SUPPLEMENTAL METHODS

List of the known genes associated with nephrotic syndrome:

Gene	Inheritance	Disease
ACTN4	AD	Familial and sporadic SRNS (usualy adult)
ADCK4	AR	SRNS
ALG1	AR	Congenital disorder of glycosylation
ANLN	AD	FSGS (mainly adult)
ARHGAP24	AD	FSGS
ARHGDIA	AR	Congenital nephrotic syndrome
CD151	AR	NS,pretibial bullous skinlesions, neurosensory deafness, bilateral lacrimal duct stenosis, nail dystrophy, and -thalassemia minor
CD2AP	AD/AR	FSGS/SRNS
CFH	AR	MPGN type II + NS
COL4A3	AR	Alport's disease/FSGS
COL4A4	AR	Alport's disease/FSGS
COL4A5	X-linked AR	Alport's disease/FSGS
COQ2	AR	Mitochondrial disease/isolated nephropathy
COQ6	AR	NS +/- sensorineural deafness; DMS
CRB2	AR	SRNS
CUBN	AR	Intermittent nephrotic range proteinuria +/- with epilepsia
DGKE	AR	Hemolytic-Uremic Syndrome + SRNS
E2F3	AD	FSGS+mental retardation (whole gene deletion)
EMP2	AR	Childhood-onset SRNS and SSNS
INF2	AD	Familial and sporadic SRNS, FSGS-associated Charcot-Marie-Tooth neuropathy
ITGA3	AR	Congenital interstitial lung disease, nephrotic syndrome, and mild epidermolysis bullosa
ITGB4	AR	epidermolysis bullosa and pyloric atresia + FSGS
KANK1	AR	SSNS
KANK2	AR	SSNS/SDNS +/- hematuria
KANK4	AR	SRNS + haematuria
LAMB2	AR	Pierson syndrome
LMNA	AD	Familial partial lipodystrophy + FSGS
LMX1B	AD	Nail patella syndrome; also FSGS without extrarenal involvement
MYO1E	AR	Familial SRNS
NUP93	AR	Childhood SRNS
NUP107	AR	Childhood SRNS
NUP205	AR	Childhood SRNS
NPHS1	AR	Congenital nephrotic syndrome/SRNS
NPHS2	AR	CNS, SRNS
NXF5	X-linked recessive	FSGS with co-segregating heart block disorder
OCRL	X-linked recessive	Dent disease2, Lowe syndrome, +/- FSGS, +/- nephrotic range proteinuria
PAX2	AD	adult onset FSGS without extrarenal manifestations
PDSS2	AR	Leigh syndrome
PLCe1	AR	Congenital nephrotic syndrome/SRNS
PMM2	AR	Congenital disorder of glycosylation
PODXL	AD	FSGS

PTPRO	AR	NS
SCARB2	AR	Action myoclonus renal failure syndrome +/-hearing loss
SMARCAL1	AR	Schimke immuno-osseous dysplasia
SYNPO	AD	sporadic FSGS (promoter mutations)
TRPC6	AD	Familial and sporadic SRNS (mainly adult)
TTC21B	AR	FSGS with tubulointerstitial involvment
WDR73	AR	Galloway-Mowat syndrome (microcephaly and SRNS)
WT1	AD	Sporadic SRNS (children—may be associated with abnormal genitalia); Denys-Drash and Frasier syndrome
XPO5	AR	Childhood SRNS
ZMPSTE24	AR	Mandibuloacral dysplasia with FSGS
MYH9	AD/assoc.	MYH9-related disease; Epstein and Fechtner syndromes
APOL1	G1, G2 risk alleles	Increased susceptibility to FSGS and ESRD in African Americans, Hispanic Americans and in individuals of African descent

Table S1. 53 genes associated with steroid-resistant nephrotic syndrome (SNRS) of congenital, childhood, or adult onset, familial and sporadic origin, different syndrome association/risk factors are shown. [1-8]

Mapping statistics - Coverage – whole exome

	Mean	Std. Error
Reads mapped to target (%)	73.16	0.25
Reads mapped to target plus 150bp (%)	82.25	0.35
Mean coverage	115.90	1.48
Accessible target bases 1x (%)	98.50	0.04
Accessible target bases 5x (%)	97.35	0.07
Accessible target bases 10x (%)	96.06	0.11
Target bases 20x (%)	92.60	0.21

