nature genetics

Mutations in *LRP2*, which encodes the multiligand receptor megalin, cause Donnai-Barrow and facio-oculo-acoustico-renal syndromes

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Donnai-Barrow syndrome is associated with agenesis of the corpus callosum, congenital diaphragmatic hernia, facial dysmorphology, ocular anomalies, sensorineural hearing loss and developmental delay. By studying multiplex families, we mapped this disorder to chromosome 2q23.3–31.1 and identified *LRP2* mutations in six families with Donnai-Barrow syndrome and one family with facio-oculo-acoustico-renal syndrome. *LRP2* encodes megalin, a multiligand uptake receptor that regulates levels of diverse circulating compounds. This work implicates a pathway with potential pharmacological therapeutic targets.

Congenital diaphragmatic hernia (CDH), a defect of diaphragm formation, has a persistently high morbidity and mortality often related to pulmonary hypoplasia. To identify genetic pathways of CDH, we study monogenic disorders in which CDH is a component, such as Donnai-Barrow syndrome (DBS; OMIM 222448). Individuals with this rare autosomal recessive disorder have major malformations, including those described above[1,2]. DBS has clinical similarity to facio-oculo-acoustico-renal (FOAR) syndrome (OMIM 227920), although the latter is typically reported as having proteinuria but lacking agenesis of the corpus callosum (ACC) and CDH[3,4].

To detect regions of identity by descent (IBD), we applied Affymetrix 10K SNP arrays to four individuals with DBS in a large consanguineous family (kindred 1) (**Supplementary Fig. 1** and **Supplementary Methods** online). The largest region of IBD (~21 Mb) was on chromosome 2q23.3–q31.1, flanked by SNPs rs1020088 and rs1362496 (data not shown). Using microsatellite markers in multiplex kindred 1 and three additional multiplex kindreds (2–4), we refined this region to ~18 Mb between D2S2299 and D2S2284 and generated a maximum two-point LOD score of 4.31 and multipoint LOD score of 6.243 (**Supplementary Fig. 2** online). The interval contains 51 known genes, including the 79-exon gene *LRP2* (low-density lipoprotein receptor–related protein 2), which encodes megalin.

We analyzed *LRP2* coding sequences and intron-exon boundaries in affected individuals from a total of seven kindreds; kindreds 1–6 were clinically diagnosed with DBS, and kindred 7 was clinically diagnosed with features of both FOAR and DBS (**Supplementary Tables 1** and **2** and **Supplementary Note** online). All affected individuals showed missense, nonsense, splice junction or frameshift mutations in evolutionarily conserved residues of both *LRP2* alleles (**Fig. 1** and **Supplementary Table 3** online). The similar phenotype observed among affected individuals, including those with homozygous frameshifts, suggests that most mutations are functionally null. These mutations were absent in NCBI and Ensembl SNP databases; additionally, we did not detect the kindred 1–3 mutations by sequencing DNA from 96 controls of similar ancestry. Thus, DBS and FOAR syndromes should be regarded as the same disorder, DBS/FOAR, and may represent the first human phenotype associated with mutations in *LRP2*.

Megalin, an endocytic transmembrane receptor of 4,655 amino acid residues, containing low-density lipoprotein receptor class A and B motifs, epidermal growth factor-like repeats and an intracellular protein-binding Asn-Pro-X-Tyr sequence, is located primarily on the apical surface of absorptive epithelia[5]. It is critical for reuptake of numerous ligands, including lipoproteins, sterols, vitamin-binding proteins and hormones[6,7]. Megalin also has a role in cell signaling by interacting with sonic hedgehog[8]. Studies in mice demonstrate megalin expression in specialized epithelium of numerous organs, including brain, kidney, and lung[5,9]. Its expression has not been examined in the diaphragm.

