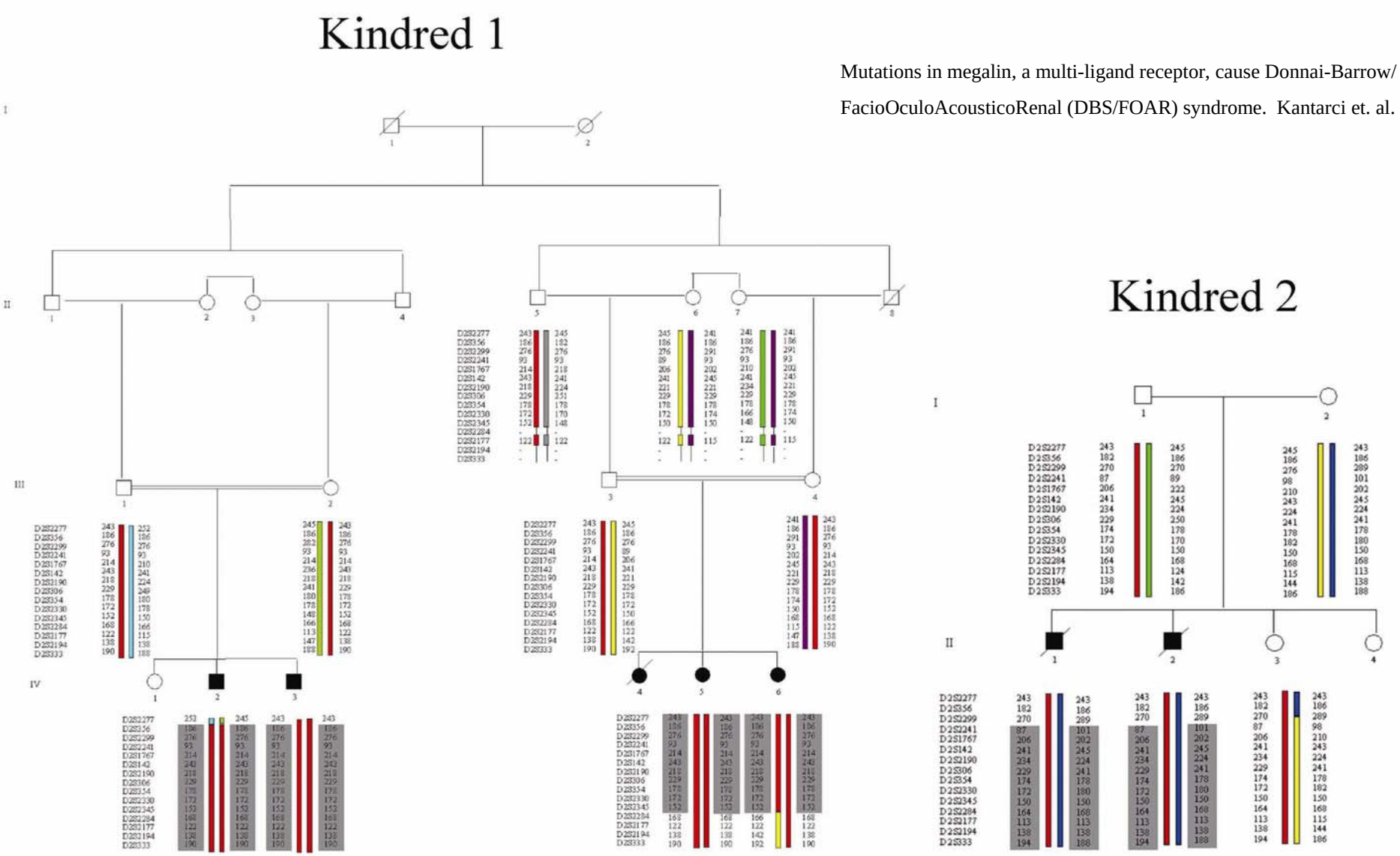
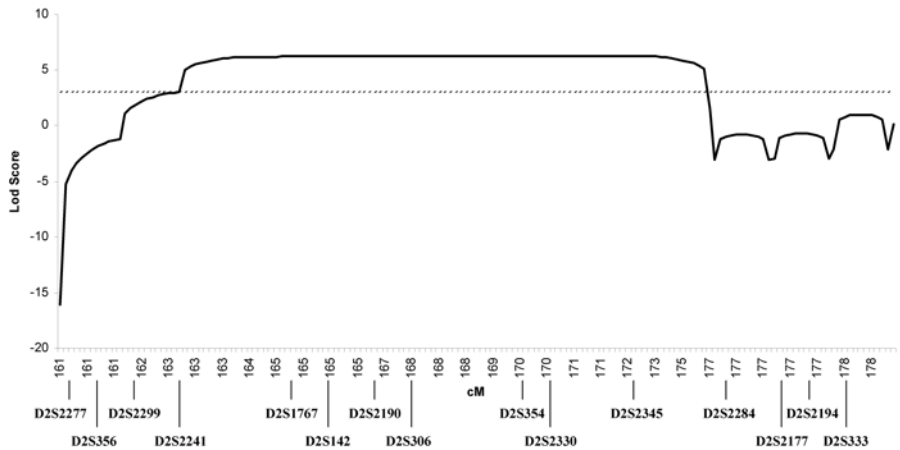
Mutations in megalin, a multi-ligand receptor, cause Donnai-Barrow/ FacioOculoAcousticoRenal (DBS/FOAR) syndrome. Kantarci et. al.



Supplementary Fig.1. Haplotype analyses in multiplex DBS/FOAR Kindreds 1 and 2

Chromosome 2q23.3-q31.1 microsatellite markers are in the following order: cen- D2S2277-D2S333-tel. Shaded haplotypes demonstrate regions of identity by descent among the affected individuals. Markers D2S2299 and D2S2284 are not identical by descent in Kindreds 2 and 1, respectively.

