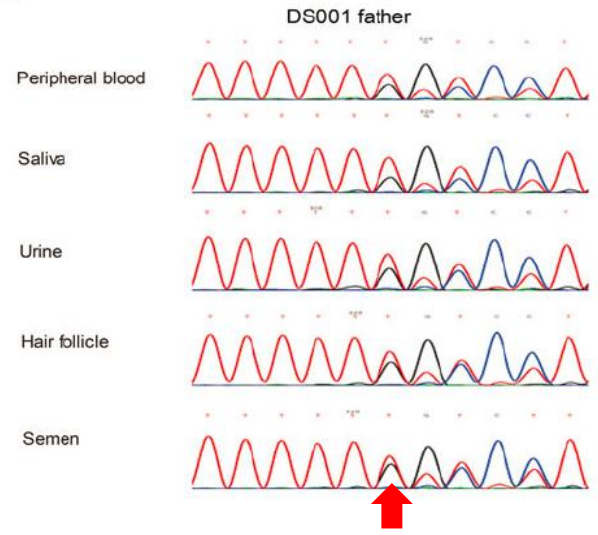
Single tail Wilcoxon rank sum test

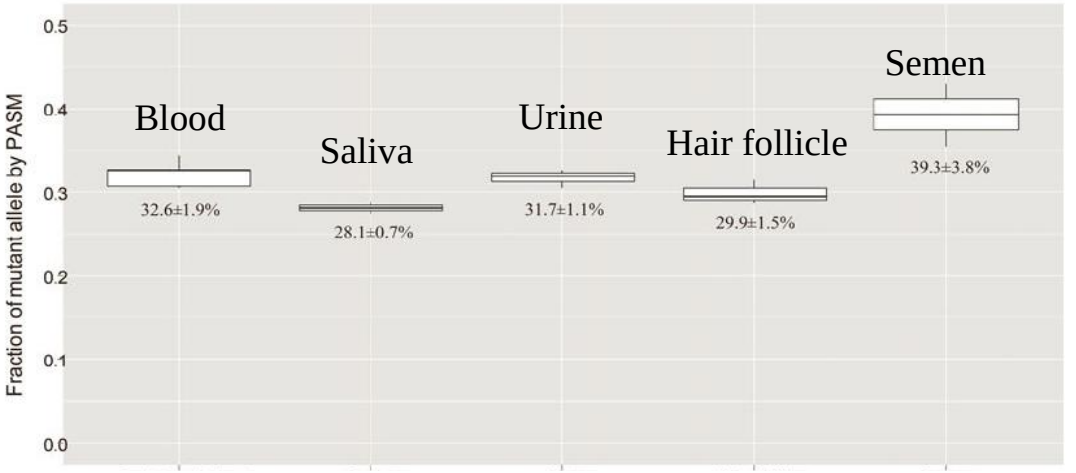
(Xu, *et al*. 2015)

Mosaicism in samples collected from multiple tissues of a same dornor









(Xu, *et al*. 2015)

Conclusion

- A considerate proportion of "*De novo*" mutations might be inherited from parental mosaicism

- Better detection methods lead to more informative results

RESEARCH ARTICLE

Human Mutation

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Amplicon Resequencing Identified Parental Mosaicism for Approximately 10% of "*de novo*" *SCN1A* Mutations in Children with Dravet Syndrome

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北京市科学技术委员会
Beijing Municipal Science & Technology Commission

Approximately 10% of "*de novo*" *SCN1A* mutations causing Dravet Syndrome are inherited from undetected parental mosaicism

