

t(X;14)(p11.4;q32.33) is recurrent in marginal zone lymphoma and up-regulates GPR34

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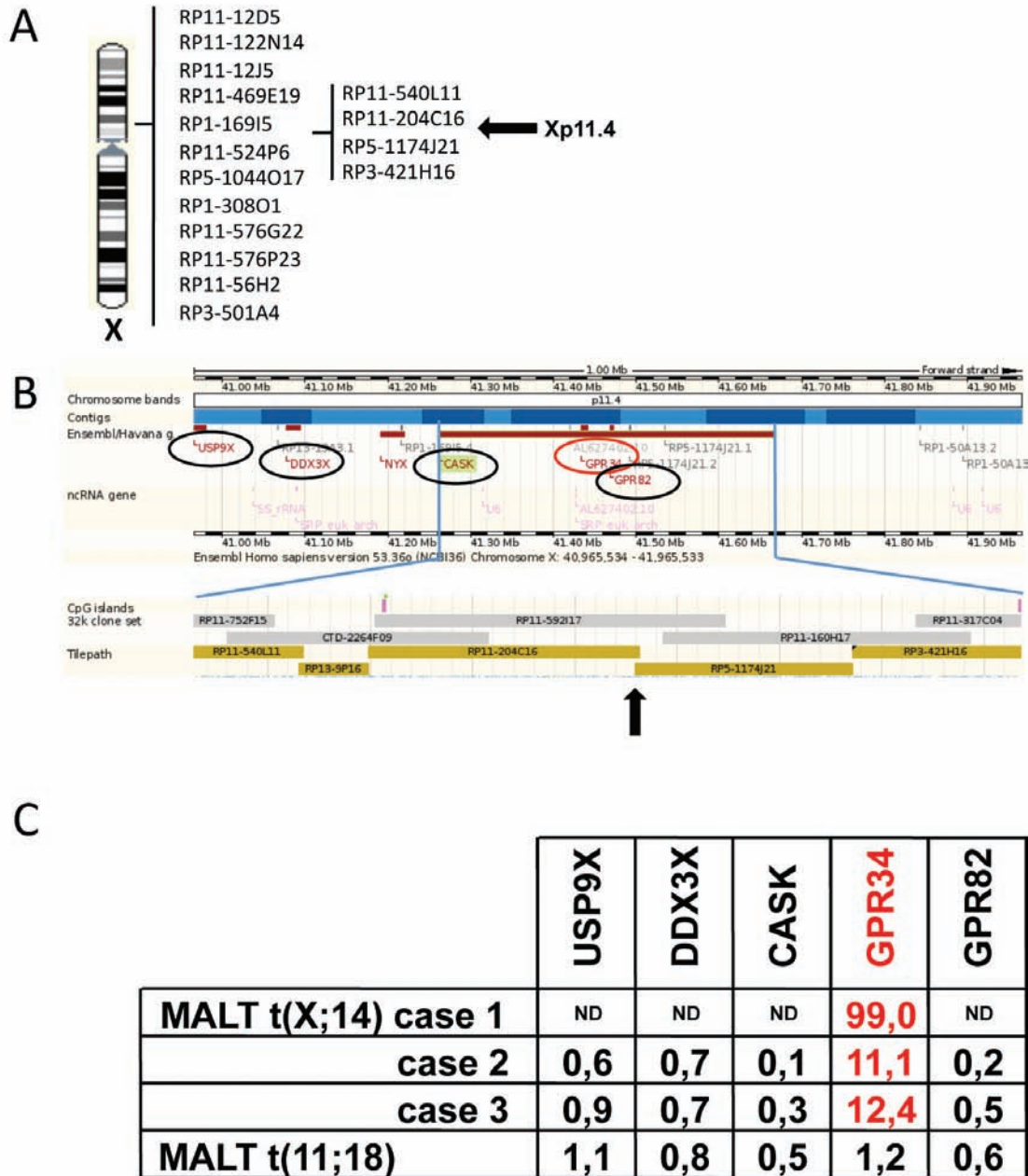
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Citation: Baens M, Finalet Ferreiro J, Tousseyn T, Urbankova H, Michaux L, de Leval L, Dierickx D, Wolter P, Sagaert X, Vandenberghe P, De Wolf-Peeters C, and Wlodarska I. t(X;14)(p11.4;q32.33) is recurrent in marginal zone lymphoma and up-regulates GPR34. *Haematologica* 2012;97(2):184-188. doi:10.3324/haematol.2011.052639

Online Supplementary Table S1.