Table S2. Coverage – whole exome

SUPPLEMENTAL RESULTS

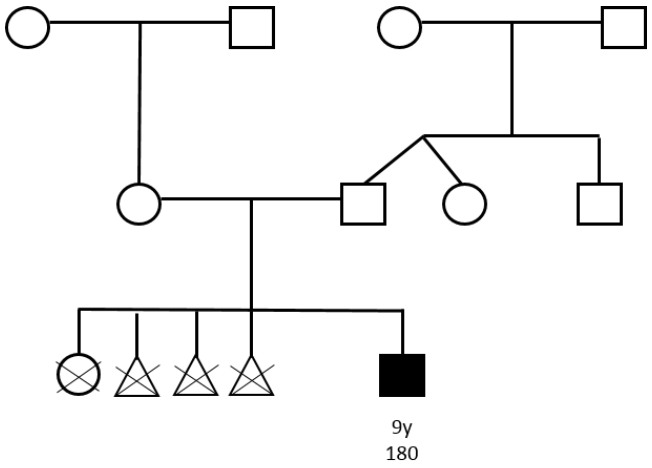

Figure S1. Family pedigree of patient 180.

Black filled square represents patient 180 diagnosed with SRNS at the age of 9 years who is a compound heterozygote forMAGI2; variant in exon 1 was inherited from the Father and in exon 20 from the Mother. Crossed triangles represent miscarriages and the crossed circle represents stillborn female. DNA samples from other members of the family were not available.

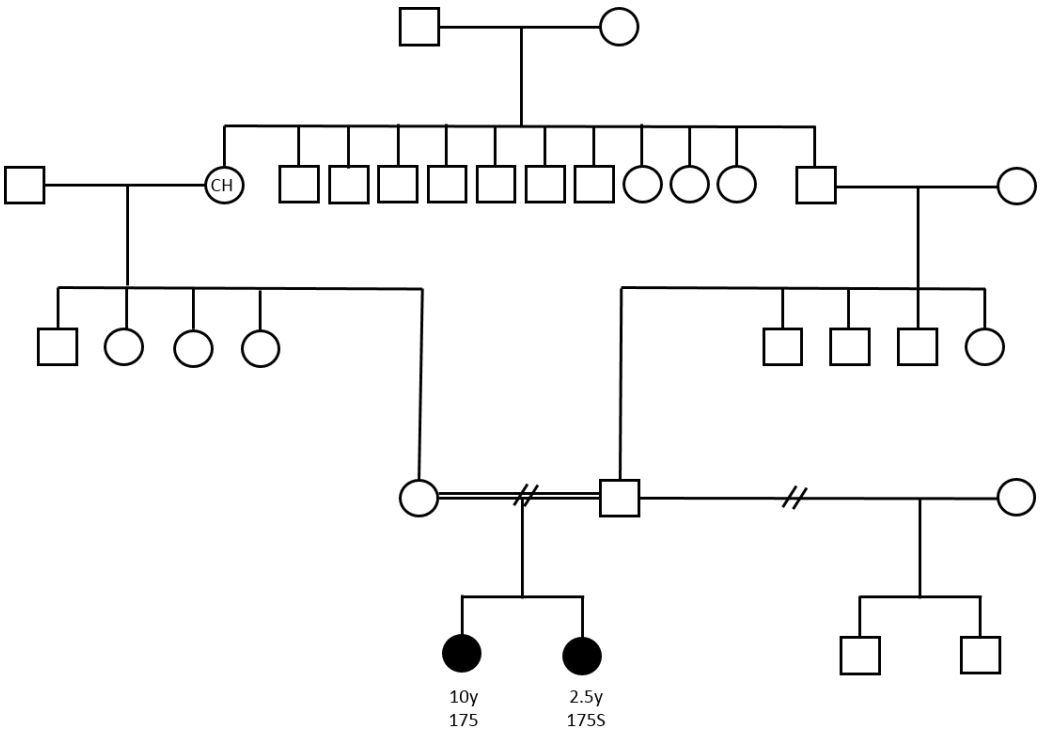

Figure S2. Family pedigree of sisters 175 and 175S.

Black filled circles represent sisters 175 and 175S whose parents are first cousins. Both siblings are homozygous for a single nucleotide deletion in exon 22 of MAGI2 gene. Mother of the siblings is heterozygous for the variant. DNA samples from other members of the family was not available.CH - Celiac and heart disease.

