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Supplemental Data

Dosage-Dependent Severity of the Phenotype in Patients with Mental Retardation Due to a Recurrent Copy-Number Gain at Xq28 Mediated by an Unusual Recombination

Joke Vandewalle, Hilde Van Esch, Karen Govaerts, Jelle Verbeeck, Christiane Zweier, Irene Madrigal, Montserrat Mila, Elly Pijkels, Isabel Fernandez, Jürgen Kohlhase, Christiane Spaich, Anita Rauch, Jean-Pierre Fryns, Peter Marynen, and Guy Froyen

Table S1. Primer sequences used for copy number analysis, expression profiling, and breakpoint analysis in the four families. All positions are based on the UCSC genome browser (NCBI Build 36.1, Hg18, March 2006).

qPCR primer sequences for mapping the aberrations and for copy number quantification

locus ID	forward primer (5' to 3')	reverse primer (5' to 3')	position
normalizer	GGTGTCCAAGCCACTGAAT	GTGACTTGGTGACTCACAGTTA	48257866
1	TGCTGGATGAATAACCACAACAC	GAAGGTCTCCAGCCATCAGAAG	152958445
2	TCCCAGCTGAGAGTCCATCTG	CACGTCTTCTCAACCTAATGGAATT	153056474
3	GGATGGGACGCTGCTACAGA	GGACATGGTATCAGGTGGACTCA	153190779
4	TAACACCCACAGCCACAT	AAGTCCGGGCTCAAATACC	153201372
5	GCCATAAAGCAGGTGTTTCCA	TTTCCATTTGGTGGCGTTTT	153208098
6	CACAGCAAATGCAGAAAACAGT	TTTCCAGGCGCAAACACT	153210170
7	TGGAGCTGAGGGAGGAT	AGACAAGGGCCAGGAAG	153228970
8	CCGCGGGTCGCACTTAC	CAAATCAGTGGCTCTCCCTCTT	153232685
9	CATGCACGCTACCAGCAGTC	AGAATGATGTGCCAGAGACGC	153263348
10	TGCTGGTTCTCACACCTT	TCAGTGGCTGCAACGAAA	153279966
11	TTGACCTGATTCCCAAATTCCT	CCCCCGACCTGCAATACC	153320372
12	CGCAGGGAAGGGTTTGTG	AAAAGAGGGTCCCAGGAACAG	153344474
13	ACGGACGTCATCTGAGTTGGT	CACCTACTGCAGATGCTGTGTCT	153414994
14	TGGGTGGTGCCAGCATCT	CAGCTTTCCGCCAGTTCCT	153485548
15	TCCAGTGTGAAAGCAGCAAG	ATGATCCAAACTCCTGAGTCC	153515781
16	GGAGTAAGATTCTGCCACCATTG	CCATCTTGTTCCAGCCTCTGA	153559191
17	CCAAATGCCGTGCCTCTTT	TGGACCACGTTGGGTATTCA	153634659

qPCR primer sequences for copy number analysis of LCR sets

locus ID	forward primer (5' to 3')	reverse primer (5' to 3')		position
K1/K2	GCCTTCCCTGAGGAATGT	CCAGGACCCTGTACCCGA		153226431
			and	153268727
L1/L2	TCCAGTGTGAAAGCAGCAAG	ATGATCCAAACTCCTGAGTCC		153451460
			and	153515781

qPCR primer sequences for gene expression analysis

gene ID	forward primer (5' to 3')	reverse primer (5' to 3')	map
FLNA	CAGATGAGGCCCAGGATCA	CATCAAACTGGTGTCCATCGA	ex1-2
GDI1	CCTGCAACGACATCAAAGACA	TTTGCGCTTCATGTTCTCAAAG	ex11
RPL10	TGCTGGTTCTCACACCTT	TCAGTGGCTGCAACGAAA	ex1
ATP6AP1	TCTGCTGCTCATTCGCC	CTCATCGTTGCCTGTGAGGA	ex4-5
HUWE1	ACAGGCCATGCAGAGCTTTAA	ATACGTTCCTCTGTACCAACAACCT	ex23-24
PORCN	CTCTGCCGACATTCCTCC	TGTGCATCTCACCCATGAGT	ex2-3
HPRT	TGACACTGGCAAAACAATGCA	GGTCCTTTTCACCAGCAAGCT	ex6-7
ACTB	CACCCTGAAGTACCCCATCG	CACGGCATCGTCACCAACTGGG	ex3
GUSB	AGAGTGGTGCTGAGGATTGG	CCCTCATGCTCTAGCGTGTC	ex2-3

Table S2. Summary of the 44K array data for duplication mapping in families 1 to 4. All positions are based on the UCSC genome browser (NCBI Build 36.1, Hg18, March 2006).