1	GPR34-f GPR34-r	5'-GAAAGGTTGCGACTATTACCAA 5'-GTGAAAGTGCTAAATGACATAATCCTC
2	GPR82-f GPR482-r	5'-ACAGAAGGAGAAGAGAGCCTATGC 5'-AATGAGACCTGCAATCTGAGAGATC
3	CASK-f CASK-r	5'-AGCTCAGATGGAATGCTTTACATG 5'-CGCTTTACGATTTCAAACACAGAT
4	USP9X-f USP9X-r	5'-CTCCACCTGAAGATGCC 5'-GGCCTGTATATGGCTGTCC
5	DDX3X-f DDX3X-r	5'-ACAAGGGTAGCAGTCGT 5'-CTACTTTGTCGGTAGTCTCTGG
6	BCL2L1-f BCL2L1-r	5'-GCAGGTGTTTTGGACAATGGA 5'-AGCTCCCGGTTGCTCTGA
7	CCND2-f CCND2-r	5'-CTGGCTAAGATCACCAACACAGA 5'-GAGGAGCACCGCCTCAATC
8	cMYC-f cMYC-r	5'-CACCACCAGCAGCGACTCT 5'-GCCTGCCTCTTTCCACAGA
9	TNFAIP3-f TNFAIP3-r	5'-CTGGAGTCTCTCAAATCTCAGG 5'-TTGTCCCAATTCATTCAGTTC
10	BIRC3-f BIRC3-r	5'-TGGGTTCAACATGCCAAGTG 5'-GGATGAACTCCTGTCCCTTTAATTCT
11	BCL2A1-f BCL2A1-r	5'-TAACACAGGAGAATGGATAAGGCA 5'-ATCCAGCCAGATTTAGGTTCAA
12	TRAF1-f TRAF1-r	5'-TGGCTGAGGCTGGAATTG 5'-GAGGTGACCTCATGCTCTT
13	IRF4-f IRF4-r	5'-GTTCTGAGGGAGCCAAA 5'-TAAGCGTTGTCATGGTGTAG
14	TRAF2-f TRAF2-r	5'-CCGTCTGTCCAGTGAT 5'-ACATTCCGGTCAGCATGAG
15	HPRT1-f HPRT1-r	5'-TGACACTGGCAAAACAATGCA 5'-GGTCCTTTTCACCAGCAAGCT

Online Supplementary Figure S1. Mapping of the Xp11.4 breakpoint. (A) Idiogram of a normal X chromosome, the applied FISH probes and the identified breakpoint. Probes distal to the breakpoint hybridized with der(14), proximal probes hybridized to der(X). (B) The Ensembl map of the involved Xp11.4 region. The arrow indicates the breakpoint mapped by FISH. Candidate genes further investigated by qRT-PCR are circled. (C) Results of qRT-PCR analysis. Note upregulation of GPR34 in all 3 analyzed cases with t(X;14), but not in MALT lymphoma with t(11;18).



