SUPPLEMENTARY INFORMATION

Phenotypic expansion of *DGKE*-associated diseases

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SUPPLEMENTARY METHODS

Patients and families.

Patient recruitment was performed at G.Gaslini Institute in Genoa Italy between 2007 and 2011. We collected peripheral blood samples for DNA isolation from the proband, her parents and her brother. The Institutional Review Board for Columbia University, and local ethic review committees in Genoa approved the study protocol.

Genotyping, Mapping and Sequencing.

Genomic DNA was purified from peripheral blood cells using standard procedures previous informed consent. Genome wide genotype was conducted on individuals 50, 51 and 58, using the Omni1-quad chips (Illumina). Genotype data were processed for quality controls using Genome Studio and PLINK softwares[1] as previously described[2]. Homozygosity mapping was performed using Homozygositymapper program (http://www.homozygositymapper.org/) as previously described[3]. Whole exome sequencing was performed on individual 58, using an Illumina HiSeq 2000, as previously described[4]. Briefly, for each capture experiment, 3 μg of genomic DNA was fragmented, linkers were ligated to the ends and a library was prepared. Genomic DNA was annealed to capture probes, and bound genomic DNA was eluted and subjected to sequencing. Next-Gen sequencing was then performed on an Illumina HiSeq 2000 machine. Sequence reads were converted to FASTQ format and mapped to the reference genome. Reads that aligned to the targeted exome were extracted and statistics on coverage were collected using a Perl script. Positions found to harbor heterozygous or homozygous variants that deviate from the reference sequence were identified and rare or novel SNPs were identified by comparison to the reference genome, 1000 Genomes data and dbSNP. Low-probability SNVs were identified by empiric methods that we have found significantly reduce false-positive calls: low-quality bases (quality scores <45), heterozygous calls based on low read coverage (<8X), variants that appear exclusively or with high frequency at the same read position on the same strand (implying a preponderance of non-independent reads), and low quality

genotype calls using samtools (<40). Quality score ≥30 for SNVs, ≥60 for indels, and coverage ≥8X identified high-quality variants.

Allelic frequencies were compared to dbSNP, 1000 genome, the NHLBI Exome Variant Server (http://evs.gs.washington.edu/EVS/), and to exomes from 45 IgAN patients ran in-house at the same time of this exome study.

Linkage analysis was performed using ALLEGRO 2.0 software[5] using an autosomal recessive model with a disease gene frequency of 0.001 and phenocopy rate of 0.001.

Sanger sequencing was performed to validate exome sequencing results in persons 50, 51, 58, and 59. Primers used to direct PCR at the *DGKE* p.K101X mutation were: Forward – GGCACCTGATCTTGTGGAC; Reverse – ACACCACTCTATGAAATCCTG.

Anti-factor H Autoantibody Test

Test for autoantibodies against factor H was performed using an ELISA assay with plates covered with purified human factor H, as previously described[6].

Kidney Biopsy

Biopsy was performed for the indication of persistent proteinuria previous informed consent and tissue processed for light microscopy and immunofluorescence microscopy according to the standard techniques.

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Supplementary Table 1. Regions of significant homozygosity identified by studying individual 58 and her parents.

Chr	Start (Mb)	End (Mb)	Start (SNP)	End (SNP)	Size (Mb)
1	76469334	87311182	rs1251531	rs493341	10.84
5	167591402	178935665	rs891933	rs6864599	11.34
9	81196948	87820596	rs1505637	rs7850866	6.62
10	54413554	77850698	rs991681	rs4746357	23.44
10	108597250	111950733	rs10786978	rs4918486	3.35
17	46081041	55158811	rs757351	rs7217371	9.08
Total					64.67

Chr = chromosome; Mb = megabases (distances are based on Hg 18); SNP = single nucleotide polymorphism.

Supplementary Table 2. Variants identified in genes of the complement and cobalamin deficiency pathways in Individual 58.