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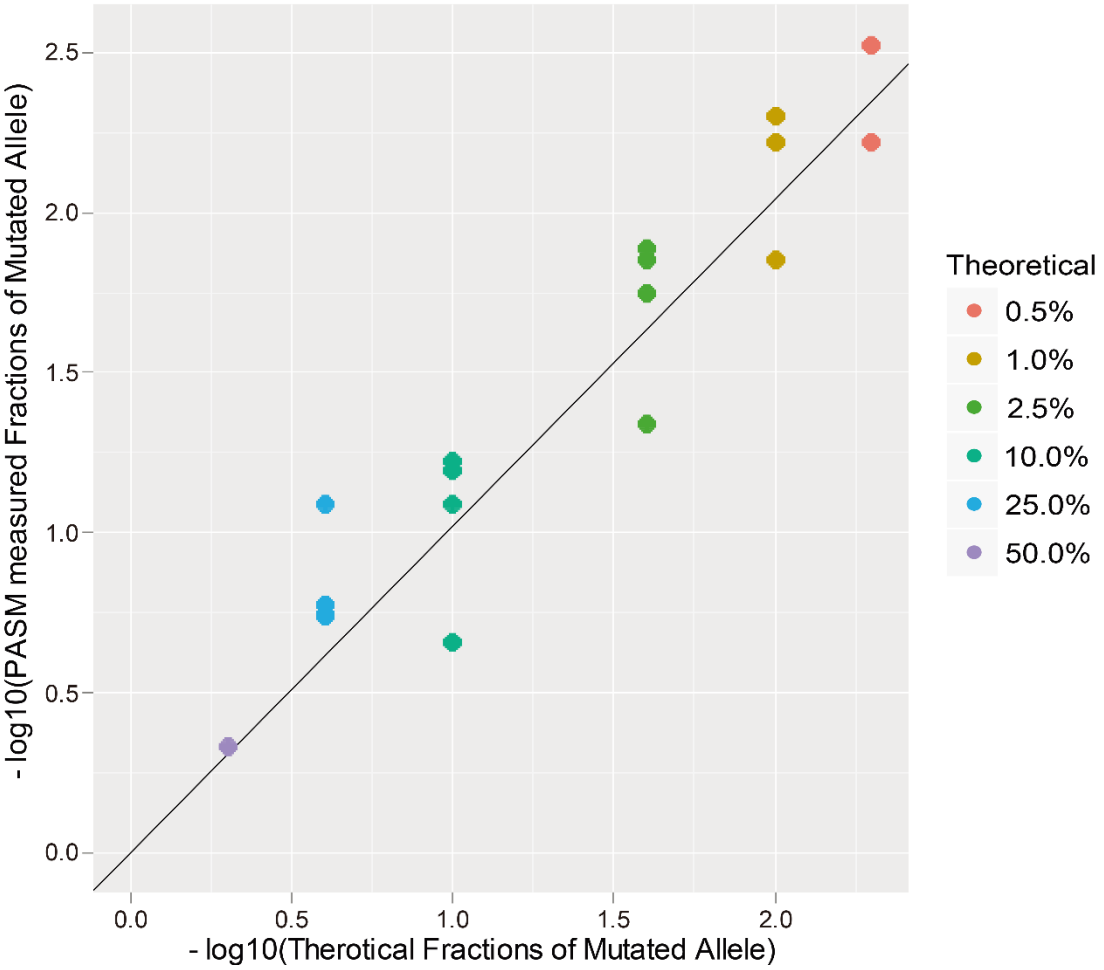
Sep 7, 2015

Appendix



(Xu, *et al*. 2015)

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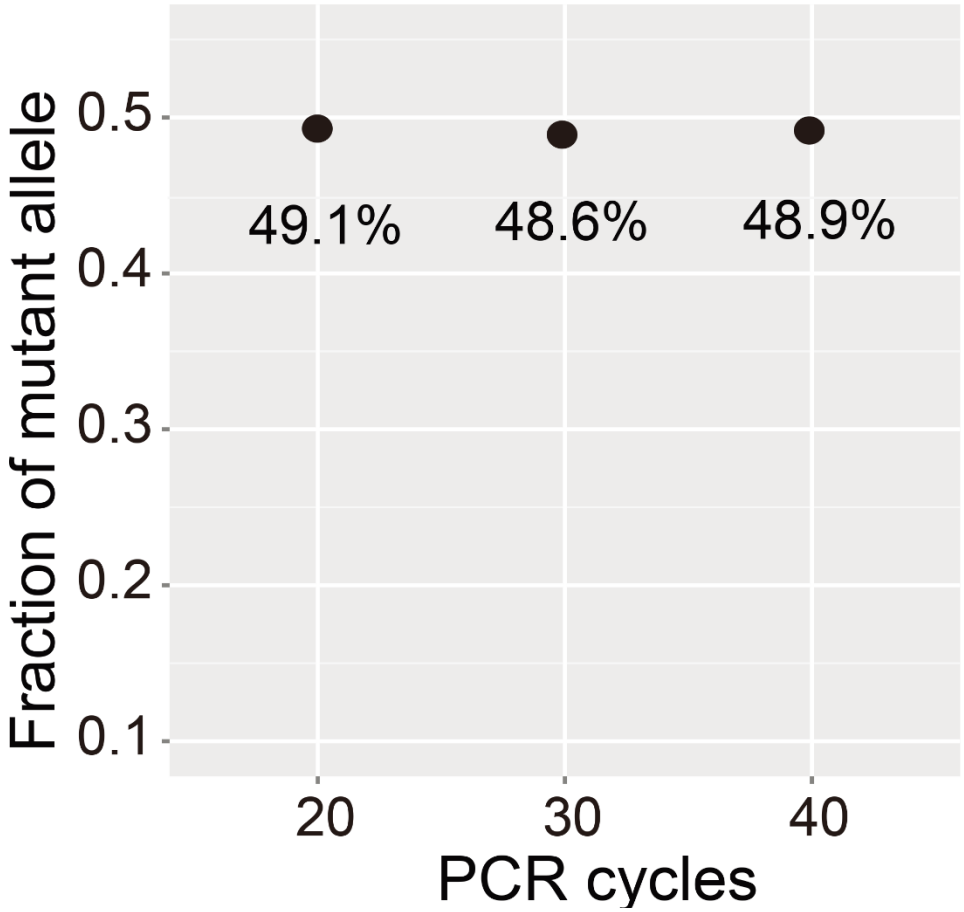