	Family 1 (IV.2)	Family 2 (II.1)	Family 3 (II.2)	Family 4 (II.1)		
Region 1 (R1)	153,063,119	153,063,119	153,075,872	153,062,921	JA-JB-JC	
	mean: -0.39	mean: -0.04	mean: 0.47	mean: -0.54		
	153,174,273	153,176,589	153,176,589	153,176,459		
Region 2 (R2)	153,218,037	153,218,037	153,218,037	153,218,037		153.217
	mean: 0.58	mean: 0.99	mean: 0.76	mean: 0.53	K1-K2	
	153,277,405	153,276,349	153,272,904	153,277,380		153.278
Region 3 (R3 and R3')	153,278,883	153,277,032	153,273,266	153,277,390	intervening region	
				mean: 0.91		
	mean: 0.97	mean: 1.43	mean: 1.15	153,308,905		
				153,308,932		
	153,440,851	153,428,103	153,443,457			
Region 4 (R4 and R4')	153,440,879	153,428,204	153,443,474	mean: 0.50		153.437
	mean: 0.63	mean: 1.07	mean: 0.82		L1-L2	
	153,535,824	153,542,213	153,535,824	153,535,824		153.531
Size	317.787	324.176	317.787	317.787		

Figure S1

Pictures of affected male individuals of family 1. The proband IV.2 and his mother III.11 (**A**), male III.10 (**B**) and III.19 (**C**) are shown.

Figure S1

Figure S2

X chromosome-specific array-CGH plots obtained for probands of **A.** Family 1 and **B.** Family 2 revealing the duplications at Xq28. DNA from two unrelated MR patients were differentially labeled and co-hybridized onto the X-array. The log2 normalized intensity ratios of the Cy5 (patient with duplication) and Cy3 (unrelated MR patient) signals are plotted (Y-axis) against the position on the X chromosome (in Mb), from Xpter to Xqter (X-axis). The duplications are visible as clones with aberrant ratios >0.3 (arrows). Other clones outside the normal interval are confirmed polymorphic clones or outliers.