Aberration Summary Report

Case I

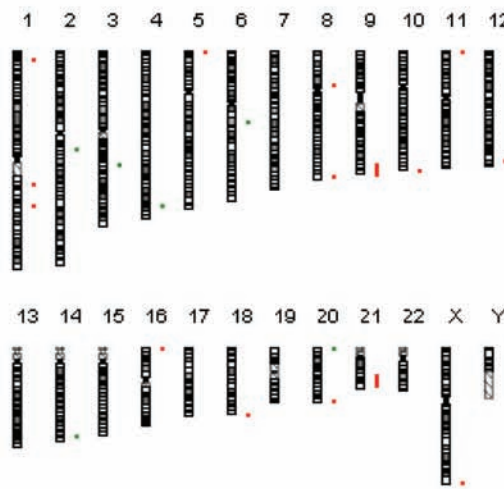
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Centralization	: ON
Bin Size	: 10
Centralization Threshold	: 6.0
Fuzzy Zero	: ON
Combine Replicates (Intra Array)	: OFF
Genome	: hg18
Aberration Filters	: minProbes = 3 AND minAvgAbsLogRatio = 0.0 AND maxAberrations = 100 AND percentPenetrance = 0
Feature Level Filters	: glsSaturated = true OR rlsSaturated = true OR glsFeatNonUnifOL = true OR rlsFeatNonUnifOL = true
Expand Non Unique Probes	: ON
Genomic Boundaries	: Not Applied

Genome Overview



Comments

Technician: _____ Date: __/__/__ Supervisor _____ Date: __/__/__

Text Summary Report for Sample Case 1_310924

Event No	Chr	Cytoband	#Probes	Amp/Del	P-value	Annotations
1	chr1:10613121-10675865	p36.22	9	1.473570	7.97E-15	PEX14, CASZ1, CASZ1
2	chr1:150822873-150853058	q21.3	6	1.528778	1.28E-16	LCE3C, LCE3B
3	chr1:174244866-174245266	q25.1	3	1.972704	1.02E-14	RFWD2
4	chr2:110190729-110563525	q13	23	-0.836511	1.00E-30	MALL, NPHP1, NCRNA00116
5	chr3:128821447-128821847	q21.3	3	-1.302441	1.74E-12	MCM2
6	chr4:173665872-173666272	q34.1	3	-3.557698	2.69E-20	GALNTL6
7	chr5:74949-2904841	p15.33	286	0.668073	4.95E-164	PLEKHG4B, LOC389257, CCDC127...
8	chr6:79035691-79080188	q14.1	3	-5.743711	1.18E-60	
9	chr8:39356395-39505456	p11.23 - p11.22	20	1.018627	3.27E-22	ADAM5P, ADAM3A
10	chr8:142062484-146043851	q24.3	491	0.828658	0.00E00	PTK2, DENND3, SLC45A4...
11	chr9:127789729-140145883	q33.3 - q34.3	1492	0.642396	0.00E00	FAM125B, FAM125B, LMX1B...
12	chr9:138516770-138542479	q34.3	4	2.730254	1.45E-14	NOTCH1
13	chr10:133759859-135062100	q26.3	171	0.978366	3.24E-133	JAKMIP3, DPYSL4, STK32C...
14	chr11:182172-3210953	p15.5 - p15.4	394	0.914841	0.00E00	SCGB1C1, ODF3, BET1L...
15	chr12:123339427-123579022	q24.31	30	1.121606	1.27E-27	FAM101A, NCOR2
16	chr14:100446410-100530313	q32.31	12	-0.624985	8.34E-19	SNORD113-1, SNORD113-2, SNORD113-3...
17	chr16:36950-5242084	p13.3	740	0.793833	0.00E00	POLR3K, SNRNP25, RHBDF1...
18	chr18:75211877-75851742	q23	72	0.776309	3.24E-57	ATP9B, NFATC1, NFATC1...
19	chr20:1516766-1539355	p13	3	-1.347955	1.44E-12	SIRPB1, SIRPB1
20	chr20:59638996-62223344	q13.33	355	0.996347	7.16E-183	CDH4, TAF4, LSM14B...
21	chr21:31288486-46914886	q22.11 - q22.3	2002	0.600348	0.00E00	KRTAP19-8, TIAM1, SOD1...
22	chrX:151804174-153542323	q28	237	0.874145	4.62E-115	ZNF185, PNMA5, PNMA3...

