Arthrogryposis multiplex congenita (AMC), a hereditary disease in swine, maps to Chromosome 5 by linkage analysis

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Abstract

Arthrogryposis multiplex congenita (AMC), defined as permanent joint contractures present at birth, is one of the most common congenital defects in piglets and other mammals. A genetic form of arthrogryposis was recently identified in Swiss Large White (LW) pigs. The disease is controlled by a single autosomal recessive allele designated as *amc*. At least 14 LW AI (artificial insemination) boars (about 25% of the Swiss population) are known to be carriers of the *amc* allele. A total of 219 pigs were used for linkage analysis, including seven founders (F_1), three F_0, 160 F_2, and 49 F_3 animals. All founder pigs were full or half sibs. Of the 219 pigs, 41 (18.7%) were found to be affected, while the remaining 178 (81.3%) were healthy. A comprehensive genome scan revealed that microsatellite *SW1987* located on pig (*Sus scrofa*) Chromosome 5 (SSC5), was linked with *AMC*. Sixteen additional SSC5 microsatellites were selected for further genotyping to generate a multipoint map covering the AMC region. Significant pairwise linkage (LOD > 6.00) was found for *AMC* and eight marker loci. The order that best fit with the data was *SW963–SW1987–SW152–AMC–*(*SW904*, *SW1094*)*–SWR1526–*(*SWR1974*, *SW310*). *AMC* was mapped by linkage analysis to the position 92 cM, between *SW152* and *SW904*/*SW1094*, which are located on SSC5 in bands q12–q23.

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Arthrogryposis [also known as arthrogryposis multiplex congenita (AMC), amyoplasmia congenita, or multiple congenital articular rigidities] was recently identified in the Swiss Large White (LW) breed and has been described in detail in Yorkshire piglets by Ely and Leipold (1979). It is one of the most common congenital defects observed in piglets and other mammals. In humans, AMC seems to have a very old origin and is estimated to occur in one of every 3000 births (Anderson 1997; Staheli et al. 1998). It is defined as permanent fixation or ankylosis of the joints and can occur in the forelimbs, hind limbs, and/or the vertebral column, which leads to various degrees of flexion or extension at birth and/or scoliosis (see Fig. 1, numbers 1 and 2). Moreover AMC may affect ligaments, skeletal muscles, the central and peripheral nervous systems, or any combination of these tissues. A shortened lower jaw (brachygnathia inferior) has also been observed in affected piglets (Fig. 1, number 3). However, cleft lip, cleft palate, hydrocephalus, or other cranial anomalies have not been linked with AMC and no malformations of internal organs have been detected.

It is generally accepted that AMC has many causes and can be classified into neurogenic and myogenic forms (Adams et al. 1962). The neurogenic forms of AMC are more common and are usually associated with spinal cord dysplasias, including various degrees of loss of ventral horn (motor) neurons and syringomyelia with fibrofatty replacement of muscles, typical of denervation atrophy (Edwards 1971). It has been proposed that prolonged prenatal immobilization for any reason will lead to fixation of the joints of the immobilized parts (Whittem 1957).

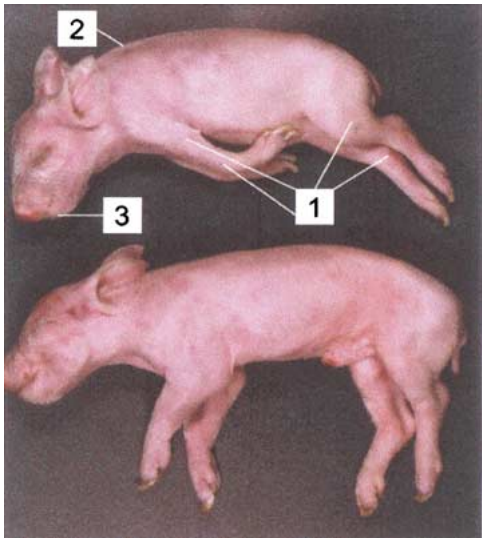

Fig. 1. Typical AMC symptoms in piglets (**Above**) Piglet affected with arthrogryposis multiplex congenita (AMC), (**Below**) Normal piglet. (1) The joints of the fore- and hind limbs are fixed, and positioned parallel to the thorax, (2) scoliosis, (3) brachygnathia inferior.

