Supplemental Tables and Figure

Senthilkumar Ramamoorthy et al: “Biallelic inactivation of the *NF1* tumor suppressor gene in juvenile myelomonocytic leukemia: Genetic evidence of driver function and implications for diagnostic work-up”

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Table S1. List of *NF1* variants identified in leukemic cells of JMML children with clinical diagnosis of NF-1 and children in the JMML-5neg group

ID	Variant	AA change	VAF (%)	Total reads	Clinvar	ACMG Annotation	ACMG class
NF01	c.7035_7040delinsTA	p.(Leu2345Phefs*50)	44	533	-	PVS1, PM2	LPATH
	c.4600C>T	p.(Arg1534*)	18	220	VCV000220152.4	PVS1, PM2, PP5	PATH
NF02	c.4569del	p.(Ser1524Alafs*50)	94	2490	-	PVS1, PM2	LPATH
NF03	c.5305C>T	p.(Arg1769*)	72	565	RCV000220916.1	PVS1, PM2, PP5	PATH
NF04	c.910C>T	p.(Arg304*)	6	679	VCV000187722.3	PVS1, PM2, PP5	PATH
NF05	c.6855C>A	p.(Tyr2285*)	98	5346	RCV000489640.4	PVS1, PM2, PP5	PATH
NF06	c.1277G>A	p.(Trp426*)	99	3063	-	PVS1, PM2	PATH
NF07	c.6642+1G>C	p.(Glu2214sp*)?	58	657	RCV000457846.1 / -1G?	PVS1, PM2	LPATH
	c.821T>G	p.(Leu274Arg)	50	1384	-	PM2, PP3 (REVEL 0.917)	VUS
NF08	c.1756_1759del	p.(Thr586Valfs*18)	97	2276	VCV000186215.4	PVS1, PM2, PP5	PATH
NF09	c.4110+1G>T	p.(Gln1370sp*)?	98	2675	VCV000582401.2	PVS1, PM2	LPATH
NF10	c.1657dup	p.(His553Profs*5)	95	1042	-	PVS1, PM2	LPATH
NF11	c.6772C>T	p.(Arg2258*)	28	282	RCV000461033.4	PVS1, PM2, PP5	PATH
	c.7189G>C	p.(Gly2397Arg)	53	1890	RCV000497028.2 (G>A)	PS1, PS3, PM2, PP3 (REVEL 0.739)	PATH
	c.2737dup	p.(Ile913Asnfs*6)	13	7414	-	PVS1, PM2	LPATH
NF12	c.574C>T	p.(Arg192*)	32	1149	VCV000040093.4	PVS1, PM2, PP5 (LOVD)	PATH
NF13	c.910C>T	p.(Arg304*)	87	918	VCV000187722.4	PVS1, PM2, PP5	PATH

