The American Journal of Human Genetics, Volume *98*

Supplemental Information

Mosaic Activating Mutations in *FGFR1* Cause Encephalocraniocutaneous Lipomatosis

James T. Bennett, Tiong Yang Tan, Diana Alcantara, Martine Tétrault, Andrew E. Timms, Dana Jensen, Sarah Collins, Malgorzata J.M. Nowaczyk, Marjorie J. Lindhurst, Katherine M. Christensen, Stephen R. Braddock, Heather Brandling-Bennett, Raoul C.M. Hennekam, Brian Chung, Anna Lehman, John Su, SuYuen Ng, David J. Amor, Jacek Majewski, Les G. Biesecker, Kym M. Boycott, William B. Dobyns, Mark O'Driscoll, Ute Moog, Laura M. McDonell, University of Washington Center for Mendelian Genomics, and Care4Rare Canada Consortium

SUPPLEMENTAL DATA

TABLE S1. Capture methods and coverage summary of exome data

	LR12-068		LR13-278			LR13-175		IN_0039		NIH_183	
	Tumor*	Scalp nevus	Unaffected skin	Scalp nevus	Eyelid dermoid	Scalp nevus	Lipoma	Scalp nevus	Unaffected skin	Scalp nevus	Blood*
Capture Method	SeqCap EZ Exome Library v2.0 kit							SureSelect All Exon V5		SeqCap EX Exome+UTR	
Mean Coverage	122X	160X	161X	160X	150X	169X	172X	106X	105X	65X	64X
% Covered > 20X	95.9	97.7	97.3	97.1	96.6	97.8	97.9	94.7	94.7	83.1	84.4
c.1638C>A (p.Asn546Lys)	0% (47)	0% (98)	35% (74)	42% (99)	54% (92)	0% (93)	0% (105)	33% (76)	23% (61)	0% (24)	0% (39)
c.1966A>G (p.Lys656Glu)	32% (127)	47% (182)	0% (219)	0% (172)	0% (181)	0% (202)	0% (205)	0% (70)	0% (90)	45% (29)	0% (40)

All DNA isolated from cultured fibroblasts cultured from biopsied tissue except those with asterisk (*), in which DNA was directly isolated from tissue without culture.

TABLE S2: Exome sequencing and variant filtering pipelines

Sample	**LR12-068, LR13-278, LR13-175**	**IN_0039**	**NIH_183**
Sequencing platform	HiSeq 2000 (Illumina)	HiSeq 2000 (Illumina)	HiSeq 2500 (Illumina)
Sequence alignment	Burrows-Wheeler Aligner	Burrows-Wheeler Aligner	Novoalign
Variant calling & annotation	Unified Genotyper, SeattleSeq Annotation Server	GATK, SAMtools, BCFtools, custom script	Shimmer, Mutect, Somatic Sniper
Filtering	missense,nonsense, & splice variants with < 1% in EVS, ExAC, & dbSNP	missense,nonsense, & splice variants with < 1% in EVS, ExAC, & dbSNP. Not seen in > 5 in-house exomes	missense, nonsense and splice variants with < 2% ClinSeq™ frequency*

*ClinSeq™ frequency is defined as the number of individuals with alternative allele frequency ≥1%, divided by the number of individuals with at least ten reads at that position. This is a population frequency based filter that is not limited to constitutional variants (as is the case with EVS, EXAC, and dbSNP), and is based on the NIH in house ClinSeq dataset (www.genome.gov/25521305)

TABLE S3: Primers used for subcloning 1408 basepair fragment containing c.1681G>A (p.Val561Met) and c.1966A>G (p.Lys656Glu)

Name	**Sequence**
FGFR1_ex14_F	CTTTGAGGTGAAGCCAAACC
FGFR1_ex15_R	ACCCCACTCCTTGCTTCTC