A number of known or suspected etiologies of AMC in piglets have been recorded; the most important of these include genetic factors, nutritional deficiencies, toxic plants, pharmacological and viral agents, and prolonged prenatal immobilization. For example, feeding pigs tobacco stalks (*Nicotiana tabacum*) between days 4 and 50 of pregnancy was reported to cause the condition (Crowe 1978). Between 40% and 50% of piglets in affected litters were deformed. Poison hemlock (*Conium maculatum*) has also been reported to cause AMC in piglets (Dyson and Wrathall 1977). Piglets affected with AMC may cause dystocia, as parturation has been reported to be prolonged by several hours and neonatal deaths from asphyxia and hypoglycemia were common.

Death in newborn piglets with AMC is believed to result from respiratory arrest due to a neurogenic problem from the respiratory center, hypoplasia of the muscles involved in respiration within the thorax, or a combination of both (Ely and Leipold 1979). Fetal atelectasis, together with amniotic fluid in the respiratory passages, is another indication of terminal respiratory failure. However, in our case, all piglets were stillborn so it was not possible to establish the origin of the disease.

Lomo (1985) suggested that the syndrome is of genetic origin and is controlled by a single autosomal recessive allele, designated by the gene symbol *amc*. The mutation responsible for AMC occurred in the boar 2401 JN Hift, which was born in 1990 and then widely used for artificial insemination (AI) in Switzerland. Its defective gene was spread through AI; however, the exact number of affected animals is unknown. Currently, at least 14 AI boars (about 25% of the AI LW boars) are known to carry this defect, resulting in considerable economic losses in the Swiss pork industry.

Therefore, the present study aims to (1) confirm the autosomal recessive inheritance of AMC in the Swiss LW population and (2) map the AMC phenotype to the porcine genome. To do this, we performed a whole-genome scan followed by a SSC5 scan with microsatellite markers.

Materials and methods

Animals. AMC was first observed in Switzerland in two litters of Swiss Large White breed pigs (Table 1, matings 1 and 2). These litters were derived from two sisters (359 HNM and 360 HNM) mated with the same sire 1077 GT (F_0 animals). Six offspring from these first two matings (one sire, 68 HNM, and five dams, 71 HNM, 72 HNM, 75 HNM, 78 HNM, and 82 HNM, F_1 founder generation) were identified and purchased. These animals formed the basis for the highly inbred AMC herd. The sire 68 HNM was then mated with ten dams: three were his sisters (Table 1, matings 3, 4, and 5), two were his half-sisters (matings 6 and 7), and five were his daughters (matings 8, 9, 10, 11, and 12), born from previous matings.

A total of 219 piglets were analyzed. Of these, the 41 diseased piglets (18.7%) were easily identifiable because they exhibited all the typical symptoms described above and shown in Fig. 1. Therefore, they had the allele pair *amc/amc*. On the other hand, it was phenotypically impossible to distinguish between healthy (*AMC/AMC*) and carriers (*AMC/amc*) in the remaining 178 normal piglets (81.3%).

Isolation and extraction of genomic DNA. Genomic DNA was extracted from EDTA-anticoagulated whole blood of living piglets at the approximate age of 2 months, according to the method of Vögeli et al. (1994). Genomic DNA was obtained from the tail of diseased piglets and those that died of normal causes according to the protocol described by Laird et al. (1991).

DNA amplification and microsatellites analysis. Polymerase chain reaction (PCR) amplification was carried out as described in Rohrer et al. (1996), in a reaction volume of 25 µl containing 100 ng of porcine genomic DNA, 10 mM Tris-HCl (pH 9), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM of each

Table 1. Summary of mating pairs, identification number, *AMC* genotype, and number of affected and normal offspring with their generation

			Offspring number			
Mating	*Father*	*Mother*	*Affected (amc/amc)*	*Normal (AMC/AMC, AMC/amc)*	*Total*	*Generation*
1	1077 GT (*AMC/amc*)	360 HNM (*AMC/amc*)	2[a]	4	6	F_1
2	1077 GT (*AMC/amc*)	359 HNM (*AMC/amc*)	1[a]	3	4	F_1
3	68 HNM (*AMC/amc*)	71 HNM (*AMC/amc*)	3	18	21	F_2
4	68 HNM (*AMC/amc*)	72 HNM (*AMC/amc*)	13	46	59	F_2
5	68 HNM (*AMC/amc*)	75 HNM (*AMC/amc*)	9	17	26	F_2
6	68 HNM (*AMC/amc*)	78 HNM (*AMC/amc*)	7	39	46	F_2
7	68 HNM (*AMC/amc*)	82 HNM (*AMC/amc*)	2	6	8	F_2
8	68 HNM (*AMC/amc*)	1154 CH (*AMC/AMC*)	0	9	9	F_3
9	68 HNM (*AMC/amc*)	1731 CH (*AMC/amc*)	2	9	11	F_3
10	68 HNM (*AMC/amc*)	1733 CH (*AMC/AMC*)	0	11	11	F_3
11	68 HNM (*AMC/amc*)	1734 CH (*AMC/amc*)	1	8	9	F_3
12	68 HNM (*AMC/amc*)	2215 CH (*AMC/amc*)	1	8	9	F_3
Total			41	178	219	