Amp=Amplification
Del=Deletion

Aberration Summary Report

Case 2

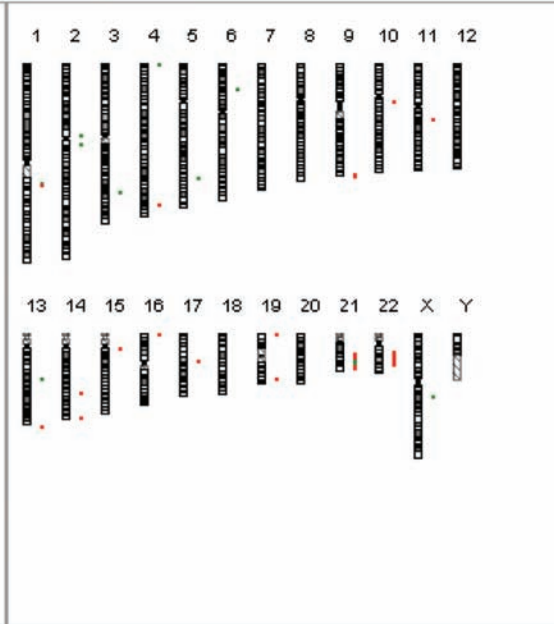
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Analysis Settings

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Centralization	: ON
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Threshold	
Fuzzy Zero	: ON
Combine Replicates (Intra Array)	: OFF
Genome	: hg18
Aberration Filters	: minProbes = 3 AND minAvgAbsLogRatio = 0.0 AND maxAberrations = 100 AND percentPenetrance = 0
Feature Level Filters	: glsSaturated = true OR rlsSaturated = true OR glsFeatNonUnifOL = true OR rlsFeatNonUnifOL = true
Expand Non Unique Probes	: ON
Genomic Boundaries	: Not Applied

Genome Overview



Comments

Technician: _____ Date: __/__/__ Supervisor _____ Date: __/__/__

Text Summary Report for Sample Case 2_246624

Event No	Chr	Cytoband	#Probes	Amp/Del	P-value	Annotations
1	chr1:148490589-148490989	q21.2	3	-0.838041	2.33E-11	
2	chr1:150822873-150853058	q21.3	6	1.347138	9.86E-42	LCE3C, LCE3B
3	chr2:88944777-89207963	p11.2	19	-0.374078	3.04E-15	
4	chr2:99664901-100131655	q11.2	54	-0.346190	1.37E-34	AFF3, AFF3
5	chr3:159418960-159442295	q25.32	4	-1.138804	4.90E-23	RSRC1
6	chr4:137382-233554	p16.3	11	-0.463531	1.42E-13	ZNF718, ZNF732
7	chr4:173665872-173666272	q34.1	3	3.179281	3.92E-53	GALNTL6
8	chr5:140203240-140216724	q31.3	4	-0.848914	9.50E-15	PCDHA1, PCDHA1, PCDHA2...
9	chr6:32595202-32630048	p21.32	4	-3.508226	6.17E-59	HLA-DRB5, HLA-DRB6
10	chr9:136460984-136472396	q34.2	5	1.414950	3.33E-31	RXRA
11	chr9:138354529-138370181	q34.3	3	1.015631	5.81E-12	GPSM1, GPSM1, GPSM1
12	chr9:138516770-138542479	q34.3	4	1.091858	2.43E-17	NOTCH1
13	chr10:46371043-47172734	q11.22	38	0.383007	5.35E-30	SYT15, SYT15, GPRIN2...
14	chr11:69191719-69192119	q13.2	3	1.373461	2.35E-20	ORAOV1
15	chr13:56658279-56673383	q21.1	3	-1.076723	1.24E-14	
16	chr13:113790951-113811196	q34	3	0.857069	3.25E-11	RASA3
17	chr14:73071204-73092218	q24.3	4	0.745657	1.86E-11	HEATR4, ACOT1
18	chr14:105023934-105041257	q32.33	6	0.909347	1.50E-16	CRIP1, C14orf80, C14orf80
19	chr15:18657188-20250086	q11.2	72	0.603750	2.02E-127	LOC727832, GOLGA8C, LOC646214...
20	chr16:511580-545156	p13.3	6	0.923999	8.08E-18	RAB11FIP3, RAB11FIP3, C16orf10...
21	chr17:34216958-34217358	q12	3	0.998284	2.87E-13	CCDC49
22	chr19:1162440-1162840	p13.3	3	1.083353	1.89E-11	STK11
23	chr19:55612843-55626737	q13.33	3	0.934678	1.89E-11	POLD1, SPIB
24	chr21:25096319-26465647	q21.2 - q21.3	138	0.289481	1.27E-60	NCRNA00158, MIRHG2, C21orf71...
25	chr21:28900932-29954544	q21.3	111	0.290144	1.34E-49	N6AMT1, RNF160, RWDD2B...
26	chr21:33450859-34211336	q22.11	111	0.374029	2.42E-79	C21orf54, IFNAR2, IFNAR2...
27	chr21:34444500-41331781	q22.11 - q22.2	812	-0.351449	0.00E00	C21orf82, C21orf82, KCNE2...
28	chr21:41561927-46914886	q22.3	778	0.361086	0.00E00	BACE2, FAM3B, MX2...
29	chr22:22677759-22725505	q11.23	10	1.000394	2.97E-45	LOC391322, GSTT1, GSTTP2
30	chr22:23475199-41167733	q11.23 - q13.2	2176	0.348479	0.00E00	PIWIL3, TOP1P2, SGSM1...
31	chr22:37688858-37715585	q13.1	6	0.949236	1.89E-11	APOBEC3A, APOBEC3B
32	chrX:77989378-82101245	q21.1	200	-0.402013	6.56E-162	P2RY10, GPR174, ITM2A...

