ORIGINAL ARTICLE

Polyhydramnios, Transient Antenatal Bartter's Syndrome, and *MAGED2* Mutations

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ABSTRACT

BACKGROUND

Three' pregnancies with male offspring in one family were complicated by severe polyhydramnios and prematurity. One fetus died; the other two had transient massive salt-wasting and polyuria reminiscent of antenatal Bartter's syndrome.

METHODS

To uncover the molecular cause of this possibly X-linked disease, we performed whole-exome sequencing of DNA from two members of the index family and targeted gene analysis of other members of this family and of six additional families with affected male fetuses. We also evaluated a series of women with idiopathic polyhydramnios who were pregnant with male fetuses. We performed immunohistochemical analysis, knockdown and overexpression experiments, and protein–protein interaction studies.

RESULTS

We identified a mutation in *MAGED2* in each of the 13 infants in our analysis who had transient antenatal Bartter's syndrome. *MAGED2* encodes melanoma-associated antigen D2 (MAGE-D2) and maps to the X chromosome. We also identified two different *MAGED2* mutations in two families with idiopathic polyhydramnios. Four patients died perinatally, and 11 survived. The initial presentation was more severe than in known types of antenatal Bartter's syndrome, as reflected by an earlier onset of polyhydramnios and labor. All symptoms disappeared spontaneously during follow-up in the infants who survived. We showed that MAGE-D2 affects the expression and function of the sodium chloride cotransporters NKCC2 and NCC (key components of salt reabsorption in the distal renal tubule), possibly through adenylate cyclase and cyclic AMP signaling and a cytoplasmic heat-shock protein.

CONCLUSIONS

We found that *MAGED2* mutations caused X-linked polyhydramnios with prematurity and a severe but transient form of antenatal Bartter's syndrome. MAGE-D2 is essential for fetal renal salt reabsorption, amniotic fluid homeostasis, and the maintenance of pregnancy. (Funded by the University of Groningen and others.)

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In the second trimester of gestation, fetal kidneys become the predominant source of amniotic fluid, which is primarily removed by the fetus swallowing it.[1] Excessive amniotic fluid (called polyhydramnios) is caused by an imbalance between its production and removal — as observed, for instance, in fetuses with esophageal atresia. Overall, polyhydramnios has a prevalence of 1 to 2% and confers an increased risk of adverse perinatal outcome, especially preterm delivery.[2] The cause of polyhydramnios remains unknown in 30 to 60% of cases.[2,3] There are only a few mendelian diseases associated with polyhydramnios, including antenatal Bartter's syndrome, a rare autosomal recessive renal tubular disorder. Antenatal Bartter's syndrome is a potentially life-threatening disease characterized by fetal polyuria, polyhydramnios, prematurity, and postnatal polyuria with persistent renal salt wasting. The known genetic causes of antenatal Bartter's syndrome directly affect the molecules that mediate salt reabsorption in the thick ascending limb of the loop of Henle.[4] Treatment includes lifelong fluid and electrolyte supplementation, as well as the use of nonsteroidal antiinflammatory drugs (NSAIDs) to inhibit excessive renal prostaglandin E_2 formation,[5] although the safety of long-term treatment with NSAIDs, especially in preterm infants, is a subject of controversy.[6]

Two case reports describing three male infants with antenatal Bartter's syndrome are of special interest because salt wasting spontaneously resolved within several weeks after birth.[7,8] In one family with three affected sons, only the younger two sons had postnatal development of polyuria.[8] This points to an overlap with another condition of unknown cause, termed acute recurrent polyhydramnios, a familial condition that has also been described in male infants.[9-11] In this study, we sought to characterize severe polyhydramnios and transient antenatal Bartter's syndrome in 15 boys from nine families, to determine the genetic basis of this disorder, and to provide insight into the pathophysiological basis of the phenotype.

METHODS

STUDY DESIGN AND OVERSIGHT

We conducted the study from April 2013 until May 2015. DNA samples were obtained with written informed consent from the patients or their guardians, as well as from unaffected family members. Clinical and biochemical data were collected retrospectively from medical charts. We studied the index family (F1) plus 6 additional families (F2 through F7, all of which had members with a transient clinical course of antenatal Bartter's syndrome) of Dutch, German, Belgian, and Turkish descent chosen from a cohort of 300 families with antenatal Bartter's syndrome. All affected infants were male and had tested negative for mutations in *SLC12A1* (encoding NKCC2), *KCNJ1* (encoding ROMK), and *BSND* (encoding barttin). We also evaluated a series of 11 women who had been counseled for idiopathic polyhydramnios during pregnancies with male fetuses. Polyhydramnios was diagnosed as an amniotic fluid index (a score indicating the amount of amniotic fluid measured on an ultrasonogram) of greater than 24 cm.[12] The study was approved by the ethics committees at the University Medical Centers in Cologne and Groningen.