NF14	c.4084C>T	p.(Arg1362*)	44	6857	VCV000000344.3	PVS1, PM2, PP5	PATH
	c.279_280delinsAA	p.(Cys93*)	47	4999	-	PVS1, PM2	LPATH
NF15	c.2033dup	p.(Ile679Aspfs*21)	41	303	RCV000204850.7	PVS1, PM2, PP5	PATH
	c.1185+1G>T	p.(Lys395sp*)?	50	5305	VCV000219572.2/G>A	PVS1, PM2	LPATH
NF16	c.2446C>T	p.(Arg816*)	93	4100	VCV000280055.4	PVS1, PM2, PP5	PATH
NF17	c.1381C>T	p.(Arg461*)	98	7106	RCV000229618.6	PVS1, PM2, PP5	PATH
NF18	c.4235G>C	p.(Arg1412Thr)	48	6934	VCV000523345.2	PM2, PM6, PP3 (REVEL 0.900), PM5	LPATH
	c.877A>G	p.(Asn293Asp)	50	1408	-	PM2, BP4 (REVEL 0.119)	VUS
NF19	c.7328_7331dup	p.(Val2445Tyrfs*4)	96	3452	-	PVS1, PM2	LPATH
NF20	c.7159_7164del	p.(Asn2387_Phe2388del)	49	1175	-	PS4, PM2, PM4, PM6, PP3, PP5	PATH
NF21	c.4600C>T	p.(Arg1534*)	98	3202	VCV000220152.4	PVS1, PM2, PP5	PATH
NF22	c.6427G>A	p.(Glu2143Lys)	99	1040	RCV000530481.1	PS4_Moderate, PM2, PP3 (REVEL 0.915), PP4	LPATH
NF23	c.1246C>T	p.(Arg416*)	54	1172	VCV000404597.3	PVS1, PM2, PP5	PATH
	c.2033del	p.(Pro678Argfs*10)	43	542	RCV000558816.4	PVS1, PM2, PP6	PATH
NF24	c.4084C>T	p.(Arg1362*)	40	42	RCV000000372.6	PVS1, PM2, PP5	PATH
	c.205-2A>G	p.(Arg69sp*)?	52	4018	RCV000808377.1	PVS1, PM2, PP5 (LOVD)	LPATH
NF25	c.499_502del	p.(Cys167Glnfs*10)	41	671	VCV000185021.3	PVS1, PM2, PP5	PATH
	c.482T>G	p.(Leu161*)	53	657	-	PVS1, PM2	LPATH
NEG01	c.339dup	p.(Leu114Alafs*13)	89	2936	-	PVS1, PM2	LPATH

NEG02	c.711_723del	p.(Pro238Trpfs*39)	46	1487	-	PVS1, PM2	LPATH
NEG03	c.3721C>T	p.(Arg1241*)	32	202	VCV000000361.3	PVS1, PM2, PP5	PATH
NEG04	c.2033dup	p.(Ile679Aspfs*21)	46	691	RCV000204850.7	PVS1, PM2, PP5	PATH
	c.6007-5A>G	p.(Ile2003Leufs*6)	53	1737	RCV000000387.6	PS3, PM2, PP1, PP3, PP5	PATH
NEG05	c.2024dup	p.(Thr676Asnfs*24)	45	217	-	PVS1, PM2	LPATH
	c.2033dup	p.(Ile679Aspfs*21)	33/16.67	228	RCV000204850.7	PVS1, PM2, PP5	PATH
NEG06	c.2033dup	p.(Ile679Aspfs*21)	84	400	RCV000204850.7	PVS1, PM2, PP5	PATH
NEG07	c.3826C>T	p.(Arg1276*)	83	4839	VCV000237556.3	PVS1, PM2, PP5	PATH
NEG08	c.2510G>A	p.(Trp837*)	48	5672	RCV000680802.1	PVS1, PM2, PP5 (LOVD)	PATH
	c.622_636del	p.(Ala208_Val212del)	52	5807	-	PM2, PM4, PP3	VUS

NF1 variants, predicted amino acid change, variant allelic frequency, read depth, Clinvar accession, and predicted pathogenicity are indicated in the table.

Table S2. The computational prediction of pathogenicity of *NF1* missense variants

Amino acid change	Coding sequence change	CADD13	SIFT	Polyphen2	Mutation Taster	FATHMM	PROVEAN	SpliceAI
p.Leu274Arg	c.821T>G	28.2	0	1	1	-1.4	-4.37	No (0.12)
p.Asn293Asp	c.877A>G	17.52	0.769	0	0.991	-0.05	-1.17	No (0)
p.Arg1412Thr	c.4235G>C	24.4	0	0.993	1	-2.48	-4.78	No (0)
p.Glu2143Lys	c.6427G>A	28.8	0.01	0.974	1	-1.74	-3.44	Donor loss (0.70)
p.Gly2397Arg	c.7189G>C	28.7	0.001	0.989	1	1.48	-6.45	Donor loss (0.54)

Five missense *NF1* variants identified in the cohort and their pathogenicity as predicted by different algorithms are listed in the table. The variants are not reported in the healthy population (gnomAD, v.2.1.1). The computational tools predicted a deleterious effect on protein function for the four missense variants p.Leu274Arg, p.Asn293Asp, p.Glu2143Lys, and p.Gly2397Arg (pathogenic score values above the threshold are highlighted by red background). The variants c.6427G>A and c.7189G>C affect the last nucleotide of exons 42 and 48, respectively. Besides causing the predicted amino acid exchange, the proximity of these variants to conserved splice sites may also impair exon splicing, as predicted by the algorithm spliceAI.

