‘Twin Research and Human Genetics’, ***One CNV Discordance in NRXN1 Observed upon Genome-wide Screening in 38 Pairs of Adult Healthy Monozygotic Twins*** , Patrik K. E. Magnusson, Donghwan Lee, Xu Chen, Jin Szatkiewicz, Setia Pramana, Shumei Teo, Patrick F. Sullivan, Lars Feuk, Yudi Pawitan

SUPPLEMENTARY MATERIALS

Plate1

P1-T1-CNV1-Ref	P1-T1-CNV2-Ref	P1-T1-CNV3-Ref	P1-T1-CNV4-Ref	**P2'-T1-CNV5-Ref**	P1-T1-CNV6-Ref	P1-T1-CNV7-Ref	P1-T1-CNV8-Ref	**P9-T1-CNV9-Ref**	P1-T1-CNV10-Ref	P1-T1-CNV10-Ref	P1-T1-Ref
P1-T2-CNV1-Ref	P1-T2-CNV2-Ref	P1-T2-CNV3-Ref	P1-T2-CNV4-Ref	**P2'-T2-CNV5-Ref**	P1-T2-CNV6-Ref	P1-T2-CNV7-Ref	P1-T2-CNV8-Ref	**P9-T2-CNV9-Ref**	P1-T2-CNV10-Ref	P1-T2-CNV10-Ref	P1-T2-Ref
P2-T1-CNV1-Ref	**P2-T1-CNV2-Ref**	P2-T1-CNV3-Ref	P2-T1-CNV4-Ref	P2-T1-CNV5-Ref	**P6-T1-CNV6-Ref**	P2-T1-CNV7-Ref	P2-T1-CNV8-Ref	P2-T1-CNV9-Ref	**P10-T1-CNV10-Ref**	P2-T1-CNV11-Ref	P2-T1-Ref
P2-T2-CNV1-Ref	**P2-T2-CNV2-Ref**	P2-T2-CNV3-Ref	P2-T2-CNV4-Ref	P2-T2-CNV5-Ref	**P6-T2-CNV6-Ref**	P2-T2-CNV7-Ref	P2-T2-CNV8-Ref	P2-T2-CNV9-Ref	**P10-T2-CNV10-Ref**	P2-T2-CNV11-Ref	P2-T2-Ref
P3-T1-CNV1-Ref	P3-T1-CNV2-Ref	**P3-T1-CNV3-Ref**	P3-T1-CNV4-Ref	P3-T1-CNV5-Ref	P3-T1-CNV6-Ref	**P7-T1-CNV7-Ref**	P3-T1-CNV8-Ref	P3-T1-CNV9-Ref	P3-T1-CNV10-Ref	**P11-T1-CNV11-Ref**	P3-T1-Ref
P3-T2-CNV1-Ref	P3-T2-CNV2-Ref	**P3-T2-CNV3-Ref**	P3-T2-CNV4-Ref	P3-T2-CNV5-Ref	P3-T2-CNV6-Ref	**P7-T2-CNV7-Ref**	P3-T2-CNV8-Ref	P3-T2-CNV9-Ref	P3-T2-CNV10-Ref	**P11-T2-CNV11-Ref**	P3-T2-Ref
P4-T1-CNV1-Ref	P4-T1-CNV2-Ref	P4-T1-CNV3-Ref	**P4-T1-CNV4-Ref**	P4-T1-CNV7-Ref	P4-T1-CNV6-Ref	P4-T1-CNV7-Ref	**P8-T1-CNV8-Ref**	P4-T1-CNV9-Ref	P4-T1-CNV10-Ref	P4-T1-CNV11-Ref	P4-T1-Ref
P4-T2-CNV1-Ref	P4-T2-CNV2-Ref	P4-T2-CNV3-Ref	**P4-T2-CNV4-Ref**	P4-T2-CNV7-Ref	P4-T2-CNV6-Ref	P4-T2-CNV7-Ref	**P8-T2-CNV8-Ref**	P4-T2-CNV9-Ref	P4-T2-CNV10-Ref	P4-T2-CNV11-Ref	P4-T2-Ref

Plate2

P1-T1-CNV1-Ref	P2'-T1-CNV2-Ref	P2'-T1-CNV3-Ref	P2'-T1-CNV4-Ref	**P2'-T1-CNV5-Ref**	P2'-T1-CNV6-Ref	P2'-T1-CNV7-Ref	P2'-T1-CNV8-Ref	**P9-T1-CNV9-Ref**	P2'-T1-CNV10-Ref	P2'-T1-CNV11-Ref	P2'-T1-Ref
P1-T2-CNV1-Ref	P2'-T2-CNV2-Ref	P2'-T2-CNV3-Ref	P2'-T2-CNV4-Ref	**P2'-T2-CNV5-Ref**	P2'-T2-CNV6-Ref	P2'-T2-CNV7-Ref	P2'-T2-CNV8-Ref	**P9-T2-CNV9-Ref**	P2'-T2-CNV10-Ref	P2'-T2-CNV11-Ref	P2'-T2-Ref
P6-T1-CNV1-Ref	**P2-T1-CNV2-Ref**	P6-T1-CNV3-Ref	P6-T1-CNV4-Ref	P6-T1-CNV5-Ref	**P6-T1-CNV6-Ref**	P6-T1-CNV7-Ref	P6-T1-CNV8-Ref	P6-T1-CNV9-Ref	**P10-T1-CNV10-Ref**	P6-T1-CNV11-Ref	P6-T1-Ref
P6-T2-CNV1-Ref	**P2-T2-CNV2-Ref**	P6-T2-CNV3-Ref	P6-T2-CNV4-Ref	P6-T2-CNV5-Ref	**P6-T2-CNV6-Ref**	P6-T2-CNV7-Ref	P6-T2-CNV8-Ref	P6-T2-CNV9-Ref	**P10-T2-CNV10-Ref**	P6-T2-CNV11-Ref	P6-T2-Ref
P7-T1-CNV1-Ref	P7-T1-CNV2-Ref	**P3-T1-CNV3-Ref**	P7-T1-CNV4-Ref	P7-T1-CNV5-Ref	P7-T1-CNV6-Ref	**P7-T1-CNV7-Ref**	P7-T1-CNV8-Ref	P7-T1-CNV9-Ref	P7-T1-CNV10-Ref	**P11-T1-CNV11-Ref**	P7-T1-Ref
P7-T2-CNV1-Ref	P7-T2-CNV2-Ref	**P3-T2-CNV3-Ref**	P7-T2-CNV4-Ref	P7-T2-CNV5-Ref	P7-T2-CNV6-Ref	**P7-T2-CNV7-Ref**	P7-T2-CNV8-Ref	P7-T2-CNV9-Ref	P7-T2-CNV10-Ref	**P11-T2-CNV11-Ref**	P7-T2-Ref
P8-T1-CNV1-Ref	P8-T1-CNV2-Ref	P8-T1-CNV3-Ref	**P4-T1-CNV4-Ref**	P8-T1-CNV5-Ref	P8-T1-CNV6-Ref	P8-T1-CNV7-Ref	**P8-T1-CNV8-Ref**	P8-T1-CNV9-Ref	P8-T1-CNV10-Ref	P8-T1-CNV11-Ref	P8-T1-Ref
P8-T2-CNV1-Ref	P8-T2-CNV2-Ref	P8-T2-CNV3-Ref	**P4-T2-CNV4-Ref**	P8-T2-CNV5-Ref	P8-T2-CNV6-Ref	P8-T2-CNV7-Ref	**P8-T2-CNV8-Ref**	P8-T2-CNV9-Ref	P8-T2-CNV10-Ref	P8-T2-CNV11-Ref	P8-T2-Ref

Plate3

P1-T1-CNV1-Ref	P9-T1-CNV2-Ref	P9-T1-CNV3-Ref	P9-T1-CNV4-Ref	**P2'-T1-CNV5-Ref**	P9-T1-CNV6-Ref	P9-T1-CNV7-Ref	P9-T1-CNV8-Ref	**P9-T1-CNV9-Ref**	P9-T1-CNV10-Ref	P9-T1-CNV11-Ref	P9-T1-Ref
P1-T2-CNV1-Ref	P9-T2-CNV2-Ref	P9-T2-CNV3-Ref	P9-T2-CNV4-Ref	**P2'-T2-CNV5-Ref**	P9-T2-CNV6-Ref	P9-T2-CNV7-Ref	P9-T2-CNV8-Ref	**P9-T2-CNV9-Ref**	P9-T2-CNV10-Ref	P9-T2-CNV11-Ref	P9-T2-Ref
P10-T1-CNV1-Ref	**P2-T1-CNV2-Ref**	P10-T1-CNV3-Ref	P10-T1-CNV4-Ref	P10-T1-CNV5-Ref	**P6-T1-CNV6-Ref**	P10-T1-CNV7-Ref	P10-T1-CNV8-Ref	P10-T1-CNV9-Ref	**P10-T1-CNV10-Ref**	P10-T1-CNV11-Ref	P10-T1-Ref
P10-T2-CNV1-Ref	**P2-T2-CNV2-Ref**	P10-T2-CNV3-Ref	P10-T2-CNV4-Ref	P10-T2-CNV5-Ref	**P6-T2-CNV6-Ref**	P10-T2-CNV7-Ref	P10-T2-CNV8-Ref	P10-T2-CNV9-Ref	**P10-T2-CNV10-Ref**	P10-T2-CNV11-Ref	P10-T2-Ref
P11-T1-CNV1-Ref	P11-T1-CNV2-Ref	**P3-T1-CNV3-Ref**	**P4-T1-CNV4-Ref**	P11-T1-CNV5-Ref	P11-T1-CNV6-Ref	**P7-T1-CNV7-Ref**	**P8-T1-CNV8-Ref**	P11-T1-CNV9-Ref	P11-T1-CNV10-Ref	**P11-T1-CNV11-Ref**	P11-T1-Ref
P11-T2-CNV1-Ref	P11-T2-CNV2-Ref	**P3-T2-CNV3-Ref**	**P4-T2-CNV4-Ref**	P11-T2-CNV5-Ref	P11-T2-CNV6-Ref	**P7-T2-CNV7-Ref**	**P8-T2-CNV8-Ref**	P11-T2-CNV9-Ref	P11-T2-CNV10-Ref	**P11-T2-CNV11-Ref**	P11-T2-Ref
NTC-CNV1-Ref	NTC-CNV2-Ref	NTC-CNV3-Ref	NTC-CNV4-Ref	NTC-CNV5-Ref	NTC-CNV6-Ref	NTC-CNV7-Ref	NTC-CNV8-Ref	NTC-CNV9-Ref	NTC-CNV10-Ref	NTC-CNV11-Ref	NTC-Ref
NTC-CNV1	NTC-CNV2	NTC-CNV3	NTC-CNV4	NTC-CNV5	NTC-CNV6	NTC-CNV7	NTC-CNV8	NTC-CNV9	NTC-CNV10	NTC-CNV11	NTC

FIGURE S1

Plates design for Taqman qPCR validation.