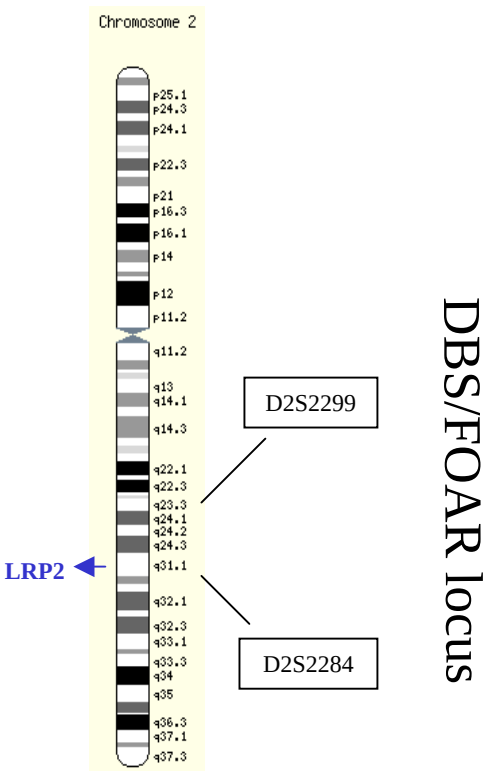
a)



b)

MARKER	cM	0.00	0.05	0.10	0.15	0.20	0.25	0.30 θ
D2S2277	160.82	-Inf	-0.72	0.10	0.42	0.54	0.55	0.51
D2S356	161.33	1.34	1.18	1.01	0.84	0.67	0.51	0.36
D2S2299	162.65	2.35	2.06	1.77	1.48	1.19	0.92	0.67
D2S2241	163.27	3.37	2.93	2.49	2.05	1.64	1.24	0.89
D2S1767	165.18	4.13	3.63	3.12	2.62	2.14	1.67	1.24
D2S142	165.19	4.26	3.79	3.30	2.80	2.31	1.82	1.35
D2S2190	167.91	3.74	3.29	2.85	2.40	1.96	1.53	1.13
D2S306	167.91	2.52	2.22	1.92	1.61	1.30	0.99	0.71
D2S354	170.27	2.28	2.00	1.72	1.44	1.17	0.91	0.66
D2S2330	171.10	4.31	3.81	3.30	2.79	2.28	1.79	1.32
D2S2345	172.64	3.83	3.43	3.00	2.57	2.13	1.70	1.27
D2S2284	177.28	-Inf	1.16	1.14	1.01	0.84	0.66	0.48
D2S2177	177.35	3.37	2.93	2.48	2.03	1.59	1.17	0.79
D2S2194	177.43	-Inf	1.61	1.51	1.28	1.01	0.74	0.50
D2S333	177.98	-Inf	2.13	1.98	1.70	1.38	1.04	0.73

c)



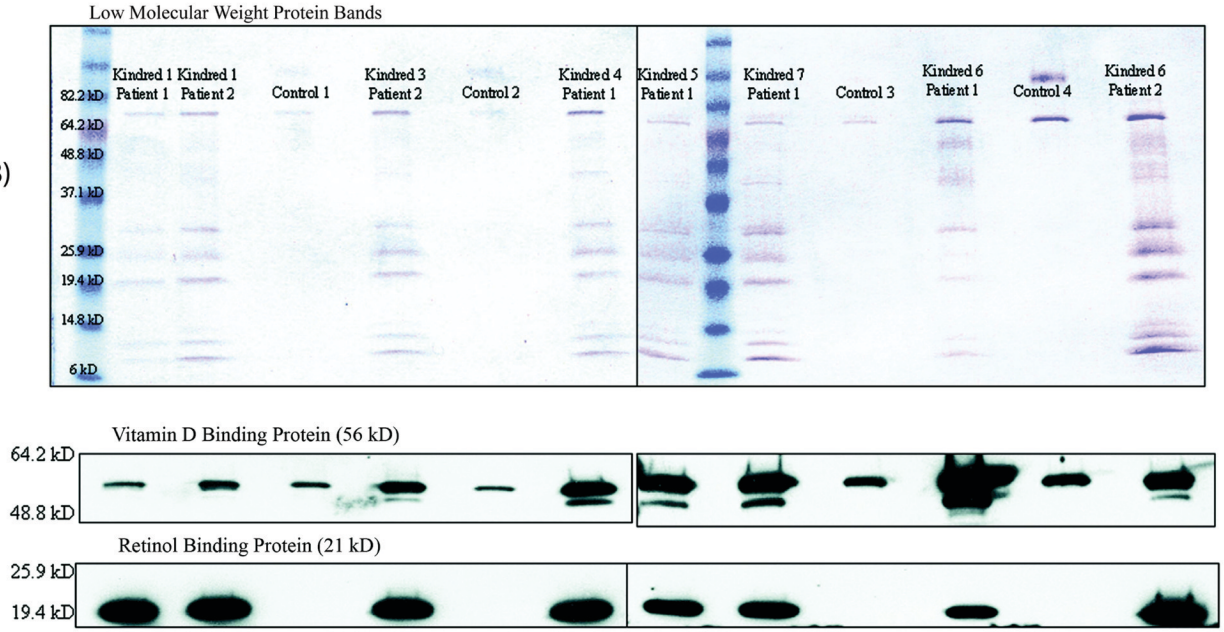
Supplementary Fig 2. LOD scores and DBS/FOAR locus

(a) Multipoint lod score analysis of chrosomosome 2q23.3-q31.1 summed across four multiplex families (Kindreds 1-4). The maximum multipoint LOD score was 6.243 between microsatellite markers D2S1767 and D2S142. **(b)** Two-point LOD score analysis summed across four multiplex kindreds generated a maximum two-point score of 4.31 at microsatellite D2S2330. **(c)** The DBS candidate interval detected by combining haplotype analyses of DBS multiplex families (Kindreds 1-4). The interval is located between flanking markers D2S2299 and D2S2284 (see **Supplementary Fig. 1**). *LRP2* is located on chromosome 2q31.1.