Table S3. List of *NF1* variants identified in leukemic cells of children with JMML and canonical *PTPN11* or *NRAS* mutations

ID	Primary variant	NF1 variant	AA change	VAF (%)	Total reads	ClinVar	ACMG Annotation	ACMG class
PTPN11-01	PTPN11;c.227A>G; p.(Glu76Gly); 45.9%	c.1236_1237insAA	p.(Ser413Asnfs*61)	24	652	-	PVS1, PM2, PP3	LPATH
PTPN11-02	PTPN11;c.227A>C; p.(Glu76Ala); 26.6%	c.7439A>G	p.(His2480Arg)	49	1175	VCV000229064.3	PM1	VUS
PTPN11-03	PTPN11;c.181G>T; p.(Asp61Tyr); 47.95%	c.271dup	p.(Glu91Glyfs*16)	10	9321	-	PVS1, PM2, PP3	LPATH
PTPN11-04	PTPN11;c.227A>C; p.(Glu76Ala); 44.3%	c.2033dup	p.(Ile679Aspfs*21)	10	328	VCV000141513.3	PVS1, PM2, PP5	PATH
PTPN11-05	PTPN11;c.181G>T; p.(Asp61Tyr); 47.42%	c.7784_7785del	p.(Lys2595Serfs*5)	17	3505	VCV000652439.1	PVS1, PM2, PP3, PP5	PATH
		c.1885G>A	p.(Gly629Arg)	11	446	VCV000068308.3	PS3, PM1, PM2, PP5	PATH
PTPN11-06	PTPN11;c.1504T>C; p.(Ser502Pro); 42.61%	c.574C>T	p.(Arg192*)	22	5270	VCV000040093.3	PVS1, PM2, PP5 (LOVD)	PATH
PTPN11-07	PTPN11;c.214G>A; p.(Ala72Thr);46.71%	c.2033dup	p.(Ile679Aspfs*21)	6	431	VCV000141513.3	PVS1, PM2, PP5	PATH

PTPN11-08	PTPN11;c.227A>G; p.(Glu76Gly); 45.9%	c.5671C>T	p.(Gln1891*)	28	2359	VCV000237577.1	PVS1, PM2, PP3, PP5	PATH
PTPN11-09	PTPN11;c.179G>T; p.(Gly60Val); 70.08%	c.6852_6855del	p.(Tyr2285Thrfs*5)	40	2937	VCV000216866.4	PVS1, PM2, PP3, PP5	PATH
NRAS-01	NRAS;c.35G>C; p.(Gly12Ala); 50.48%	c.1802G>A	p.(Arg601Gln)	52	6805	VCV000404478.2	PM2	VUS
NRAS-02	NRAS;c.38G>A; p.(Gly13Asp); 47.08%	c.2033dup	p.(Ile679Aspfs*21)	19	401	VCV000141513.3	PVS1, PM2, PP5	PATH
		c.7701dup	p.(Lys2568Glnfs*9)	58	911	-	PVS1, PM2, PP3	LPATH
NRAS-03	NRAS;c.38G>A; p.(Gly13Asp); 47.14%	c.2065G>A	p.(Val689Met)	39	248	VCV000404577.2	PM1, PM2	VUS

The primary *PTPN11* or *NRAS* variants*, NF1* variants, resulting amino acid change, variant allelic frequency, read depth, Clinvar accession, and the predicted pathogenicity of the variants are indicated in the table.