Triplicate runs (labeled with red color) and eight internal controls (labeled with dark gray color) for each interesting CNV (CNV1 to CNV11, CNV2 and CNV5 are in the same pair) were performed on three 96-wells plates, each column represents one CNV, each row for one individual. For example, P1-T1-CNV1-Ref means pair1-twin1-CNV1 combined with reference assay; P1-T1-Ref (labeled with light blue) means independent reference assay for twin1 in pair1; NTC-CNV1-Ref (labeled with light gray) means no template control (NTC) for CNV1 combined with reference assay; NTC-CNV1 means no template control for CNV1 without the reference assay.

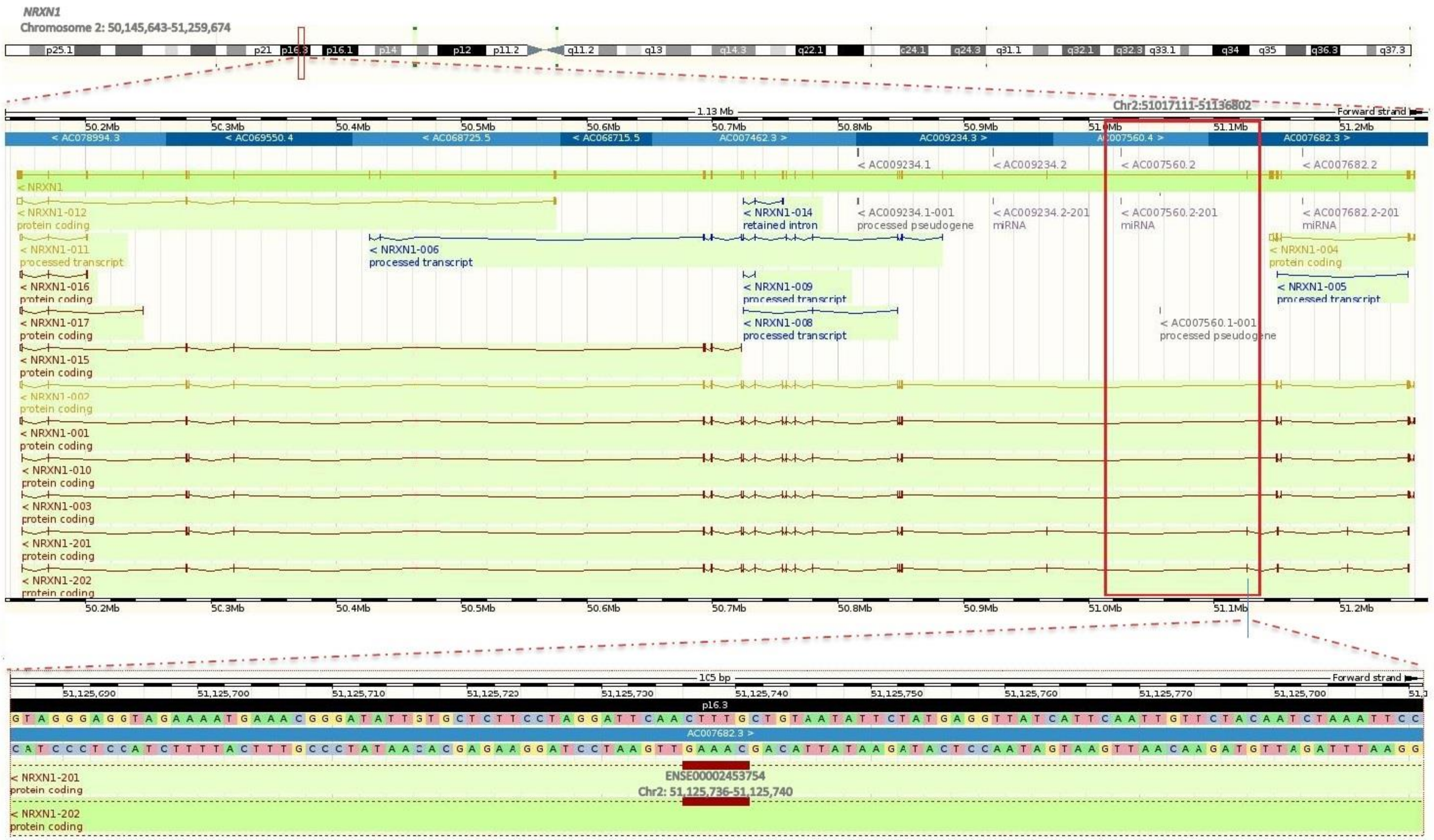


FIGURE S2

Location of the top ranked MZ CNV discordance in relation to the exon/intron structure of *NRXN1* gene (GRCh37/hg19 from Ensembl)

The deletion is labeled by a red rectangular frame. The 5bp exon (Chr2: 51125736-51125740) it involves is included in transcript nrxn1-201 and nrxn1-202 shown at the bottom of the figure.

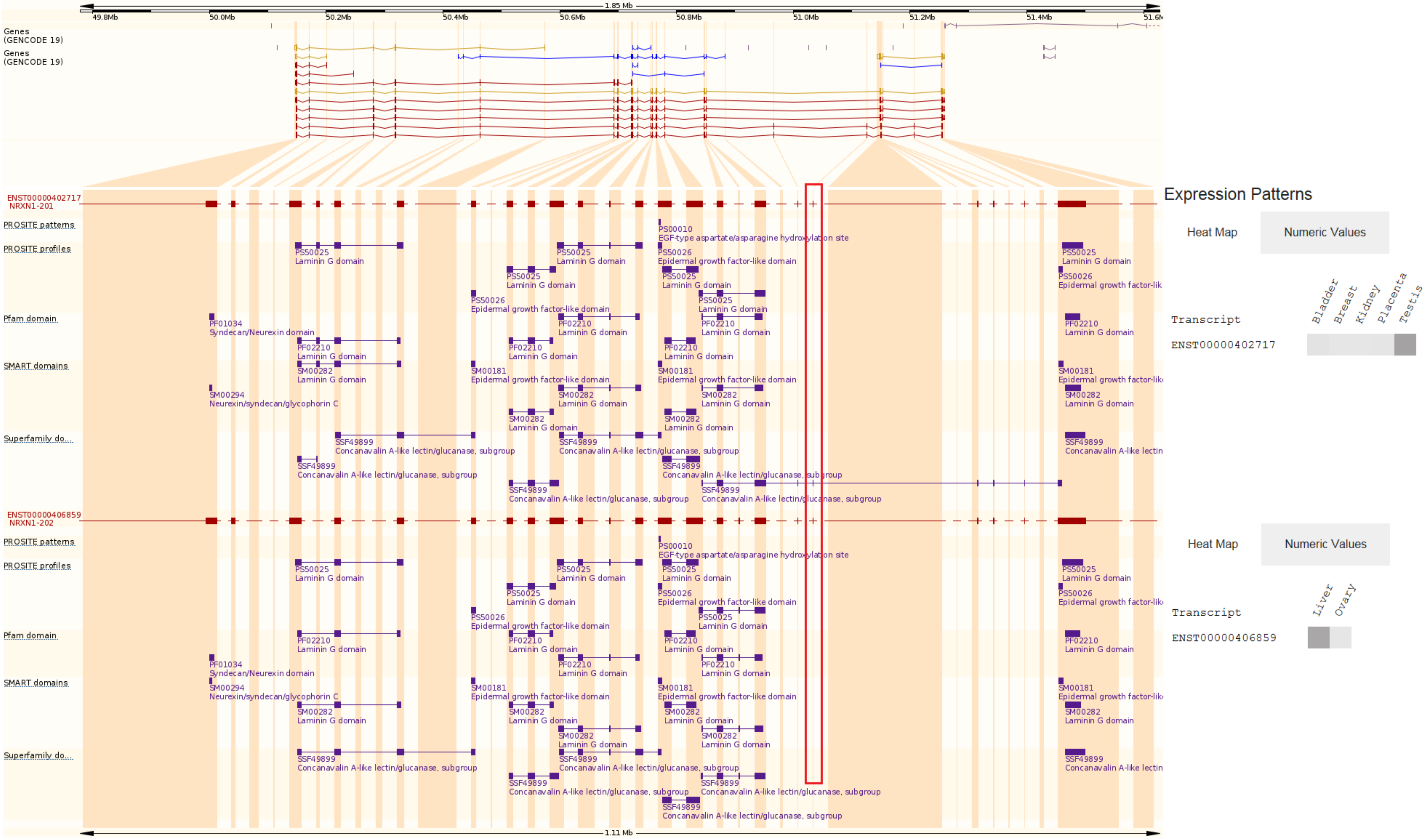


FIGURE S3

Two transcripts (nrxn1-201 and nrxn1-202) including 5bp exon of *NRXN1* gene (GRCh37/hg19 from Ensembl and expression pattern from C-It-Loci)