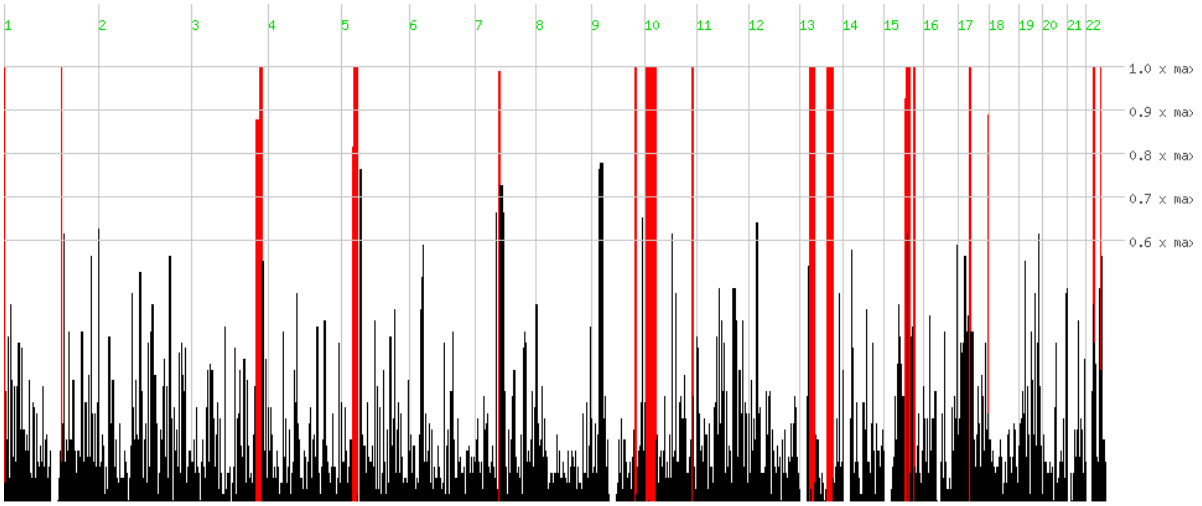

Figure S3. Homozygosity plot.

Homozygosity mapping (http://www.homozygositymapper.org/) was performed for individual 175 since the parents are first cousins. Homozygosity plot is shown with chromosome numbers presented on x-axis and score on y-axis. Red bars represent regions of homozygosity, which reach maximum significance (height of red bar) on chromosomes: 1, 3, 5, 9, 10, 13, 15, 17and 22. Only 6 homozygous variants with MAF <0.01 were found within the homozygosity regions, none of these variants seemed however like a good SRNS gene candidate. One (*AKR1C1*, RS139588200) was found 9 times as a homozygote in ExAC and therefore not considered causative. Four other (*GABRD*, *POLR2M*, *RAD51D* and *NUP155*) were either found to be heterozygous or not present in the other affected sibling. One variant (*IGLL5* RS201956362 - not checked with Sanger sequencing here) affects not conserved amino acid and the substituted threonine is present in many other species at this position and therefore was not considered as likely causative in this patient.