Table S4. Analysis of loss of heterozygosity of the *NF1* locus in leukemic cells of children with JMML and clinical diagnosis of NF-1

ID	SNP Array	Molecular karyotype	Suggested first hit	Suggested second hit	First hit confirmed in fibroblasts / buccal cells	Interpretation
NF02	UPD of (almost) the whole q arm of chr. 17	arr[GRCh37] 17q11.2q25.3(28870726_81041938)x2 hmz	Point mutation c.4569del	UPD 17q	Not done / no material	Biallelic LOF in the leukemic cells demonstrated with a pathogenic point mutation followed by UPD 17q. The point mutation could be germline (clinical diagnosis of NF-1), but this was not proven.
NF03	UPD of (almost) the whole q arm of chr. 17	arr[GRCh37] 17q11.1q25.3(25309336_81041938)x2 hmz	Point mutation c.5305C>T	UPD 17q	Not done / no material	Biallelic LOF in the leukemic cells demonstrated with a pathogenic point mutation followed by UPD 17q. The point mutation could be germline (clinical diagnosis of NF-1), but this was not proven.
NF04	Typical 1.4 Mb *NF1* microdeletion (type 1 deletion) in an apparent heterozygous status	arr[GRCh37] 17q11.2(29055565_30395625)x1	Probably *NF1* microdeletion	Possibly point mutation c.910C>T (cave: low VAF in JMML DNA)	Not done / no material	Likely NF-1 related JMML with a heterozygous *NF1* microdeletion (type 1) in leukemic cells that may represent a germline mutation. The variant on the second allele remains unclear. The significance of the nonsense mutation remains doubtful, as its VAF is too low for what we expect for a second hit in a JMML sample and was not detectable in the JMML exome.

NF05	UPD of (almost) the whole q arm of chr. 17	arr[GRCh37] 17q11.2q25.3(26337447_81041938)x2 hmz	Point mutation c.6855C>A	UPD 17q	Not done / no material	Biallelic LOF in the leukemic cells demonstrated with a pathogenic point mutation followed by UPD 17q. The point mutation could be germline (H6), but this was not proven.
NF06	UPD of (almost) the whole q arm of chr. 17	arr[GRCh37] 17q11.2q25.3(26139558_81041938)x2 hmz	Point mutation c.1277G>A	UPD 17q	Not done / no material	Biallelic LOF in the leukemic cells demonstrated with a pathogenic point mutation followed by UPD 17q. The point mutation could be germline (clinical diagnosis of NF-1), but this was not proven.
NF08	UPD of (almost) the whole q arm of chr. 17	arr[GRCh37] 17q11.2q25.3(26400969_81041938)x2 hmz	Point mutation c.1756_1759del	UPD 17q	Not done / no material	Biallelic LOF in the leukemic cells demonstrated with a pathogenic point mutation followed by UPD 17q. The point mutation could be germline (clinical diagnosis of NF-1), but this was not proven.
NF09	Type 1 deletion	Published data (Steinemann et al.)	NF1 microdeletion	Point mutation c.4110+1G>T	Yes: copy number loss chr17:28889920-30501044; absence of c.4110+1G>T	Biallelic LOF in leukemic cells strongly suggested with a *NF1* microdeletion and a likely pathogenic point mutation as the secondary event. The *NF1* microdeletion is suggested to be germline.
NF10	Microdeletion encompassing the *NF1* locus (atypical 2.5 Mb deletion) in an apparent heterozygous status	arr[GRCh37] 17q11.2(27944929_30458834)x1	Undetermined	Undetermined	Not done / no material	Biallelic LOF in leukemic cells strongly suggested with a *NF1* microdeletion and a likely pathogenic point mutation on the non-deleted allele. Chronology of events undetermined. One of the changes could be germline (clinical diagnosis of NF-1), but this was not proven.