A)

		U creat (mg/ml)	Uca/Ucr (mg/mg)	Uglu (g%)	U PO4 (mg/dl)	U Prot/U Creat
normal values		*not def*	*(0-0.27)*	*< 0.05*	*variable*	*(0-0.2 mg/mg)*
Control 1	8F	0.85	0.16	neg	89.5	0.15
Control 2	8M	0.82	0.02	neg	107.3	0.21
Control 3	8M	1.42	0.11	neg	99.7	0.10
Control 4	7M	0.6	0.20	neg	59.4	0.20
Control 5	5F	0.93	0.15	neg	100.3	0.18
Kindred 1, Patient IV-5	6F	1.05	0.09	neg	111.2	2.34
Kindred 1, Patient IV-6	3F	0.86	0.03	neg	57	2.36
Kindred 2, Unaffected Sibling II-3	7F	0.59	0.07	neg	47.9	0.29
Kindred 2, Unaffected Sibling II-4	2F	0.57	0.16	neg	56.4	0.88
Kindred 3, Patient 2	6F	0.33	0.46	neg	44.6	2.00
Kindred 3, Unaffected sibling	3F	0.38	0.44	neg	51.8	0.24
Kindred 4, Patient 1	19M	1.18	0.17	neg	89.5	0.98
Kindred 5, Patient 1	16F	0.65	0.13	neg	53.8	1.29
Kindred 6, Patient 1	0.5F	0.24	0.68	neg	42.1	2.17
Kindred 6, Patient 2	7F	0.07	1.94	0.148	34.5	5.00
Kindred 7, Patient 1	12M	0.7	0.31	neg	75.3	1.80

B)



Supplementary Fig. 3. Urine analyses in DBS/FOAR patients, siblings and controls.

A) Biochemical urinalyses. All affected children demonstrate proteinuia. The clinically unaffected siblings in Kindred 2, who carry the paternal mutant allele, demonstrate intermediate urinary protein levels. The unaffected sibling in Kindred 3 carries the paternal mutant allele. Lack of glycosuria in seven of eight patients is evidence for a specific, rather than broad, proximal tubular defect. Urinary glucose in a single child in Kindred 6 maybe indicative of confounding renal pathology. Some patients also demonstrate hypercalciuria.

B) Protein electrophoresis and Western blot.

Upper panel- Electrophoresis analyses of patient and control urine specimens demonstrate low molecular weight protein bands in affecteds not evident in controls; 68kD band likely represents albumin.

Lower panels- Western analyses show positive bands when probed with antibodies for DBP (52 kD) and RBP (21kD) with a greater intensity in affecteds than in controls.

Supplementary Methods

DNA extraction

DNA was extracted from blood samples using the Puregene DNA Purification Kit (Gentra Systems, Minneapolis, MN) and from a fibroblast cell line using standard phenol-chloroform methods. Previously extracted fetal DNAs were amplified using the GenomePlex® Whole Genome Amplification Kit (Sigma, St Louis, MO). All DNA was quantified with the NanoDrop ND-Spectrophotometer (NanoDrop Technologies, Wilmington, DE). Sequence analysis failed on poor quality DNA extracted from one fetus belonging to Kindred 3 and from one belonging to Kindred 4. DNA was not available from members of the second multiplex family reported by Donnai and Barrow.

Linkage Analysis

A genome-wide screen was performed on four affected members of Kindred 1 using GeneChip Human Mapping 10K SNP arrays (Affymetrix, CA), which contains 11,550 SNPs with genome scan resolution of 0.31 cM. The data were analyzed using DNA Chip Analyzer (dChip) software (http://www.biostat.harvard.edu/complab/dchip/snp.htm). Homozygosity mapping was performed by identifying regions that were homozygous and had identical genotypes in all affected individuals.

The homozygous chromosome 2q23.3-q31.1 region was refined and narrowed using fluorescently labeled microsatellite markers on an ABI Prism 3130xl genetic analyzer using GeneFinder (Applied Biosystems) software. The following markers were used in multiplex Kindreds 1-4: D2S2277, D2S356, D2S2299, D2S2241, D2S1767, D2S142, D2S2190, D2S306, D2S354, D2S2330, D2S2345, D2S2284, D2S2177, D2S2194, & D2S333.

Lod score calculations were carried out using only the microsatellite data assuming an autosomal recessive genetic model, complete penetrance, a frequency of disease-associated allele of 0.001, and equal allele frequencies. Multipoint lod scores were calculated using Allegro (version 2.0)[1]. SuperLink (version 1.5) (http://bioinfo.cs.technion.ac.il/superlink/) was used to calculate two-point lod scores with a theta ranging from 0.0 – 0.5 (**Supplementary Fig. 2**).

Sequence analysis of genes

A list of candidate genes in the region of homozygosity was generated using UCSC Genome Browser (http://genome.ucsc.edu/). The coding regions and 50-bp flanking intronic regions for eleven of 51 candidate genes, including *LRP2*, were bidirectionally sequenced in one affected member from Kindreds 1-3; subsequently, only *LRP2* was sequenced in Kindreds 4-7 (SeqWright, Houston, TX and in-house). The primers (**Supplementary Table 2)** for sequence analysis were designed using Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and were synthesized by Sigma-Genosys (The Woodlands, TX). Sequence results were analyzed using Sequencher™ DNA sequencing software (Gene Codes, Corporation, Ann Arbor, MI). We

confirmed the mutations discovered in the *LRP2* gene and performed genotyping analyses in all family members with available DNA. Sequencing was carried out by fluorescent dye-terminator chemistry.