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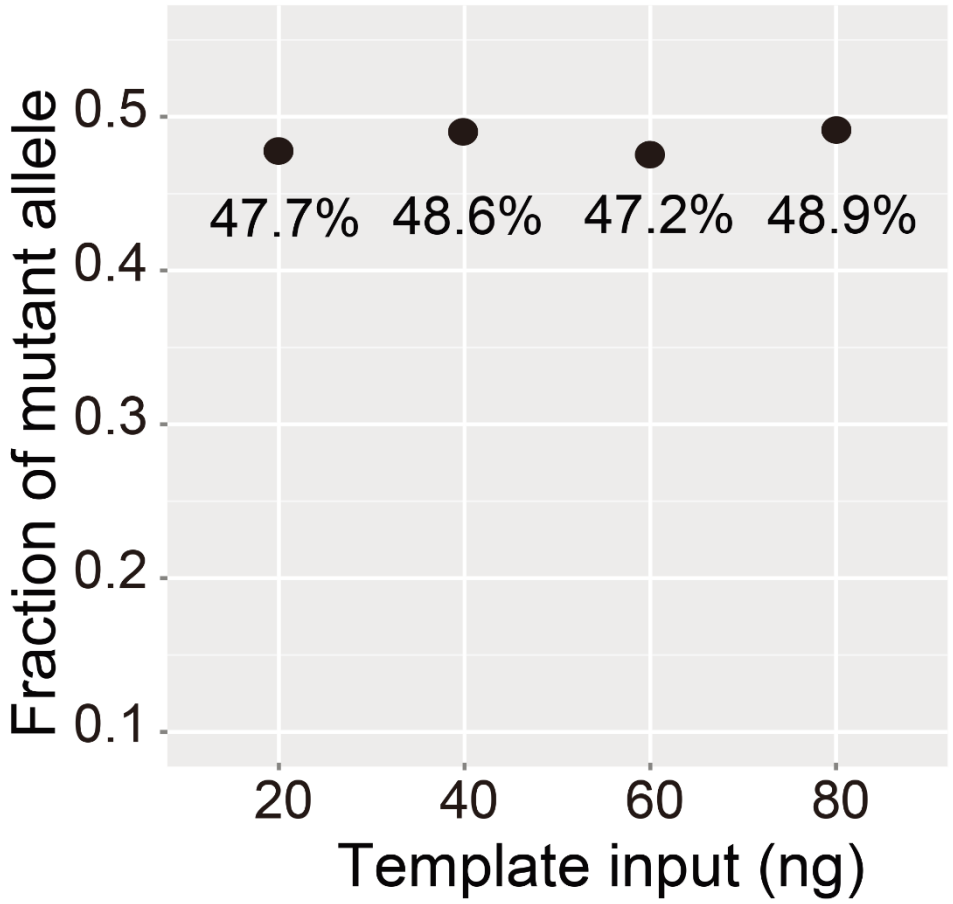
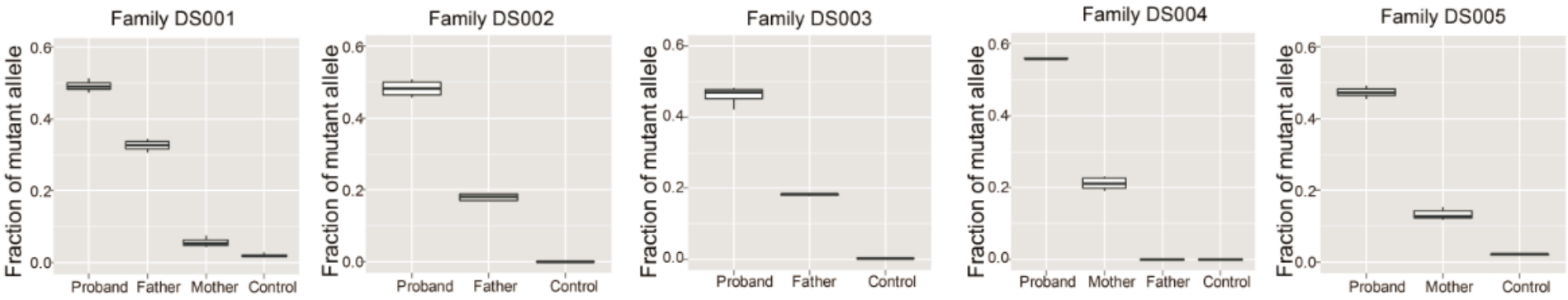


(Xu, *et al*. 2015)

