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A novel model of polyhydramnios: amniotic fluid volume is increased in aquaporin 1 knockout mice

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Objective: To test the hypothesis that amniotic fluid volume is increased in aquaporin 1 knockout mice.

Study design: Transgenic mice deficient in aquaporin 1 protein were generated by targeted gene disruption, as described previously. After a cesarean section was performed, intact, individual gestational sacs were removed from the uterus and weighed. Amniotic fluid volume, osmolality, and fetal and placental weights were determined. Data were analyzed by a 1-way analysis of variance for ranks; Dunn's post hoc test was used to analyze significant trends.

Results: Analysis of 16 litters showed 35 wild-type, 52 heterozygote, and 33 aquaporin 1 knockout mice. The knockout mice had a greater volume of amniotic fluid and lower amniotic fluid osmolality than their wild-type and heterozygote counterparts. There were no significant differences in fetal or placental weights among the groups.

Conclusions: Aquaporin 1 null fetuses produce a greater volume of more dilute amniotic fluid. Our findings show that aquaporin 1 water channels in fetal membranes may contribute to amniotic fluid volume regulation. We speculate that idiopathic polyhydramnios may be associated with a deficiency of aquaporin 1 channels in human fetal membranes. Transgenic aquaporin 1 knockout mice provide a unique animal of polyhydramnios.

The specific mechanisms underlying water transport from the amniotic cavity across the fetal membranes have yet to be elucidated. Nevertheless, the movement of water between the amniotic fluid and the fetal circulation is an important clinical issue because amniotic fluid provides a buffer for normal fetal growth, movement, and development. Both reduced (oligohydramnios) and excessive amounts of amniotic fluid (polyhydramnios) can result in significant perinatal morbidity and mortality. Therefore, understanding the molecular pathways that mediate water movement across the membranes

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overlying the placenta is crucial to understanding the mechanisms of amniotic fluid regulation.

The maintenance of amniotic fluid homeostasis represents a delicate balance between amniotic fluid production and removal. The major source of fluid entering the amniotic cavity is fetal urine,[1] with an additional minor contribution from fetal lung liquid.[2] The two primary routes of amniotic fluid removal are fetal swallowing[3] and absorption from the amniotic cavity into the fetal blood vessels of the chorionic plate. The latter route is referred to as the intramembranous (IM) pathway.[4,5]

Flow across the IM pathway primarily involves the membranes lining the fetal surface of the placenta.[6] The fetal membranes are a complex bilayer composed of the inner amnion lining the amniotic cavity and the outer chorion, which directly abuts the fetal surface of the placenta as well as the underlying decidua. The amnion is composed of a single layer of cuboidal epithelium and an underlying connective tissue stroma. The chorion is primarily composed of reticular fibers and 8-10 layers of trophoblast cells. Under normal conditions, IM water flow progressively increases throughout gestation; by term, up to 400 mL of fluid per day are transferred from the amniotic cavity across the fetal membranes into the fetal circulation.[7]

However, the exact molecular mechanisms underlying water transport across the fetal membranes are not completely understood. In vitro studies of human amnion and chorion demonstrated water permeability constants much greater than those that would be expected if water movement were due to simple osmosis.[8] This type of rapid transcellular water flux is facilitated by aquaporins (AQPs), membrane proteins that function as water channels and are found in a variety of cells that exhibit high water permeability.[9]

Hence, channel-mediated transport of water across the fetal membranes is likely to contribute to water movement between the amniotic cavity and fetal circulation. We recently demonstrated that AQP1 messenger ribonucleic acid (mRNA) and protein are expressed in amnion epithelium from the chorionic plate and reflected regions of the fetal membranes.[10] Although AQPs are found in many tissues in a variety of organs, it has been difficult to link their expression with specific phenotypic abnormalities.[11,12] Because there are no selective AQP inhibitors suitable for in vivo use, the physiologic significance of individual AQPs has been addressed by analyzing the phenotype of AQP1 knockout (KO) mice generated by targeted gene disruption. These models provide a method to test hypotheses regarding AQP function.

Therefore, we utilized an AQP1 KO mouse model to test our hypothesis that AQP1 in the fetal membranes is necessary for the maintenance of normal amniotic fluid volume. If AQP1 is not present, we anticipate that amniotic fluid would accumulate because the fluid would not be able to leave the amniotic cavity. Hence, we postulated that the AQP1 KO fetuses will have a larger volume of dilute amniotic fluid.

Methods

Transgenic mice

The AQP1 KO (null) mice (generously provided by Dr. Alan S. Verkman) were produced by breeding heterozygous mice in which 1 allele contained a disrupted AQP1 gene. Time-dated litters from 16-19 days' gestation (normal gestation = 21 days) were examined.

Surgery

The pregnant mice were anesthetized, and a cesarean section was performed. Intact, individual gestational sacs were carefully removed from the uterus and weighed. The protocols for this study were approved by the University of California, San Francisco Committee on Animal Research.

Measurements

After each sac weight was obtained, it was punctured with a microcapillary tube, and the fluid was drained into a cuvette. The sac was reweighed and the difference in weight allowed calculation of the amniotic fluid volume. The amniotic fluid collected from individual gestational sacs was measured for osmolality using an osmometer (Precision Instruments, Boston, MA). Placental and fetal weights were recorded. The tail of each pup was removed to establish genotype by polymerase chain reaction analysis. Reverse transcription–polymerase chain reaction was performed on fetal membranes to confirm presence or absence of AQP1 mRNA transcripts.

Data analysis

Because the data were not normally distributed, a one-way analysis of variance on ranks was used to determine differences in amniotic fluid volume, osmolality, and weight differences among the 3 groups of mice. If a significant interaction was found, Dunn's post hoc test was utilized. Statistical significance was accepted at $P < .05$.

Results

Genotype analysis of 16 litters showed a quasi-Mendelian distribution of 35 wild-type (WT), 52 heterozygote (HZ), and 33 KO mouse fetuses. The AQP1 KO mouse fetuses did not differ from WT fetuses in physical appearance or utero survival.

As shown in the Table, the KO mice had a greater volume of amniotic fluid and lower amniotic fluid osmolality than their WT counterparts. The KO pups