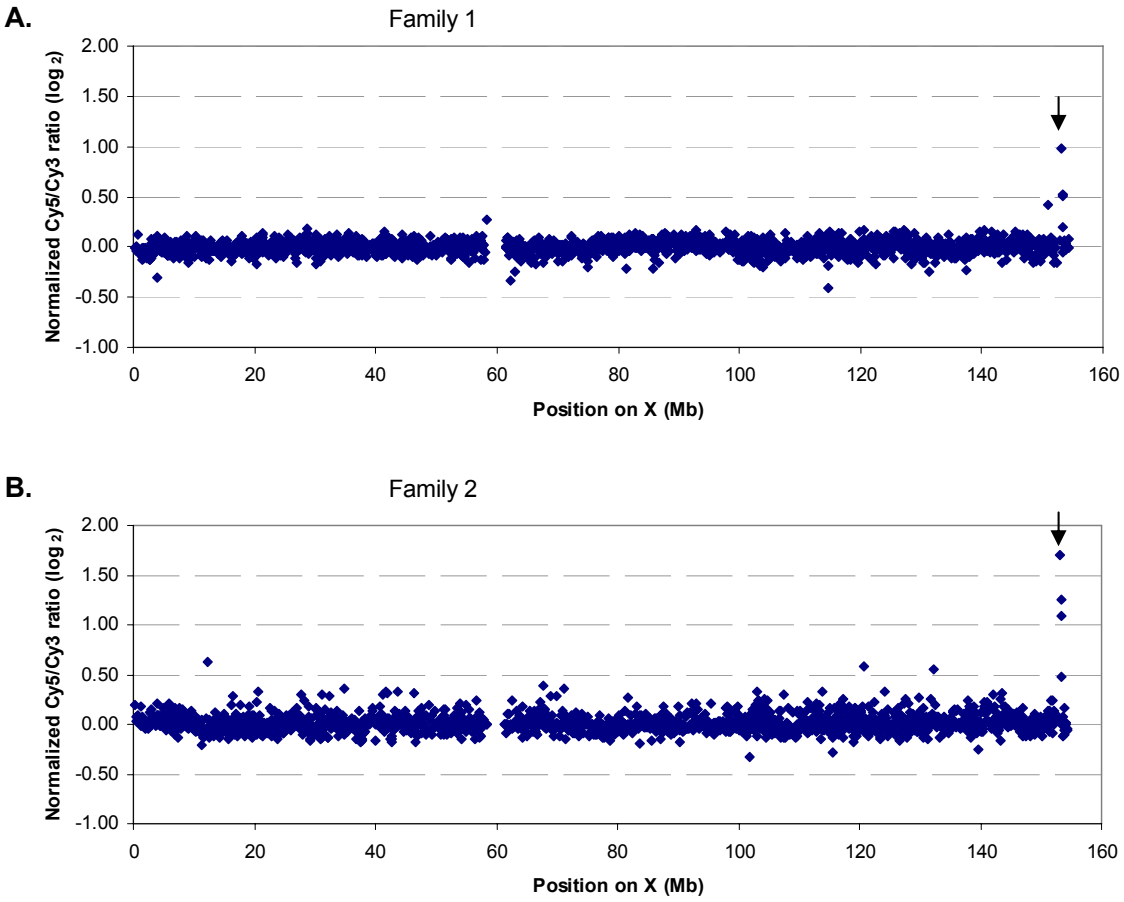

Figure S2

Figure S3

Relative copy number (indicated by the numbers above each bar) at several loci within the R3 region in the probands of the four families (Fam) as determined by qPCR, normalized to the *PORCN* locus on the X chromosome and relative to two male control samples (Co1 and Co2). The data represent the average of at least 4 independent experiments and standard deviations are indicated.

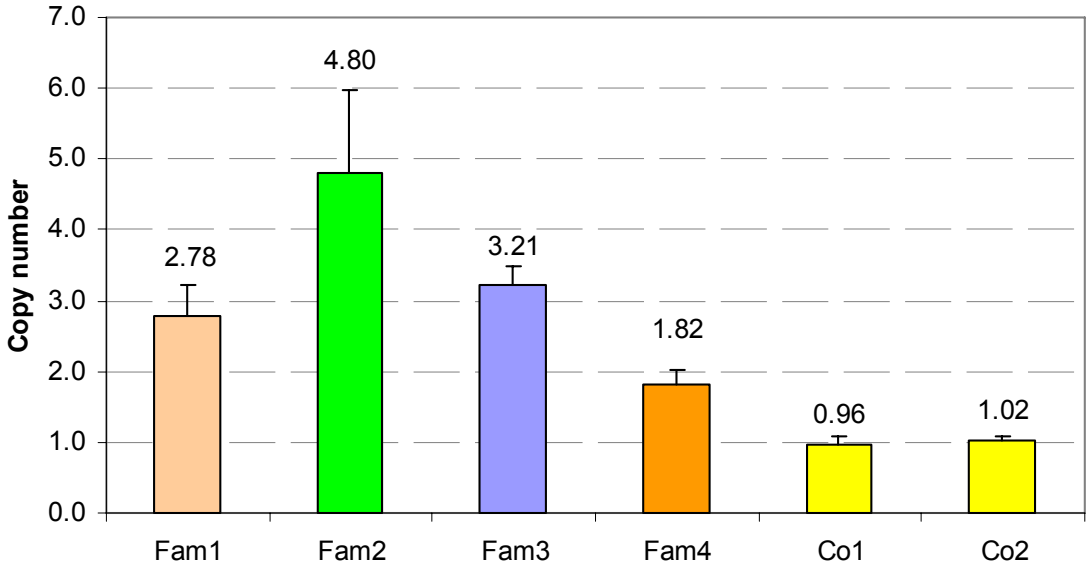

Figure S3

Figure S4

Alternative inversion polymorphism-dependent recombination model to explain the triplication found in families 1 and 3. In Step 1, an inversion takes place in between the members of the K- and L-LCRs, which results in the repositioning of K2 and L1. This relocation then allows NAHR between sister chromatids resulting in duplication of the intervening sequence R3 (Step 2: NAHR1). In Step 3a (or 3b) a second NAHR will yield triplication (or quadruplication) of this intervening sequence (NAHR2).