Quantification of Sanger sequencing detectable mosaicism by PASM



		Fractions of mutated allele measured by PASM in peripheral blood			
Number	Variants	Proband	Mother	Father	Negative control
DS001	c.1118 del T	49.19±1.92%	5.64±1.62%	32.61±1.88%	0.79±0.16%
DS002	c.4351C>A	48.30±2.40%		18.05±1.05%	0.03±0.00%
DS003	c.2593 C>T	46.10±2.65%		18.21±0.32%	0.17±0.02%
DS004	c.5003C>G	56.07±0.37%	21.15±1.86%	0.00±0.01%	0.00±0.01%
DS005	c.4302G>A	47.36±2.55%	13.26±1.42%		0.28±0.26%

(Xu, *et al*. 2015)

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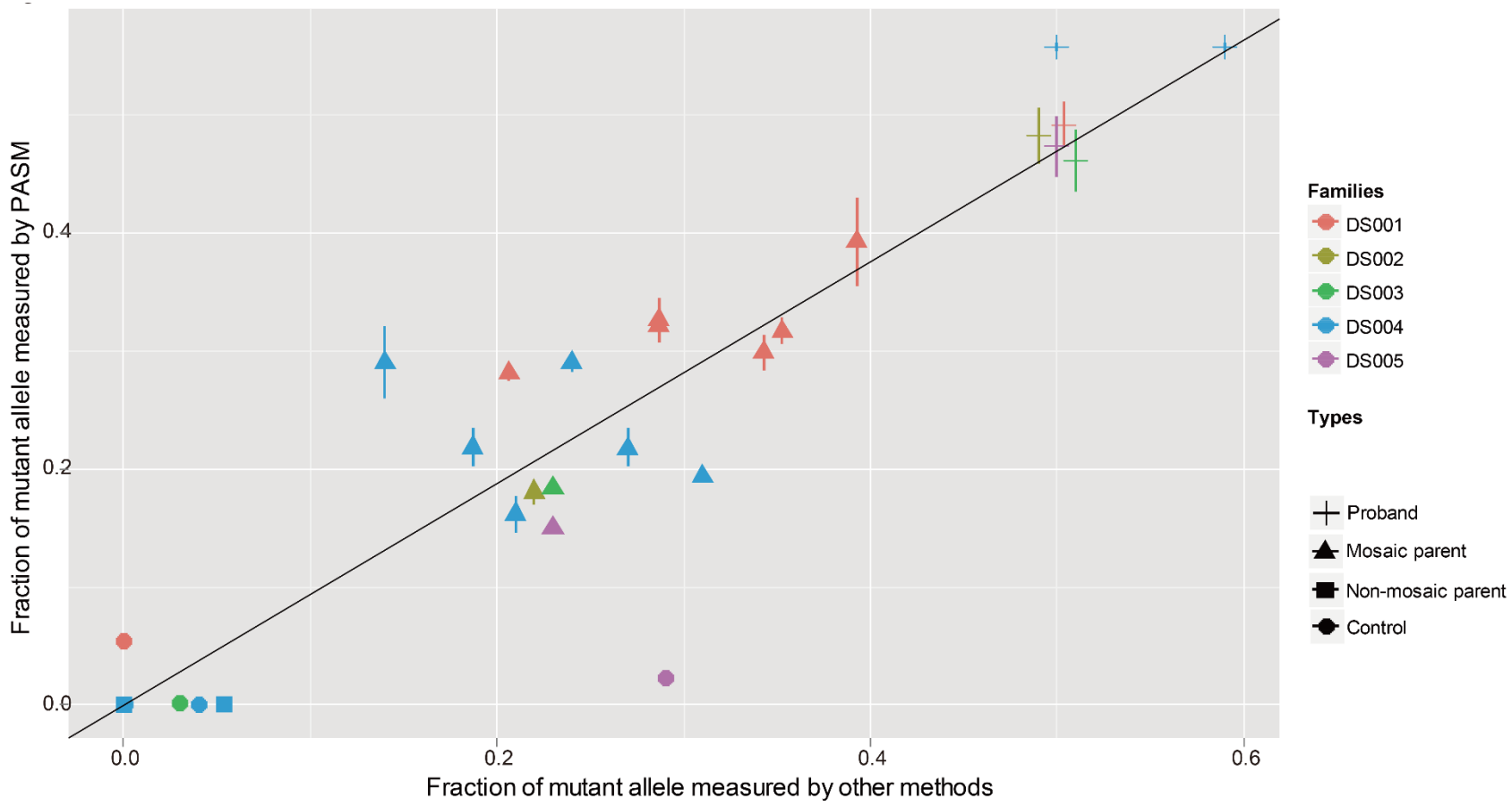