LABORATORY ANALYSIS

Standard methods were used to analyze electrolyte and creatinine levels and solute concentrations (osmolalities) in urine and blood. Plasma aldosterone and plasma renin concentrations were measured with the use of radioimmunologic assays.[13] Urinary prostaglandin E_2 (PGE_2) was measured by gas chromatography–tandem mass spectrometry in cooled 24-hour urine samples, as described previously.[14]

GENETIC ANALYSIS

We performed whole-exome sequencing of DNA from Patient F1.III-1 and his mother (F1.II-2), as described previously.[15] Splicing was analyzed in vitro with a minigene (pSPL3 splicing) assay.[16] See the Supplementary Appendix, available with the full text of this article at NEJM.org, for further details of the genetic analysis methods, as well as for details of the immunohistochemical analysis, the studies of manipulating gene expression in a cell line, and interactome analyses.

RESULTS

INDEX FAMILY

The first pregnancy of Family Member F1.I-2 was complicated by severe polyhydramnios that was diagnosed at 19 weeks of gestation and resulted in preterm delivery of a stillborn male (F1.II-1) at 22 weeks (Fig. 1A). Two subsequent pregnan-

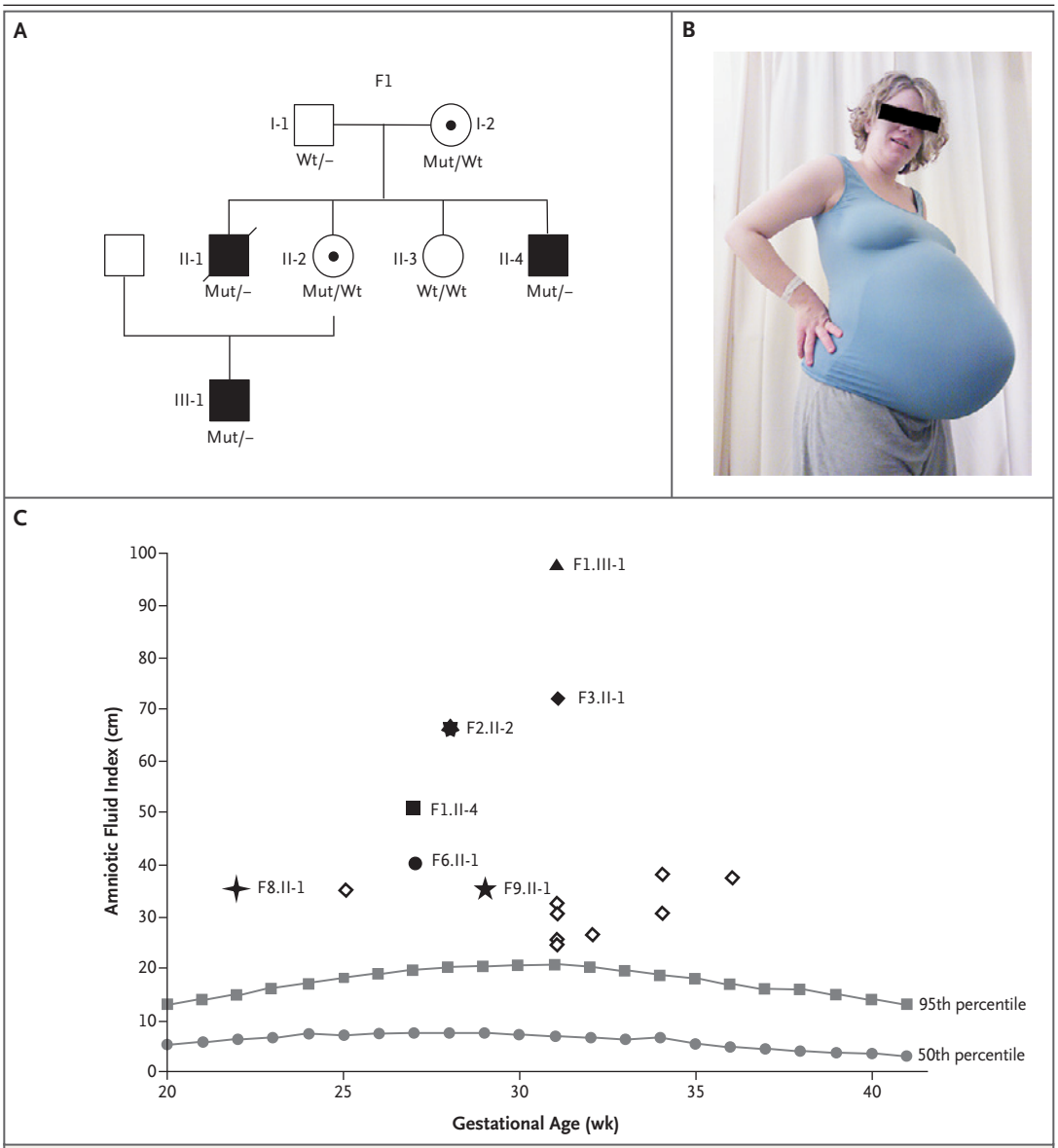

Figure 1. Clinical and Genetic Characteristics of the Index Family and Amniotic Fluid Index in Patients with Mutations in *MAGED2*.

