Lethal Cystic Kidney Disease in Amish Neonates Associated With Homozygous Nonsense Mutation of *NPHP3*

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Background: Nephronophthisis is a group of genetically heterogeneous autosomal recessive cystic kidney disorders with a wide spectrum of severity and age of onset. We present a clinical and genetic study of a lethal form of nephronophthisis in neonates.

Study Design: Clinical and genetic investigations of a case series.

Setting & Participants: 12 affected offspring born to consanguineous parents from the Old Order Amish community.

Outcomes: In this extended pedigree, the disorder is particularly severe; affected individuals survive only hours or days, with the cause of death invariably respiratory distress.

Results: Cystic kidneys were confirmed in 11 infants and suspected in an additional individual who had 2 affected siblings. Although the renal aspect of the phenotype was a consistent feature in all affected individuals, additional pulmonary, cardiac, and urinary tract abnormalities are variable parts of this syndrome. Physical mapping of the causative mutation in this extended Amish pedigree highlighted a 475-kilobase candidate region on chromosome 3 that contains the *NPHP3* gene. Sequence analysis of this gene showed a cytosine to thymine substitution in exon 15 (c.2104C→T) that cosegregated with the disease status. This substitution is predicted to lead to premature termination at position 702 of the protein product (p.Arg702X).

Limitations: Because of the severe nature of this disease, few affected infants underwent full clinical evaluation.

Conclusion: The presence of congenital malformations in the case series confirms the crucial role of NPHP3 in early embryonic development of the kidneys and urinary tract. The study also highlights the subtle variations in phenotypic expression in a cohort of patients with the same mutation in *NPHP3*.
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INDEX WORDS: *NPHP3*; nephronophthisis; Amish; c.2104C→T.

Nephronophthisis is a group of rare autosomal recessive inherited cystic kidney disorders with a broad clinical spectrum, ranging from lethality in utero to clinically undetected until adulthood.[1] Clinically, nephronophthisis typically presents with progressive renal failure and is the most common genetic cause of end-stage renal failure in the first 3 decades of life. Mutations have been identified in a group of 9 genes (*NPHP1* to *NPHP9*) that encode the nephrocystin proteins. The nephrocystins are expressed in primary cilia, basal bodies, and/or centrosomes.[2] Expression patterns of these genes strongly suggest that the cyst formation in these disorders is, at least in part, a consequence of defective cilia function. *NPHP1* is the most common site of mutation in patients with nephronophthisis and is responsible for 21% to 34% of patients with chronic renal failure in the first 3 decades of life. Mutations in *NPHP3* are far less common and originally were identified in a small cohort of patients with an adolescent form of nephronophthisis.[3] More recently, mutation of *NPHP3* has been identified in isolated sibships with more severe earlier onset polycystic kidney disease with end-stage renal failure, often in the first decade of life and sometimes during the neonatal period.[4]

In the present study, we present a clinical and genetic study of a severe form of nephronophthisis, lethal in neonates born to consanguineous parents from a single Old Order Amish community. We present an overview of the clinical presentations of 12 affected individuals that de-

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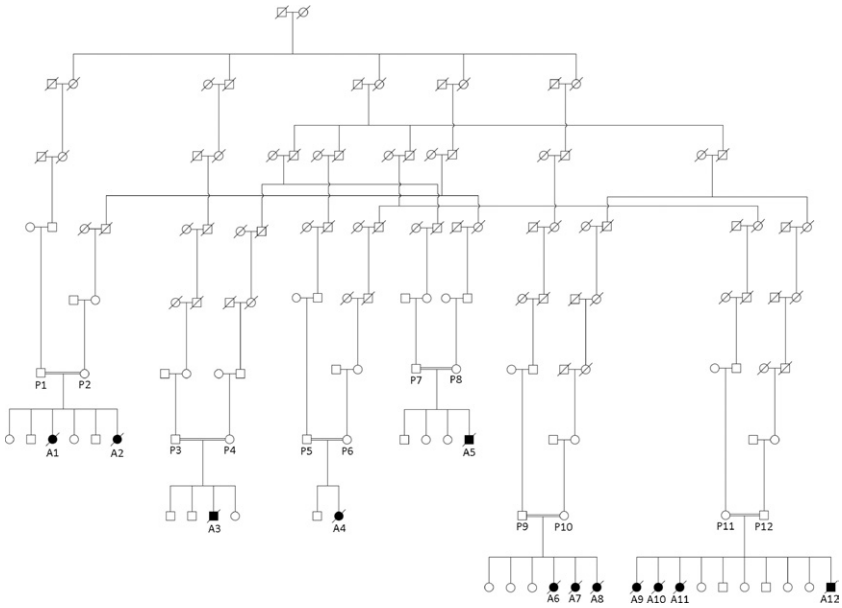