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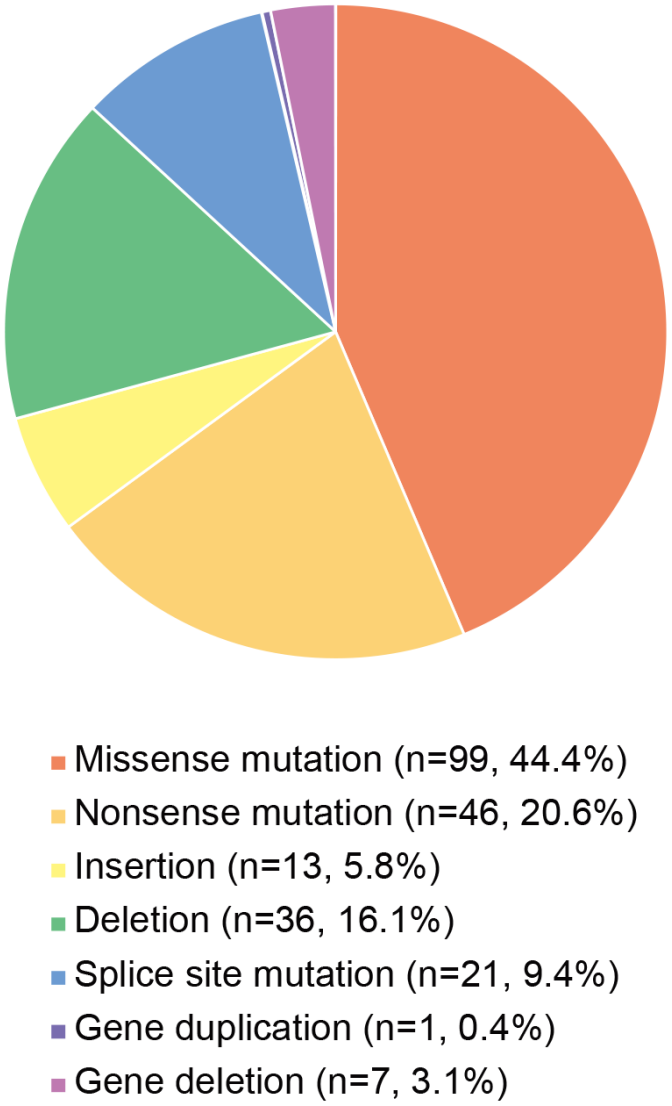

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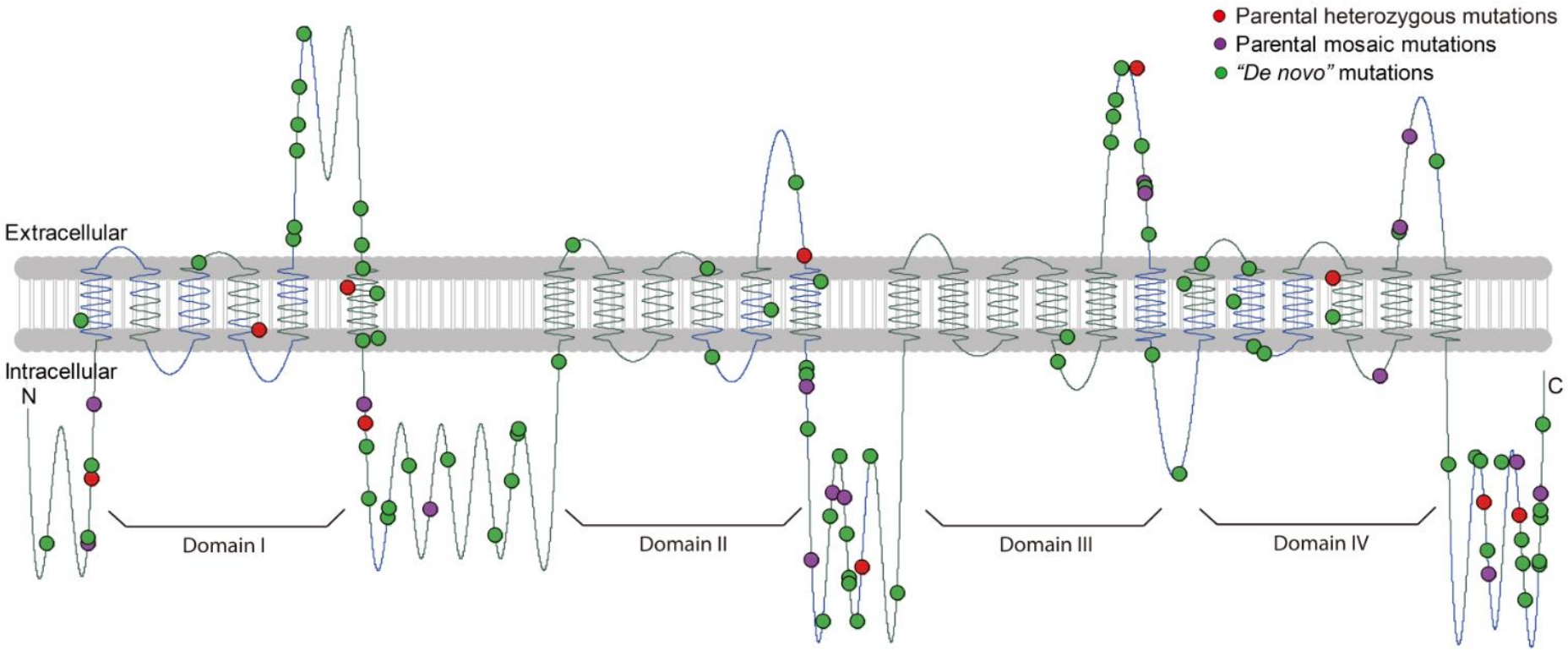