NF12	Typical 1.4 Mb *NF1* microdeletion (type 1 deletion) in an apparent heterozygous status; additionally, mosaic trisomy 8 (fraction 20-40%)	arr[GRCh37] 17q11.2(28997791_30386515)x1, 8p23.3q24.3(158048_146295771)x2-3	NF1 microdeletion	Point mutation c.574C>T	Yes; copy number loss chr17:29119495-30228792; absence of c.574C>T	Biallelic LOF in leukemic cells demonstrated with a *NF1* microdeletion and a pathogenic point mutation as the secondary event. The *NF1* microdeletion is suggested to be germline.
NF13	Typical 1.2 Mb *NF1* microdeletion (type 2 deletion) in an apparent heterozygous status	arr[GRCh37] 17q11.2(29103297_30294925)x1	NF1 microdeletion	Point mutation c.910C>T	Yes; copy number loss chr17:29096017-30498238; absence of c.910C>T	Biallelic LOF in leukemic cells demonstrated with a *NF1* microdeletion and a pathogenic point mutation as the secondary event. The *NF1* microdeletion is suggested to be germline.
NF16	UPD of (almost) the whole q arm of chr. 17	arr[GRCh37] 17q11.2q25.3(28836600_81041938)x2 hmz	Point mutation c.2446C>T	UPD 17q	c.2446C>T not covered; absence of UPD 17q	Biallelic LOF in the leukemic cells demonstrated with a pathogenic point mutation followed by UPD 17q. The point mutation could be germline (clinical diagnosis of NF-1), but this was not proven.

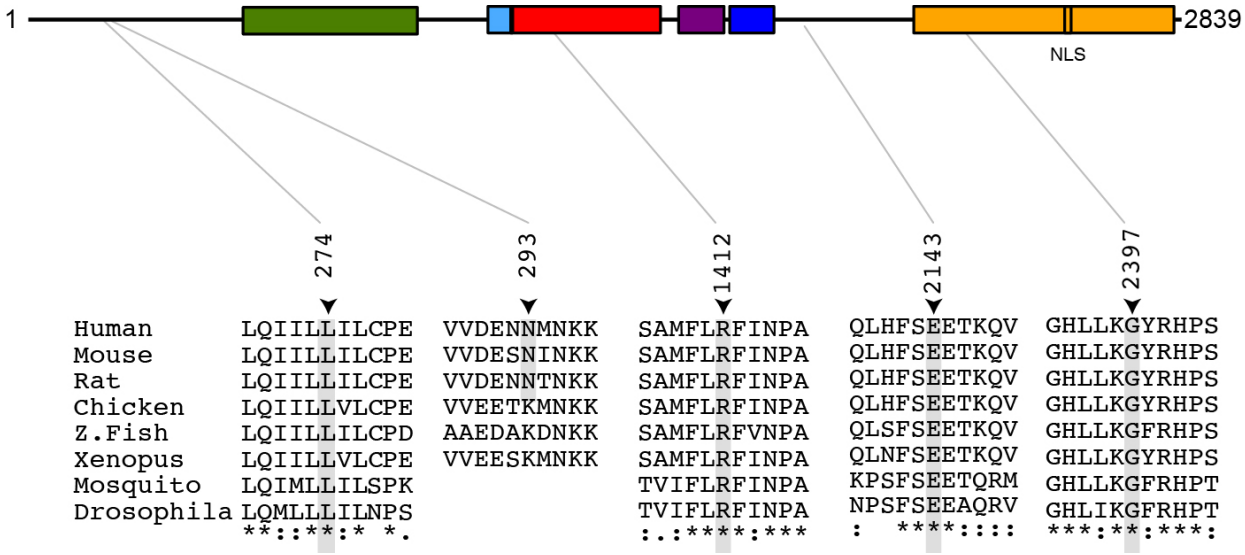
NF17	UPD of (almost) the whole q arm of chr. 17	arr[GRCh37] 17q11.2q25.3(26739139_81041938)x2 hmz	Point mutation c.1381C>T	UPD 17q	Not done / no material	Biallelic LOF in the leukemic cells demonstrated with a pathogenic point mutation followed by UPD 17q. The point mutation could be germline (clinical diagnosis of NF-1), but this was not proven.
NF19	UPD of (almost) the whole q arm of chr. 17	arr[GRCh37] 17q11.2q25.3(27801069_81041938)x2 hmz	Point mutation c.7328_7331dup	UPD 17q	Not done / no material	Biallelic LOF in the leukemic cells strongly suggested with a likely pathogenic point mutation followed by UPD 17q. The point mutation could be germline (clinical diagnosis of NF-1), but this was not proven.
NF21	UPD of (almost) the whole q arm of chr. 17	arr[GRCh37] 17q11.2q25.3(26985248_81041938)x2 hmz	Point mutation c.4600C>T	UPD 17q	Not done / no material	Biallelic LOF in the leukemic cells demonstrated with a pathogenic point mutation followed by UPD 17q. The point mutation could be germline (clinical diagnosis of NF-1), but this was not proven.
NF22	UPD of (almost) the whole q arm of chr. 17	arr[GRCh37] 17q11.1q25.3(25309336_81041938)x2 hmz	Point mutation c.6427G>A	UPD 17q	Not done / no material	Biallelic LOF in the leukemic cells strongly suggested with a likely pathogenic point mutation followed by UPD 17q. The point mutation could be germline (clinical diagnosis of NF-1), but this was not proven.