To determine if *LRP2* mutations discovered in affected individuals were normal variants, the Ensembl (http://www.ensembl.org/index.html) and NCBI dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/index.html0) databases were searched. The specific mutations present in Kindreds 1-3 were sequenced in DNA from 96 control samples (DNA Polymorphic Technologies, Alameda, CA) consisting of subjects of Northern European (80%) or Middle-Eastern (20%) ancestry. The ClustalW software (http://www.ebi.ac.uk/clustalw/) was used for the missense mutations to perform multiple sequence alignments among different species.

Urinary Protein Electrophoresis and Western Blots

Non-timed random spot urine samples were collected from eight children with DBS/FOAR, three phenotypically-normal siblings, and five age-matched healthy controls. Urine samples were shipped on dry ice and maintained at –80C until evaluation. Urine was examined for glucose, calcium, phosphorus, creatinine, and total protein by the Massachusetts General Hospital clinical chemistry laboratory (analyzers used available upon request). Polyclonal rabbit anti-human antibodies to retinol-binding protein (RBP) and vitamin-D binding protein (DBP) were obtained from Dako (Carpinteria, CA). Sensitivity and specificity of the primary antibodies were confirmed using dot blots and western analysis of solutions spiked with the correlating antigen. RBP antigen was obtained from Fitzgerald Industries (Concord, MA) and DBP antigen was obtained from Calbiochem (San Diego, CA). An equal amount of total protein for each patient and control was loaded on Invitrogen 4-20% tris-glycine gels which were run at 125V for ~2 hours and stained with Coomassie blue. Following membrane wash, goat anti-rabbit secondary antibody (Jackson Immunoresearch Laboratories, West Grove, PA) with a horseradish peroxidase conjugate (dilution 1:20000 with 5% powdered milk in PBST) was applied. The West Pico Chemiluminescent Kit (Pierce Biotechnology, Rockford, IL) was used for detection. The western blots performed on urine samples (lower panel of Fig. 2) show increased urinary excretion of vitamin D binding protein (52 kD) and retinol-binding protein (21kD) in DBS/FOAR patients compared the low levels normally present in controls[2, 3].

MRI surface reconstruction and visualization

We utilized the Oxford FSL tools (http://www.fmrib.ox.ac.uk/fsl/) for preprocessing of MRI images and brain tissue segmentation. The outer cortical surface was reconstructed using the in-house developed tools[4]. The IBM OpenDX toolkit (http://www.research.ibm.com/dx/) was used for surface rendering.

1. Gudbjartsson, D.F., Thorvaldsson, T., Kong, A., Gunnarsson, G. & Ingolfsdottir, A. Allegro version 2. *Nature Genetics* **37**, 1015-1016 (2005).
2. Smith, G.C., Winterborn, M.H., Taylor, C.M., Lawson, N. & Guy, M. Assessment of retinol-binding protein excretion in normal children. *Pediatr Nephrol* **8**, 148-50 (1994).
3. Lauridsen, A.L. et al. Sensitive automated ELISA for measurement of vitamin D binding protein (Gc) in human urine. *Clin Chem* **51**, 1016-8 (2005).

4. Liu, T., Shen, D., & Davatzikos, C. Deformable registration of cortical structures via hybrid volumetric and surface warping. *Neuroimage* **22**, 1790-801 (2004).

Supplementary Notes: ***Clinical Information on DBS/FOAR Kindreds 1-7***

Phenotypic details for all patients are summarized in **Supplementary Table 1**.

A) Kindred 1 – A New Family with DBS from the United Arab Emirates
We recruited a large inbred family from the United Arab Emirates who had five children diagnosed with DBS (**Supplementary Fig. 1**). The proband (IV-4) had characteristic craniofacial features but died of complications secondary to CDH. Of the four surviving children with DBS, one has right-sided diaphragmatic eventration and left pulmonary hypoplasia, while two have documented agenesis of the corpus callosum. Chromosome analyses on the four survivors, and 1 Mb array-based comparative genomic hybridization (Spectral GenomechipTM V1.2 arrays, Spectral Genomics, Inc., Houston, TX) on two survivors, were normal (data not shown). Both sets of parents of the affected children are entirely healthy. Brain imaging on IV-6 demonstrated numerous abnormalities including hypogenesis of the corpus callosum (most notably involving the rostrum and splenium), partially empty sella turcica, small pons (**Fig. 2b**), extensive periventricular nodular heterotopia (**Fig. 2c**), small optic nerves and chiasm, small ocular colobomas (**Fig. 2d**), and malformation of the left horizontal semicircular canal and vestibule (**Fig. 2e**). Cortical surface reconstruction of the same patient (**Fig. 2h & i**) was abnormal compared to an age-matched control (**Fig. 2f & g**).

The four surviving children from this Kindred carried a homozygous c.7564T>C transition in *LRP2* exon 41, leading to substitution of a basic polar (imidizole) histidine for an uncharged aromatic nonpolar tyrosine at amino acid position 2522 (Y2522H) (**Supplementary Table 1** and **Fig. 1**). Each parent was heterozygous for this mutation, inheriting it from the heterozygous grandfather II-5 (**Supplementary Fig. 1**)

B) Kindreds 2 and 3 – DBS Families Previously Reported by Chassaing et. al. (2003)[2]
The two affected patients in Kindred 2, Patient 1 and Patient 2, (II-1 and II-2, respectively in **Supplementary Fig. 1**) had typical prenatal features of DBS, including brain malformations in both and CDH in Patient 2. The parents are a healthy non-consanguineous couple who subsequently gave birth to two healthy daughters. Patient 1 in Kindred 3, prenatally diagnosed with CDH and partial ACC, is deceased. At the time of original publication, her surviving sister (Patient 2) had hypertelorism, arrested development of the corpus callosum, high myopia, sensorineural hearing loss, and proteinuria. Currently at six years of age, the sister is legally blind but is learning Braille, has mild developmental delay, diminished serum retinol level, and abnormal renal function with evidence of a complex non-acidotic proximal tubulopathy (including low molecular weight proteinuria, elevated beta-2 microglobulin, and hypercalciuria without glycosuria). These healthy non-consanguineous parents now have a healthy daughter.

