Aquaporin-4 Gene Variation and Sudden Infant Death Syndrome

SIRI H. OPDAL, ÅSHILD VEGE, ARNE STRAY-PEDERSEN, AND TORLEIV O. ROGNUM

Institute of Forensic Medicine [S.H.O., Å.V., A.S.P., T.O.R], University of Oslo, Oslo 0027, Norway; Pathology Clinic [S.H.O., T.O.R.], Rikshospitalet University Hospital, Oslo 0027, Norway

ABSTRACT: The purpose of this study was to investigate the aquaporin-4 (AQP4) gene in cases of sudden infant death syndrome (SIDS) and controls and to elucidate the hypothesis that a genetically determined disturbed water homeostasis in the brain is involved as a predisposing factor in SIDS. The single nucleotide polymorphisms (SNPs) rs2075575, rs4800773, rs162004, and rs3763043 in the AQP4 gene were investigated in 141 SIDS cases and 179 controls. For each SIDS case, a brain/body weight ratio was calculated. The study revealed an association between the T allele and the CT/TT genotypes of rs2075575 and SIDS (C *versus* T, $p < 0.01$; CC *versus* CT/TT, $p = 0.03$). For the other three investigated SNPs, there were no differences in genotype frequencies between SIDS cases and controls. For the SNP rs2075575, it was also found an association between brain/body weight ratio and genotype in the SIDS cases aged 0.3–12 wk ($p = 0.014$, median ratio CC 10.6, CT/TT 12.1). In conclusion, this study indicates that rs2075575 may be of significance as a predisposing factor for SIDS, and that the CT/TT genotypes are associated with an increased brain/body weight ratio in infants dying from SIDS during the vulnerable period from birth up to 3 mo of age. (*Pediatr Res* 68: 48–51, 2010)

Sudden infant death syndrome (SIDS) is the sudden unexpected death in an infant, which remains unexplained after a thorough investigation of the history, the circumstances of death, and an adequate autopsy (1). Knowledge of risk factors such as prone sleeping position, parental smoking, and overheating has dramatically reduced the occurrence (2–4). However, a large proportion of infants who succumb to SIDS most likely have an underlying genetic predisposition, and it has been indicated that genes involved in the development of the brain and in the serotonergic network is of importance (5–10). According to our version of the three-hit model for SIDS, the fatal triangle (11), this genetic predisposition constitute one corner. The two others are a vulnerable developmental stage of the immune system and the CNS, and environmental risk factors, such as prone sleeping, maternal smoking, or a slight infection. If all factors coincide, a vicious circle is triggered, eventually leading to severe hypoxia, coma, and death (12).

Several studies report findings of increased brain weight and edema in SIDS (13–15). Aranda *et al.* (13) investigated the brain weight of 208 SIDS cases and found that for term infants the brains were heavier than reference values, when matched by age and body length, which suggests that the enlarged brains were present from birth rather than a result of disproportional postnatal growth. The finding of increased brain weight in SIDS is confirmed by Kadhim *et al.* (13), who speculates that the excessive brain weight might reflect abnormal cerebral development and could be detrimental to vital neural control.

One might speculate that the enlarged brain seen in some SIDS victims are due to a disturbance of the development of the water homeostasis in the brain. The most important proteins with regard to water transport are the aquaporins (AQP), which provide a way of rapid transport of water across the plasma membrane. Aquaporin-4 (AQP4) is the major water channel in the brain and spinal cord, expressed at fluid-tissue barriers (blood-brain and brain-cerebrospinal fluid) (16). AQP4 plays a significant role in the development of brain edema. When exposed to acute water intoxication and ischemic stroke, APQ4 knock-out mice show less brain tissue water content, better neurologic outcome, and also improved survival than wild-type mice (17). The gene encoding AQP4 is located on chromosome 18, and mutations giving reduced water permeability and gain-of-function mutations have been detected (18,19). It has also been indicated that specific variants of the AQP4 gene may contribute to brain edema. A recent study of 10 SNPs in the AQP4 gene in patients with middle cerebral artery occlusion disclosed an association between the SNP rs9951307 and severe brain edema (20).

