

— Supplementary Material —

Low efficacy of genetic tests for the diagnosis of primary lymphedema prompts novel insights into the underlying molecular pathways

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List of diagnostic and candidate genes

Table S1. List of diagnostic genes for primary lymphedema. Name of the gene, OMIM number, gene-phenotype relationship, relative reference and inheritance, involved pathway, relative reference and effect on the pathway are reported. References are from OMIM, Kegg, or literature.

Gene	OMIM	Gene-Phenotype relationship	Reference	Inheritance	Pathways	Reference	Effect
ADAMTS3	605011	Hennekam lymphangiectasia-lymphedema syndrome 3	618154	AR	VEGFR3 pathway	[24]	Activation
AKT1	164730	Proteus syndrome, somatic	176920		Ras pathway	map04014	Inactivation
					PI3K/AKT pathway	map04151	Activation
BRAF	164757	Noonan syndrome 7	613706	AD	MAPK pathway	map04010	Activation
CBL	165360	Noonan syndrome-like disorder with or without juvenile myelomonocytic leukemia	613563	AD	Ras pathway	[116]	Inactivation
CCBE1	612753	Hennekam lymphangiectasia-lymphedema syndrome 1	235510	AR	VEGFR3 pathway	[21]	Activation
CELSR1	604523	Lymphatic malformation 9	619319	AD	Planar cell polarity	[117]	Activation
EPHB4	600011	Capillary malformation-arteriovenous malformation 2	618196	AD	Ras pathway	[118]	Inactivation
		Lymphatic malformation 7	617300	AD			
FAT4	612411	Hennekam lymphangiectasia-lymphedema syndrome 2	616006	AR	Planar cell polarity	[89]	Activation
FLT4	136352	Lymphatic malformation 1	153100	AD	Ras pathway	map04014	Activation
					PI3K/AKT pathway	map04151	Activation

FOXC2	602402	Lymphedema-distichiasis syndrome	153400	AD	Transcription factors	[75]	Activation
GATA2	137295	Emberger syndrome	614038	AD	Transcription factors	[12]	Activation
GJA1	121014	Oculodentodigital dysplasia	164200		Lymphatic valve development	[91]	Activation
GJC2	608803	Lymphatic malformation 3	613480	AD	Lymphatic valve development	[92]	Activation
HGF	142409	Primary lymphedema	[8]		Ras pathway	map04014	Activation
					PI3K/AKT pathway	map04151	Activation
HRAS	190020	Costello syndrome	218040	AD	Ras pathway	map04014	Activation
					PI3K/AKT pathway	map04151	Activation
KIF11	148760	Microcephaly with or without chorioretinopathy, lymphedema, or mental retardation	152950	AD	PI3K/AKT pathway	[119]	Activation
KRAS	190070	Noonan syndrome 3	609942	AD	Ras pathway	map04014	Activation
					PI3K/AKT pathway	map04151	Activation
NRAS	164790	Noonan syndrome 6	613224	AD	Ras pathway	map04014	Activation
					PI3K/AKT pathway	map04151	Activation
PIEZO1	611184	Lymphatic malformation 6	616843	AR	PI3K/AKT pathway	[120]	Activation
					Lymphatic valve development	[94]	Activation
PIK3CA	171834	CLAPO syndrome, somatic	613089		Ras pathway	map04014	Activation
		CLOVE syndrome, somatic	612918		PI3K/AKT pathway	map04151	Activation
PTPN11	176876	Noonan syndrome 1	163950	AD	Ras pathway	map04014	Activation
					PI3K/AKT pathway	[121]	Activation
PTPN14	603155	Choanal atresia and lymphedema	613611	AR	VEGFR3 pathway	[25]	Activation
RASA1	139150	Capillary malformation-arteriovenous malformation 1	608354	AD	Ras pathway	map04014	Inactivation
RIT1	609591	Noonan syndrome 8	615355	AD	Ras pathway	[50]	Activation
SHOC2	602775	Noonan syndrome-like with loose anagen hair 1	607721	AD	Ras pathway	map04014	Activation
SOS1	182530	Noonan syndrome 4	[122]	AD	Ras pathway	map04014	Activation
					PI3K/AKT pathway	map04151	Activation
SOX18	601618	Hypotrichosis-lymphedema-telangiectasia syndrome	607823	AR	Transcription factors	[12]	Activation
		Hypotrichosis-lymphedema-telangiectasia-renal defect syndrome	137940	AD			
VEGFC	601528	Lymphatic malformation 4	615907	AD	Ras pathway	map04014	Activation
					PI3K/AKT pathway	map04151	Activation

Table S2. List of candidate genes for primary lymphedema. Name of the gene, OMIM number, gene-phenotype relationship, relative reference and inheritance, involved pathway, relative reference and effect on the pathway are reported. References are from OMIM, Kegg, or literature.