Amp=Amplification
Del=Deletion

Aberration Summary Report

Case 3

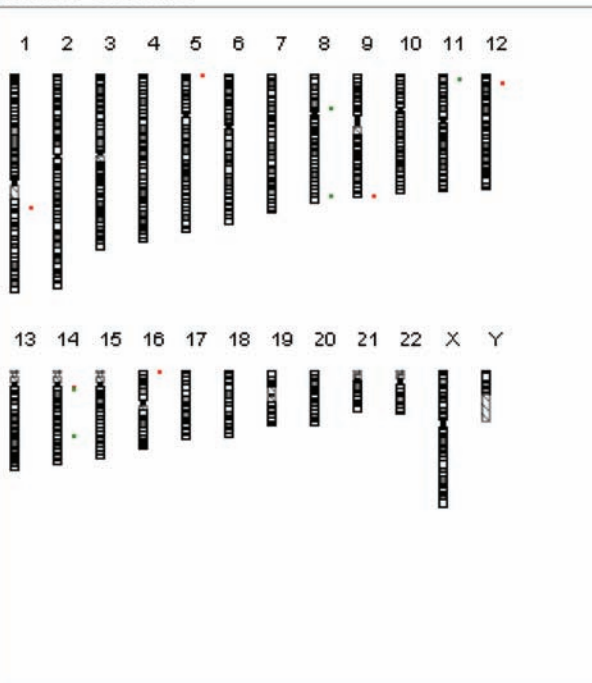
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Analysis Settings

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Centralization	: ON
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Fuzzy Zero	: ON
Combine Replicates (Intra Array)	: OFF
Genome	: hg18
Aberration Filters	: minProbes = 3 AND minAvgAbsLogRatio = 0.0 AND maxAberrations = 100 AND percentPenetrance = 0
Feature Level Filters	: glsSaturated = true OR rlsSaturated = true OR glsFeatNonUnifOL = true OR rlsFeatNonUnifOL = true
Expand Non Unique Probes	: ON
Genomic Boundaries	: Not Applied