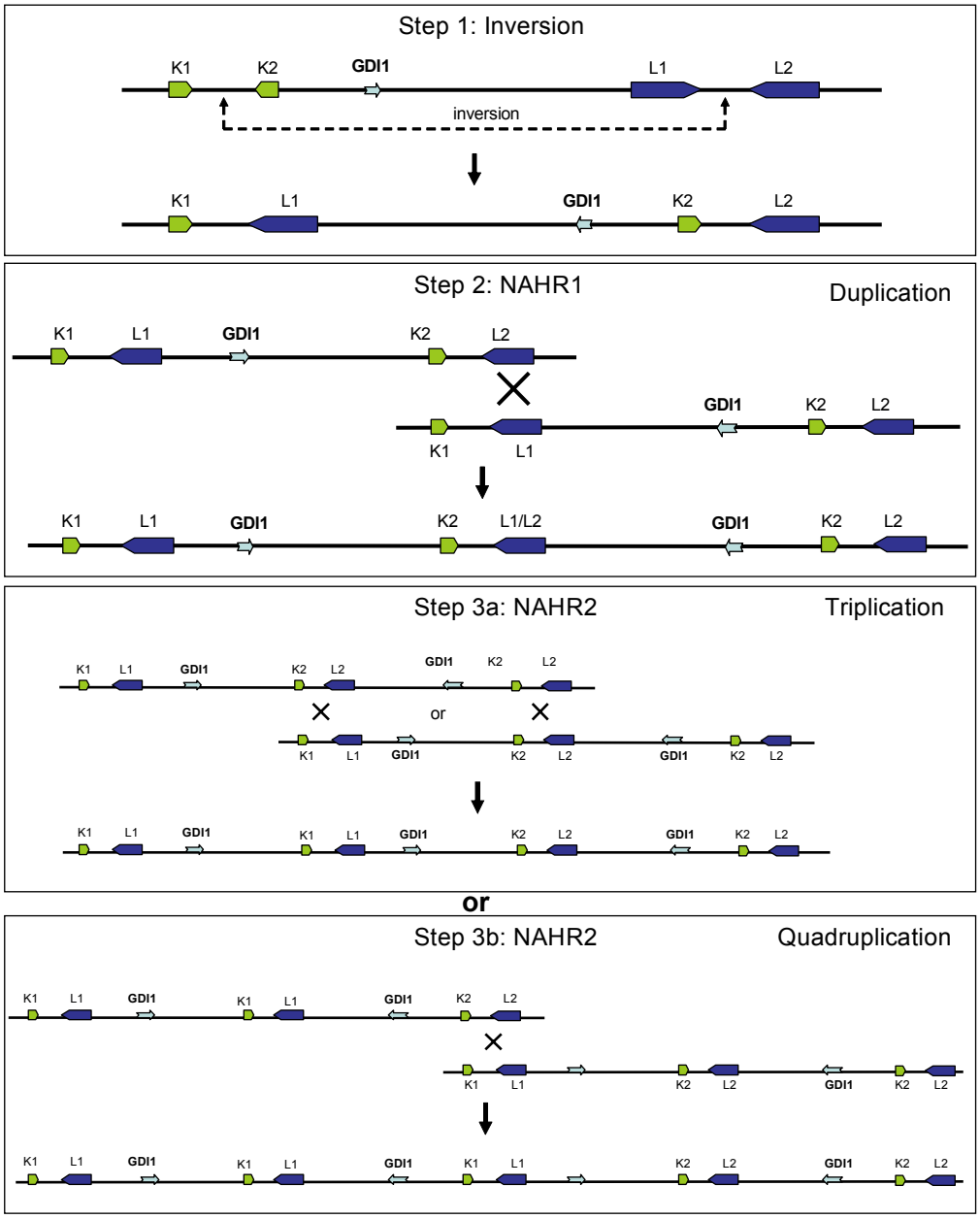

Figure S4