Panel A shows a pedigree of Family F1, in which pregnancies with male fetuses were complicated by early-onset and severe polyhydramnios. A stillborn male (F1.II-1) was delivered at 22 weeks of gestation; two subsequent pregnancies (with female fetuses) were uneventful. In the mother's last pregnancy, a male infant (F1.II-4) was delivered at 27 weeks. The genotypes are shown beneath each symbol; Mut denotes the mutant *MAGED2* allele, and Wt wild type. Squares denote male family members, circles female family members, solid symbols affected family members, symbols with a dot unaffected carriers, and symbols with a line through them deceased family members. During the first pregnancy of Family Member F1.II-2, shown in Panel B, early-onset severe polyhydramnios (amniotic fluid index [a score indicating the amount of amniotic fluid measured on an ultrasonogram], 98 cm) occurred and led to birth of a preterm male infant (F1.III-1) after 31 weeks of gestation. Panel C shows the amniotic fluid index for each patient with *MAGED2* mutations (labeled symbols), as well as for patients with idiopathic polyhydramnios who did not have *MAGED2* mutations (diamonds); the 50th percentile (gray circles) and 95th percentile (gray squares) for values during normal pregnancy are shown for comparison.

cies (with female fetuses) were uneventful. The mother's last pregnancy was again complicated by early-onset (at 19 weeks) severe polyhydramnios (amniotic fluid index, 51 cm) that led to preterm delivery, at 27 weeks, of a male infant (F1.II-4). Immediately after birth, progressive polyuria

developed in the infant (maximum, 140 ml per kilogram per hour), with salt loss necessitating intravenous fluid infusion. Severe hypercalciuria also developed, which caused medullary nephrocalcinosis. Despite the surprising disappearance of the clinical symptoms within 3 to 4 weeks, antenatal Bartter's syndrome was suspected, and the patient was treated with supplemental electrolytes and indomethacin for 1 year. At the last follow-up, when the patient was 17 years of age, he showed no clinical signs of renal salt loss and had normal urinary concentrating and diluting capacities.

During the first pregnancy of his sister (F1.II-2), early-onset (at 19 weeks of gestation) severe polyhydramnios (amniotic fluid index, 98 cm) (Fig. 1B and 1C) occurred and led to birth of a preterm male infant (F1.III-1) after 31 weeks of gestation. Again, neonatal polyuria was observed, which peaked at 50 ml per kilogram per hour and normalized within 1 week. At last follow-up, at 2 years of age, the patient had normal tubular and glomerular function.

GENETIC ANALYSIS

Because polyhydramnios occurred exclusively in pregnancies with male offspring, we filtered the results obtained by whole-exome sequencing analysis of DNA from Patient F1.III-1 and his mother for rare shared X-chromosomal variants (minor-allele frequency, ≤0.001). Both the mother and the son carried a nonsense mutation that resulted in a premature stop codon in *MAGED2* (c.1038C→G, p.Y346*), which encodes melanoma-associated antigen D2 (MAGE-D2).

Targeted Sanger sequencing of DNA from family members showed cosegregation of p.Y346* in all affected males and their mothers (Fig. 1A). Subsequent sequencing of *MAGED2* in six additional families with transient antenatal Bartter's syndrome and 11 women who had had idiopathic polyhydramnios while pregnant with male fetuses identified mutations that were specific to each family in all affected males with transient antenatal Bartter's syndrome (F2 through F7) (Fig. 2A) and to two families with idiopathic polyhydramnios (F8 and F9). In total, seven truncating mutations (two nonsense, two frameshift, and three splice-site mutations) and two nontruncating mutations (one missense and one in-frame deletion) were identified (Fig. 2B, and Tables S1 and S2 in the Supplementary Appendix), none of which was present in 110 persons of European descent without a family history of polyhydramnios or in public databases (1000 Genomes [http://1000genomes.org] and ExAC [http://exac.broadinstitute.org]).

TRANSIENT ANTENATAL BARTTER'S SYNDROME

Polyhydramnios was recognized early during pregnancy, at 19 to 20 weeks of gestation (Table 1; for individual data, see Table S1 in the Supplementary Appendix), and was deemed to be severe (amniotic fluid index, >35 cm) (Fig. 1C).[2] All the infants were born preterm, seven of them extremely preterm (at <28 weeks).[17] The onset of polyhydramnios and labor occurred several weeks earlier than in known types of Bartter's syndrome. Two fetuses died in utero without a recognizable cause on autopsy, and one infant died from extreme prematurity.

In the preterm babies, polyuria lasted from 3 days to 6 weeks and ended at 30 to 33 weeks of gestational age. Hypercalciuria was initially present, but it normalized in parallel with the resolution of renal salt and water losses. Nephrocalcinosis was noted in six patients and persisted in four patients. In the neonatal period, hyponatremia, hypokalemia, and elevated levels of renin and aldosterone were noted; these subsequently resolved or normalized. Five patients were treated with indomethacin for up to 9 years.