C) Kindreds 4 and 5 –DBS Families Previously Reported by Donnai & Barrow(1993) [1]
The cardinal report, after which this disorder is named, described two multiplex families with a novel constellation of anomalies; an additional unrelated child was reported in a

"note added in proof". CDH and brain malformations were present in all those diagnosed with DBS. Kindred 4 contained three children affected with DBS, the sole survivor of which is now 19 years old. The single affected girl in Kindred 5, corresponds to the patient added "in proof", and is currently 16 years of age. Brain MRI scan (**Fig. 2a**) shows ACC.

D) Kindred 6- A new DBS family from Qatar
Two female siblings born to healthy first cousin parents were recently diagnosed with DBS; additional clinical characterization is in progress.

E) Kindred 7- Patient with FOAR-DBS syndrome previously reported by Devriendt et al (1998)[4]
This patient, initially reported in 1998, had CDH, hypertelorism, a large anterior fontanelle, high myopia, sensorineural hearing loss, macrocephaly, and "non-selective glomerular proteinuria" without glycosuria or aminoaciduria. At the time of publication in 1998, the authors noted the phenotypic overlap between FOAR and DBS. The patient, now 12 years old, demonstrates profound hearing loss, blindness secondary to retinal detachment, moderate mental retardation, diminished serum retinol level, normal physical growth, and normal renal function.

Additional Patients
Two further patients whose phenotypes resembled DBS/FOAR were sequenced for *LRP2* coding regions but no mutations were found (data not shown); further review of the phenotypes demonstrated distinct craniofacial dysmorphology in comparison to classic DBS/FOAR.

Mutations in megalin, a multi-ligand receptor, cause Donnai-Barrow/ FacioOculoAcousticoRenal (DBS/FOAR) syndrome. Kantarci et. al.

Supplementary Table 1- Phenotype in DBS/FOAR syndrome patients.

Kindred 1: A New Family from the United Arab Emirates (see also **Supplementary Fig. 1**)

Pt	Sex	Status	Congenital Diaphragmatic Hernia	Omphalocele	Hyper-telorism	High myopia	Anomalies of Corpus Callosum	Large Anterior Fontanelle	Sensori-neural Hearing Loss	Develop-mental Delay	Miscellaneous
IV-4	F	Neonatal death	R CDH	No	Yes	Unk	Unk	Yes	Unk	Unk	Large prominent eyes
IV-5	F	6 yrs	No	No	Yes	Yes	Partial agenesis	Yes	Yes	Speech delay	Macrocephaly; iris coloboma; large prominent eyes; retinal dystrophy; short sternum; small R auricular pit
IV-6	F	3 yrs	R focal diaphragm eventration; L lung hypoplasia	Yes	Yes	Yes	Partial agenesis	Yes	Yes	Yes	Large corneal diameters; retinal dystrophy; scoliosis; rib & vertebral anomalies; short sternum; hypoplastic left pulmonary artery; L preauricular tag
VI-2	M	12 yrs	No	Yes	Yes	Yes	Unk* (Dilated lateral ventricles)	Yes	Yes	Yes	
IV-3	M	6 yrs	No	Yes	Yes	Yes	Unk* (Dilated lateral ventricles)	Yes	Yes	Yes	Albinism

Kindreds 2 and 3: Previously reported by Chassaing et al, 2003[2]
Kindreds 4 and 5: Previously reported by Donnai & Barrow, 1993[1]; updated by Gripp K. et. al., AJMG 68: 441-4; 1997.

Pt	Original Pt #	Sex	Status	CDH	Omphal-ocele	Hyper-telorism	High myopia	ACC	Large AF	HL	Dev del	Misc
Kind-2 Pt 1 (II-1, Sup.Fig 1)	1 Chassaing	M	22 wk fetus	No	No	Yes	Unk	Yes	Yes	Unk	Unk	Prominent eyes; arachnoid cyst
Kind-2 Pt 2 (II-2, Sup. Fig 1)	2 Chassaing	M	25 wk fetus	L CDH	No	Yes	Unk	Partial ACC	Yes	Unk	Unk	
Kind-3 Pt 1	3 Chassaing	F	26 wk fetus	CDH	No	Unk	Unk	Partial ACC	Unk	Unk	Unk	
Kind-3 Pt 2	4 Chassaing	F	6 yrs	No	Yes	Yes	Yes	Partial ACC	No	Yes	Yes- mild delay	Retinal dystrophy; "legally blind"; proteinuria (excess beta-2 microglobulin); hypercalciuria; diminished DMSA renal uptake c/w proximal tubular defect. Serum retinol = 210 ug/L (300-650)
Kind-4 Pt 1	1 Donnai	M	19 yrs	L CDH	"small exom-phalos"	Yes	Yes	Yes	Yes	Yes	Yes	Prominent eyes; L iris coloboma; L retinal detachment; seizures
Kind-4 Pt 2	2 Donnai	F	21 wk fetus	L CDH	Umbilical hernia	Yes	Unk	Yes	Yes	Unk	Unk	
Kind-4 Pt 3	3 Donnai	F	17 wk fetus	L CDH	Yes	Yes	Unk	Yes	Yes	Unk	Unk	DORV; VSD
Kind-5 Pt 1	"added in proof" Donnai	F	16 yrs	R CDH	No	Yes	Yes	Yes	Yes	Yes	Yes	VSD; PDA; gross attenuation retinal pigment epithelium; seizures

Kindred 6: A New Family from Qatar

Kind-6 Pt 1	New family	F	5 mo	L CDH	Yes	Yes- severe hyper-telorism	Unk	Yes	Yes	Yes	Unk	
Kind-6 Pt 2	New family	F	7 yrs	No	No	Yes- severe hyper-telorism	Yes	Unk	Unk	Yes- severe HL	Yes- mild delay	Heart murmur, mild interdigital webbing
Kindred 7: DBS/FOAR Family previously reported by Devriendt et al, 1998[4]												
Kind-7 Pt 1	case report Devriendt	M	12	L CDH	No	Yes	Yes	No	Yes	Yes	Yes	Non-selective glomerular proteinuria without glycosuria or amino aciduria. Serum retinol = 238 ug/L (300-650)

ACC- anomaly of corpus callosum, AF- anterior fontanelle, CDH- congenital diaphragmatic hernia, Dev del- developmental delay, DMSA- dimercaptosuccinic acid, DORV- double outlet right ventricle, F- female, HL- hearing loss, L- left, M- male, PDA- patent ductus arteriosus, R- right, Unk- unknown, VSD- ventriculoseptal defect.