Table S5. Analysis of loss of heterozygosity of the *NF1* locus in leukemic cells of children in the JMML-5neg group

ID	SNP Array	Molecular karyotype	Suggested first hit	Suggested second hit	First hit confirmed in fibroblasts / buccal cells	Interpretation
NEG01	Typical 1.4 Mb *NF1* microdeletion (type 1 deletion) in an apparent heterozygous status	arr[GRCh37] 17q11.2(29013191_30391813)x1	Undetermined	Undetermined	Absence of c.339dup in buccal cells; no clear evidence of the deletion	Biallelic LOF in leukemic cells strongly suggested with a *NF1* microdeletion and a likely pathogenic point mutation on the non-deleted allele. Chronology of events undetermined. The point mutation was excluded as a germline event.
NEG02	No LOH	Normal	Unclear	Point mutation c.711_723del (VAF 20% on genomic *NF1* sequencing)	First hit unclear, point mutation c.711_723del absent	Possibly NF1-related JMML with one likely pathogenic *NF1* mutation in leukemic cells that probably represents a somatic event. The variant on the other allele remains undetected.
NEG05	No LOH; monosomy 7 mosaic (fraction 50-70%)	arr[GRCh37] 7p22.3q36.3(43360_159119707)x1-2	Point mutation c.2024dup	Point mutation c.2033dup	c.2024dup not covered	Two likely pathogenic variants in the leukemic cells, proven to be in trans; the variant showing lower allele frequency is suggested to be the second event. Germline status remained unclear (site of variants not covered by germline exome, no material left).

NEG06	Microdeletion encompassing the *NF1* locus (atypical 1.8 Mb deletion) in an apparent heterozygous status	arr[GRCh37] 17q11.2(28467244_30243583)x1	Undetermined	Undetermined	Not done / no material	Biallelic LOF in leukemic cells demonstrated with a *NF1* microdeletion and a pathogenic point mutation on the non-deleted allele. Chronology of events and germline status remain undetermined.
NEG07	Typical 1.4 Mb *NF1* microdeletion (type 1 deletion) in an apparent heterozygous status	arr[GRCh37] 17q11.2(29012966_30369402)x1	Undetermined	Undetermined	Not done / no material	Biallelic LOF in leukemic cells demonstrated with a *NF1* microdeletion and a pathogenic point mutation on the non-deleted allele. Chronology of events and germline status remain undetermined.

Figure S1. Evolutionary conservation of missense NF1 variants



A sequence alignment of five *NF1* missense variants and flanking regions across 8 species is shown. The functional regions of neurofibromin are color-coded as in Figure 1.