IDIOPATHIC POLYHYDRAMNIOS AND *MAGED2* MUTATIONS

Because the first son of Family F3 presented with polyhydramnios only,[8] we studied a series of 11 women who had idiopathic polyhydramnios while pregnant with male fetuses and found *MAGED2* mutations in two additional families (F8 and F9) (Fig. 2A). We identified a missense mutation in one fetus (F8.II-1), who died at 22 weeks of gestation, and a heterozygous intronic mutation in the mother of a second family. The boy in the second family (F9.II-1), who was born after 29 weeks of gestation, survived without transient antenatal Bartter's syndrome. Aberrant splicing was shown in vitro; this splicing led to the generation of a premature stop codon (Fig. S1 in the Supplementary Appendix).

EXPRESSION OF MAGE-D2 IN HUMAN KIDNEY

We observed prominent tubular expression of MAGE-D2 in the human fetal renal cortex (Fig. S2 in the Supplementary Appendix). In both fetal

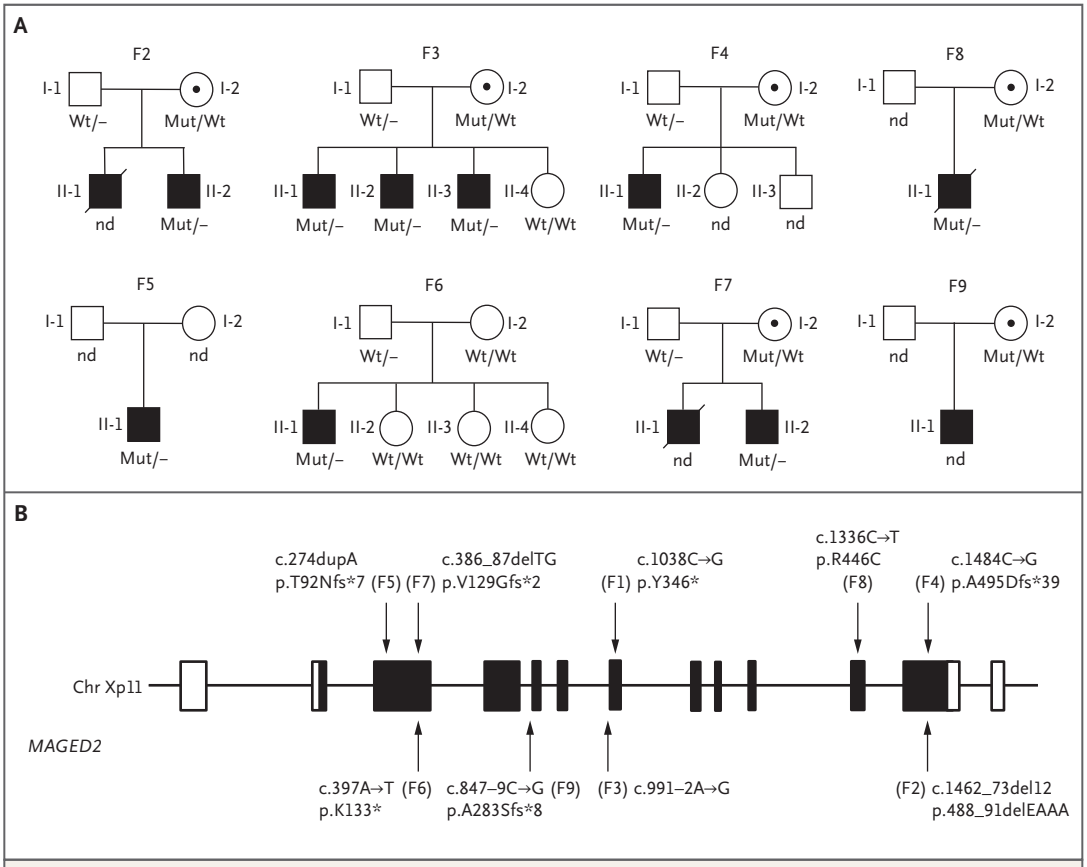

Figure 2. Genetic Characteristics of Patients with Transient Antenatal Bartter's Syndrome and Idiopathic Polyhydramnios with Mutations in *MAGED2*.

Panel A shows the pedigrees of six additional families with transient antenatal Bartter's syndrome (F2 through F7) and of two families with idiopathic polyhydramnios that occurred during pregnancies with male fetuses (F8 and F9); *MAGED2* mutations were found in all affected males. The abbreviation nd denotes no data. Panel B shows the seven truncating mutations and two nontruncating mutations that were identified in *MAGED2*; mutations are shown at the nucleotide level and protein level for each family. The splice-site mutation in Family F3 has not been confirmed in vitro.

and adult kidney, MAGE-D2–positive tubules also bound anti-uromodulin (UMOD) antibody, which supported the conclusion that MAGE-D2 is expressed in the thick ascending limb of the loop of Henle (Fig. S3 in the Supplementary Appendix). We also found that MAGE-D2 is expressed in tubules outside the thick ascending limb of the loop of Henle in both fetal and adult kidney.