* Studies of adequate resolution to exclude morphological abnormalities of the corpus callosum have not been performed to date.

Mutations in megalin, a multi-ligand receptor, cause Donnai-Barrow/ FacioOculoAcousticoRenal (DBS/FOAR) syndrome. Kantarci et. al.

Supplementary Table 2. *LRP2* PCR primer pair sequences

Exon #	Forward primer	Reverse Primer
1	5′-GGTAGAAAACGGGATGCCTCG-3′	5′-GGCTTCGCGAAGCAACCTGAG-3′
2	5′-CTTCCCACGGATGTTCAAAT-3′	5′-AACCCCTACCTCAGAAGGGA-3′
3	5′-TCCACAGACTCTAGTATAAGG-3′	5′-TGAATGAGATTGCTGGCATAGG-3′
4	5′-TGGTGGTAGCCAGGTACTTTAC-3′	5′-GCCAAGAGATTGCAATAACAAG-3′
5	5′-TGACTTGCATATAGTAATCACTC-3′	5′-TCAAACTACCAACATGAATTC-3′
6	5′-AGCACACCACTGCTTAATAAG-3′	5′-AAAAGAGGTACATTGGTGG-3′
7	5′-TCTAAGGGATTAAATTGATATAG-3′	5′-TCAACTCCAGCCAATCCTGAGG-3′
8	5′-GTAGATCATTTATAGATTTCTC-3′	5′-TCTTGGGGTGCTTTGTTATGC-3′
9	5′-TGTACATCTACTTCATGCTTAC-3′	5′-TTATGACAGACCATGACGACTC-3′
10	5′-TGTTTATTTAGACATGGGCAAC-3′	5′-ATGATAAAGCCCTGCCAACAAC-3′
11	5′-AAATCAGGTAAGACAACCTAG-3′	5′-AGAAAACAAAGGAACTTTCCC-3′
12	5′-GCATGAAATTTCTGAAAATTGAG-3′	5′-TGGCTTCTAGAGTATACAGCC-3′
13	5′-AGCTGGATAAAATTTCCATTG-3′	5′-AAAGCAATATTTTAATTCTGG-3′
14	5′-TCTATTCCGTATTTGTGACAAG-3′	5′-AATGCCACCCAAATCACATAAG-3′
15	5′-TAATCCATTACTCTATTTCACC-3′	5′-AGACAGGGAGCTGGTCCAGTTC-3′
16	5′-TTGCTCTGTGGATGCATTTATC-3′	5′-GATACTGAGTTTTAATTATCAAG-3′
17	5′-AATATGCTCTAACTCTGACAC-3′	5′-TCTTATATTTGCTGAACTTACC-3′
18	5′-ACAAAATTAATTGCAGTATCTG-3′	5′-AGGGCAAGCAGTACCAGTTTCC-3′
19	5′-GTTTAATAAATGTGTCAACTG-3′	5′-GTGGCCAGCTTTTCTGGGATTG-3′
20	5′-AGATGATCGTACTTGCAAGAAG-3′	5′-TACCAGGAGCTAGACCTTTAAG-3′
21	5′-GTCTGAAAAATGTTTTGTCCTG-3′	5′-GATATTTTTCCCATATAAATAGC-3′
22	5′-CCTTATTATATACAATGCCTC-3′	5′-ACAATGAAAATTGCATGAGCC-3′
23	5′-TGATTCAAAAGAATTTTGAACC-3′	5′-AACAGGCCCCGGTGTGTGATG-3′
24	5′-TCTCAGTGTTTAGTCATTTTAGC-3′	5′-TTTGAGGATACTTTCCCCCAG-3′
25	5′-TTGTTACTATAAACCTACTATTG-3′	5′-GAACTGAAATCCTTCCTACAAAG-3′
26	5′-ATCATATTGAAGGTGGCATGAG-3′	5′-CAAGTCGATCTAACTCCTAATC-3′
27	5′-TGGAATTACACCTTCTTTCTG-3′	5′-ACCATGAGAGCTACCCAGATG-3′
28	5′-AAGTAATTATAGGACAAGTTAG-3′	5′-ATTTTGCATATCAGCAACATGC-3′
29	5′-ACCAGAGATGATTTATTTTAAG-3′	5′-TGATCTGACAATGTCTATGAC-3′
30	5′-ATAATGACATGTTAAAGGAAAC-3′	5′-ATGTATTGTCCAAACAAATGG-3′
31	5′-TGTACTGGGGCCTCTGGAAGGAC-3′	5′-ACCTGAGGAATCAAAGGACAC-3′
32	5′-AATGCTGCAATGTCATTTTAGC-3′	5′-TTTCCCTTTTACTCACATGAC-3′
33	5′-TAAGGACTGTTCAAGAAATAC-3′	5′-ATGATTCCAAAATCCACGTGAG-3′
34	5′-TGCAAATCCTCTCTCATCCATC-3′	5′-TGTTGACATATGAAATCAGAAG-3′
35	5′-TCAGCCTTTGCCCATTGTATTG-3′	5′-AACACTGGTACAAGTTACCAAG-3′
36	5′-GGATAGAGGGCTGTATAAATG-3′	5′-TTGTATGCACGTGTATGTACGT-3′
37	5′-TAACTCAGCTACTATATGTTGG-3′	5′-TTGATTTAAAGGGAAAGGAAAG-3′
38	5′-TTGGGAATGAGGGTTTGAGAG-3′	5′-AATGGTCTTAATAATTGTCTAG-3′