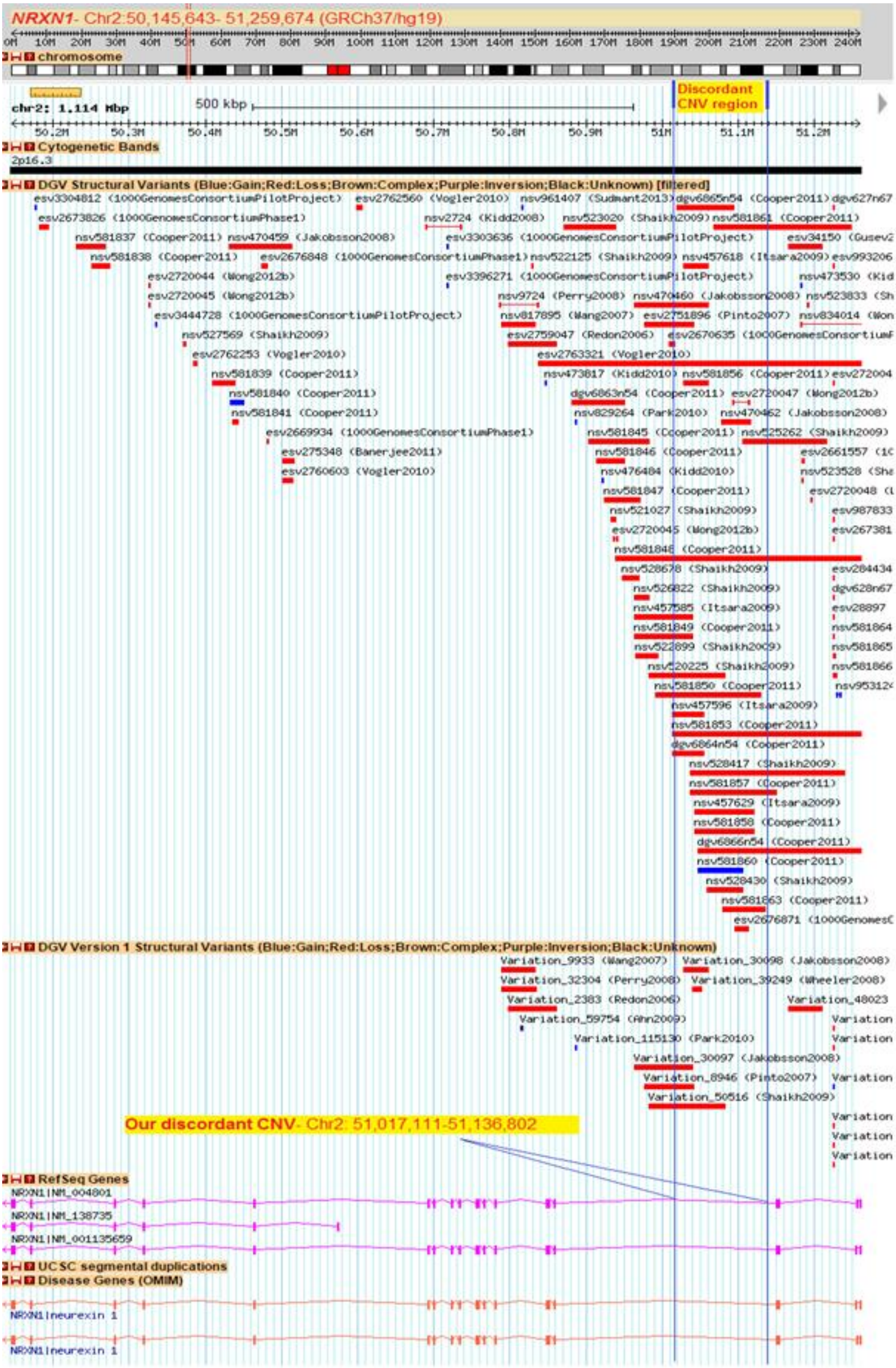
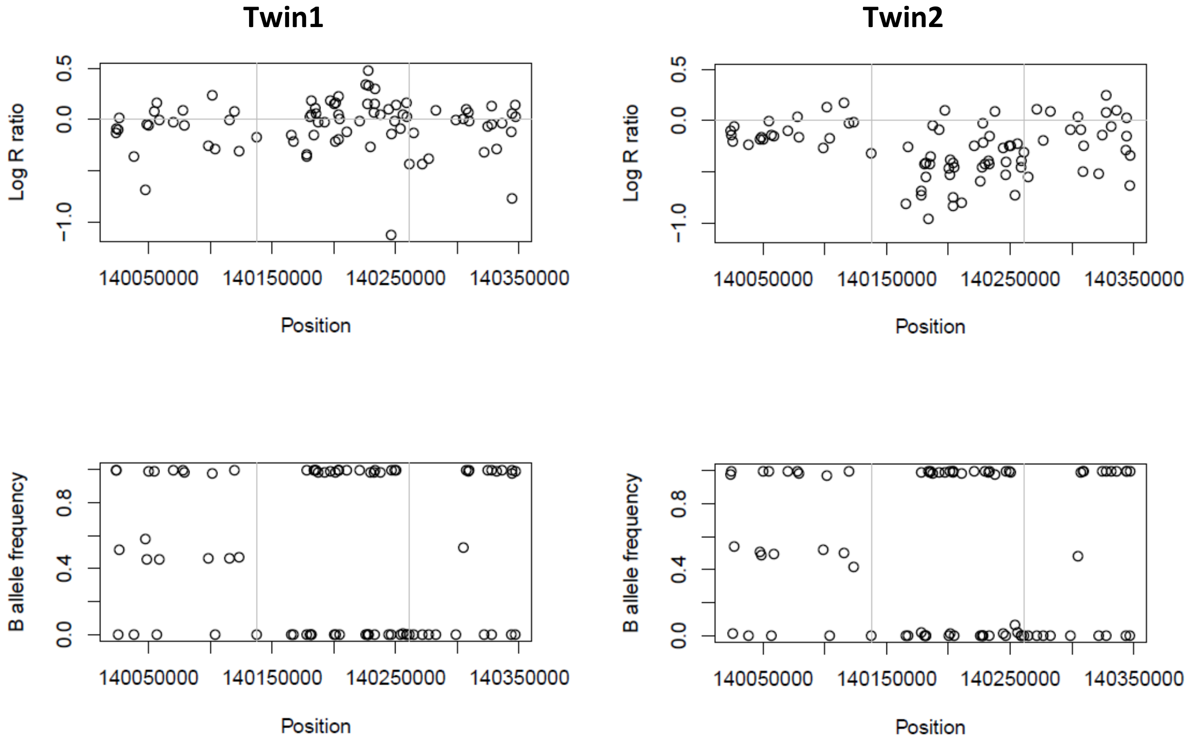


FIGURE S4

Recurrence of CNVs in *NRXN1* gene reported from Database of Genomic Variants archive (DGVa)

The region of the validated discordant CNV overlapped with a large proportion of previously reported CNVs in *NRXN1* gene.