Megalin knockout mice have high perinatal mortality owing to respiratory insufficiency, with their lungs described as 'emphysematous' and 'atelectatic'[10]. A small number of surviving megalin-null mice, as well as those with a conditional knockout affecting the kidneys, demonstrate proteinuria with elevated levels of megalin

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Received 13 February; accepted 24 May; published online 15 July 2007; doi:10.1038/ng2063

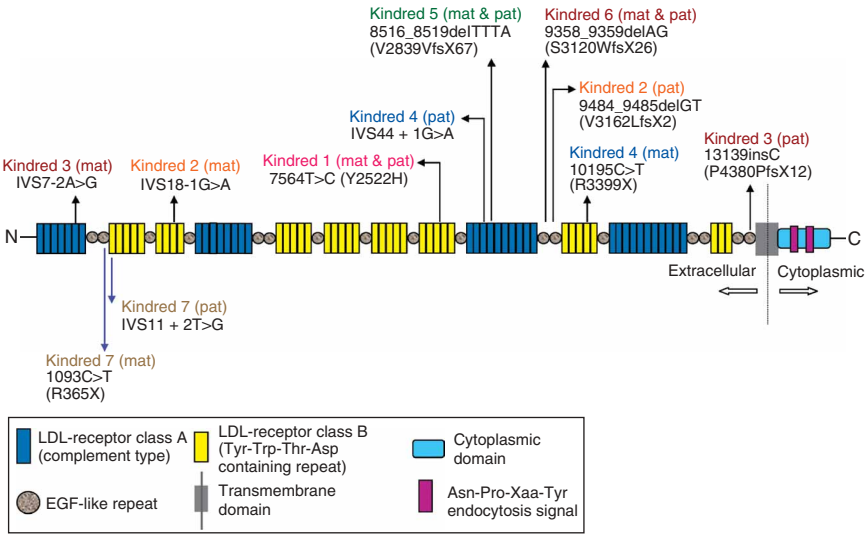

Figure 1 Schematic representation of *LRP2* mutations and corresponding megalin domains. *LRP2* sequencing uncovered missense, nonsense, frameshift or splice junction mutations in individuals with DBS/FOAR from seven kindreds. Mutations are indicated adjacent to the megalin extracellular domains that they affect; mutation 'hotspots' or genotype-phenotype correlations are not apparent. Mat, maternally inherited mutation; pat, paternally inherited mutation (**Supplementary Table 3**).

ligands, including retinol-binding (RBP) and vitamin D–binding (DBP) proteins[11]. Comparably, urine samples from eight individuals with DBS/FOAR show proteinuria, including increased spillage of RBP and DBP (**Supplementary Fig. 3** online). This characteristic proteinuria in all affected individuals tested provides strong evidence that the *LRP2* mutations have a negative impact on megalin function in the renal proximal tubule and that elevated urinary RBP and DBP can serve as valuable diagnostic and surrogate markers for DBS/FOAR.

The megalin-null mice also demonstrated forebrain and olfactory bulb anomalies, ACC, mild holoprosencephaly, microophthalmia or anophthalmia, and craniofacial dysmorphology[10]. Review of magnetic resonance imaging (MRI) scans available from three unrelated individuals with DBS/FOAR (**Fig. 2a–e**) showed that none had holoprosencephaly, but all had corpus callosum anomalies (agenesis in kindred 5; hypoplasia involving the splenium and rostrum in kindreds 1 and 3) and enlarged globes with small colobomas at the optic nerve heads. Comparison of cortical surface recontructions between one affected individual who had periventricular nodular heterotopia along the lateral ventricles and one age-matched control (**Fig. 2f–i**) demonstrated disrupted frontal lobe development with an unrecognizable, abnormally placed central sulcus. Based on previously published and recent reports with adequate imaging information, all clinically diagnosed individuals with DBS have either callosal agenesis (58%) or hypoplasia (42%); by contrast, individuals clinically diagnosed with FOAR have macrocephaly but not ACC.