Genome Overview



Comments

Technician: _____ Date: __/__/__ Supervisor _____ Date: __/__/__

Text Summary Report for Sample Case 3_367294

Event No	Chr	Cytoband	#Probes	Amp/Del	P-value	Annotations
1	chr1:150822873-150853058	q21.3	6	1.221685	1.70E-26	LCE3C, LCE3B
2	chr5:739092-878490	p15.33	14	0.838228	2.93E-19	TPPP, ZDHC11
3	chr8:39356395-39505456	p11.23 - p11.22	20	-3.791313	1.26E-189	ADAM5P, ADAM3A
4	chr8:137762218-137919508	q24.23	7	-1.039435	1.05E-27	
5	chr9:136456120-136472396	q34.2	6	1.355736	4.62E-27	RXRA
6	chr11:5742276-5756608	p15.4	3	-0.995910	1.06E-12	OR52N5
7	chr12:9528390-9613274	p13.31	4	1.240498	2.25E-23	
8	chr14:18624183-19490830	q11.1 - q11.2	36	0.486447	5.00E-33	POTEG, P704P, OR4Q3...
9	chr14:21448435-22052999	q11.2	75	-0.364990	1.66E-41	
10	chr14:73071204-73092218	q24.3	4	-1.122229	5.29E-19	HEATR4, ACOT1
11	chr16:511580-545156	p13.3	6	1.057486	2.96E-13	RAB11FIP3, RAB11FIP3, C16orf10...

Amp=Amplification
Del=Deletion

Aberration Summary Report

Case 4

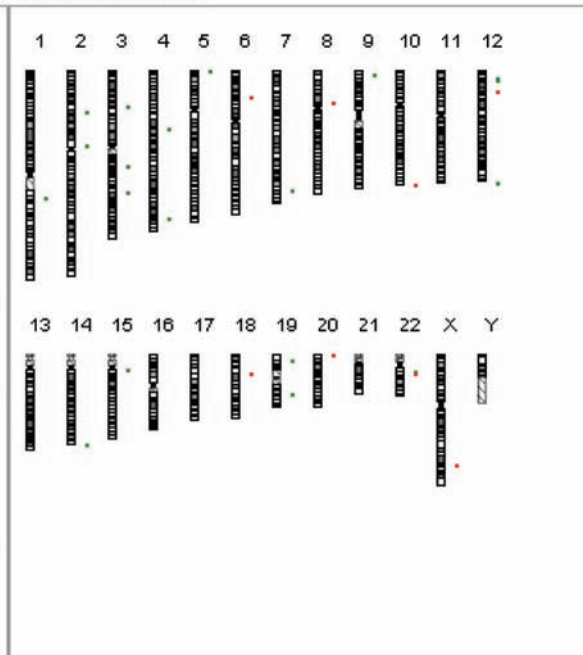
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Analysis Settings

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Centralization :	ON
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Centralization :	6.0
Threshold :	
Fuzzy Zero :	ON
Combine Replicates (Intra Array) :	OFF
Genome :	hg18
Aberration Filters :	minProbes = 3 AND minAvgAbsLogRatio = 0.0 AND maxAberrations = 100 AND percentPenetrance = 0
Feature Level Filters :	glsSaturated = true OR rlsSaturated = true OR glsFeatNonUnifOL = true OR rlsFeatNonUnifOL = true
Expand Non Unique Probes :	ON
Genomic Boundaries :	Not Applied