[a]DNA not analyzed.

deoxynucleotide, 0.4 μM of fluorescently labeled forward and normal reverse primers (microsatellite markers), and 1.25 U of Taq DNA-Polymerase (Pharmacia Biotech, Uppsala, Sweden). PCR profile was 95°C for 5 min followed by 35 cycles of 95°C for 30 sec, 55–62°C for 30 sec, and 72°C for 30 sec. The final elongation was at 72°C for 7 min. Using a Genescan-350 TAMRA size standard, samples diluted in formamide were analyzed on a 377 ABI sequencer (Applied Biosystems, Foster City, CA, USA). Results were evaluated with ABI 672 Genescan program and genotype software (version 2.1, Applied Biosystems).

Microsatellite markers

Full-genome screening. Forty-eight evenly spaced fluorescently labeled (TET, FAM, or HEX) microsatellite markers were used to perform a full-genome scan of 130 animals. Depending on the length of the chromosomes, two to five well-informative microsatellites for each chromosome, distributed over the entire swine genome and spread at intervals of approximately 40 cM apart, were selected from Rohrer et al. (1996).

Chromosome 5 screening. After the initial full-genome linkage analysis with 5 microsatellite markers spread out on the entire SSC5 (*ACR*, *SW1482*, *SW2*, *SW1987*, and *SW995*), 12 additional polymorphic markers (*SW2425*, *SW1071*, *S0005*, *TNFR*, *SW963*, *SW152*, *SW904*, *SW1094*, *SWR1526*, *SWR1974*, *SW310*, and *SW1200*), in close proximity to marker *SW1987*, and 89 more pigs were added for further genotyping. This allowed us to generate a multipoint map that covers the AMC region. The details of each microsatellite are available at http://www.marc.usda.gov/genome/genome.html.

Each animal was manually haplotyped, and each genotype was checked for plausible parentage and double recombinants. In the case of disputable or unclear genotypes, animals were re-evaluated and, if necessary, regenotyped. This permitted us to precisely locate *AMC*, as described in Results (Table 2).

Linkage analysis. Marker linkage maps were computed with Crimap software version 2.4 (Green et al. 1990) by using the flips and all options to determine the best ordering of the markers and the fixed option to obtain the map distances.

Results

Recessive inheritance of AMC. Of the 219 descendants studied, 41 piglets (18.7%) exhibited severe arthrogryposis. Of these, only 199 (Table 1, matings 1–7, 9, and 11 and 12) were born from heterozygous parents. The χ^2 test, which was calculated from the segregation data, showed that the observed ratios of the *AMC* vs. *amc* alleles did not deviate significantly from the 3:1 ratio of healthy to diseased piglets that would be expected to result from two heterozygous parents ($\chi^2 = 1.1$; $0.2 < p < 0.3$; 1 df).

Markers and linkage mapping. The preliminary genome scan revealed a correlation between the *AMC* locus and the allele *165* (165 = size in bp) of microsatellite *SW1987* located on pig Chromosome

Table 2. Genotypes and haplotypes of four important recombinant offspring (two healthy and two with AMC), reported with their parents and mating number, tested with seven polymorphic microsatellites