Given their phenotypic similarity to individuals with DBS/FOAR, we sequenced *LRP2* in three families with Chudley-McCullough syndrome (OMIM 604213) and observed that this syndrome is not allelic. Overlapping syndromes such as acrocallosal syndrome (OMIM 200990) may belong to a family of 'megalinopathies' due to mutations in the gene encoding megalin or an interacting gene in the same pathway. The mechanism(s) by which *LRP2* mutations cause DBS/FOAR is currently unknown. Megalin is not reported to be expressed in fibroblasts or lymphoblasts, and accordingly, we were unable to detect either protein or mRNA by immunohistochemistry or RT-PCR in these tissues from individuals with DBS/FOAR (kindreds 1, 3, and 7) or from controls (data not shown). Immunohistochemistry for megalin in kidney sections from a week 25 fetus with DBS/FOAR (kindred 2, subject 2) demonstrated a staining pattern comparable to that of a control of matched gestational age, suggesting that the functional defect does not require absent protein. The characteristic pattern of DBS/FOAR anomalies may result from impaired megalin endocytosis and subsequent failure to deliver lipophilic compounds during

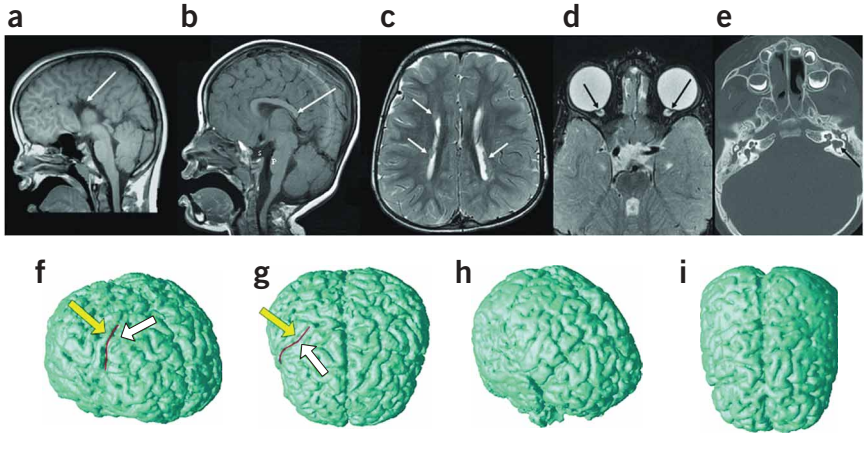

Figure 2 MRI abnormalities in individuals with DBS/FOAR. (**a**) Sagittal T1-weighted magnetic resonance image of subject 1, kindred 5, showing ACC with associated absence of cingulate gyrus. (**b**) Sagittal T1-weighted magnetic resonance image of subject IV-6, kindred 1, showing arrested development of corpus callosum with truncation of posterior body, absence of splenium and rostrum of corpus callosum, partially empty sella (s) and small-appearing pons (P). (**c**,**d**) Axial FSE T2-weighted magnetic resonance image of subject IV-6, kindred 1, showing subependymal nodular heterotopia (**c**) and enlarged globes with small colobomas at the optic nerve heads (**d**). (**e**) Axial computed tomography of temporal bones of subject IV-6, kindred 1, showing globular malformation of the left horizontal semicircular canal and vestibule. Abnormalities are indicated by arrows in **a**–**e**. (**f**–**i**) Brain cortical surface reconstruction from spoiled gradient-echo (SPGR) structural magnetic resonance image. **f** shows a frontal right view and **g** shows a top view for a 4-year-old control. Central sulcus, precentral gyrus and postcentral gyrus are labeled by a red curve, a white arrow and a yellow arrow, respectively. **h** shows a frontal right view and **i** shows a top view for subject IV-6, kindred 1, with DBS/FOAR. Note abnormally developed frontal lobe and unrecognizable abnormally placed central sulcus.

ontogeny[12]. Megalin ligands (such as vitamin A (retinol) and cholesterol) are critical for normal embryonic development. Extensive data on teratogenic and genetic animal models, as well as from recently published accounts of affected individuals with *STRA6* mutations, confirm a role of vitamin A in diaphragm and lung development[13,14]. In addition, disruption of megalin–sonic hedgehog interactions may contribute to abnormal brain and lung development[12,15]. Our data raise the possibility that ligand-selective supplementation, such as with vitamin A analogs, may be valuable in preventing or mitigating some of the specific birth defects seen in DBS/FOAR. Finally, examination of a larger cadre of affected individuals with isolated or nonsyndromic malformations, such as CDH or ACC, can help determine whether megalin pathway defects have a role in their pathogenesis and whether this pathway contains putative therapeutic targets.