TABLE S4: Sequences for *FGFR1* smMIPs

Name	Sequence
FGFR1_01	GAGCTCTGGCTCTGGCACGGGCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNGGGTGTCGGGAAAGCTGGGGG
FGFR1_02	CACGCCCTCCCCAGACTCCACTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNTTTCCCGACACCCGGAGCTCTACGT
FGFR1_03	GGGCCCCTCCTCCCTGCTCAGGGAGGTGCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNATGAGAGAAGACGGAA
FGFR1_04	CCCACTGCGTGCACGCACCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNGTACATGATGATGCGGGACTGCTGGC
FGFR1_05	CGTCTCCTGGAGATGGATACTCTCTAGTCCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNCCTGGTTGGAGGTCAA
FGFR1_06	GCAAATGGGCGGAGAGCCACAGGGTGTTACTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNTGGTGCAGTTACTGGG
FGFR1_07	TGAGCCAGGCCTTGGGGCACTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNTGGGAGATCTTCACTCTGGGCGG
FGFR1_08	CGCATGGACAAGCCCAGTAACTGCACCAACTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNAACACCCTGTGGCTCT
FGFR1_09	TGGCCCCAGGCAGGGCCATGACTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNTCTCTATCCCACACCTCCCTGGCA
FGFR1_10	CCTCTGTCACCAGGACATTCCTGGCTGCCCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNCACTCCTTGCTTCTCA
FGFR1_11	CGCACGGGACATTCACCACATCGACTACTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNCAGAGCCTTCCAGCTC
FGFR1_12	GGGTTGTGGCTGGGGTTGTCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNAAGACTAGGGGGGCTCTGTTCCCAC
FGFR1_13	AGCAGCTCTCCTCCAAGGACCTGGTGTCCCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNTCTTCTCCTGTGCCTG
FGFR1_14	CCACCCCCAGCAGCACACCCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNGGGGCCTCCGGGCCTGCAGGTACTCC
FGFR1_15	GCTAGGGAAGGGGGTTAAGAGAGGCTGCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNGGGAAGCATAAGAATA
FGFR1_16	CGCAGGATGGTGGGTGCCGGCCAGACTGCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNCAAGTAAATGAGTCTCA
FGFR1_17	CCCATTCCAAGCAAACAGCAGGCCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNTGGGAGAGGGCTGCTTTGGG
FGFR1_18	CGTGTGACCAAAGTGGCTGTGAAGATGTCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNAACTTCACCAGCCCCAA
FGFR1_19	CGAACCAGAAGAACCCCAGAGTTCATGGACTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNAATGCCTTCAAAAAGT
FGFR1_20	GCAAGGAGGGGGGACGGGGTGACTCCTCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNTCATACTCAGAGACCC
FGFR1_21	TGCACACTCAGCACCACCCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNATCTCCTGCATGGTGGGGTCGGTCATC
FGFR1_22	GGTACCAAGAAGAGTGACTTCCACAGCCACTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNCACTGACTCAGCCCTG
FGFR1_23_SNPa	AGGCC**G**GCAGTGATGACCTCGCCCCTGTACTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNCATGGTTCTTCTCCCT
FGFR1_23_SNPb	AGGCC**A**GCAGTGATGACCTCGCCCCTGTACTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNCATGGTTCTTCTCCCT
FGFR1_24_SNPa	CGTGCC**C**GTGGCGAGGGCAGGACATCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNCTAGGGAAGCTCTTCTC
FGFR1_24_SNPb	CGTGCC**T**GTGGCGAGGGCAGGACATCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNCTAGGGAAGCTCTTCTC
FGFR1_25	GGGGAGACACAGAGGCAGGAGAGCTGCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNGTGCTTGGCGGGTAAC
FGFR1_26	CGGAAGCAAAATGGACAAGCACAGGACCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNGAGTGATGGGAGAGTC
FGFR1_27	CGTCACTGGGGCTTTGGGGTCAGCTACACTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNACACACACTCCATCTCA
FGFR1_28	AGTAACAGAGGTCACAAAGTGGAGGTGAGCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNTGGGAAGGAGACCACT
FGFR1_29	CGGGGGCTCAAGTTCCTGTGGGCAGCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNGCACATCCAGTGGCTAAAG
FGFR1_30	CGGTGAGGGGACCGCTCTGTGGACTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNAATCTTGCTCCCATTCACCTC
FGFR1_31	GGGTGGGCTCACCTGCGCCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNTTACACATGAACTCCACGTTGCTACC
FGFR1_32	AAGAGCCAGGCTTGGAGAACACAGCCCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNTGGACTCTGTGGTGCCCT
FGFR1_33	GGCAACTACACCTGCATTGTGGAGACTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNAGGTGCCACGGGGTGC
FGFR1_34	AGGGGAGGCCGAGTTAGGAAGTCCTGATTCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNCAAATGCCCTTCCAGT
FGFR1_35	GGGACCCCAAACCCCACACCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNAGCTGCTCCTCTCCACCCTGCCT
FGFR1_36	GGGAAGAAGAAGGGGCACTGAGGTTCCTCCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNCAGACCCAAAGGGCAG
FGFR1_37	GGACACCCTCCCCATGGGGATCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNTTTCTCCTCTGAAGAGGAGTCA
FGFR1_38_SNPa	GGGCAC**G**GAGTCCTGCACCTCCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNTGGCAGAGAGGGCTGGAGGGGG
FGFR1_38_SNPb	GGGCAC**A**GAGTCCTGCACCTCCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNTGGCAGAGAGGGCTGGAGGGGG
FGFR1_39	TGCTCTGCACATCGTCCCCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNTGGTGTCACTGCCCGAGGGGCTGCTG
FGFR1_40	TCAACTGGCTGCGGGACGCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNTCTCTCGCCCCTTGGCTTCCCTTC
FGFR1_41	CGTAATAAAAAAACCTCTGCAGAGGGCCCCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNTTTTGGGGCTCTCTCC
FGFR1_42	GGGCAGCCTGGACTCCTGGGCCTTGGGACTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNTCACCAACCTCTAACT
FGFR1_43	GGATGTGGAGCTGGAAGTGCCTCCTCTCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNTCCCCTACCTAGACCCT
FGFR1_44	CCCTCTGATGAGTGGGAAACTGAGATGTGCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNCTATCTCACCAGACAC

Sequences of all 47 smMIPs used in this study are listed. The string of five N's represents the degenerate molecular tag. Three smMIPs overlapped a common SNP, so smMIPs complimentary to both alleles (in red) were used, and labeled "a" and "b"

TABLE S5: Coverage depth at the two *FGFR1* mutation sites for each sample sequenced by smMIPs

Cohort	Individual	Tissue	c.1638C>A (p.Asn546Lys)	c.1966A>G (p.Lys656Glu)
Exome sequencing	**LR13-278**	Unaffected Skin	50/160 (31%)	0/22
		Scalp Nevus	52/108 (48%)	0/12
		Eyelid Dermoid	42/76 (55%)	low coverage
		Blood	0/153	0/29
		Saliva	0/27	0/28
	IN_0039	Scalp Nevus	30/83 (36%)	low coverage
		Saliva	0/114	low coverage
		Buccal	0/40	low coverage
		Blood	0/51	low coverage
Tissue biopsy available	**LR14-261**	Scalp Nevus	110/199 (55%)	0/67
		Saliva	0/36	low coverage
	LR04-090	Unaffected skin	0/119	0/22
		Saliva	0/228	0/36
		Blood	0/119	0/23
	LR09-120	Scalp Nevus	0/35	0/19
		Saliva	0/124	0/24
	IN_0025	Lipoma	0/117	0/22
		Blood	0/211	0/26
		Saliva	0/94	0/25
Blood/Saliva Only	**LR04-093**	Blood	0/152	0/39
	LR09-252	Saliva	0/105	0/28
	LR14-210	Blood	0/227	0/49

Low coverage was defined as less than 10 independent reads