EXPRESSION OF NKCC2 AND NCC IN FETAL KIDNEY

Because of the phenotypic overlap between transient Bartter's syndrome and antenatal Bartter's syndrome caused by NKCC2 defects, we analyzed expression of NKCC2 in fetal kidney from Patient F1.II-1 and from control kidneys (21 and 23 weeks of gestation). In fetal controls, NKCC2 localized predominantly at the apical membrane of tubular epithelial cells (Fig. 3A). In contrast, NKCC2 expression was reduced and virtually absent from the apical cell membrane in Patient F1.II-1. Instead, NKCC2 staining was predominantly cytoplasmic and colocalized with a marker of the endoplasmic reticulum.

Because impaired NKCC2 expression cannot fully account for the severity of transient Bartter's syndrome, we also analyzed the expression of the sodium chloride cotransporter NCC, a crucial component of salt reabsorption in the distal convoluted tubule that was previously shown to be compensatorily up-regulated in a mouse model of antenatal Bartter's syndrome.[18] Like NKCC2, NCC was absent from the apical membrane in the patient's kidney tubules (Fig. 3B). Instead, intracellular retention of NCC

Table 1. Clinical and Postnatal Biochemical Characteristics of Patients with *MAGED2* Mutations.*

Characteristic	Normal Value	Patients with Mutations	
		Median (Range)	No. of Patients
Clinical data			
Onset of polyhydramnios — wk of gestation		19 (19–20)	10
Amniotic fluid index — cm†	<24	51 (36–98)	7
Gestational age at delivery — wk	40	28 (22–34)	15
Duration of polyuria — wk		4.5 (0.5–6)	8
Nephrocalcinosis — no. of patients			6 of 10
Duration of mineral supplementation — mo		6 (1–36)	8
Duration of indomethacin treatment — yr		2 (1–9)	6
Blood data			
Sodium — mmol/liter	134–146	128 (123–133)	9
Chloride — mmol/liter	97–110	94 (84–100)	9
Potassium — mmol/liter	4–6.5	2.9 (2.8–3.3)	9
Bicarbonate — mmol/liter	22–29	28 (24–28)	6
Renin — ng/ml/hr	4–23	69 (11–320)	6
Aldosterone — ng/dl	25–213	1735 (107–4260)	6
Urine data			
Maximal calcium:creatinine ratio‡	<0.8	5 (2.9–14.3)	8
PGE_2 — ng/hr/1.73 m^2	4–27	85 (8.4–243)	5

* To convert the values for aldosterone to picomoles per liter, multiply by 27.74. To convert the values for renin to nanograms per liter·second, multiply by 0.2778. PGE_2 denotes prostaglandin E_2.
† The amniotic fluid index is a score indicating the amount of amniotic fluid measured on an ultrasonogram.
‡ The levels of calcium and creatinine used to calculate the ratio were measured in milligrams per deciliter.

was observed in the patient sample. Unlike the expression of NKCC2 and NCC, UMOD expression was clearly discernible in the apical membrane in the sample from the patient (Fig. 3C).

EFFECT OF MAGE-D2 ON NKCC2, NCC, AND UMOD

On the basis of the intracellular retention of NKCC2, we hypothesized that a loss of MAGE-D2 may cause retention of NKCC2 and its degradation in the endoplasmic reticulum, resulting in diminished total and cell-surface expression. We therefore analyzed the effects of MAGE-D2 on the stability and biosynthetic processing of NKCC2, using a cycloheximide decay assay in HEK293T cells transiently expressing NKCC2. In cells transfected with MAGE-D2 small interfering RNA (Fig. 4A), we observed a faster rate of decay of the immature NKCC2 protein, which was associated with significantly lower levels of mature NKCC2 protein. To confirm the specificity of these findings, we investigated the effects of MAGE-D2 overexpression, which increased the half-life of immature NKCC2 (Fig. 4B) and accelerated its maturation, resulting in higher levels of the mature and hence membrane-expressed NKCC2.[19] These results were confirmed by the increase in cell-surface expression and activity of NKCC2 when it was coexpressed with MAGE-D2 (Fig. 4C and 4D). MAGE-D2 overexpression enhanced the expression of total and cell-surface NCC but did not affect the expression of UMOD, a finding consistent with the immunohistochemical findings (Fig. S4A and S4B in the Supplementary Appendix).

INTERACTOME OF MAGE-D2

To determine the mechanism by which MAGE-D2 regulates renal salt reabsorption, we analyzed the MAGE-D2 interactome and compared the interactors of wild-type versus mutant MAGE-D2 (p.R446C). Gs-alpha (also called GNAS) and Hsp40 (also called DNAJB1) interacted with wild-type MAGE-D2 but not with mutant MAGE-D2 (Fig. S5A and S5B in the Supplementary Appen-

dix). The interaction of MAGE-D2 with Gs-alpha was further confirmed in HEK293T cells with the use of endogenous MAGE-D2 immunoprecipitated with two different polyclonal antibodies with nonoverlapping epitopes (Fig. S5C and S5D in the Supplementary Appendix).