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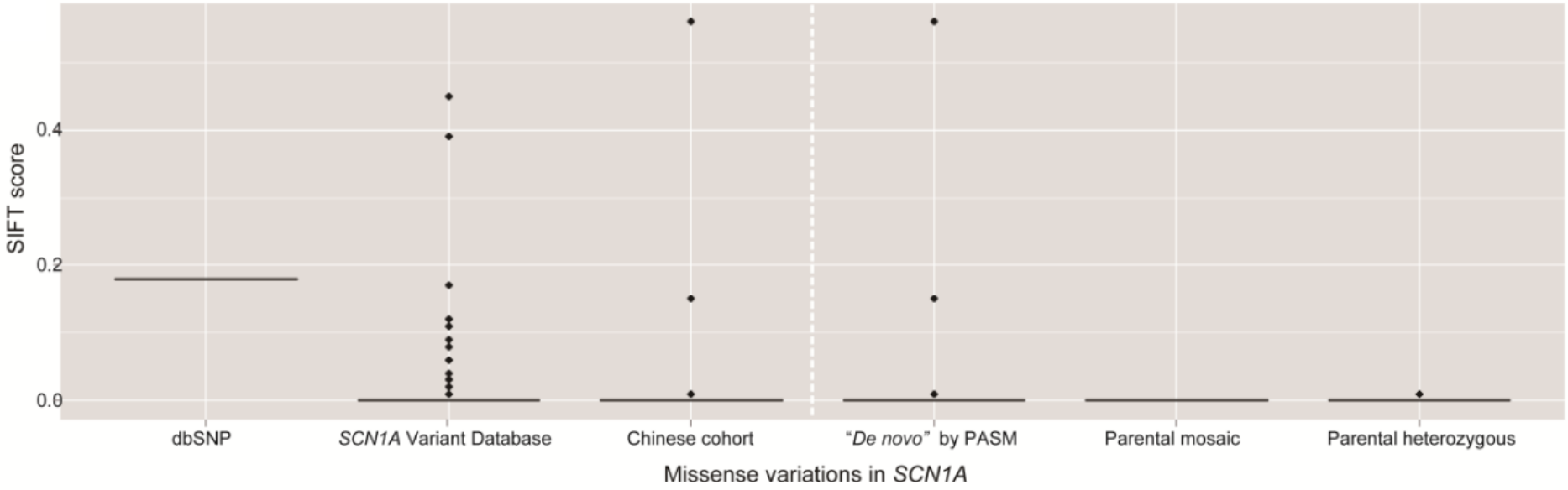

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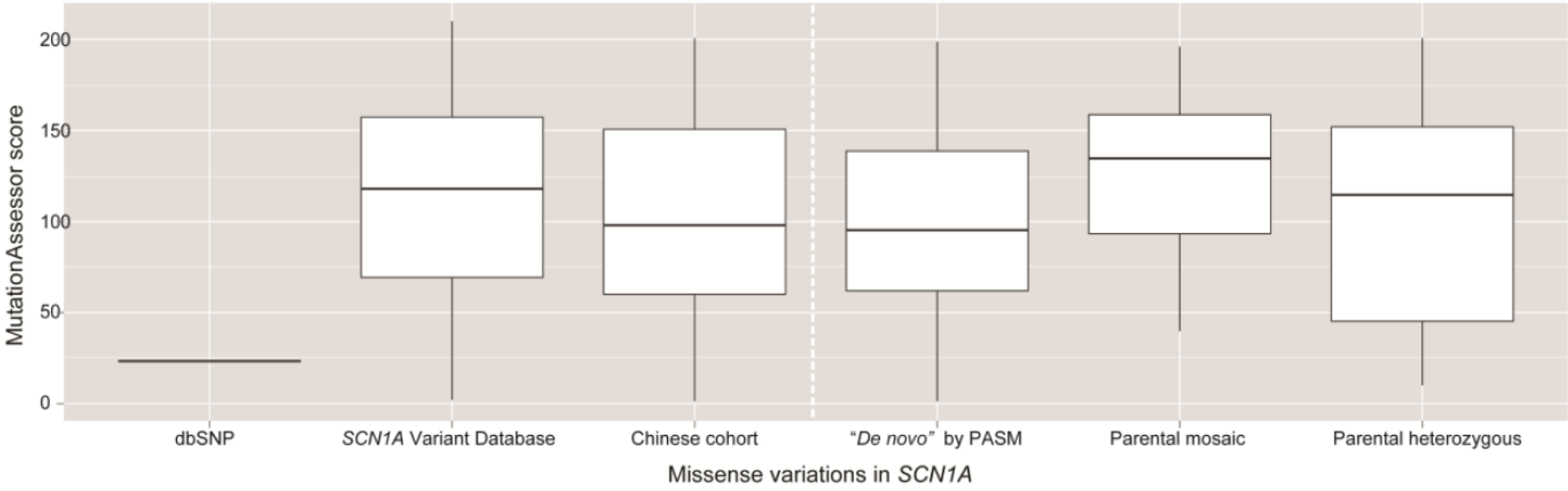