Gene	OMIM	Gene-Phenotype relationship	Reference	Inheritance	Pathways	Reference	Effect
ACKR2	602648	Lymphatic vascular development	[105]		Macrophage recruitment/activation	[105]	Activation
ADM	103275	Lymphatic vascular development	[123]		Ras pathway	[124]	Activation
		Predisposition to secondary lymphedema	[125]		PI3K/AKT pathway	[124]	Activation
					Vascular smooth muscle contraction	map04270	Activation
ANGPT2	601922	Lymphatic malformation 10	619369	AD	Ras pathway	map04014	Inactivation
					PI3K/AKT pathway	map04151	Inactivation
ANGPTL4	605910	Lymphatic vascular development	[126]		Rho and cytoskeletal remodelling pathway	[127]	Activation
					PI3K/AKT pathway	[128]	Inactivation
ARAF	311010	Noonan syndrome	H01738		Ras pathway	map04010	Activation
		Noonan syndrome and related disorders	H00523		Vascular smooth muscle contraction	map04270	Activation
ARAP3	606647	Lymphatic vascular development	[129,130]		Rho and cytoskeletal remodelling pathway	map04015	Activation
					PI3K/AKT pathway	[130]	Activation
PPP1R13B	606455	Lymphatic vascular development	[131]		NFkB pathway	[132]	Inactivation
CALCRL	114190	?Lymphatic malformation 8	618773	AR	Ras pathway	[124]	Activation
					PI3K/AKT pathway	[124]	Activation
					Vascular smooth muscle contraction	map04270	Activation
CDH5	601120	Lymphedema	[133]		Rho and cytoskeletal remodelling pathway	[134]	Activation
					Mechanotransduction	[99]	Activation
					PI3K/AKT pathway	[79]	Activation
					β -catenin pathway	[79]	Activation
CDK5	123831	Lymphatic vascular development	[78]		Transcription factors	[78]	Activation
CYP26B1	605207	Lymphedema	[102]		RA pathway	[102]	Activation
DCHS1	603057	Lymphatic valve development	[90]		Hippo pathway	map04392	Activation
EFNB2	600527	Lymphatic valve development	[135]		Lymphatic valve development	[135]	Activation
EMILIN1	130660	Lymphatic valve development	[136]		Lymphatic valve development	[136]	Activation
FABP4	600434	Lymphedema	[137]		Macrophage recruitment/activation	[106]	Activation
FLT1	165070	Lymphatic vascular development	[138]		Ras pathway	map04014	Activation
					PI3K/AKT pathway	map04151	Activation
FOXC1	601090	Lymphatic vascular development	[139]		Transcription factors	[140]	Activation
FOXC2-AS1		Lymphedema	[75]		Transcription factors	[75]	Activation
GDF2	605120	Lymphatic vascular development	[141]		Transcription factors	[82,142]	Inactivation
		Lymphatic valve development	[141]				
GJA4	121012	Lymphatic valve development	[142]		Lymphatic valve development	[142]	Activation
		Predisposition to secondary lymphedema	[143]				
HHEX	604420	Lymphatic vascular development	[144]		Transcription factors	[144]	Activation