DISCUSSION

We examined an X-linked disease entity characterized by acute, early-onset, severe polyhydramnios; prematurity; perinatal mortality; and transient renal salt-wasting. We identified mutations in *MAGED2* in nine families, including those originally described in the 1990s.[7,8] In developing and adult kidneys, we found expression of MAGE-D2 in the thick ascending limb of the loop of Henle and distal tubules. In a fetus with transient antenatal Bartter's syndrome, we found reduced expression of the two critical sodium chloride cotransporters NKCC2 and NCC (which are expressed in the thick ascending limb of the loop of Henle and distal convoluted tubules), which explains the massive salt loss. We observed that, in a cultured renal-cell line, MAGE-D2 promotes NKCC2 and NCC expression and activity and that MAGE-D2 interacts specifically with Hsp40 and Gs-alpha. MAGE-D2 may thereby control the maturation of membrane proteins in the endoplasmic reticulum and enhance apical expression and activity of sodium chloride cotransporters.

Our results indicate that MAGE-D2 is critical for the maintenance of normal pregnancy. The fetal genotype is both necessary and sufficient for the full obstetrical and perinatal phenotype, as evidenced by Family F6, in which a de novo mutation in *MAGED2* was found in the fetus. Antenatal Bartter's syndrome with sensorineural deafness caused by mutations in *BSND* was hitherto considered to be the form of antenatal Bartter's syndrome with the most severe initial presentation.[20,21] However, the severe consequences of fetal MAGE-D2 loss are illustrated by the fact that the onset of severe polyhydramnios was earlier in fetuses with MAGE-D2 loss than in fetuses with *BSND* mutations (median, 19 vs. 22 weeks of gestation), as was the age at delivery (median, 28 vs. 32 weeks of gestation). The severity of the polyhydramnios caused by mutated *MAGED2* is further illustrated by a comparison of the amniotic fluid index in patients with "idiopathic" polyhydramnios who had *MAGED2* muta-

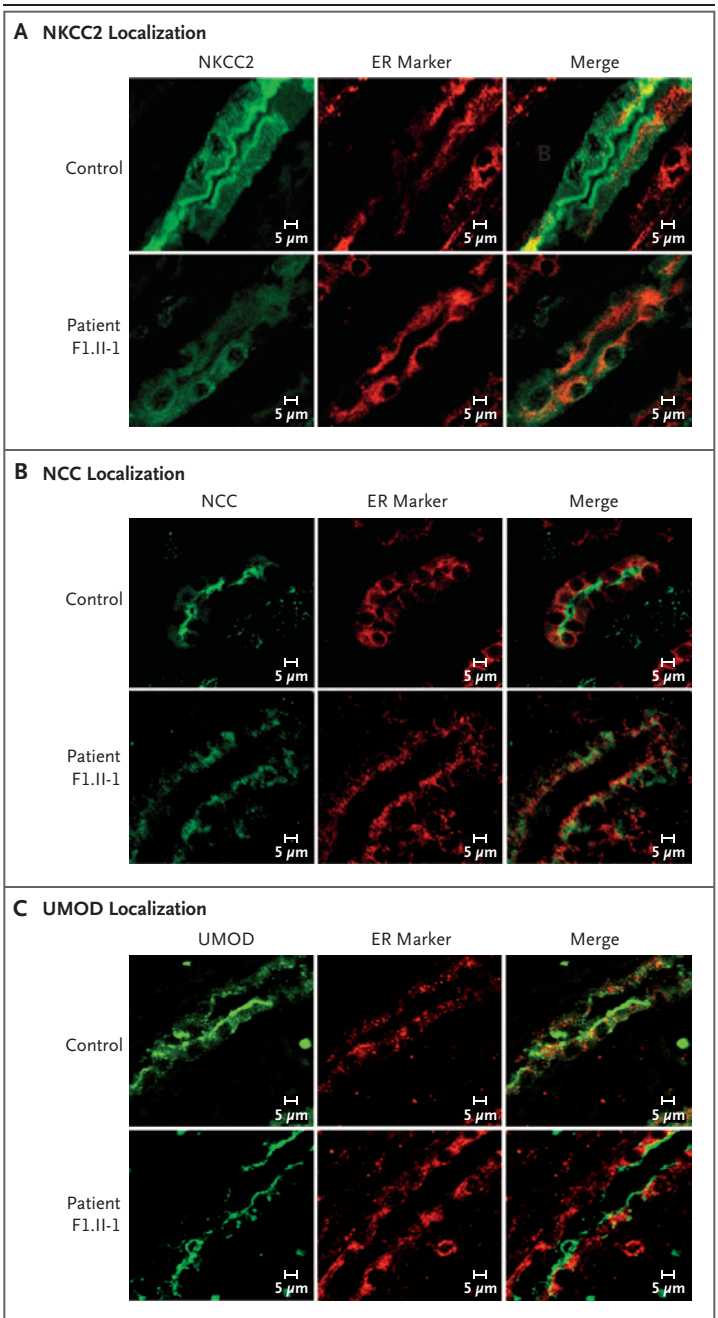

Figure 3. Altered Expression of NKCC2 and NCC in Fetal Renal Tissue from a Patient with Transient Antenatal Bartter's Syndrome.