Mating (see Table 1)	*Animal*	*SW963*	*SW1987*	*SW152*	*AMC*	*SW904*	*SW1094*	*SWR1974*	*AMC location*
3	Father 68 HNM	164/168	167/165	167/177	AMC/amc	182/180	152/172	159/153	
3	Mother 71 HNM	168/168	165/165	177/167	*amc*/*AMC*	182/172	152/154	159/153	
3	F_2: 1735 CH (normal)	168/168	165/165	177/167	*amc*/*AMC*	180/↑**182**	172/**152**	153/**159**	**A**
9	Father 68 HNM	164/168	167/165	167/177	*AMC*/amc	182/180	152/172	159/153	
9	Mother 1731 CH	168/168	165/165	177/167	*amc*/*AMC*	180/172	172/154	153/153	
9	F_3: 80 (AMC)	168/168	165/165	177/177	*amc*/*amc*	↑**182**/180	**152**/172	**159**/153	**A**
12	Father 68 HNM	164/168	167/165	167/177	AMC/*amc*	182/180	152/172	159/153	
12	Mother 2215 CH	168/162	165/165	177/167	*amc*/*AMC*	180/180	172/172	153/153	
12	F_3: 3289 CH (normal)	**168**/168	**165**/165	**177**↑/177	*AMC*/*amc*	182/180	152/172	159/153	**B**
6	Father 68 HNM	164/168	167/165	167/177	*AMC*/amc	182/180	152/172	159/153	
6	Mother 78 HNM	168/162	165/165	177/167	*amc*/*AMC*	172/180	152/172	163/153	
6	F_2: 66 (AMC)	**164**/**162**	**167**/**165** ↑ ?	**167**↑/177	amc/*amc*	180/172	172/152	153/163	**B**

The first allele shown for each offspring is the father's allele (underlined) and the second is derived from the mother (boxed). The last column indicates the location of the *AMC* locus based on the recombination site (location A: *AMC* locus located toward the centromere from *SW904/SW1094*; location B: *AMC* locus located toward the telomere from *SW152*).
F_2/F_3: offspring generation. ↑: Site of recombination. ?: Uncertain whether the recombination occurred at this site or at the next microsatellite. **Bold**: recombined chromosomal piece. Genotype *amc/amc*: diseased piglets. Genotypes *AMC/amc* and *amc/AMC*: normal piglets.

5. The logarithm of odds (LOD) scores between *AMC* and 43 microsatellite markers located on the entire swine genome, except SSC5, ranged from 0.00 to 0.35 and therefore did not nearly approach the significant value of 3.00.

Porcine *AMC* mapped to SSC5, and significant pairwise linkage to eight markers was identified. For each of these markers, the two-point recombination frequencies and LOD scores greater than 6.00 are as follows: *SW963* (0.12, 8.3), *SW1987* (0.07, 10.5), *SW152* (0.01, 14.9), *SW904* (0.03, 16.8), *SW1094* (0.03, 14.9), *SWR1526* (0.03, 13.3), *SWR1974* (0.03, 15.3), and *SW310* (0.03, 13.3). In Fig. 2 the sex-averaged linkage map of this study with the most likely order of the microsatellites and *AMC* on SSC5 and the comparative physical maps of SSC5 and HSA12 are shown. The distance between *SW152* and *AMC* is 2.5 cM and the distance between *SW904/SW1094* and *AMC* is 2.4 cM.

Position of AMC according to recombinants. The chromosome scan also revealed that the allele *177* of microsatellite *SW152* cosegregated with the recessive allele *amc*, whereas the allele *167* cosegregated with the normal *AMC* allele. Only two recombinant offspring were found: number 66 (Table 2, mating 6), a diseased swine with the genotype $SW152^{167/177}$, and number 3289 CH (Table 2, mating 12), a healthy swine with the genotype $SW152^{177/177}$. Table 2 provides a summary of four decisive recombinant animals (two normal and two diseased) which were manually haplotyped. Table 2 also summarizes seven polymorphic microsatellites, which are informative with respect to the location of the *AMC* locus. The results shown in Table 2 strengthen the results of the linkage analysis (Fig. 2) and suggest that the locus order on the q-arm of SSC5 most probably is centromere–*SW152* (location B, animals 3289 CH and 66)–*AMC*–*SW904/SW1094* (location A, animals 1735 CH and 80).

Discussion

To our knowledge, this study is among the first to investigate AMC in swine on a genetic level by specifically utilizing linkage analysis and gene mapping. Our findings provide important evidence suggesting that the *AMC* gene, which is responsible for arthrogryposis disease in pigs, is inherited in an autosomal recessive manner. The mutated gene is located on SSC5, most likely between *SW152* and *SW904/SW1094* at position 92 cM based on our linkage analysis.