The purpose of this study was to investigate AQP4 gene variation in SIDS, and further to relate the genotypes to brain weight. Our hypothesis is that a genetically determined disturbed development of the water homeostasis in the brain is involved as a predisposing factor in SIDS. We speculate that the genetic markers investigated in this study may contribute to an up-regulation of AQP4 molecules in the perivascular membranes in the brain, and thus edema and increased brain weight. To our knowledge this has not been investigated in SIDS before.

PATIENTS AND METHODS

Subjects. Included in this study were 141 SIDS cases and 179 controls (Table 1). The SIDS cases were classified according to the criteria used in the Nordic SIDS study (21,22), and were all pure SIDS cases, in which the autopsy and clinical information do not reveal any cause of death. The protocols included evaluation of the circumstances of death, review of

Abbreviations: AQP4, Aquaporin 4; **HWE,** Hardy-Weinberg equilibrium; **SIDS,** sudden infant death syndrome; **SNP,** single nucleotide polymorphism

Table 1. *Survey of the subjects included in this study*

	Number of cases	Sex, male/female	Age, median (range)
SIDS	141	86/55	5 wk (0.3–111 wk)
Infant controls	36	18/18	68 wk (2–150 wk)
Adult controls	143	94/49	46 y (11–91 y)

Table 2. *Distribution of SNP genotypes and alleles in SIDS cases and controls*

SNP	Genotype /allele	SIDS, $N = 141$	Controls, $N = 179$	p*
rs2075575	TT	66 (46.8%)	65 (36.3%)	0.05
	CT	53 (37.6%)	68 (38%)	
	CC	22 (15.6%)	46 (25.7%)	
	T	185 (65.6%)	198 (55.3%)	<0.01
	C	97 (34.2%)	160 (44.7%)	
rs4800773	GG	62 (44%)	74 (41.3%)	0.68
	AG	57 (40.4%)	70 (39.1%)	
	AA	22 (15.6%)	35 (19.6%)	
	G	181 (64.2%)	218 (60.9%)	0.39
	A	101 (35.8%)	140 (39.1%)	
rs162004	GG	54 (38.3%)	81 (45.3%)	0.22
	GC	72 (51.1%)	74 (41.3%)	
	CC	15 (10.6%)	24 (13.4%)	
	G	180 (63.8%)	236 (65.9%)	0.58
	C	102 (36.2%)	122 (34.1%)	
rs3763043	AA	77 (54.6%)	91 (50.8%)	0.60
	AG	52 (36.9%)	67 (37.4%)	
	GG	12 (8.5%)	21 (11.7%)	
	A	206 (73%)	249 (69.6%)	0.33
	G	76 (27%)	109 (30.4%)	

* 3 × 2 table for genotypes; 2 × 2 table for alleles.

medical and family history, radiographic examination, toxicology, and a thorough autopsy with extensive histologic and microbiologic examinations, including a neuropathologic examination. Cases with medium-chain acyl-CoA dehydrogenase A985G mutation or mutations in long QT syndrome-related genes were excluded (23,24).

Information regarding brain and body weight for the SIDS cases and infant controls was available from the autopsy report. Measurement of brain weight was done immediately after taking the brain out of the scull. For each case, a brain/body weight ratio was calculated. Information regarding risk factors was obtained from the parents, either by the doctor who first examined the infant or by using a questionnaire. The questionnaire was sent to the parents one to several months after the death, and it was voluntary to answer (4). Thus, information is missing in some of the cases. Nicotine exposure, based on self-report from the mother, was known in 78 cases; of these, 48 were exposed to nicotine *via* maternal smoking. Sleeping position when found dead was known in 120 cases, of which 80 were found prone, either with the head right down or to one side. Information about infection before death was known in 119 cases; of these, 52 cases had a slight upper airway infection the last week of life.

Adult and infant controls were randomly chosen from autopsies performed at the Institute of Forensic Medicine, University of Oslo, in the years 1984–2007, and all subjects were Caucasians, from the south-eastern part of Norway. Eighty-four subjects had suffered violent death, 35 cases had died from intoxication, and 60 had died due to a disease. The study has been reviewed and approved by The National Committees for Research Ethics in Norway.

Gene analysis. The investigated single nucleotide polymorphisms (SNPs) were chosen using the website www.hapmap.org. Based on calculations of taqSNPs, the SNPs rs4800773, rs162004, rs2075575, and rs6753043 were chosen for investigation. DNA was extracted from blood or spleen, using standard methods. The SNPs was genotyped by TaqMan SNP genotyping assay (Applied Biosystems, Foster City, CA) and Mx3000p real-time PCR machine (Stratagene, La Jolla, CA), according to the manufacturers instructions.