39a	5′-AACATATAGTGACCCAGACTTC-3′	5′-CTGGCTTGGAAGATCTTTTTC-3′
39b	5′-TGGCAGTCGTTACCCAACTCC-3′	5′-CATTAGAAACACTCAGTCATC-3′
40	5′-GTCCTGCAAGTAGACACAATG-3′	5′-TCTGTAGCCATAGTTTTATAAG-3′
41	5′-TTAAAGGACTATGAGGTTCCCTG-3	5′-ATTCTGGCAATGTCTATCTGG-3′
42	5′-TTGCAGCTTGTGTAAACAATG-3′	5′-AGGAGAATTTGAATTTTTTCTCC-3′
43	5′-GCTTCTTAGGGAGCATTTAATG-3′	5′-TGCCTAGGCACACATTTCAAAC-3′
44	5′-TGGAAGGTAAATTCAAAGTGTC-3′	5′-ATATAATAATTTCATCTCCTG-3′
45	5′-CTTCCAGTGGTTTGAGTTCCAC-3′	5′-ATGTGAAATGACAATTGAAATC-3′
46	5′-TTGGTAACTATTATCTAGCTC-3′	5′-ACAGGAAGTCCAAGCATTCAGC-3′
47	5′-GCTCTGTTTCAGGTTTTCTAG-3′	5′-TCAGTGTCCATTCAATTACAGAG-3′
48	5′-TTTCCTTTCATACTTAGAATG-3′	5′-AAGCCACTAAAAGCATCATGGG-3′
49	5′-AAATGGTTGACCTGGCATTTAG-3′	5′-TTTATGGTAACTTTGTAGTTAG-3′
50	5′-TGTTGACTTTGAACCATTATAG-3′	5′-TTCCTATCAAGATGAAAACATG-3′
51	5′-TTGCCTGAAGAAAGGTGACTTC-3′	5′-ACCAAGTAGGAAAAGCAGAAG-3′
52	5′-TGGAGAAAAGGTGTGTATTTTG-3′	5′-AATGACTTCCAACAGACAGGCC-3′
53	5′-TCTAGGAGGTAGAATTTTATTG-3′	5′-ACAAGTGCAACAAACTTTTTG-3′
54	5′-TTTTACTAAGAGGTGATGAG-3′	5′-AATTAACCAAAATCTTGTCTC-3′
55	5′-TCTAGGGCCACGAGAGAGCTC-3′	5′-CTTTGAACTGGTTTTTAGTTC-3′
56	5′-TAAGTCCTCTAAAAAGAAATC-3′	5′-ATCAGCTGAAAAAGAAAGCTC-3′
57	5′-TTGCTTGGTAACATGGTCATTG-3′	5′-TAAACAAAGAAAAGATGACATG-3′
58	5′-ATGAATGTTGCTGGCATTCCTC-3′	5′-TGACCAGCAGAAAGAAAGAGATC-3′
59	5′-ACCCAGGGCACTATGGTCAG-3′	5′-TTGTTTACAAGGCTAACGCAGA-3'
60	5′-ATTTGCTAACCACAAGTTGTG-3′	5′-TCAGGTGAACTGATCATTATC-3′
61	5′-AAGGCCACTATACATCTTGATG-3′	5′-ACACCAACGCTGATCCTGTGTC-3′
62	5′-TGTAAGGTGTATCTGCTGATTG-3′	5′-ATGGTCTTGTAAACAATTATG-3′
63	5′-AAGGGAGACTGGGAAATGTAG-3′	5′-GACTGGAATTCAAGCAAAAAG-3′
64	5′-CCTCTCCAAGGCTTATGGAAAG-3′	5′-GACCTAGAACCATGAAACCATG-3′
65	5′-TCATTGCTTATTTTCATCTGTAC-3′	5′-ACTATGAGAAGCTGAAGTTTC-3′
66	5′-ATTGATTAACAGAGATAGTCAG-3′	5′-ATGCTGGATTGGAAGAGTTTC-3′
67	5′-ATATTATAACAACAGTGAGGAG-3′	5′-TGCCCAGCTGCACCCATGGCTG-3′
68	5′-ATGATAGGTATCTGAGCAGCTG-3′	5′-TGAAGGACAATTTCAGTTAAAC-3′
69	5′-CACCCAGGTCCCTGAGATAAAC-3′	5′-ACTATATAAGCCATTTCCTAAG-3′
70	5′-CATTGTATGAGGGAATAATTC-3′	5′-TTTCATGCACCAAGAGATTAGC-3′
71	5′-ACACGTGGACGAGAGGTATTG-3′	5′-ATCCCTTAACCCTCCTTCCAG-3′
72	5′-GCTTTGACAGGGAGACTAATTG-3′	5′-TGTAAGAATTAGTGAACTGGTG-3′
73	5′-CAGGATACATTGTCTGTTCAAC-3′	5′-GGCTAGAACCACTCCTTCAAAG-3′
74	5′-TGAATGCTGTCACAGCTTTTC-3′	5′-TGGAATCGGTGAACTGCGTATTG-3′
75	5′-ATTACATTTGAGGAAGATTTG-3′	5′-CTTATTCTGAGAGGCCTAATAC-3′
76	5′-GTGAGGAAGGCACCTTTCTTTG-3′	5′-ACCACTCACTATGCTTCCTTC-3′
77	5′-AGAACAAGATGGCAAAAAGAG-3′	5′-ACACATCTTGAGATCTTTGAG-3′
78	5′-ATGGTCCCAGGTCCCAAATTG-3′	5′-TCATAAAGAAGACTATTTGTGG-3′
79	5′-GTTAAATTGGACACACACTTAG-3′	5′-TTACCTTCAGACAACTTCAGTG-3′

Supplementary Table 3. *LRP2* mutations in DBS/FOAR patients[♦].