A. PennCNV



B. Taqman qPCR

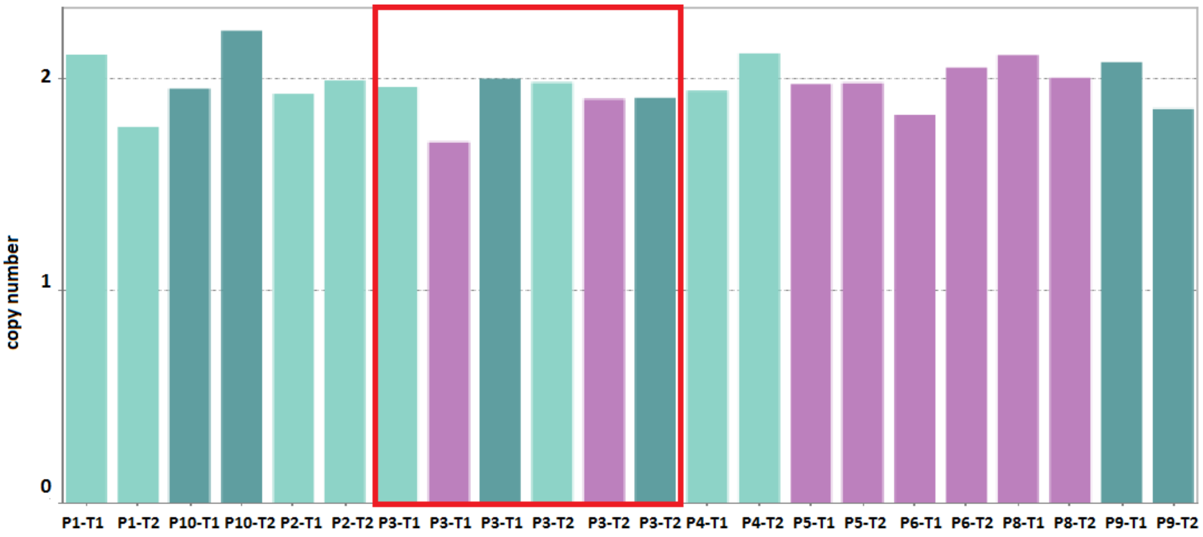
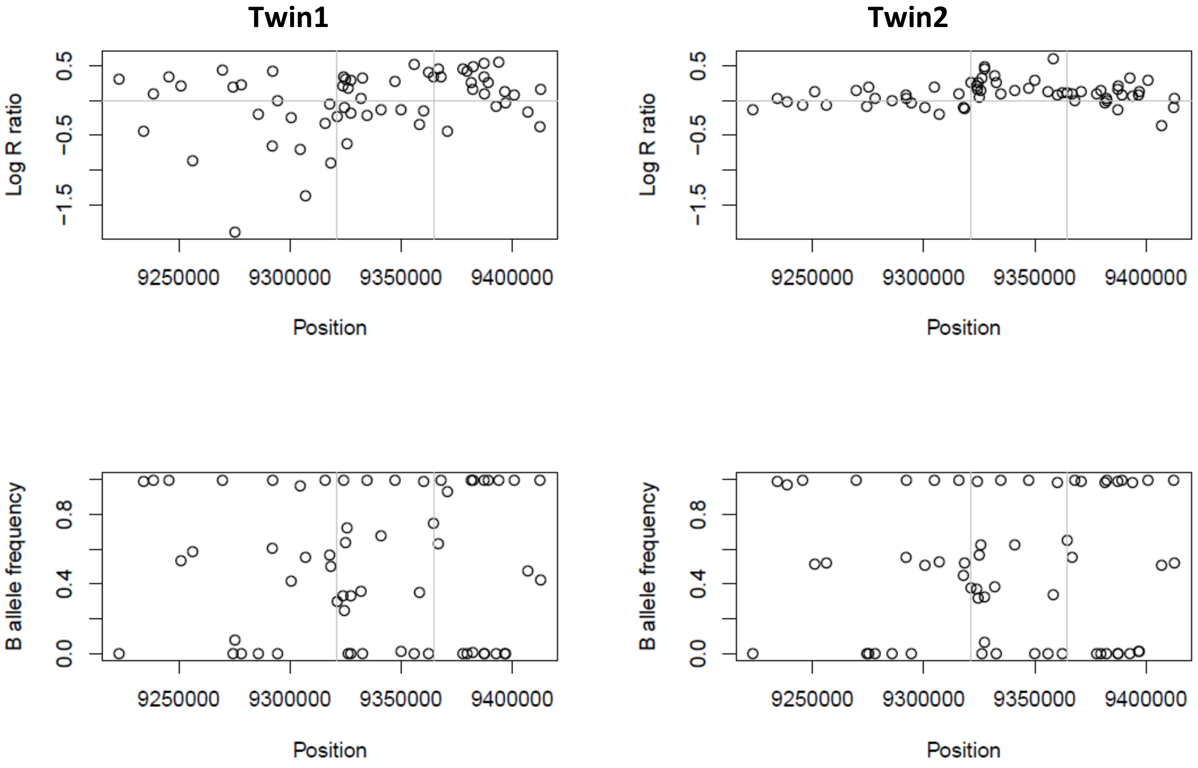


FIGURE S5

Results from PennCNV estimation and qPCR validation attempts for discordant CNV3

(A). Log R ratio (LRR) and B allele frequency (BAF) of discordant CNV3 (Chr5:140137644-140261235) from PennCNV; (B). Copy number detected from qPCR signal, both of the twin members carry two copies in this region. The color-coding of the bars indicates replicate (on 3 different 96-well plates). The red box shows the triplicate runs for the MZ pair in which the CNV implicated by PennCNV is tested. Bars outside the red box are control samples for the particular CNV in question.

A. PennCNV



B. Taqman qPCR

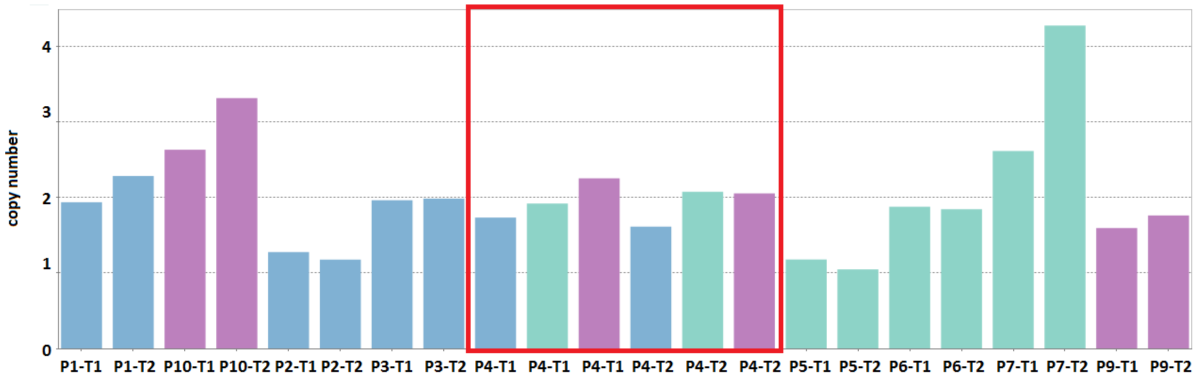
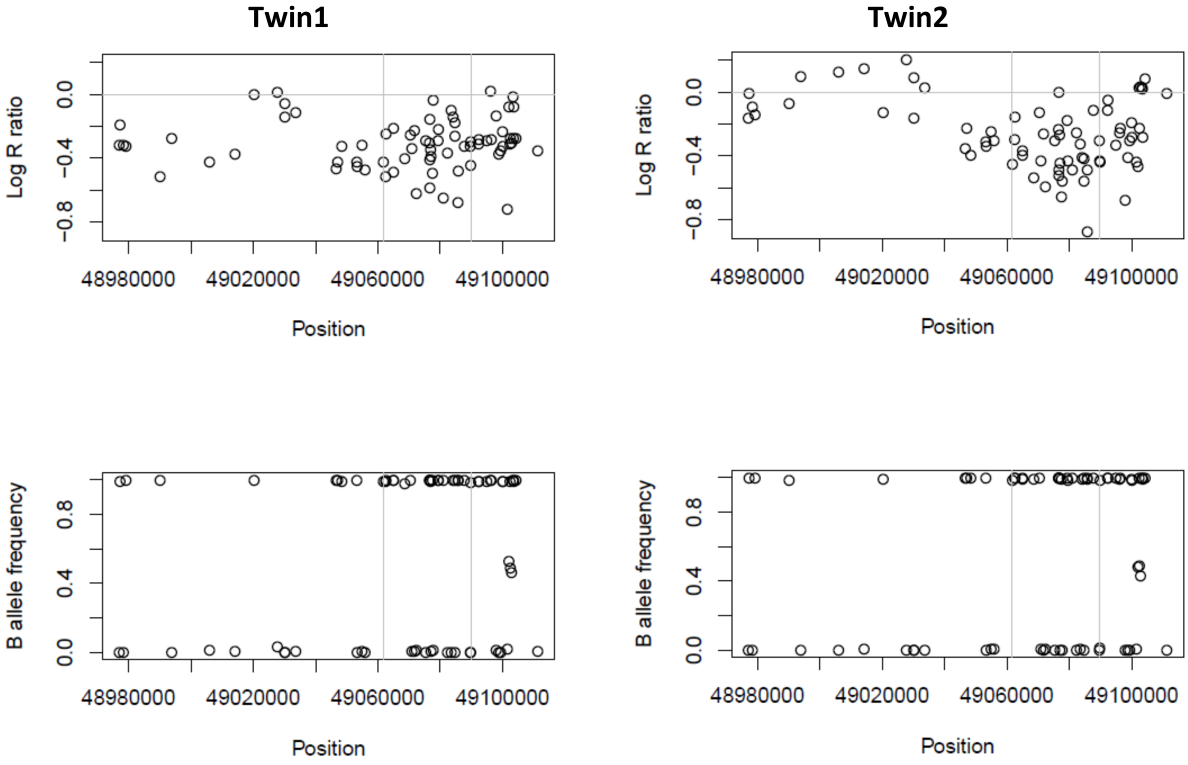


FIGURE S6

Results from PennCNV estimation and qPCR validation attempts for discordant CNV4

(A). Log R ratio (LRR) and B allele frequency (BAF) of discordant CNV4(Chr1:9321241-9364634) from PennCNV; (B). Copy number detected from qPCR signal, both of the twin members carry two copies in this region. The color-coding of the bars indicates replicate (on 3 different 96-well plates). The red box shows the triplicate runs for the MZ pair in which the CNV implicated by PennCNV is tested. Bars outside the red box are control samples for the particular CNV in question.