Individuals with DBS/FOAR were first recruited and clinically characterized by their geneticists, who, with appropriate consent, provided samples to be analyzed in our study ('Gene Mutations and Rescue in Human Diaphragmatic Hernia'); the protocol for this study is approved annually by the Massachusetts General Hospital Institutional Review Board.

Note: Supplementary information is available on the Nature Genetics website.

ACKNOWLEDGMENTS

We thank the many families who supported this project. We thank D. Altshuler, D. Brown, M. Daly, J. Gusella, J. Ingelfinger, M. MacDonald and T.E. Willnow for discussions; L. Blakemore, J. Graham, Y. Lacassie, E. McPherson, A. Paterson, D. Powell, J. Tchinda Ndjuiken, D. Gleason, L. Mitova, K. O'Brien, and T. Manganaro for advice and assistance; L. Holmes for reporting the first family with FOAR and for encouraging this study from its inception; L. Javois and T. Hewitt for conceiving the US National Institute of Child Health and Human Development Birth Defects Initiative and encouraging and supporting this work. G.C.M.B. is a Wellcome Trust Senior Clinical Research Fellow. C.A.W. and R.S.H. are supported by US National Institute for Neurological Disorders and Stroke grant R37 NS35129. C.A.W. is an Investigator of the Howard Hughes Medical Institute. P.K.D., S.K., M.L., D.T.M., K.M.N., B.R.P. and M.K.R. are supported by R01 HD55150-01. K.M.N. is also supported by the 2006 American College of Surgeons' Resident Research Award.

AUTHOR CONTRIBUTIONS

S.K. designed and conducted experiments, interpreted data and prepared manuscript. K.M.N. designed and conducted experiments (urinary, immunostaining and RT-PCR), interpreted data and prepared manuscript. L.A.-G., G.C.M.B., E.B., N.C., D.D., K.D. and A.T. were clinical collaborators (recruiting DBS/FOAR kindreds and providing phenotypic information, patient samples and results of laboratory testing). G.C.M.B. prepared the manuscript as well. R.S.H. interpreted Affymetrix SNP array mapping and microsatellite marker genotyping and performed LOD score calculations. C.R. interpreted MRI scans. T.L. performed and analyzed MRI surface reconstructions of the brain. M.K.R. was the study coordinator, designed and implemented infrastructure, obtained institutional review board approval and consents, and assisted in conducting experiments. D.T.M. designed and interpreted (urinary) experiments, and designed and implemented infrastructure. M.L. designed and interpreted megalin expression studies. P.K.D., B.R.P. and C.A.W., who contributed equally to this work, conceived the project, designed infrastructure, supervised design of experiments and data interpretation, and prepared the manuscript.

COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

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1. Donnai, D. *et al. Am. J. Med. Genet.* **47**, 679–682 (1993).
2. Chassaing, N. *et al. Am. J. Med. Genet. A.* **121**, 258–262 (2003).
3. Holmes, L.B. *et al. J. Pediatr.* **81**, 552–555 (1972).
4. Devriendt, K. *et al. J. Med. Genet.* **35**, 70–71 (1998).
5. Christensen, E.I. *et al. Nat. Rev. Mol. Cell Biol.* **3**, 256–266 (2002).
6. Raila, J. *et al. J. Nutr.* **135**, 2512–2516 (2005).
7. Nykjaer, A. *et al. Cell* **96**, 507–515 (1999).
8. McCarthy, R.A. *et al. J. Biol. Chem.* **277**, 25660–25667 (2002).
9. Fisher, C.E. *et al. Dev. Biol.* **296**, 279–297 (2006).
10. Willnow, T.E. *et al. Proc. Natl. Acad. Sci. USA* **93**, 8460–8464 (1996).
11. Leheste, J.R. *et al. Am. J. Pathol.* **155**, 1361–1370 (1999).
12. McCarthy, R.A. *et al. J. Cell Sci.* **116**, 955–960 (2003).
13. Kluth, D. *et al. J. Pediatr. Surg.* **25**, 850–854 (1990).
14. Pasutto, F. *et al. Am. J. Hum. Genet.* **80**, 550–560 (2007).
15. Unger, S. *et al. Am. J. Pathol.* **162**, 547–555 (2003).