Gene	Chr	Position	Type	Genotype	Reference allele	Alternative allele	rsID	AAF (dbSNP)	AAF (1000 genomes)	MAF (EVS)
C1R	Chr12	7244956	intronic	Het	A	T	rs12422724	0.37	0.41	0.39
	Chr12	7244962	intronic	Het	T	G	rs12426834	0.37	0.41	0.39
C1S	Chr12	7170336	missense	Het	G	A	rs12146727	0.12	0.13	0.13
		7175872	intronic	Het	T	C	rs11838267	0.11	0.14	0.13
C1QA	Chr1	22965438	synonymous	Het	A	G	rs172378	0.55	0.40	0.37
C1QB	Chr1	-	-	-	-	-	-	-	-	-
C1QC	Chr1	-	-	-	-	-	-	-	-	-
C1QL2	Chr2	119915249	synonymous	Hom	T	C	rs7556873	0.64	0.70	0.25
	Chr2	120704164	indel	Hom	C	-	rs186933518		0.98	0.02
C1qTNF6	Chr22	37581383	missense	Het	C	T	rs7290488	0.12	0.18	0.18
C1qTNF9B	Chr13	24465717	missense	Het	A	G	rs3864970	0.37	0.36	-
		24465743	synonymous	Hom	A	G	rs3864971	0.98	0.98	0.0002
		24470913	intronic	Het	T	G	rs7317337	0.37	0.37	0.36
		24471039	synonymous	Het	T	G	rs77896939	0.50	0.37	-
		24471048	synonymous	Het	C	T	rs75960895	-	-	-
C1qTNF9	Chr13	24895240	synonymous	Hom	G	A	rs200205073	-	0.62	-
		24895559	missense	Hom	A	G	rs79586693	-	0.73	-
		24895684	synonymous	Hom	A	G	rs2862239	0.87	0.87	0.07
C2	Chr6	-	-	-	-	-	-	-	-	-
C3	Chr19	6677989	synonymous	Hom	G	A	rs17030	0.51	0.52	0.48
		6679511	intronic	Hom	C	T	rs2277984	0.49	0.52	0.48
		6690771	intronic	Het	C	T	rs3745567	0.09	0.13	0.12
		6693387	intronic	Het	G	A	rs389404	0.17	0.13	0.12
		6696342	intronic	Het	A	G	rs2287848	0.78	0.62	0.38
		6696496	intronic	Hom	T	C	rs2287847	0.18	0.76	0.25
		6696557	intronic	Het	C	G	rs2287846	0.60	0.62	0.38
		6696597	intronic	Het	G	A	rs2287845	0.69	0.62	0.38

		6696691	intronic	Het	G	T	rs2355315	0.69	0.62	0.38
		6696699	intronic	Het	G	A	rs2253756	0.73	0.62	0.38
		6697406	synonymous	Hom	A	G	rs423490	0.79	0.76	0.25
		6697829	intronic	Het	G	T	rs366510	0.70	0.62	0.38
		6702157	synonymous	Het	C	G	rs428453	0.67	0.62	0.38
		6702598	intronic	Hom	G	A	rs406514	0.82	0.76	0.25
		6713175	intronic	Het	G	C	rs11085197	0.45	0.19	0.20
C4A	Chr6	-	-	-	-	-	-	-	-	-
C4B	Chr6	-	-	-	-	-	-	-	-	-
C4BPA	Chr1	207288897	intronic	Het	T	C	rs12031629	0.51	0.59	0.42
		207297680	synonymous	Het	T	C	rs1126618	0.85	0.83	0.17
		207304900	missense	Het	T	C	rs4844573	0.48	0.37	0.35
C5	Chr9	123760086	indel	Het	T	-	novel	-	-	-
		123769200	missense	Hom	C	T	rs17611	0.36	0.47	0.45
		123780005	synonymous	Hom	G	A	rs25681	0.35	0.47	0.45
C6	Chr5	41158863	synonymous	Hom	G	A	rs6866352	0.99	1	-
		41199959	missense	Hom	G	T	rs1801033	0.58	0.65	0.37
C7	Chr5	40949997	intronic	Hom	C	T	rs1450656	0.66	0.64	0.35
		40955561	missense	Hom	G	C	rs1063499	0.51	0.60	0.39
		40964816	intronic	Het	C	A	rs7713884	0.29	0.23	0.23
C8A	Chr1	-	-	-	-	-	-	-	-	-
C8B	Chr1	57395251	intronic	Hom	T	C	rs605648	0.86	0.86	0.14
		57422484	missense	Hom	C	T	rs1013579	0.98	0.97	0.03
C8G	Chr9	139839904	synonymous	Het	T	G	rs2071006	0.56	0.53	0.46
		139840521	intronic	Hom	T	C	rs41307442	0.12	0.14	0.14
		139840543	missense	Hom	A	G	rs7850844	0.95	0.94	0.05
		139841212	intronic	Het	T	C	rs41309980	0.89	0.14	0.14
C9	Chr5	39342308	intronic	Het	C	T	rs476569	0.63	0.47	0.50
		39364554	missense	Het	G	A	rs700233	0.32	0.43	0.41
CD55	Chr1	207504669	intronic	Hom	G	A	rs2184476	0.66	0.26	0.29
CD59	Chr11	-	-	-	-	-	-	-	-	-
CFB	Chr6	-	-	-	-	-	-	-	-	-