A. PennCNV



B. Taqman qPCR

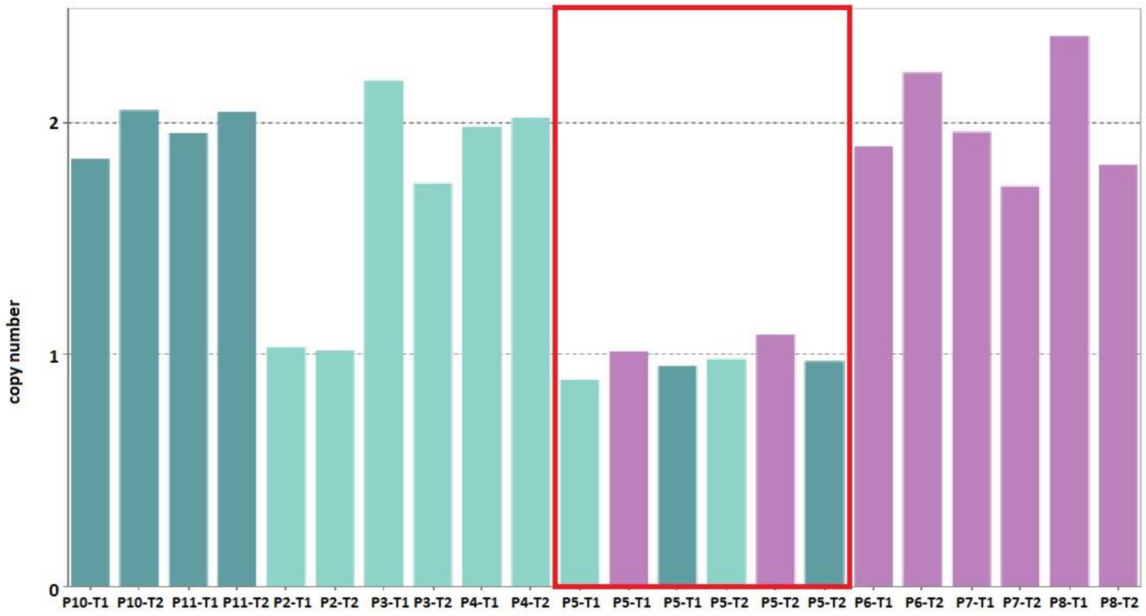
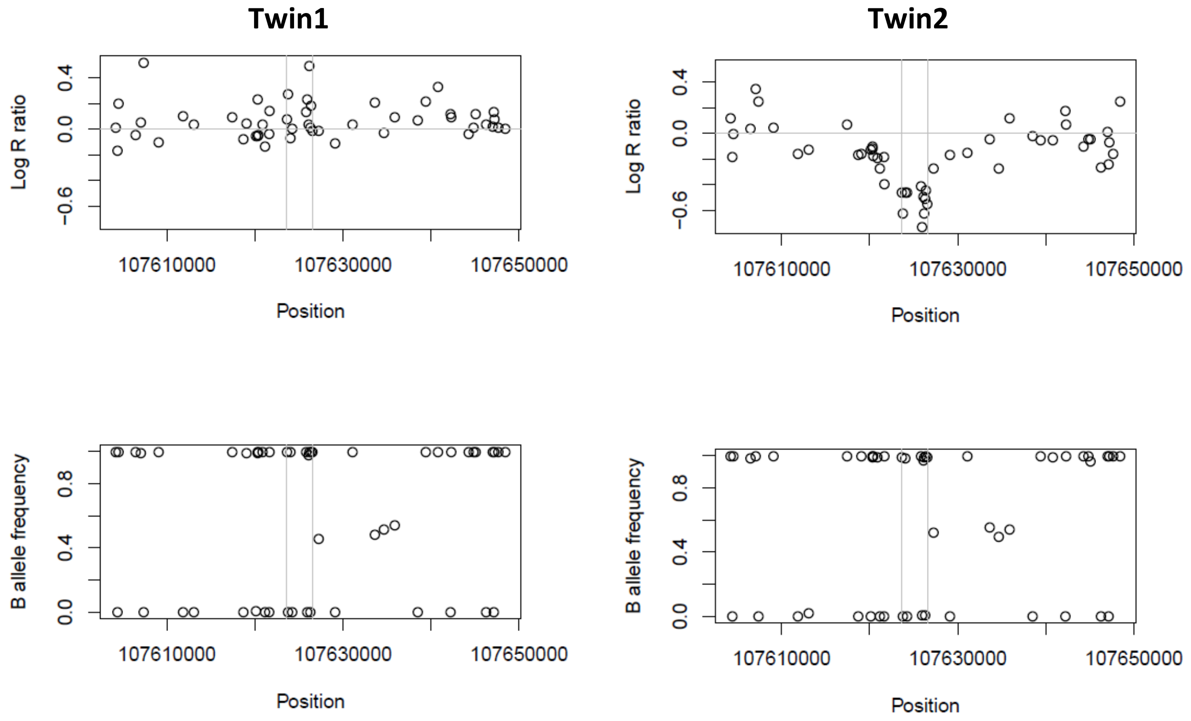


FIGURE S7

Results from PennCNV estimation and qPCR validation attempts for discordant CNV5

(A). Log R ratio (LRR) and B allele frequency (BAF) of discordant CNV5(Chr19:49061724-49089795) from PennCNV; (B). Copy number detected from qPCR signal, both of the twin members carry one copy in this region. The color-coding of the bars indicates replicate (on 3 different 96-well plates). The red box shows the triplicate runs for the MZ pair in which the CNV implicated by PennCNV is tested. Bars outside the red box are control samples for the particular CNV in question.

A. PennCNV



B. Taqman qPCR

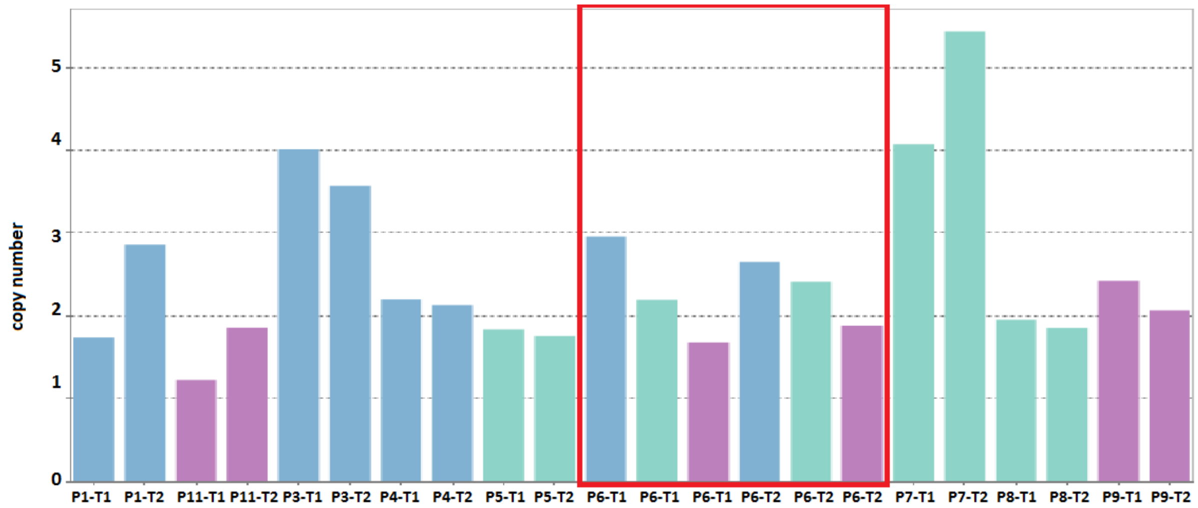
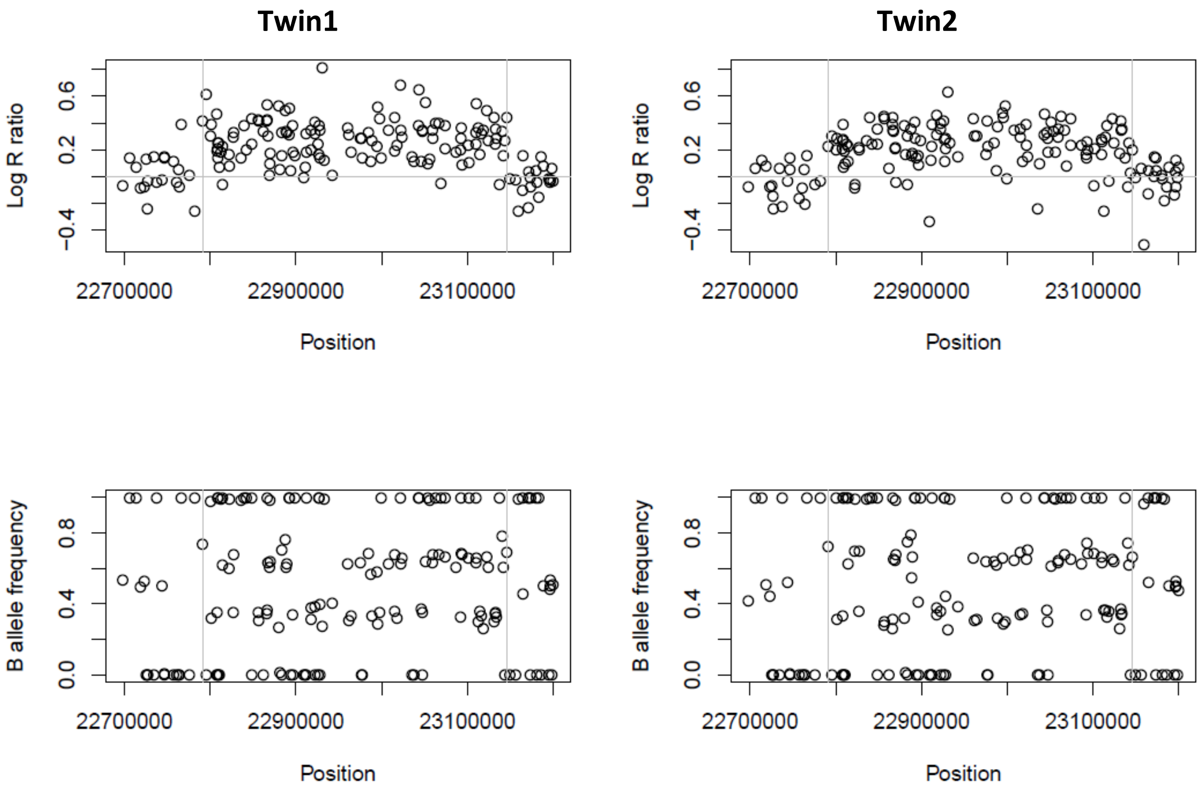


FIGURE S8

Results from PennCNV estimation and qPCR validation attempts for discordant CNV6

(A). Log R ratio (LRR) and B allele frequency (BAF) of discordant CNV6(Chr9:107623626-107626542) from PennCNV; (B). Copy number detected from qPCR signal. The color-coding of the bars indicates replicate (on 3 different 96-well plates). The red box shows the triplicate runs for the MZ pair in which the CNV implicated by PennCNV is tested. Bars outside the red box are control samples for the particular CNV in question.