Data analysis. The χ^2 test was used for comparing the frequencies of the different polymorphisms between the diagnosis groups, as well as for correlations between risk factors and genotype. In cases of small numbers, the Fisher's exact test was used. When investigating the relationship between the brain/body weight ratio and genotypes, the cases were divided into age groups, and the Mann-Whitney U test were used for investigating the relationship between brain/body weight ratio and genotypes. These analyses were implemented using SPSS version 16.0 (SPSS, Chicago, IL). The Hardy-Weinberg equilibrium (HWE) test was performed using a web-based HWE calculator (http://www.oege.org/software/hwe-mr-calc.shtml) (25). Power calculation was performed using PS: Power and Sample Size Calculation (http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize) (26).
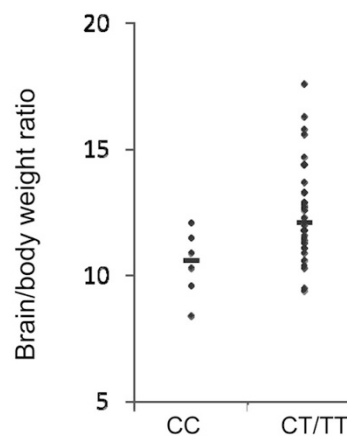
RESULTS

There was a tendency that the genotype distribution for the SNP rs2075575 differed between SIDS cases and controls (Table 2). The T allele and the CT/TT genotypes were more frequent in SIDS; 65.6% of the SIDS cases had a T allele, and 84.4% had the CT or TT genotype, compared with 55.3% and 74.3%, respectively, in the controls (C *versus* T, $p < 0.01$, CC *versus* CT/TT, $p = 0.03$). After Bonferroni correction, only the association between the rs2075575 T allele and SIDS remains significant, with 50% power at a significance level of 5%. For the other three investigated SNPs (rs4800773, rs162004, and rs6753043), there were no differences in allele or genotype frequency between SIDS cases and controls (Table 2). There were no differences in genotype frequencies between infant and adult controls, these two groups were

Figure 1. rs2075575 genotype *vs* brain/body weight ratio, actual ratios and median, in 46 SIDS cases up to 12 wk of age, $p = 0.014$.

therefore pooled. Two of the SNPs were not in HWE in the controls; rs2075575 ($\chi^2 = 9.6$, $p < 0.005$) and rs4800773 ($\chi^2 = 5.73$, $p < 0.05$).

When investigating the relationship between brain/body weight ratio and genotype, the SIDS cases were subdivided into age groups: 0.3–12 wk (46 cases), 12.5–52 wk (83 cases), and 52.5–111 wk (12 cases). A significant correlation was found only for rs2075575, and only in the age group 0.3–12 wk. In this group, the SIDS cases with the CT/TT genotypes had a significantly higher brain/body weight ratio than the SIDS cases with the CC genotype ($p = 0.014$; median ratio: CC 10.6, CT/TT 12.1; Fig. 1).

Forty-eight of the SIDS victims had been exposed to maternal smoking. For the SNP rs2075575, there was a tendency that more SIDS victims that had been exposed to maternal smoking had the genotype CT/TT, 65.7% compared with

Table 3. *rs2075575 genotype versus maternal smoking, in cases of SIDS*

Genotype	Exposed to maternal smoking		
	Yes	No	*p*
CC	4 (36.4%)	7 (63.6%)	0.09
CT/TT	44 (65.7%)	23 (34.3%)	

34.3% of the cases not exposed to nicotine ($p = 0.09$; Table 3). No other relationships between AQP4 genotypes and risk factors for SIDS were found.

DISCUSSION

The main finding of this study is the association between the T allele and the CT/TT genotypes of the SNP rs2075575 and SIDS, indicating that this SNP may be of significance as a predisposing factor for SIDS. The SNP is located in the promoter region of the AQP4 gene, in a region without any known transcription factor binding sites (27), and it is thus unlikely that it alters the function of the AQP4 protein. One may, however, speculate that the SNP is in linkage disequilibrium with one or several other polymorphisms, either in the promoter or in the coding regions of the gene, which do influence AQP4 expression and function.