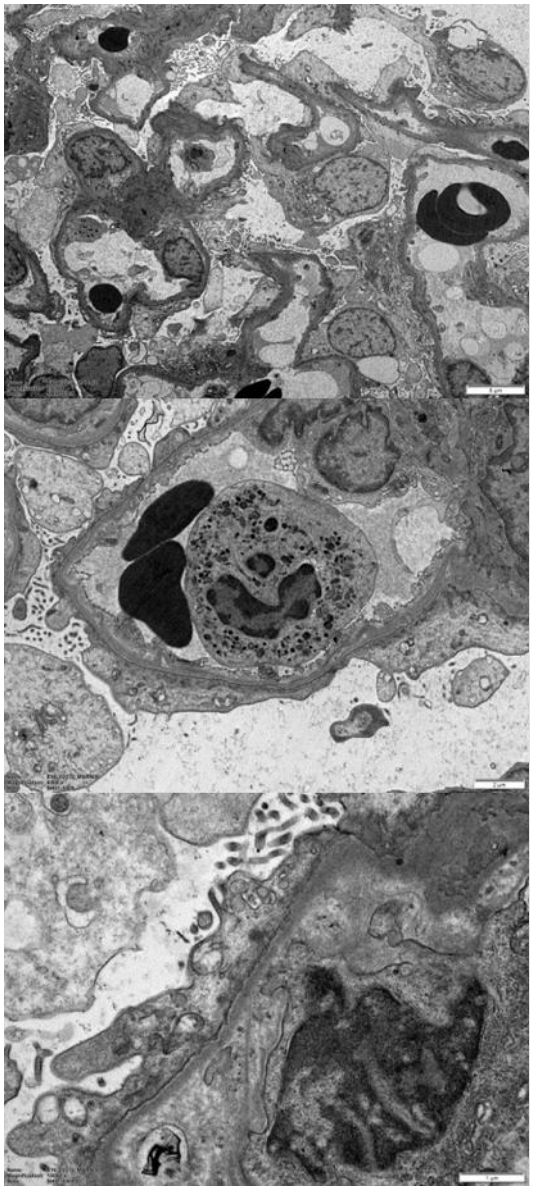

Figure S4. Electron microscopy images of a renal biopsy sample from patient 180.

One glomerulus was examined by electron microscope method. The mesangial area is normal and normocellular with no evidence of mesangial electron dense deposits. There are no peripheral electron dense deposits. The capillary loops are open and the endothelial fenestration is intact. The majority of the capillary loops have normal GBM (glomerular basement membrane). Diffuse podocyte foot process fusion is seen on the outer surface of the GBM.