HOXD10	142984	Lymphatic vascular development	[145]		Rho and cytoskeletal remodelling pathway	[145]	Inactivation
IKBKG	300248	Ectodermal dysplasia and immunodeficiency 1	300291	XLR	Ras pathway	map04014	Activation
					PI3K/AKT pathway	map04151	Activation
					NFkB pathway	[146]	Activation
ITGA5	135620	Lymphatic vascular development	[147]		PI3K/AKT pathway	map04151	Activation
ITGA9	603963	Lymphatic valve development	[148]		PI3K/AKT pathway	map04151	Activation
LCP2	601603	Predisposition to secondary lymphedema	[149]		Rho and cytoskeletal remodelling pathway	[150]	Activation
LPAR1	602282	Lymphedema	[151]		PI3K/AKT pathway	map04151	Activation
LPAR2	605110	Lymphedema	[151]		PI3K/AKT pathway	map04151	Activation
LPAR3	605106	Lymphatic vascular development	[152]		Ras pathway	[153]	Activation
		Lymphatic vascular development	[152]		PI3K/AKT pathway	map04151	Activation
LPAR4	300086	Lymphatic vascular development	[153]		PI3K/AKT pathway	map04151	Activation
LYVE1	605702	Lymphedema	[154]		PI3K/AKT pathway	map04151	Activation
LZTR1	600574	Noonan syndrome 10	616564	AD	Ras pathway	[155]	Inactivation
		Noonan syndrome 2	605275	AR			
MAP2K1	176872	Noonan syndrome and related disorders	H00523		Ras pathway	map04014	Activation
					Vascular smooth muscle contraction	map04270	Activation
MAP2K2	601263	Noonan syndrome and related disorders	H00523		Ras pathway	map04014	Activation
					Vascular smooth muscle contraction	map04270	Activation
MAP4K4	604666	Lymphatic vascular development	[156]		Ras pathway	[157]	Inactivation
MET	164860	Lymphedema	[27]		Ras pathway	map04014	Activation
					PI3K/AKT pathway	map04151	Activation
NFATC1	600489	Lymphatic valve development	[158]		Transcription factors	[158]	Activation
NOTCH1	190198	Lymphatic vascular development	[77]		Transcription factors	[77]	Inactivation
NPPA	108780	Lymphatic vessels contraction and permeability	[159]		Vascular smooth muscle contraction	map04270	Activation
NPPB	600295	Lymphatic vessels contraction and permeability	[159]		Vascular smooth muscle contraction	map04270	Activation
NR2F2	107773	Lymphatic vascular development	[77]		Transcription factors	[77]	Activation
NRP1	602069	Lymphedema	[160]		Rho and cytoskeletal remodelling pathway	[127]	Activation
NRP2	602070	Lymphedema	[160]		Rho and cytoskeletal remodelling pathway	[127]	Activation
PDPN	608863	Lymphedema	[161]		Rho and cytoskeletal remodelling pathway	[60]	Activation
PLCG2	600220	Lymphatic vascular development	[162]		Ras pathway	map04014	Activation
PLXNA1	601055	Lymphatic valve development	[163]		PI3K/AKT pathway	[164]	Inactivation
					Rho and cytoskeletal remodelling pathway	[164]	Inactivation
PPP1CB	600590	Noonan syndrome-like disorder with loose anagen hair 2	617506	AD	Hippo pathway	hsa04390	Activation
					Ras pathway	[165]	Inactivation
					Vascular smooth muscle contraction	map04270	Inactivation
PROX1	601546	Lymphedema	[74]		Ras pathway	[38]	Activation

RAF1	164760	Noonan syndrome 5	611553	AD	Ras pathway	map04014	Activation
					Vascular smooth muscle contraction	map04270	Activation
RAMP2	605154	Lymphatic vascular development	[123]		Ras pathway	[124]	Activation
					PI3K/AKT pathway	[124]	Activation
					Vascular smooth muscle contraction	map04270	Activation
RELN	600514	Lissencephaly 2 (Norman-Roberts type)	257320	AR	PI3K/AKT pathway	map04151	Activation
RORC	602943	Lymphedema	[83]		Transcription factors	[83]	Activation
S1PR1	601974	Lymphatic vascular development	[61]		Ras pathway	[166]	Activation
					PI3K/AKT pathway	[166]	Activation
					NFkB pathway	[166]	Activation
S1PR2	605111	Lymphatic vessels contraction	[167]		Ras pathway	[166]	Activation
					Rho and cytoskeletal remodelling pathway	[166]	Activation
					PI3K/AKT pathway	[166]	Activation
					NFkB pathway	[166]	Activation
S1PR3	601965	Lymphatic vascular development	[168]		Rho and cytoskeletal remodelling pathway	[166]	Activation
S1PR4	603751	Lymphatic vascular development	[152]		Ras pathway	[166]	Activation
S1PR5	605146	Lymphatic vascular development	[152]		Ras pathway	[166]	Activation
SDC4	600017	Lymphatic vascular development	[169]		Planar cell polarity	[170]	Activation
SEMA3A	603961	Lymphedema	[171]		Rho and cytoskeletal remodelling pathway	[172]	Activation
SMARCA4	603254	Lymphatic vascular development	[173]		Transcription factors	[174]	Activation
SOS2	601247	Noonan syndrome 9	616559	AD	Ras pathway	map04014	Activation
SOX17	610928	Lymphatic vascular development	[175]		Transcription factors	[175]	Activation
SPRED1	609291	Lymphatic vascular development	[38]		Ras pathway	[38]	Inactivation
SPRED2	609292	Noonan syndrome-like phenotype	[176]		Ras pathway	[38]	Inactivation
SVEP1	611691	Lymphedema	[104]		Cell adhesion	[104]	Inactivation
					PI3K/AKT pathway	[177]	Activation
SYK	600085	Lymphedema	[137]		PI3K/AKT pathway	map04151	Activation
PECAM1	173445	Lymphatic valve development	[100]		Mechanotrasduction	[100]	Activation
TACR1	162323	Lymphedema	[178]		Ras pathway	[179]	Activation
TIE1	600222	Lymphatic malformation 11	619401	AD	PI3K/AKT pathway	[79]	Inactivation
					Ras pathway	[180]	Inactivation
VANGL2	600533	Lymphedema	[89]		Planar cell polarity	[65]	Activation
					Rho and cytoskeletal remodelling pathway	[65]	Activation
VCAM1	192225	Lymphedema	[137]		NFkB pathway	map04064	Activation