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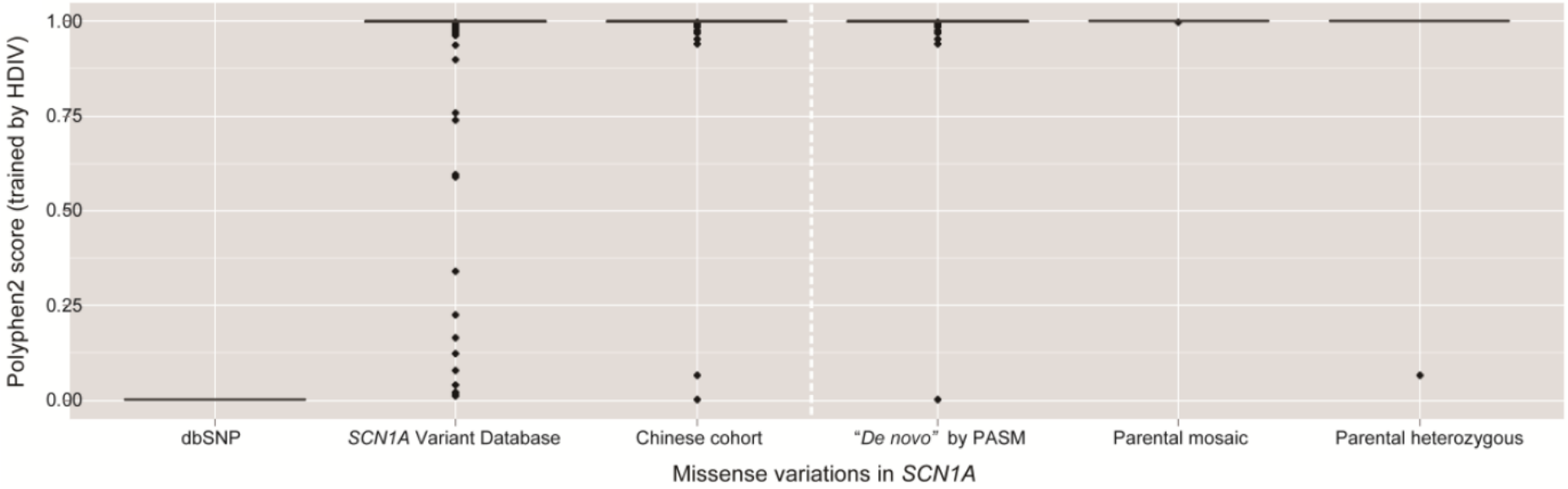

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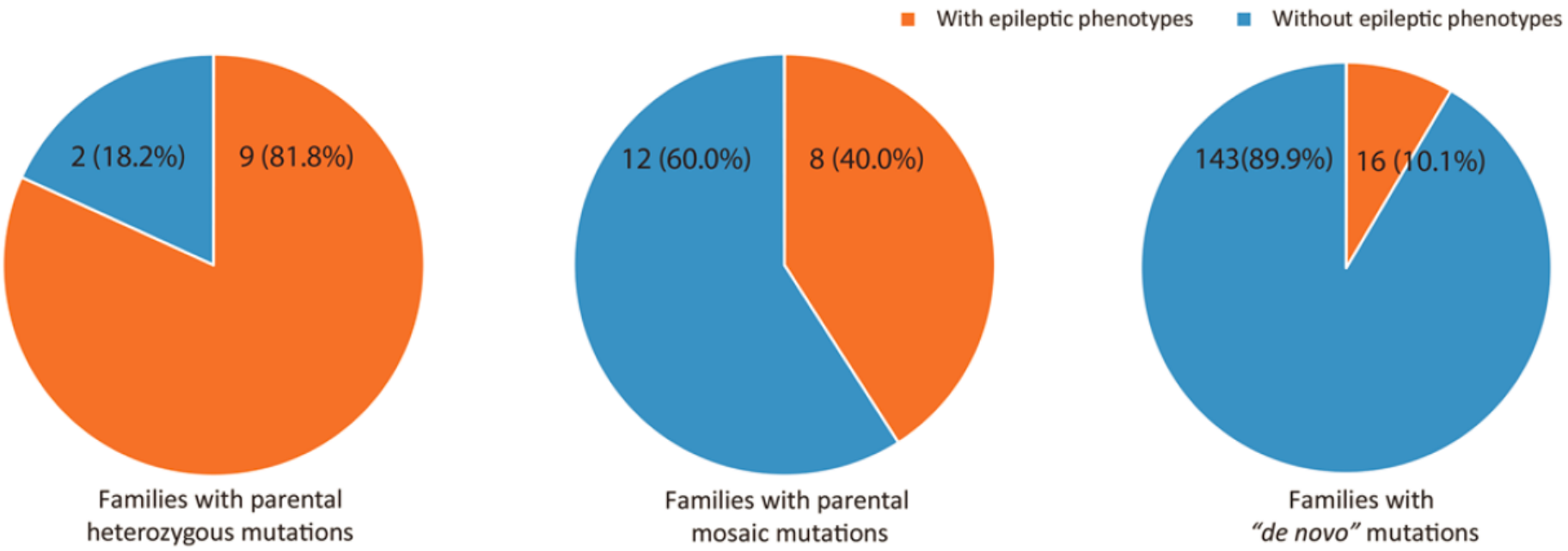