CFD	Chr19	-	-	-	-	-	-	-	-	-
CFH	Chr1	196642233	missense	Het	G	A	rs800292	0.42	0.26	0.22
		196654324	synonymous	Hom	A	C	rs1061147	0.65	0.63	0.38
		196659237	missense	Hom	C	T	rs1061170	0.66	0.63	0.38
		196682947	synonymous	Het	G	A	rs2274700	0.57	0.41	0.40
		196695742	synonymous	Het	A	G	rs3753396	0.22	0.18	0.17
		196709774	missense	Het	G	T	rs1065489	0.17	0.18	0.17
		196712577	intronic	Het	T	C	rs513699	0.04	-	-
		196712586	synonymous	Het	C	T	rs61822181	0.11	-	-
CFHR1	Chr1	196797238	missense	Hom	C	T	rs425757	0.35	0.34	0.24
		196797244	missense	Hom	C	G	rs113811987	0.31	0.29	0.23
		196797292	missense	Hom	G	C	rs388862	0.30	0.28	-
		196797357	synonymous	Hom	A	G	rs76835795	0.50	0.30	0.20
		196799691	synonymous	Het	G	A	rs56411312	0.12	0.16	0.15
		196801042	synonymous	Hom	G	T	rs4230	0.48	0.43	0.41
		196801078	synonymous	Hom	A	T	rs61743621	0.40	0.43	0.40
CFHR3	Chr1	196743964	5'UTR	Het	C	A	rs446868	0.33	0.22	-
		196757392	synonymous	Hom	C	T	rs61735322	0.62	0.42	-
CFHR4	Chr1	196884258	synonymous	Het	A	T	rs150845796	0.10	0.06	-
		196884290	intronic	Het	T	A	rs200244837	0.14	-	-
		196887344	synonymous	Het	A	G	rs202205962	0.002	-	0.0002
CFHR5	Chr1	196946869	intronic	Het	T	A	rs3748557	0.54	0.25	0.24
	Chr1	196967354	missense	Het	G	A	rs35662416	0.02	0.03	0.007
CFI	Chr4	110678925	missense	Hom	T	G	rs11098044	0.98	1	0.0009
		110681505	synonymous	Hom	C	T	rs2298749	0.30	0.26	0.26
CFP	ChrX	-	-	-	-	-	-	-	-	-
FCN3	Chr1	-	-	-	-	-	-	-	-	-
ITGAL	Chr16	30505513	intronic	Hom	A	G	rs4350585	0.73	0.69	0.34
		30510571	intronic	Hom	C	T	rs1557672	0.34	0.39	0.36
ITGAM	Chr16	31273129	intronic	Hom	T	C	rs3764327	0.72	0.70	0.28
		31276811	missense	Het	G	A,T	rs1143679	0.09	0.13	0.11
		31283164	intronic	Het	G	A	rs35314490	0.13	0.14	0.13