Genome Overview



Comments

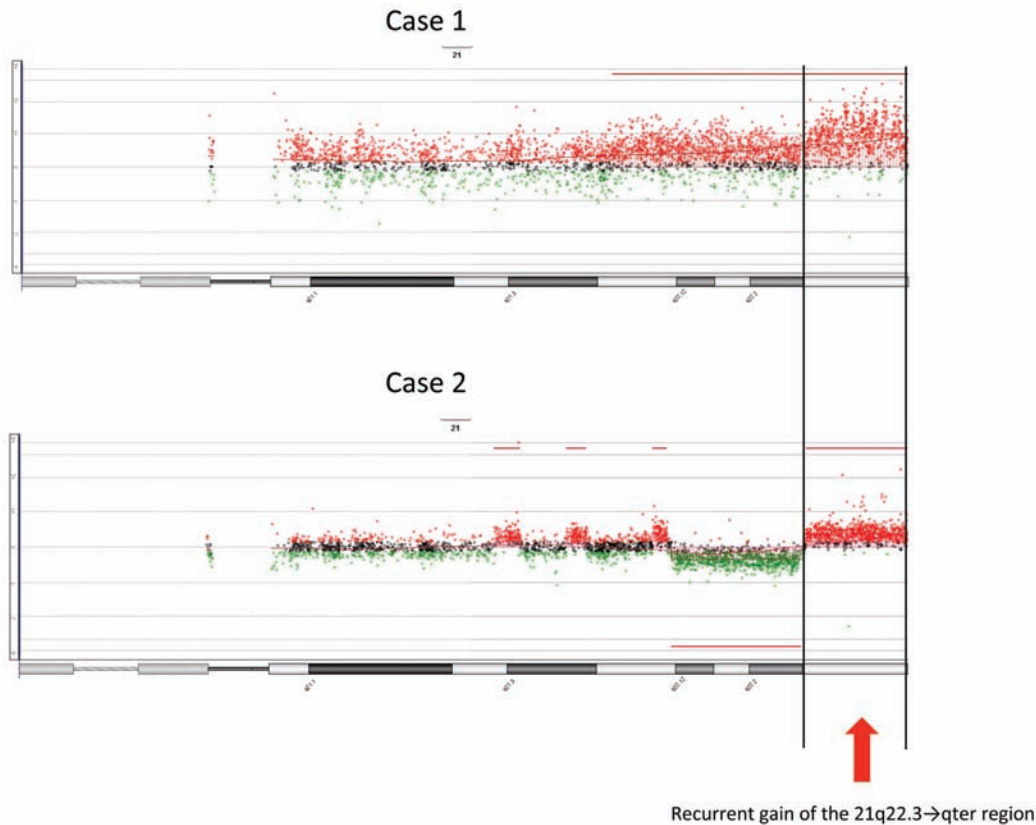
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Text Summary Report for Sample Case 4_221165

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1	chr1:150822873-150848709	q21.3	5	-1.243157	2.78E-30	LCE3C
2	chr2:48949349-48997540	p16.3	3	-1.079864	2.01E-16	
3	chr2:88924773-89275285	p11.2	25	-1.325821	2.26E-160	
4	chr3:42619765-42767956	p22.1	24	-0.545422	2.52E-36	NKTR, ZBTB47, KBTBD5...
5	chr3:113294781-115105925	q13.2 - q13.31	186	-0.624400	0.00E00	C3orf52, GCET2, SLC9A10...
6	chr3:142461403-143207163	q23	74	-0.599888	6.99E-129	ACPL2, ZBTB38, RASA2...
7	chr4:69057535-69114268	q13.2	6	-1.622061	7.40E-45	UGT2B17
8	chr4:69138837-69166014	q13.2	4	-5.081631	8.69E-79	
9	chr4:173665872-173666272	q34.1	3	-3.566994	2.20E-23	GALNTL6
10	chr5:763294-826244	p15.33	7	-0.926392	7.97E-28	
11	chr6:32567182-32630048	p21.32	6	1.382051	1.60E-36	HLA-DRB5, HLA-DRB6
12	chr7:141413152-141438704	q34	3	-0.879869	3.42E-12	MGAM
13	chr8:39356395-39505456	p11.23 - p11.22	20	0.565358	3.59E-33	ADAM5P, ADAM3A
14	chr9:5151945-6548642	p24.1	143	-0.604052	1.01E-250	INSL6, INSL4, RLN2...
15	chr10:135092839-135252719	q26.3	22	0.544447	5.45E-34	LOC619207, CYP2E1, SYCE1...
16	chr12:10463818-10475125	p13.2	3	-0.952976	1.82E-13	KLRC3, KLRC3, KLRC2
17	chr12:11404453-11431087	p13.2	4	-1.229780	2.22E-25	
18	chr12:24303134-25346828	p12.1	96	0.446262	1.63E-97	SOX5, C12orf67, BCAT1...
19	chr12:131731046-131943422	q24.33	30	-0.562142	8.20E-48	POLE, PXMP2, PGAM5...
20	chr14:105040857-105041257	q32.33	3	1.017069	3.23E-12	
21	chr14:105314054-105857393	q32.33	63	-1.213717	0.00E00	KIAA0125, ADAM6
22	chr14:105447169-105461612	q32.33	3	-0.398602	7.89E-12	KIAA0125
23	chr14:105545345-105623270	q32.33	9	-1.941099	1.29E-15	
24	chr15:19435300-20080135	q11.2	29	-0.538197	1.24E-42	LOC727924, OR4M2, OR4N4...
25	chr18:23010498-23010898	q11.2	3	0.882987	2.95E-12	CHST9
26	chr19:6909553-6964560	p13.2	6	-1.025796	2.19E-35	EMR4P, FLJ25758
27	chr19:47934435-48449748	q13.31	24	-0.518788	3.33E-46	PSG3, PSG8, PSG8...
28	chr20:1516766-1539355	p13	3	1.084775	1.59E-16	SIRPB1, SIRPB1
29	chr22:21433564-21576029	q11.22	16	-0.554373	5.81E-34	
30	chr22:23994418-24233684	q11.23	26	0.536513	1.02E-27	IGLL3, LRP5L, LRP5L
31	chrX:130336371-130857405	q26.2	37	0.887394	1.86E-132	OR13H1, LOC286467