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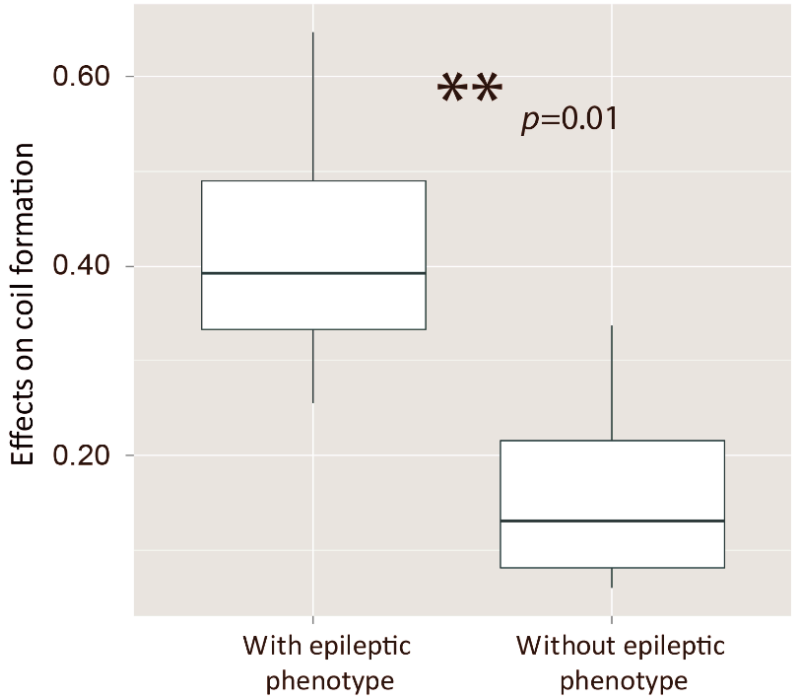
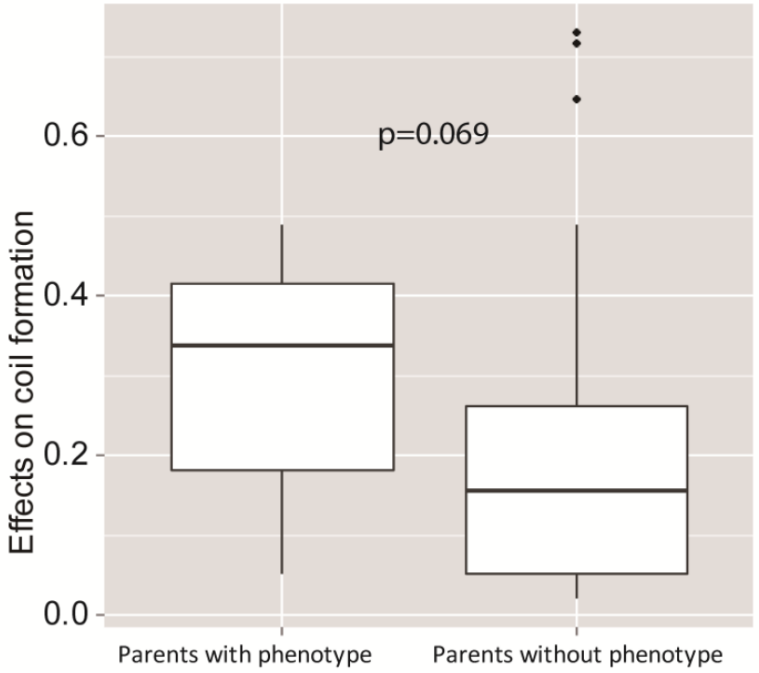


Mosaic mutations by PASM



"*De novo*" mutations by PASM

(Xu, *et al*. 2015)