		31283323	intronic	Het	C	G	rs35472514	0.25	0.14	0.12
		31335906	intronic	Het	T	C	rs41476751	0.23	0.18	0.16
		31336888	missense	Het	C	T	rs1143683	0.13	0.18	0.16
		31342608	intronic	Hom	T	C	rs7188189	0.95	0.89	0.09
		31343005	missense	Het	C	T	rs1143678	0.14	0.18	0.15
ITGAX	Chr16	31374535	missense	Het	C	G	rs2230429	0.33	0.35	0.37
ITGB2	Chr21	46311813	synonymous	Hom	A	G	rs235326	0.76	0.71	0.33
		46313442	synonymous	Hom	G	T	rs2230529	0.22	0.25	0.25
		46314907	missense	Hom	T	A	rs235330	1.0	1	0.0002
		46320403	intronic	Het	C	T	rs5030670	0.12	0.17	0.16
		46330674	synonymous	Het	C	A	rs11088969	0.20	0.20	0.21
LMBRD1¶	Chr6	-	-	-	-	-	-	-	-	-
MBL2	Chr10	-	-	-	-	-	-	-	-	-
MASP2	Chr1	11087524	synonymous	Hom	G	A	rs1782455	0.68	0.84	0.17
		11090916	missense	Hom	C	A	rs12711521	0.61	0.83	0.19
MCP	Chr1	207941191	intronic	Hom	G	T	rs112060920	0.88	0.78	0.22
MMAA¶	Chr4	-	-	-	-	-	-	-	-	-
MMAB¶	Chr12	109994870	missense	Het	A	T	rs9593	0.44	0.44	0.47
MMACHC¶	Chr1	45973928	synonymous	Het	G	A	rs2275276	0.47	0.49	0.42
MTR¶	Chr1	-	-	-	-	-	-	-	-	-
MTRR¶	Chr5	7869235	UTR 5’	Het	T	C	rs72716536	0.17	0.11	0.10
MTRR¶	Chr5	7870973	missense	Het	A	G	rs1801394	0.43	0.46	0.45
MTRR¶	Chr5	7878179	missense	Het	C	T	rs1532268	0.30	0.34	0.36
MTRR¶	Chr5	7878192	synonymous	Het	T	C	rs161870	0.22	0.12	0.12
MTRR¶	Chr5	7885959	missense	Het	A	G	rs162036	0.23	0.13	0.12
MTRR¶	Chr5	7897191	missense	Het	C	T	rs10380	0.19	0.10	0.10
MTRR¶	Chr5	7897283	synonymous	Het	G	A	rs12347	0.21	0.12	0.12
MTRR¶	Chr5	7897319	synonymous	Het	G	A	rs1802059	0.30	0.34	0.36
PIGA	ChrX	-	-	-	-	-	-	-	-	-
SERPING1	Chr11	57379170	intronic	Hom	A	G	rs2511988	0.51	0.68	0.32
		57381989	missense	Hom	G	A	rs4926	0.20	0.26	0.28
THBD	Chr20	-	-	-	-	-	-	-	-	-

C2orf25[¶]	Chr2	-	-	-	-	-	-	-	-	-

For dbSNP and 1000 genome database the alternative allele frequency is given, whereas the minor allele frequency is displayed for the Exome variance server data. [¶] Genes implicated in cobalamin deficiency pathway.

AAF = Alternative allele frequency; Chr = chromosome; Hom = homozygous; Het = heterozygous; MAF = minor allele frequency; rsID = reference SNP identifier. Websites: dbSNP: http://www.ncbi.nlm.nih.gov/projects/SNP/; 1000 genomes: www.1000genomes.org; Exome variant server: http://evs.gs.washington.edu/EVS/.