		Allele 1 (paternal)			Allele 2 (maternal)			
Kindred	**Country**	**Mutation**	**Exon/ Intron**	**Domain / repeat**	**Mutation**	**Exon/ Intron**	**Domain / repeat**	**Parental Consanguinity**
Kindred 1	United Arab Emirates	c.7564T>C[*&+] (p.Y2522H)	41	LDL-receptor class B 27	c.7564T>C[*&+] (p.Y2522H)	41	LDL-receptor class B 27	Yes
Kindred 2	France	c.9484_9485delGT* (p.V3162LfsX2)	50	EGF-like 12	IVS18-1G>A*	18	LDL-receptor class B 8	No
Kindred 3	France	c.13139insC* (p.P4380PfsX12)	72	EGF-like 17	IVS7-2A>G*	7	LDL-receptor class A 6	No
Kindred 4	United Kingdom	IVS44+1G>A	44	LDL-receptor class A 18	c.10195C>T (p.R3399X)	53	LDL-receptor class B 33	No
Kindred 5	United Kingdom	c.8516_8519delTTTA (p.V2839VfsX67)	45	LDL-receptor class A19	c.8516_8519delTTTA (p.V2839VfsX67)	45	LDL-receptor class A 20	?
Kindred 6	Qatar	c.9358_9359delAG (p.S3120WfsX26)	50	EGF-like 11	c.9358_9359delAG (p.S3120WfsX26)	50	EGF-like 11	Yes
Kindred 7[#]	Belgium	IVS11+2T>G	11	LDL-receptor class B 1	c.1093C>T (p.R365X)	10	EGF-like 2	No

***** The IVS7-2A>G, IVS18-1G>A, Y2522H, V3162LfsX2, and P4380PfsX12 mutations were not found in 96 ethnically-matched normal controls. We did not sequence the R3399X, V2839VfsX67, IVS11+2T>G, and R365X mutations in normal controls.

[#] The proband carried an additional paternally-inherited G598R missense variation in exon 14, which was neither previously reported in the NCBI dSNP database nor found among 96 control samples sequenced. Although ClustalW demonstrated that this variation changed an evolutionarily-conserved residue, the upstream mutations are likely to cause protein truncation.

[&] Polymorphism Phenotype (PoyPhen; http://genetics.bwh.harvard.edu/pph/) and Sort Intolerant from Tolerant (SIFT; http://blocks.fhcrc.org/sift/SIFT.html) were used to predict the impact of Y2522H on *LRP2;* no data were generated using either software program.

[+] Clustal W2 in LRP2

AA position	2522	
LRP2 mutation	**H**	
Human	C Q G Y L **Y** W A D W D T H A K I E R A T L G G N F R V P I V N S S L V	**2551**
Chimp	C Q G Y L **Y** W A D W D T H A K I E R A T L G G N F R V S I V N S S L V	**911**
R.macaque	C Q G Y L **Y** W A D W D M H A K I E R A T L G G N F R V P I V N S S L V	**2506**
Mouse	C R G Y M **Y** W T D W G T N A K I E R A T L G G N F R V P I V N T S L V	**2334**
Rat	C R G Y M **Y** W T D W G T N A K I E R A T L G G N F R V P I V N T S L V	**2550**
Dog	C R G Y M **Y** W T D W G T N A K I E R A T L G G N F R V P I V N S S L V	**2530**
Chicken	C R G Y M **Y** W T D W S S N A K I E R A T L G G N F R T P I V S T N L V	**2538**
X tropicalis	C R G Y M **Y** W T D W G T N A K I E R A T L G G N F R T A I V N T S L V	**2521**

♦*Kindred 1*- Affecteds: homozygous T to C transition at nt 7564 in exon 41 leads to substitution of polar histidine for non-polar tyrosine at amino acid position 2522 (Y2522H). Parents are heterozygous carriers.
Kindred 2- Affecteds: compound heterozygotes for a 2-bp GT deletion (c.9484_9485delGT) in exon 50 and an IVS18-1G>A splicing mutation. The GT deletion causes a frame shift creating an early stop codon (p.V3162LfsX2), while the IVS18-1G>A mutation affects transcript splicing by altering the acceptor site. Two healthy sisters are heterozygous carriers for the 2-bp GT deletion.
Kindred 3- Affecteds: surviving patient has a 1-bp C insertion at nt 13139 (c.13139insC) in exon 72 causing frame shift and creating a stop codon (p.P4380PfsX12). The 2nd mutation, IVS7-2A>G, affects transcript splice acceptor site. One healthy sib is a heterozygous carrier for the 1-bp C insertion.
Kindred 4- Affecteds: compound heterozygotes for an IVS44+1G>A splicing mutation which alters transcript splice donor site, and a C to T transition at nt 10195 (c.10195C>T) in exon 53 which creates a stop codon at amino acid position 3399 (p.R3399X).
Kindred 5- Affected: homozygous 4-bp deletion (c.8516_8519delTTTA) in exon 45 causes a frame shift mutation (p.V2839VfsX67). Each parent is a heterozygous mutation carrier.
Kindred 6- Affecteds: homozygous 2-bp AG deletion (c.9358_9359delAG) in exon 50 causes frameshift and creates early termination codon (p.S3120Wfs26). Parents and three healthy sibs are heterozygous carriers; one healthy sib is not a carrier.
Kindred 7- Affected: An IVS11+2T>G splicing mutation alters the transcript splice donor site, and a C to T transition at nt 1093 (c.1093C>T) in exon 10 produces a premature stop at position 365 (p.R365X).

+ClustalW alignment in *LRP2* – Kindred 1 mutation (T to C transition at nt 7564 in exon 41) changes evolutionarily conserved tyrosine residue to histidine at amino acid position 2522 (Y2522H).