Panel A shows the localization of NKCC2 predominantly at the apical membrane of tubular epithelial cells in fetal controls, whereas in Patient F1.II-1, NKCC2 expression was lower and virtually absent from the apical cell membrane. In the patient, NKCC2 staining was predominantly cytoplasmic and colocalized with a marker of the endoplasmic reticulum (ER). Panel B shows the localization of NCC, which was absent from the apical membrane in the patient's kidney tubules, in contrast to the control; in the patient, intracellular retention of NCC was observed. Panel C shows the expression of uromodulin (UMOD) immunoreactive protein, which was clearly discernible in the apical membrane in the sample from the patient.

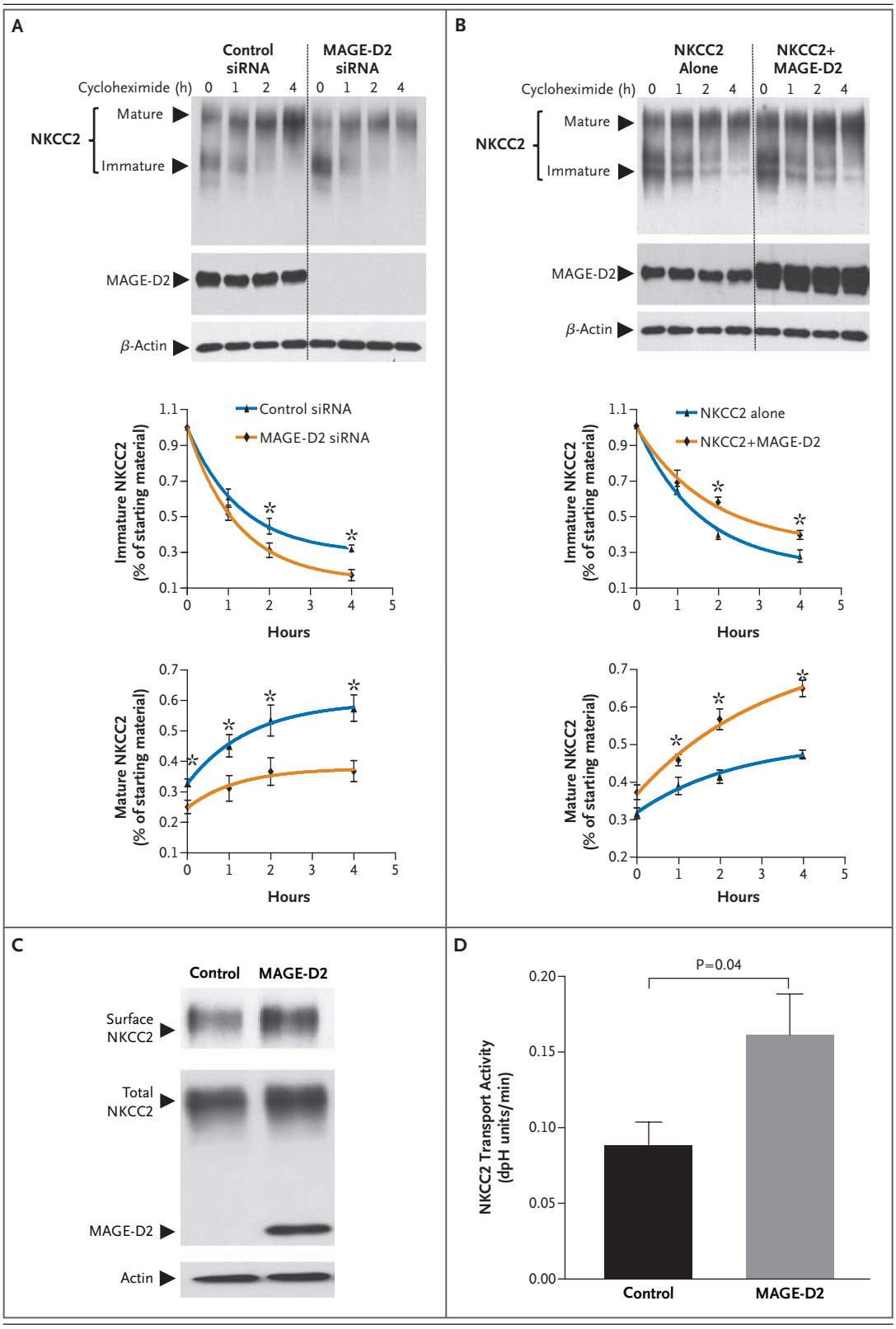

Figure 4 (facing page). Promotion of NKCC2 Biosynthesis, Cell-Surface Expression, and Activity by MAGE-D2.