Amp=Amplification
Del=Deletion

Online Supplementary Figure S3. Example of aCGH analysis. Chromosome 21 imbalances in cases 1 and 2 with the indicated 21q22.3→qter region gained in both cases.



Online Supplementary Figure S4. Results of qRT-PCR analysis.

Quantitative RT-PCR analysis of expression of NF- κ B target genes in t(X;14) MALT lymphoma cases and GPR34 expressing BJAB cells

A

case #	GPR34	BCL2L1	CCND2	MYC	TNFAIP3	BIRC3	BCL2A1	TRAF1	IRF4	TRAF2
t(X;14) case 1	99,0	4,0	4,0	1,1	2,2	1,7	2,0	2,2	25,4	2,0
t(X;14) case 2	11,8	1,3	3,2	0,8	2,2	0,7	3,1	3,1	7,8	2,2
t(X;14) case 3	12,2	1,0	1,9	1,4	2,3	1,7	2,6	4,3	5,2	3,3
t(11;18)	1,6	0,8	1,8	1,6	1,5	2,4	1,9	4,2	3,9	1,6
spleen	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0

Fold changes in expression relative to normal (non-reactive) spleen cDNA using HPRT1 as a reference control.

B

cell line	GPR34	BCL2L1	CCND2	MYC	TNFAIP3	BIRC3	BCL2A1	TRAF1	IRF4	TRAF2
BJAB + GPR34 clone 1	41,5	1,1	-	0,9	0,7	0,8	0,8	0,5	1,0	0,9
BJAB + GPR34 clone 2	42,5	1,2	-	1,1	1,0	1,5	1,9	0,5	1,0	1,3
BJAB + GPR34 clone 3	39,0	1,2	-	1,0	0,6	0,8	0,9	0,6	0,6	0,5
BJAB + GPR34 clone 4	797,9	1,2	-	0,8	0,8	0,9	1,0	0,3	0,7	0,7
BJAB + CARD11-L232LI	3,7	1,6	-	1,2	8,2	4,0	2,3	4,4	17,3	0,7
BJAB + API2-MALT1	4,2	1,8	-	2,4	7,3	11,3	3,1	8,2	4,5	0,9
BJAB	1,0	1,0	-	1,0	1,0	1,0	1,0	1,0	1,0	1,0

Fold changes in expression relative to BJAB cDNA using HPRT1 as a reference control.

Online Supplementary Figure S5. Immunoblot analysis of BJAB cells and the BJAB clones expressing GPR34 with antibodies that specifically recognize phosphorylated I κ B α . Left: unstimulated cells. Right: cells stimulated with PMA/ionomycin for 15'. Blots were stripped and re-probed with antibodies detecting phosphorylated p42/p44 MAPK (ERK) and β -actin (loading control).