A. PennCNV



B. Taqman qPCR

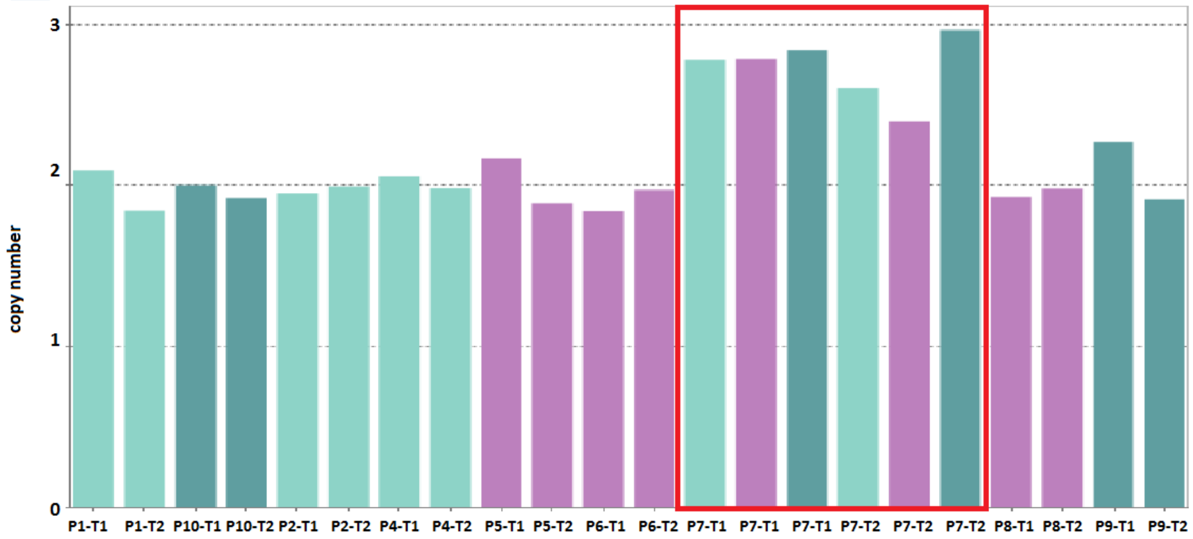
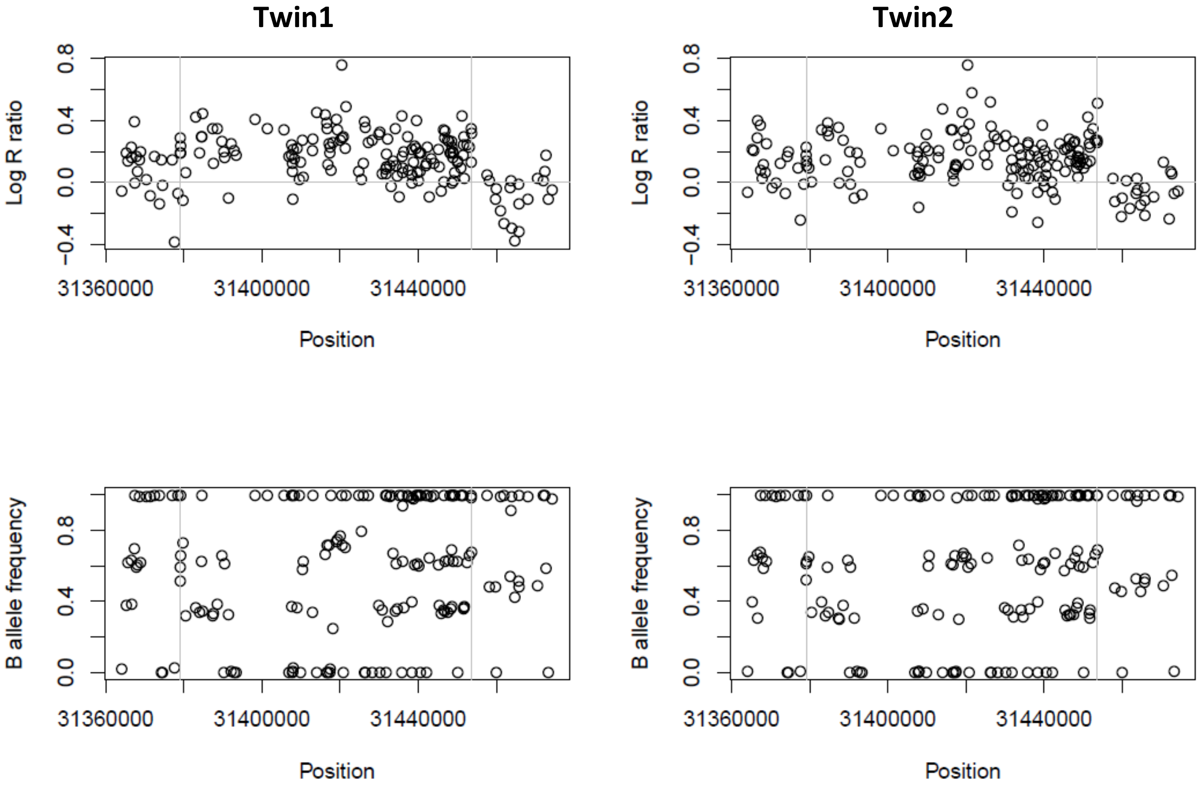


FIGURE S9

Results from PennCNV estimation and qPCR validation attempts for concordant CNV7

Both the PennCNV and qPCR support the concordant CNV within the seventh MZ Pair. (A). Log R ratio (LRR) and B allele frequency (BAF) of concordant CNV7(Chr21:22790919-23145950) from PennCNV; (B). The color-coding of the bars indicates replicate (on 3 different 96-well plates). The red box shows the triplicate runs for the MZ pair in which the CNV implicated by PennCNV is tested. Bars outside the red box are control samples for the particular CNV in question.

A. PennCNV



B. Taqman qPCR

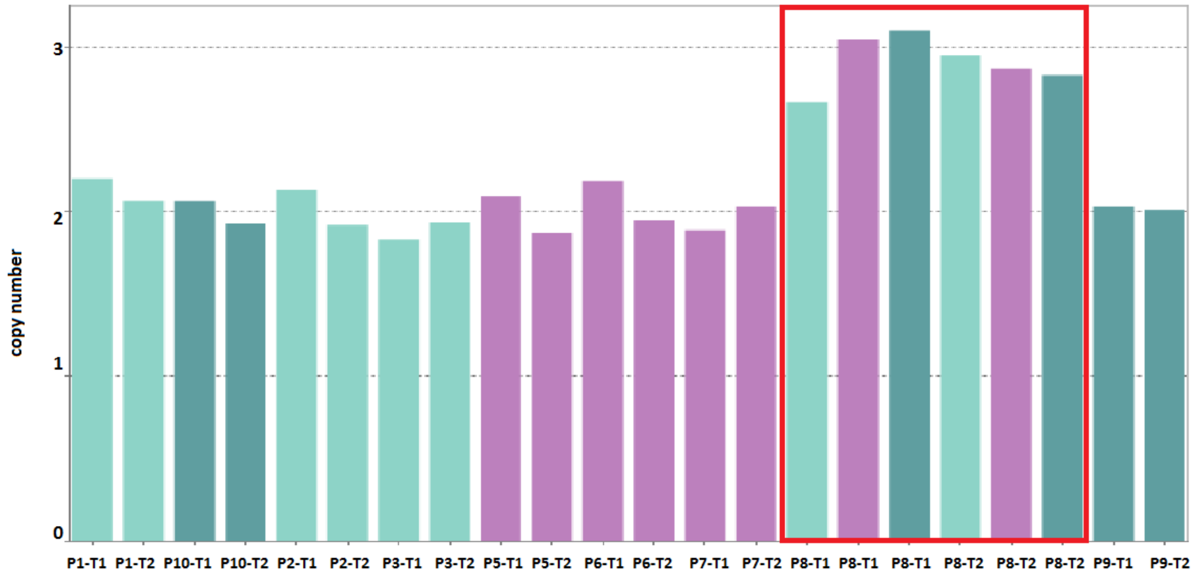
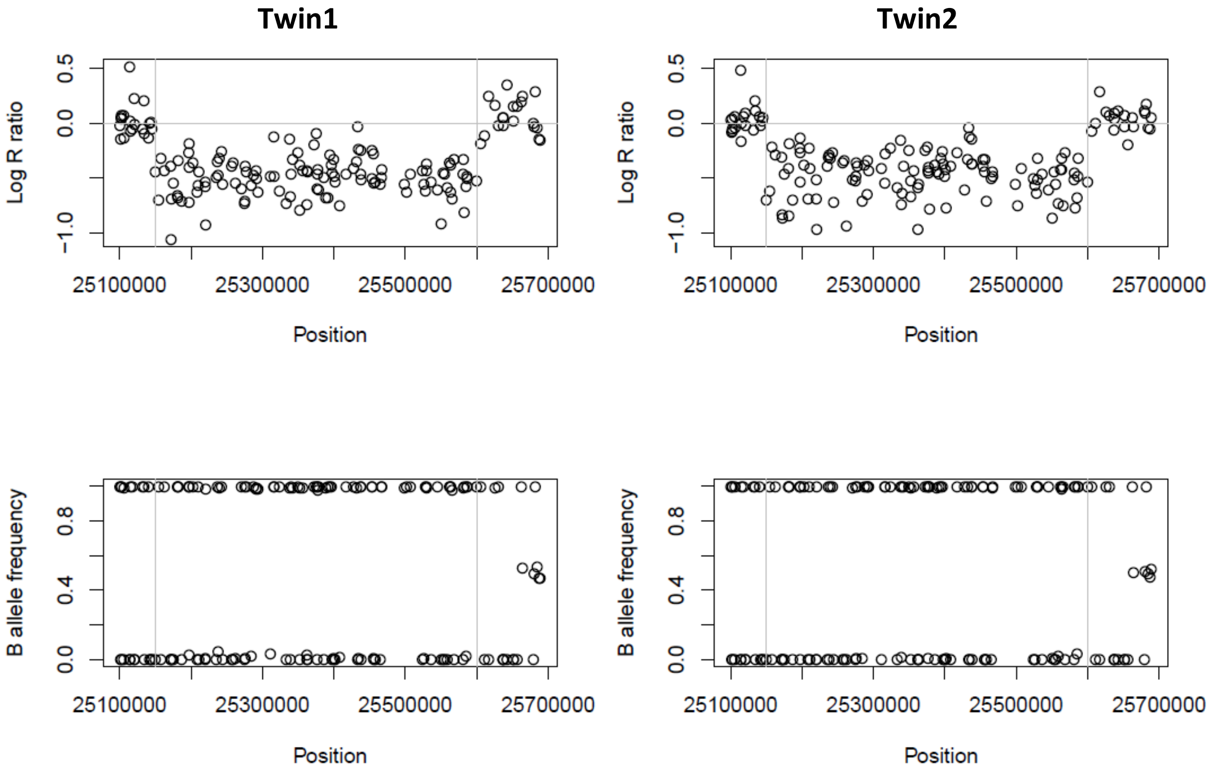