The finding of deviation from HWE in the controls for two of the SNPs, as well as a low power, may be a limitation of the study. Deviations from HWE can be due to inbreeding, population stratification or selection, genotyping errors, or may happen by chance (25). In this study, inbreeding or population stratification is not a likely explanation, because the controls are in HWE in two of the investigated SNPs, and the same controls are in HWE in several SNPs in interleukin genes (28). Genotyping errors are also unlikely; the genotyping was performed using well-established techniques.

This study also revealed a relationship between the genotypes of rs2075575 and brain/body weight ratio in infants dying from SIDS in the vulnerable period between 0.3 and 12 wk after birth. The infants with a genotype including the T allele (CT or TT) had a higher brain/body weight ratio than the cases with the CC genotype ($p = 0.014$; Fig. 1). This finding confirms that variation in the AQP4 gene may contribute to brain edema, as indicated by Kleffner *et al.* (20). Because the T-allele was associated with increased brain weight, it may be speculated that genotypes with this allele predisposes for development of edema, which ultimately leads to death.

Of the 36 infant controls included in this study, only 15 were below 1 y and only 6 were in the age range 0.3–12 wk. Because of this low number, it was not possible to test whether the association between genotype and brain/body weight ratio found in SIDS is also present in infants dying from other causes than SIDS, which is a limitation of this study. Because the brain/body weight ratio decreases from 12.7% at birth to ~2% in adults (29), it is necessary both to subdivide the cases according to age and to have age-matched controls.

The other interesting observation is the possible relationship between the rs2075575 genotype and maternal smoking, because maternal smoking tended to be overrepresented among SIDS cases with CT/TT genotypes (Table 3). However, the results must be treated with caution because a small number of cases. It is shown that nicotine induces edema (30), and one may speculate that nicotine, in combination with the 2075575 CT/TT genotypes, induces a less effective AQP4 protein synthesis and thus a disturbed water homeostasis.

In humans, it is shown that the vascular coverage of AQP4 increased from <5% at 16 wk GA to 63% at 20–26 wk GA, followed by plateauing until birth (31). This is consistent with rat experiments, which showed no labeling for AQP4 at 14–21 d gestation, and further a postnatal development of AQP4, from ~2% of adult levels at day 7 to ~60% of adult levels after 4 wk, indicating that the amount of AQP4 protein continues to increase until adulthood (32). The early development of AQP4 in humans point toward the existence of a close relationship between water transport regulation and brain development (31). In the first months of life, even a slight delay in AQP4 maturation may interfere with neural function, possibly *via* dysregulation of extracellular ion concentrations. One may speculate that the T allele, or another allele or genotype in linkage disequilibrium with the T allele, induces a delayed maturation of the processes underlying water and ion homeostasis. Such delay may predispose for an increased water flow into the brain tissue, and thus an abnormal brain/body weight ratio, which may be particularly unfavorable the first months of life.

Neuropathological examinations of the brain, included as a part of the autopsy, did not disclose any findings that could support the theory of a possible disturbed development of water homeostasis in SIDS. Macroscopic brain edema was noted in approximately one-third of the SIDS cases included in this study; however, this is not specific for SIDS but might be seen in different causes of death. Immunohistochemical studies of AQP4 expression in the brain would be important, and also more suitable, to clarify a possible relation between AQP4 gene variants and brain edema in SIDS. This, together with studies of electrolytes, should be subjects for further studies. The latter is of importance since increased water flow into the brain also may be due to hyponatremia, an issue that has not been investigated in cases of SIDS before.

In conclusion, the present findings fit with the hypothesis of a fatal triangle in SIDS, indicating that rs2075575 may be of significance as a predisposing factor for SIDS. The CT/TT genotypes tended to be associated with maternal smoking and were associated with an increased brain/body weight ratio in infants dying from SIDS during the especially vulnerable period from birth up to 3 mo of age. One might speculate that rs2075575 is part of a genetic pattern that are important for the expression of AQP4 in the brain and thereby also for the development of water homeostasis. Better knowledge regarding this may represent a way to develop strategies to prevent SIDS; it may be predicted that AQP4 inhibitors or down-regulators would be useful to reduce cytotoxic brain edema caused by hypoxia, ischemia, or meningitis (33).

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