Immunohistochemistry with Santa Cruz MAGI2 antibody

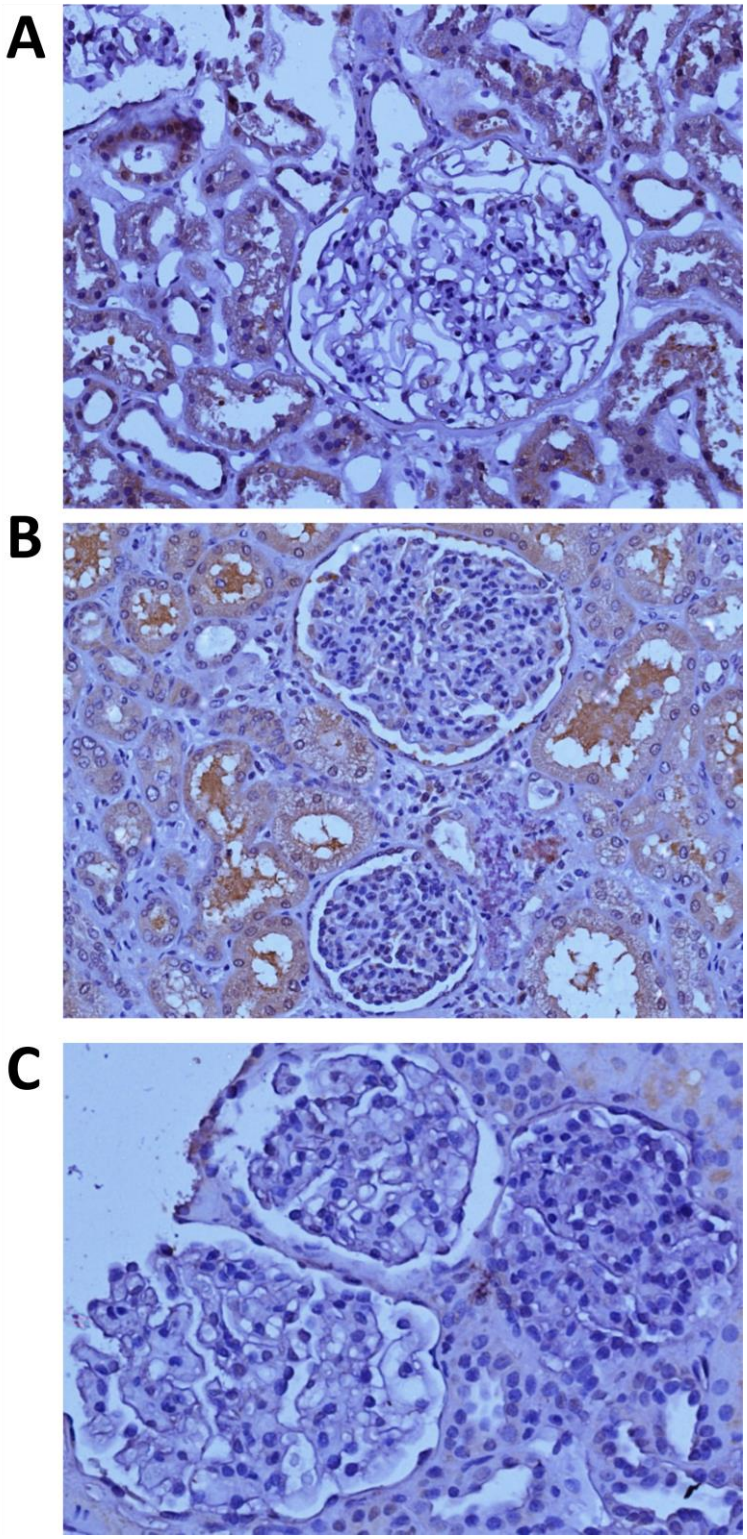

Figure S5. MAGI2 (Santa Cruz) staining

Immunohistochemical staining with human anti-MAGI2 antibody (Santa Cruz) is shown (20x). A - A kidney which was not suitable for transplant was used as a control. B – A nephrectomy specimen obtained from individual 175 (homozygous mutation in *MAGI2*). C – Biopsy specimen obtained from individual 180 (compound heterozygous mutation in *MAGI2*) shows relative absence of MAGI2.

gene and variant	dbSNP144	Phred scaled variant quality	ExAC frequency				Additional significant information*
			Allele count	Allele Number	Number of homozygotes	Allele freq	
KTN1:NM_001079521:exon40:c.3697C>A:p.Leu1233Met	rs372815686	170	.	.	.	.	Both variants are inherited from Mother (in cis conformation) and therefore not considered as likely causative here. Kinectin 1 (Kinesin Receptor). Mice homozygous for a targeted null mutation or a floxed allele exhibit no discernable phenotype; mice are viable and fertile up to one year of age. Expression: cells in glomeruli – medium/high; cells in tubules – medium/high
KTN1:NM_001079521:exon6:c.964-7A>G	rs569480873	192	6	120590	0	4.98E-05	
MAGI2:NM_012301:exon1:c.64_71del:p.Arg22Glyfs*7	No	217	.	.	.	.	This compound heterozygote is in trans. Deletion inherited from the Father and insertion from the Mother.Membrane Associated Guanylate Kinase, WW And PDZ Domain Containing 2. Mice homozygous for a knock-out allele die within 24 hours of birth and possess hippocampal neurons with altered dendritic spine morphology. Homozygotes for different knock-out allele die shortly after birth, exhibiting anuria, increased plasma creatinine levels, and abnormal podocyte morphology. Expression: cells in glomeruli – medium; cells in tubules – low
MAGI2:NM_012301:exon20:c.3526_3533dup:p.Glu1178Aspfs*9	No	217	.	.	.	.	
MCC:NM_001085377:exon1:c.58_63dup:p.Gly20_Gly21dup	No	217	.	.	.	.	Both variants are inherited from Mother (in cis conformation) and therefore not considered as likely causative here. Mutated In Colorectal Cancers (MCC); Mice homozygous for hypomorphic or null mutations are viable and fertile with no gross abnormalities. Expression: cells in glomeruli – not detected/medium; cells in tubules – not detected/high
MCC:NM_001085377:exon6:c.994G>A:p.Glu332Lys,	RS185322500	156	5	121250	0	4.12E-05	
MICALCL:NM_032867:exon3:c.1412_1413insTCC:p.Thr471_Ala472insPro (H)	No	80.4	.	.	.	.	Similar inframe insertion around this region are present in ExAC and therefore this variant is less likely to be significant here. MICAL C-Terminal Like (MICALCL). *Variant not checked with Sanger sequencing.* Expression: cells in glomeruli – low; cells in tubules –high/medium
ZIC5:NM_033132:exon1:c.1248_1262del:p.Pro420_Pro424del	rs778996938	217	.	.	.	.	Deletion inherited from Father. Similar inframe insertion/deletions around this region are present in ExAC and therefore this variant is less likely to be significant here. p.Ser610Phe inherited from the Mother. Zic Family Member 5; Homozygous null mice display postnatal lethality and reduced life spans with exencephaly, abnormal cerebral cortex and diencephalon morphology, abnormal gait and posture, and impaired growth. Expression: cells in glomeruli – not available; cells in tubules – not available
ZIC5:NM_033132:exon2:c.1829C>T:p.Ser610Phe	rs201876139	184	63	121024	0	0.00052	

Table S3. Rare variants found in patient 180.