FIGURE S10

Results from PennCNV estimation and qPCR validation attempts for concordant CNV8

Both the PennCNV and qPCR support the concordant CNV within the eighth MZ Pair. (A). Log R ratio (LRR) and B allele frequency (BAF) of concordant CNV8(Chr6:31379109-31453640) from PennCNV; (B). Copy number detected from qPCR signal. The color-coding of the bars indicates replicate (on 3 different 96-well plates). The red box shows the triplicate runs for the MZ pair in which the CNV implicated by PennCNV is tested. Bars outside the red box are control samples for the particular CNV in question.

A. PennCNV



B. Taqman qPCR

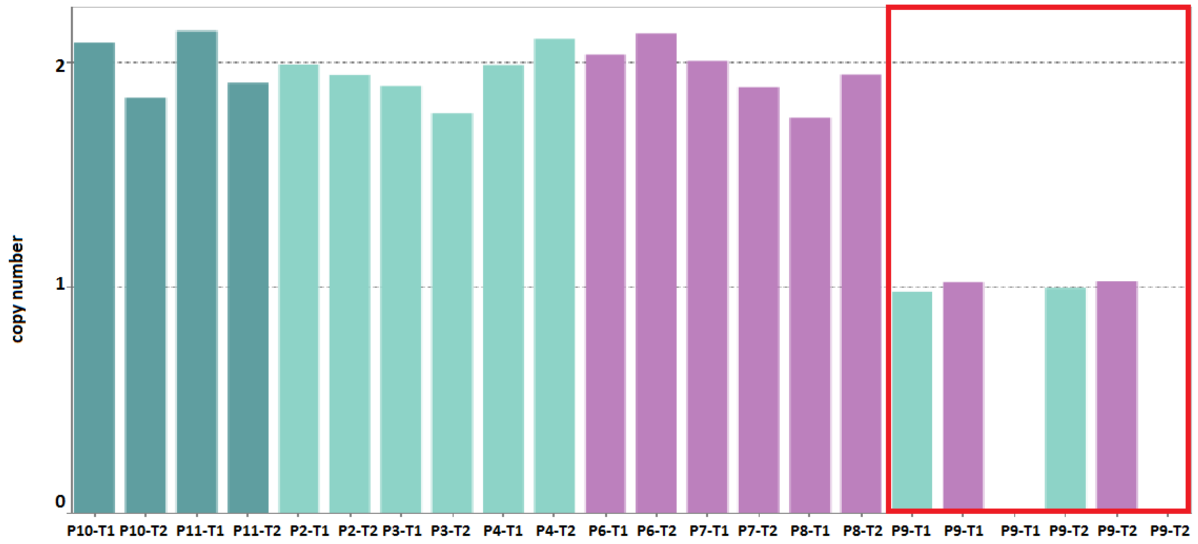
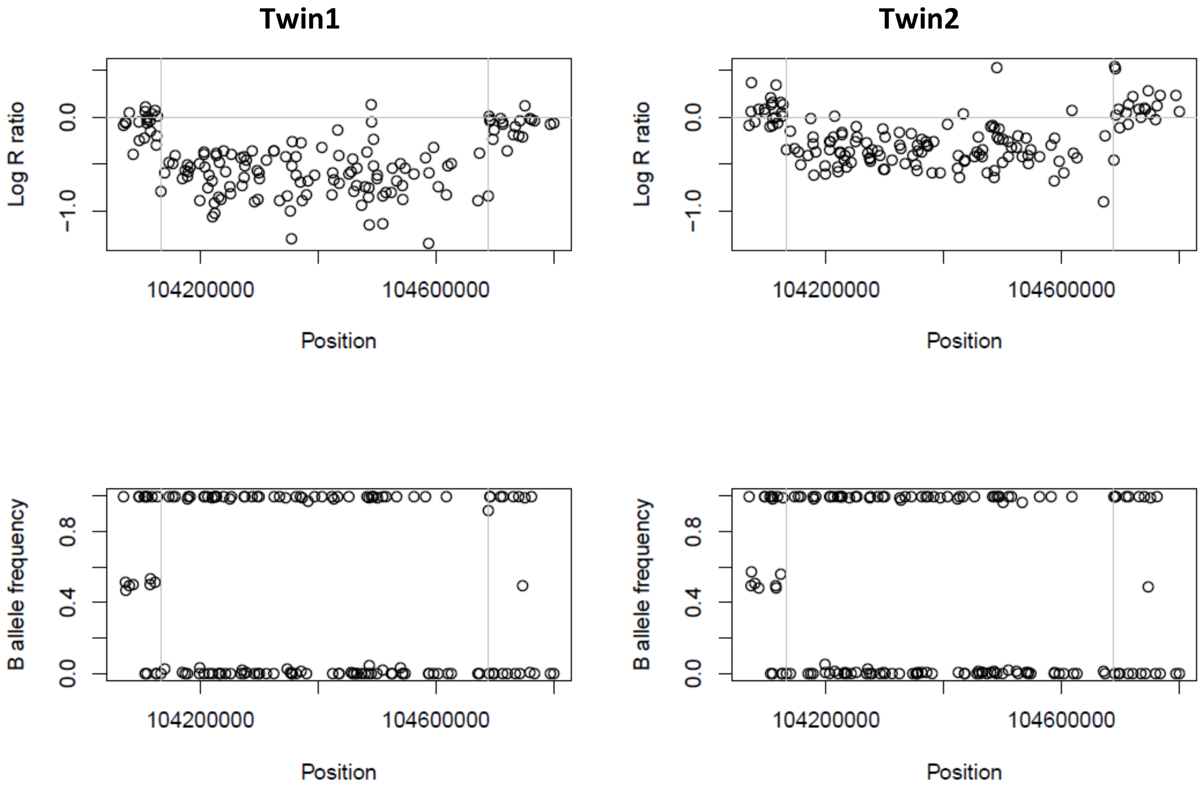


FIGURE S11

Results from PennCNV estimation and qPCR validation attempts for concordant CNV9

Both the PennCNV and qPCR support the concordant CNV within the ninth MZ Pair. (A). Log R ratio (LRR) and B allele frequency (BAF) of concordant CNV9(Chr11:25150091-25599986) from PennCNV; (B). Copy number detected from qPCR signal. The color-coding of the bars indicates replicate (on 3 different 96-well plates). The missing bars represent the failure to detect the signals from qPCR. The red box shows the triplicate runs for the MZ pair in which the CNV implicated by PennCNV is tested. Bars outside the red box are control samples for the particular CNV in question.