A QTL interval mapping approach (Haley et al. 1994) was also applied to the data to validate if a chromosome scan method gives the same results as

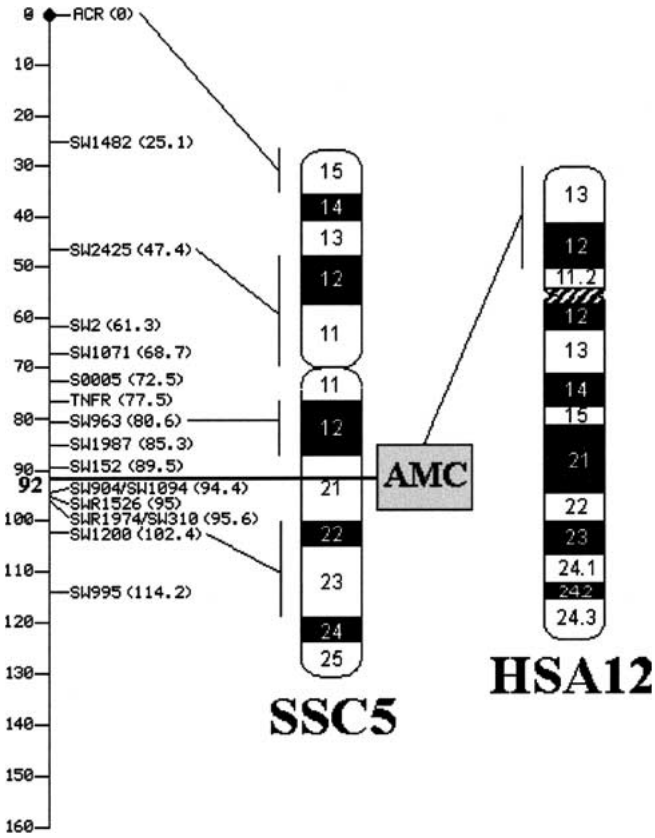

Fig. 2. Sex-averaged genetic linkage maps with genetic position in Kosambi cM of microsatellite markers and the *AMC* gene on swine Chromosome 5 (SSC5) and comparative physical map of swine Chromosome 5 (SSC5) with human chromosome 12 (HSA12). The shaded box and the indicated vertical line on HSA12 summarize results of bidirectional painting reported by Goureau et al. (2001).

typical linkage analysis when the ''trait'' is strictly under monogenic mode of inheritance. A statistically significant QTL could be mapped on SSC5 in the region where *AMC* (relative position 92 cM) was located.

The calculated ordering of markers compares well to previous swine linkage maps, despite the fact that the estimated genetic distances and recombination rates were not completely consistent. This can most easily be explained by the examination of different breeds and genetically diverse family material and the high level of inbreeding within our AMC family. In all cases but one, the map order of the markers was the same as that shown in the MARC map (http://www.genome.iastate.edu/maps/marcmap.html). The exception was that in our map *SW1094* is located at the same position as *SW904* on SSC5 (Fig. 2), whereas in the MARC map *SW1094* is located more toward the telomere. However, our results are consistent with the findings of Cirera et al. (2003) and Hawken et al. (1999).

The same haplotype associated with *AMC* in our experimental herd was also detected in 20 diseased piglets from different farms and in 11 AI-boar carriers. These pigs were all descended from the same founder boar 2401 JN Hift. Thus, it should be possible to develop a marker-based test to identify heterozygous carrier animals in susceptible LW families.

The microsatellites *SW152* and *SW904/SW1094* are located between the physically mapped microsatellites *SW963* and *SW1200* (http://www.genome.iastate.edu/maps/marcmap.html) and therefore between bands q12 and q23. This region is likely to correspond to an evolutionarily conserved region on human Chromosome 12, bands p13–p12 (http://www.toulouse.inra.fr/lgc/pig/compare/SSC.htm) (Fig. 2, physical maps). Some genes and ESTs physically assigned to the SSC5 bands q12–q23 are orthologous on human Chromosome 12p13–p12, as shown by chromosome painting between human and pig (Goureau et al. 2001; Cirera et al. 2003). Consequently, the porcine *AMC* gene has its human counterpart on HSA 12p13–p12 and human genes located there represent positional candidate genes for AMC.

Many distinct forms of AMC are known in humans, which is consistent with the variety of symptoms associated with the disease. Not all of these forms have a genetic origin, nor have they all been mapped to a specific human chromosome. Table 3 lists the 20 most common genetic forms of AMC that have been mapped to a human chromosome and shows their chromosomal locations.

Based on comparative mapping, the genes reported to cause the human forms of arthrogryposis that are not located on Chromosome 12 are not considered to be the most likely candidates for swine *AMC*. However, it would be wrong to exclude potentially appropriate genes based solely on comparative mapping because minor chromosomal rearrangements could always occur. Therefore, in pigs we analyzed the most similar form of AMC found in humans: the AMC neurogenic form (*AMCN*; Shohat et al. 1997). We evaluated five microsatellite markers (*SWR2516*, *SW1650*, *SW1026*, *S0370*, *SWR345*) on the pig chromosome (SSC2) that corresponds to HSA5, but we did not find any correlation between these markers and *amc* (LOD scores between 0.00 and 0.18).