H – homozygous, * - Additional information obtained from GeneCards (http://www.genecards.org/) and Mouse GenomeInformatics (http://www.informatics.jax.org/), ExAC - The Exome Aggregation Consortium (http://exac.broadinstitute.org/), The Human Protein Atlas (http://www.proteinatlas.org/)

gene and variant	dbSNP144	Phred scaled variant quality	ExAC frequency				Additional significant information*
			Allele count	Allele Number	Number of homozygotes	Allele freq	
AKR1C1:NM_001353:exon5:c.509G>A:p.Arg170His (H) [HM]	RS139588200	222	912	121406	9	0.007512	Present 9 times as a homozygote in individuals without a kidney phenotype. Variant not considered significant and thus not checked for presence in 175S. Aldo-Keto Reductase Family 1, Member C1. Expression: cells in glomeruli – not detected; cells in tubules – high
GABRD:NM_000815:exon3:c.C184C>T:p.Pro62Ser (H) [HM, EA]	No	222	.	.	.	.	Variant present within a homozygosity run. This variant is present as a **heterozygote** in the 175S. Gamma-Aminobutyric Acid (GABA) A Receptor, Delta. Expression: cells in glomeruli – not detected; cells in tubules – not detected
IGLL5:NM_001178126:exon3:c.523G>A:p.Ala175Thr (H) [HM]	RS201956362	222	177	121084	0	0.001462	Substitution affects weakly conserved amino acid, Thr present in several species in this position. Variant not considered significant and thus not checked for presence in 175S. Immunoglobulin Lambda-Like Polypeptide 5. Expression: cells in glomeruli – not detected; cells in tubules – high
MAGI2:NM_012301:exon22:c.3998delG:p.Gly1333Alafs*141 (H) [EA]	No	150	.	.	.	.	Patient 175S is **also homozygous** for this deletion. Membrane Associated Guanylate Kinase, WW And PDZ Domain Containing 2. Mice homozygous for a knock-out allele die within 24 hours of birth and possess hippocampal neurons with altered dendritic spine morphology. Homozygotes for different knock-out allele die shortly after birth, exhibiting anuria, increased plasma creatinine levels, and abnormal podocyte morphology. Expression: cells in glomeruli – medium; cells in tubules – low
NUP155:NM_153485:exon6:c.723G>A:p.Gln241Gln (H) [HM, EA]	No	222	.	.	.	.	Variant present within a homozygosity run. This variant is **not** present in patient 175S. Nucleoporin 155kDa. Expression: cells in glomeruli – medium/low; cells in tubules – medium/low
POLR2M:NM_015532:exon2:c.606G>A:p.Ala202Ala (H) [HM, EA]	RS139384982	222	4	121362	0	0.00003296	Variant present within a homozygosity run. This variant is present only as a **heterozygous** in the sister - 175S. Polymerase (RNA) II (DNA Directed) Polypeptide M. Expression: cells in glomeruli – not available; cells in tubules – not available
RAD51D:NM_002878:exon1:c.26G>C:p.Cys9Ser (H) [HM, EA]	RS140825795	222	42	119626	0	0.0003511	Variant present within a homozygosity run. This variant is **not** present in the sister-175S. RAD51 Paralog D. Expression: cells in glomeruli – not available; cells in tubules – not available
SKOR1:NM_001258024:exon5:c.775A>C:p.Lys259QGln [EA]	RS776369126	215	1	110882	0	0.000009019	Both variants are present also in the sister 175S and their mother thus both variants are on the **same allele**. SKI Family Transcriptional Corepressor 1. Expression: cells in glomeruli – not detected; cells in tubules – not detected
SKOR1:NM_001258024:exon2:c.80G>C:p.Arg27Thr [EA]	No	174	.	.	.	.	

Table S4. Rare variants found in patient 175

H – homozygous, * - Additional information obtained from GeneCards (http://www.genecards.org/) and Mouse Genome Informatics (http://www.informatics.jax.org/), ExAC - The Exome Aggregation Consortium (http://exac.broadinstitute.org/), The Human Protein Atlas (http://www.proteinatlas.org/). HM – variants within

homozygosity stretches, EA – variants found through exome analysis. *AKR1C1* and *IGLL5* were not checked with Sanger sequencing. Only *MAGI2* deletion was found in both siblings (175 and 175S).

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