A. PennCNV



B. Taqman qPCR

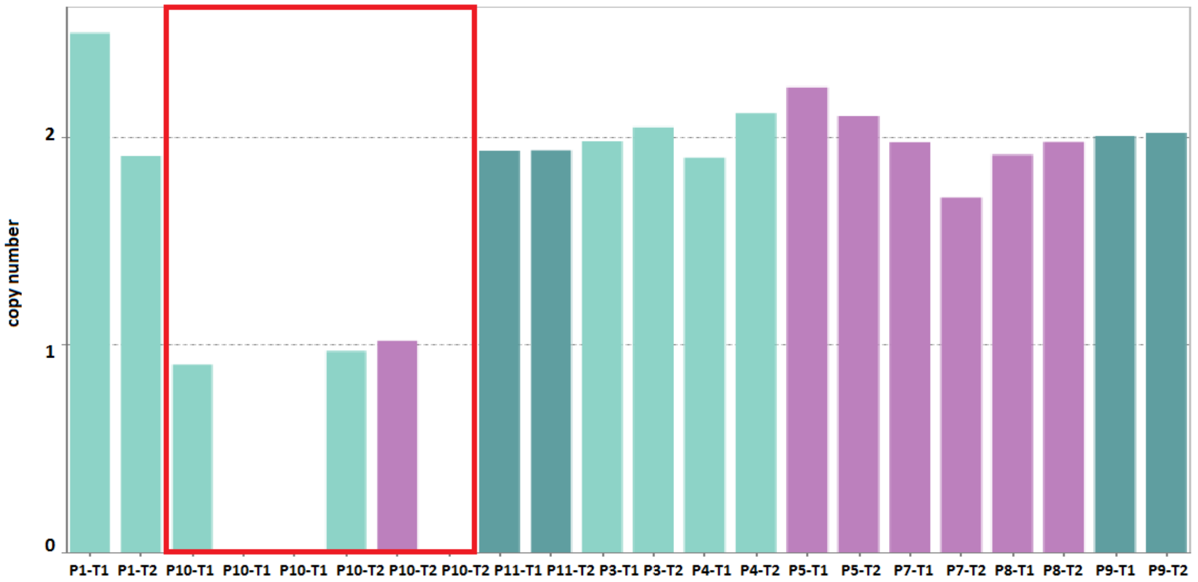
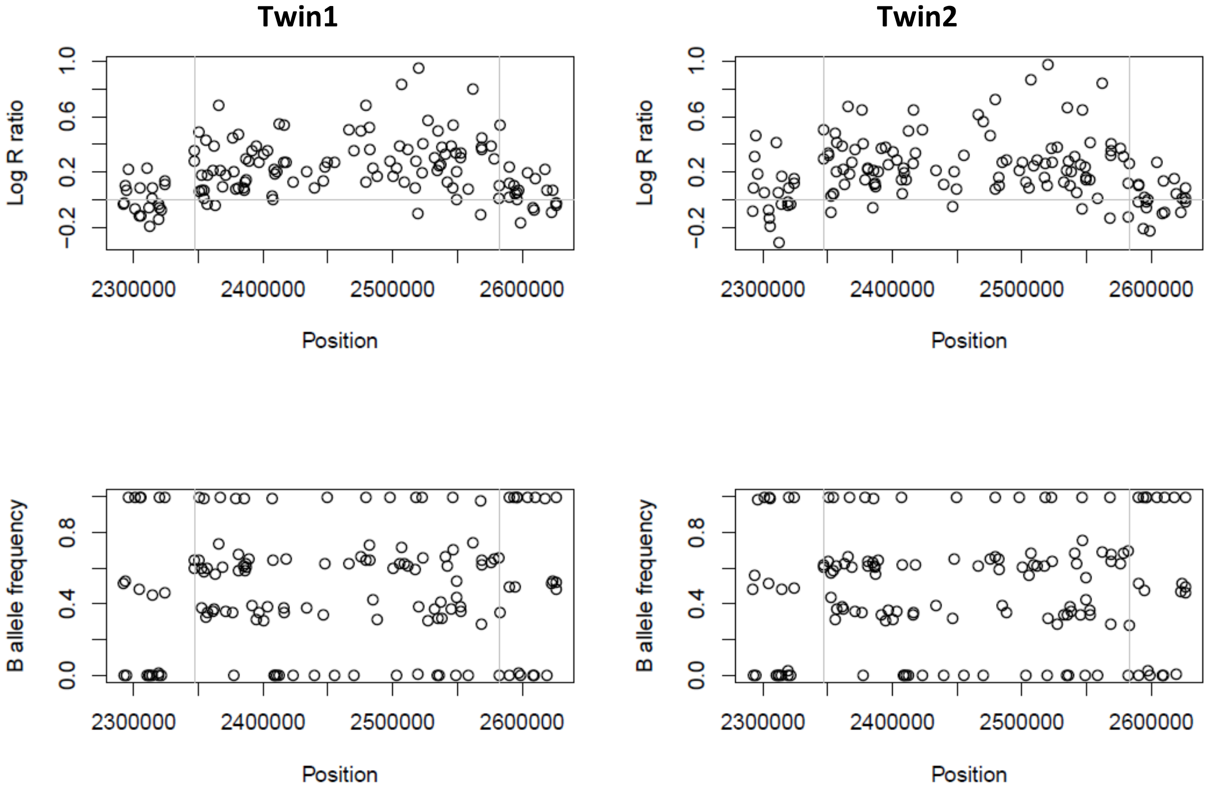


FIGURE S12

Results from PennCNV estimation and qPCR validation attempts for concordant CNV10

Both the PennCNV and qPCR support the concordant CNV within the tenth MZ Pair. (A). Log R ratio (LRR) and B allele frequency (BAF) of concordant CNV10(Chr5:104133164-104688954) from PennCNV; (B). Copy number detected from qPCR signal. The color-coding of the bars indicates replicate (on 3 different 96-well plates). The missing bars represent the failure to detect the signals from qPCR. The red box shows the triplicate runs for the MZ pair in which the CNV implicated by PennCNV is tested. Bars outside the red box are control samples for the particular CNV in question.

A. PennCNV



B. Taqman qPCR

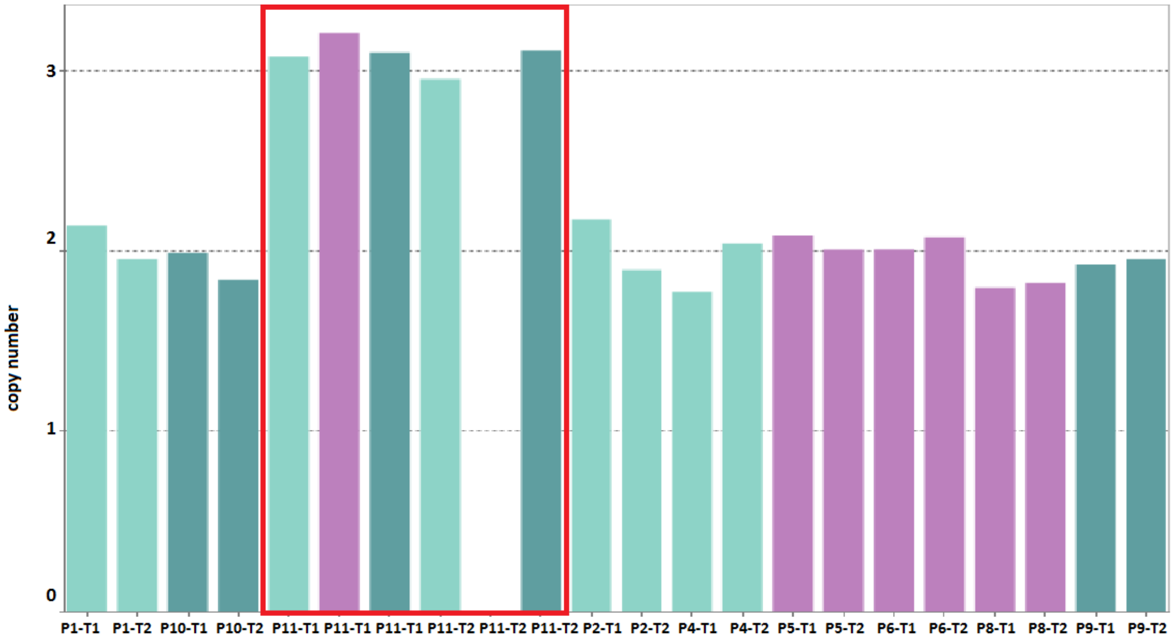


FIGURE S13

Results from PennCNV estimation and qPCR validation attempts for concordant CNV11

Both the PennCNV and qPCR support the concordant CNV within the eleventh MZ Pair. (A). Log R ratio (LRR) and B allele frequency (BAF) of concordant CNV11(Chr8:2346867-2582764) from PennCNV; (B). Copy number detected from qPCR signal. The color-coding of the bars indicates replicate (on 3 different 96-well plates). The missing bars represent the failure to detect the signals from qPCR. The red box shows the triplicate runs for the MZ pair in which the CNV implicated by PennCNV is tested. Bars outside the red box are control samples for the particular CNV in question.

TABLE S1
Taqman qPCR system for validation

Reaction components	**Volume per well(μl)**
2X TaqMan® Genotyping Master Mix	10
TaqMan® Copy Number Assay, 20X working stock	1
TaqMan® Copy Number Reference Assay, 20X	1
Nuclease-free water	4
Genomic DNA 5ng/ul	4

Note: Amplification procedure: 95°C 10min, 40 cycles of 95°C 15s, and 60°C 60s.

TABLE S2
Summary of copy numbers estimated from PennCNV and qPCR

CNV[a]	Chr[b]	N_{SNPs}[c]	PennCNV[d]		qPCR[e]
			Copy number	Confid. score	Copy number
CNV1-T1/T2-P1	2	41	2/1	-/124	2/1
CNV2-T1/T2-P2	2	50	1/2	98/-	2/2
CNV3-T1/T2-P3	5	41	2/1	-/74	2/2
CNV4-T1/T2-P4	1	20	2/3	-/66	2/2
CNV5-T1/T2-P5	19	33	2/1	-/52	1/1
CNV6-T1/T2-P6	9	11	2/1	-/50	2/2
CNV7-T1/T2-P7	21	124	3/3	535/380	3/3
CNV8-T1/T2-P8	6	137	3/3	466/379	3/3
CNV9-T1/T2-P9	11	116	1/1	458/428	1/1
CNV10-T1/T2-P10	5	109	1/1	430/249	1/1
CNV11-T1/T2-P11	8	99	3/3	387/358	3/3

Note: [a] The eleven top ranked CNVs sorted by confidence score from PennCNV; [b] Chromosome number; [c] Number of SNP markers supporting each CNV; [d]Copy numbers in the two members of the twin-pair estimated from PennCNV and the corresponding confidence scores; [e]Copy numbers as judged by the qPCR assay. CNVs 1-6 were selected for discordance and CNVs 7-11 were selected for concordance within MZ twin pairs as estimated from PennCNV.