To assess the stability and maturation of NKCC2, 12 to 14 hours after transfection of the cotransporter, cycloheximide was added to block protein synthesis; at various times after the addition of cycloheximide, NKCC2 levels were monitored by means of Western blotting. In cells transfected with MAGE-D2 small interfering RNA (siRNA), the immature NKCC2 protein had a higher rate of decay, which was associated with a significantly decreased maturation of NKCC2 protein (Panel A). MAGE-D2 overexpression was found to increase the half-life of immature NKCC2 (Panel B) and accelerate its maturation, resulting in increased levels of the mature and therefore membrane-expressed NKCC2. I bars represent the mean ±SE. Asterisks indicate significant differences ($P<0.05$). These results were confirmed by the increase in cell-surface expression and activity of NKCC2 when coexpressed with MAGE-D2 (Panels C and D). The bars and T bars in Panel D represent the mean ±SE rates of cell pH recovery from the ammonium-induced alkaline load.

tions with those who did not have such mutations (Fig. 1C). The absence of polyuria in F3.II-1 and F9.II-1 raises the question of whether previously reported cases of acute recurrent polyhydramnios might also be attributed to *MAGED2* mutations.[9-11]

Apart from our previous study showing that MAGE-D2 is prominently expressed in the developing mouse and human kidney,[22] studies of MAGE-D2 have been conducted primarily in the context of proliferation and tumor biology.[23,24] In contrast to several MAGEs that have been shown to promote ubiquitylation by activating RING ligases, little is known about the precise function of MAGE-D2.[25] We therefore sought to identify its binding partners and showed that MAGE-D2 interacts with Hsp40 and Gs-alpha.

Hsp40 is a cytoplasmic chaperone that interacts with NCC (and NKCC2 [unpublished observations]) and regulates its biogenesis in the endoplasmic reticulum.[26] Our observation that MAGE-D2 promotes NCC and NKCC2 biogenesis in a heterologous expression system that induces endoplasmic reticulum–associated degradation[27] is consistent with Hsp40 being a binding partner of MAGE-D2.

Gs-alpha is activated by G protein–coupled receptors and promotes the generation of cyclic AMP (cAMP) by activating adenylate cyclases. Our finding that MAGE-D2 promotes both total and cell-surface expression of NKCC2 and NCC is compatible with its binding to Gs-alpha. First, heterozygous loss of *GNAS*, which encodes Gs-alpha, reduces the expression of NKCC2 total protein.[28] Second, cAMP is a key regulator of cell-surface expression of NKCC2 and also enhances the activity of NCC.[29,30]

An intriguing aspect is the transient character of the renal phenotype, which is characterized by the spontaneous resolution of polyuria, a decrease in the concentrations of renin and aldosterone, and a decrease in urinary prostaglandin E_2 levels. Five patients in our study were given long-term treatment with NSAIDs and supplemental minerals in spite of their clinical and biochemical recovery; genetic diagnosis facilitated by our findings may help patients avoid such long-term treatment and may lead to better clinical management.

We do not have an explanation for the transient nature of the phenotype and can only speculate about the mechanisms involved. First, we hypothesize that increases in the sensitivity of adenylate cyclase activity to vasopressin, which has been described during renal development in several species,[31,32] permit expression of NKCC2 and NCC independent of MAGE-D2 beyond a certain stage of renal development. In addition, or alternatively, higher levels of oxygenation in the kidney during gestation may promote synthesis of NKCC2 and NCC: antenatally, the renal blood supply is just one fifth of postnatal values, which results in a remarkable degree of tissue hypoxia, especially in the medulla, where NKCC2 is expressed.[33-35] Both tissue hypoxia and heterologous expression of membrane proteins (i.e., NKCC2 in HEK293T cells) induce endoplasmic reticulum–associated protein degradation.[27,36]

We speculate that MAGE-D2 binding to the cytoplasmic chaperone Hsp40 protects NKCC2 and NCC from endoplasmic reticulum–associated degradation mediated by Hsp40. This could explain the (constitutive) requirement of MAGE-D2 for proper expression of NCC and NKCC2 in our cell system, in contrast to the oxygen-dependent — and, hence, time-dependent — function of MAGE-D2 during gestation.

We have shown that loss-of-function mutations in *MAGED2* caused a previously unrecognized

distinct phenotype of transient renal salt-wasting, which promoted early and severe polyhydramnios and therefore prematurity in association with substantial perinatal mortality. Our data indicate that MAGE-D2 has a role in positively regulating the expression of membrane proteins, which points to new avenues for restoring the trafficking of defective membrane proteins in human diseases. We speculate that the identification of loss-of-function mutations in *MAGED2* in the mothers of male fetuses with acute, early, and severe polyhydramnios may result in the avoidance of unnecessary diagnostic measures in pregnant women and of potentially harmful treatment of preterm babies.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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APPENDIX

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