Piglets born to dams that during pregnancy were fed toxic plants, such as *Nicotiana tabacum* (burley tobacco; Crowe 1978), *Conium maculatum* (poison hemlock; Forsyth et al. 1996; Dyson and Wrathall 1977; Lopez et al. 1999), or *Datura stramonium* (Jimsonweed; Leipold et al. 1973), exhibited the same symptoms as the diseased piglets observed in our

Table 3. Twenty common forms of arthrogryposis multiplex congenita in human, their location on the human genome, and their gene symbol

Disease name	*Human chromosome*	*Gene symbol*
Arthropathy–camptodactyly syndrome	1q24–q25	CACP (PRG4)[2]
Popliteal pterygium syndrome	1q32–q41	PPS1[1]
Lethal spinal muscular atrophy	5q12.2–q13.3	SMA1[2]
Congenital contractual arachnodactyly	5q23–q31	CCA (FBN2)[1]
Diastrophic dysplasia	5q32–q33.1	DTD[2]
AMC neurogenic	5q35	AMCN[2]
Saethre–Chotzen syndrome	7p21	SCS (TWIST1)[1]
Distal arthrogryposis type IA	9p21–q21.2	AMCD1[1]
Distal arthrogryposis type IA	9p13.2–p13.1	AMCD1 (TPM2)[1]
Cerebrooculofacioskeletal syndrome	10q11	ERCC6[2]
Saethre–Chotzen syndrome	10q26	SCS (FGFR2)[3]
Distal arthrogryposis type 2B, Freeman–Sheldon syndrome variant	11p15.5	TNNI2[1]
Distal arthrogryposis type 2B, Freeman–Sheldon syndrome variant	11p15.5	TNNT3[1]
Cerebrooculofacioskeletal syndrome	13q33	ERCC5[3]
Symphalangism proximal	17q21–q22	SYM1[1]
Niemann–Pick disease, Type C1	18q11–q12	NPC1[2]
Cerebrooculofacioskeletal syndrome	19q13.2–q13.3	ERCC2[3]
Lethal multiple pterygium syndrome	X linked	LMPS[2]
Infantile spinal muscular athrophy with arthrogryposis	Xp11.3–q11.2	AMCX1[2]
Arthrogryposis X-linked type V	Xq23–q27	AMCX5[2]

The gene in which the mutation occurred is shown in parentheses, along with its mode of inheritance (1 = dominant, 2 = recessive, 3 = unknown)

study. Thus, we hypothesized that candidate genes must be correlated with the functionality of some ion channels or receptors (probably nicotinic) present in brain, muscle, or spinal cord. The mechanisms underlying the toxicity of these compounds have not been completely delineated. It appears that the toxic compounds, piperidine alkaloids in most cases, such as coniine or coniceine (Lopez et al. 1999), block ion channels either directly or through their actions on a receptor. This, in turn, inhibits fetal movement and leads to the typical observed symptoms (Panter et al. 1988). Potentially interesting and promising positional candidate genes located on Chromosome 12p13–p12 include a potassium voltage-gated channel (*KCNA*) and a calcium channel (*CACNA*).

Maternal autoantibodies specific for fetal antigens could be another cause of AMC, as such antibodies could inhibit ion channel function in the fetal AChR, as reported in swine by Vincent et al. (1995) and in humans by Matthews et al. (2002). Furthermore, as proposed by Lopez et al. (1999), it is possible that the toxic compounds may affect regulation of amniotic fluid levels, thereby leading to malformations. If the volume of amniotic fluid is increased, it can exert high levels of pressure on the fetus; if it is decreased, the placenta itself may compress the fetus, which could also lead to the symptoms of AMC.

In conclusion, delineating the genetic factors responsible for AMC will allow us to identity key proteins and enzymes leading to the disease. It will also enable us to reduce the incidence of AMC-diseased pigs and carrier animals by using genetic marker-assisted election. The successful isolation of key proteins and enzymes could be the basis for designing new supplemental substances that may prevent diseases such as AMC. The swine represents a particularly attractive animal model for studying the common genetic forms of human AMC because, physiologically, AMC disease in humans and pigs

are very similar. Therefore, the chromosomal region carrying positive QTL alleles of SSC5 identified in this study and heavily marked by microsatellites may provide an important road map for future studies in humans.

Acknowledgments

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