Figure 1. Simplified extended pedigree shows the common ancestry of the 12 affected cases.

fine this syndrome and report the identification of the causative mutation in *NPHP3*.

METHODS

Case Ascertainment

These families came to our attention during an ongoing genetic survey of the Old Order Amish population for inherited disorders. Medical information for the affected individuals was obtained from their families or primary care physicians. Parents gave informed local research ethics committee–approved consent to participate in the study.

Molecular Genetics

DNA was extracted from blood samples from a single affected infant and 12 obligate carrier parents (P1 to P12) by using the QIAamp DNA extraction kit (Qiagen). Genotypes were generated using the GeneChip Human Mapping 50K Array Hind 240 (Affymetrix) and the GeneChip Genotyping Analysis Software (GTYPE) 4.0 (Affymetrix). Confirmatory microsatellite genotyping was performed by using polymerase chain reaction and polyacrylamide gel electrophoresis as previously described.[5] Primers for sequence analysis were positioned flanking each of the 27 coding exons and associated splice junctions of the *NPHP3* gene for amplification by means of polymerase chain reaction. Subsequent bidirectional sequencing of DNA from the affected individual and a single obligate carrier patient was performed using the BigDye terminator 3.1 cycle sequencing kit (Applied Biosystems). DNA from 100 control chromosomes of European ancestry was assessed for the exon 15 variant by means of polymerase chain reaction and sequencing.

RESULTS

Clinical Overview

Twelve infants were identified in 6 families (Fig 1) with lethal neonatal nephronophthisis (9 lived $<$ 24 hours). All 12 parents descend from a common ancestral couple who lived in this community during the 19th century. The pedigree is consistent with autosomal recessive inheritance of a founder mutation (Fig 1). Neither obligate carrier parents nor unaffected sibs showed evidence of kidney abnormalities or renal dysfunction.

Because of the severe nature of this disease, few affected infants had complete medical evaluations, and no autopsy information is available. However, information for events surrounding the birth and phenotypic presentations of these children has been collected and collated (listed in Table 1). In most cases, mothers had low weight gain during pregnancy and noted oligohydramnios. Gestation was 34 to 36 weeks in 10 pregnancies and lasted 28 weeks in another. The remaining infant (A4) was carried to term and also had the greatest birth weight (7 lbs 13 oz). Birth presentation was breech in 8 infants. Birth weight was less than 5 lbs in 7 newborns, 5 to 6 lbs in 3, and greater than 7 lbs in only 2. The majority lived only a few hours, but 1 (A4) lived 38 days, another (A5) lived 29 days, and 1 (A7) lived 2 days.

Table 1. Clinical Presentation and Birth Details of the 12 Affected Individuals

						Clinical Presentation					
Patient	Birth Weight	Gestation	Oligohydramnios	Birth Position	Lifespan	Cystic Kidneys	Supporting Clinical Evidence	Bladder	Low-Set Ears	Pulmonary	Other Features
A1	4 lbs 13 oz	36 wk	Yes	Breech	3.5 h	Yes	Ultrasound: bilateral cystic kidneys	No	Yes	Single lung	
A2	4 lbs 3 oz	36 wk	Yes	Breech	3.5 h	Yes	Computed tomographic scan: bilateral cyst-like densities	No	Yes	Single lung	
A3	7 lbs	36.5 wk	—	Normal	14 h	Yes	—	Yes	Yes	Small collapsed lungs	
A4	7 lbs 13 oz	Term	Yes	Normal	38 d	Yes	Ultrasound: bilateral cystic, dysplastic kidneys	Yes	Yes	Small lungs; respiratory distress	Left ventricular hypertrophy, hypertension
A5	4 lbs 12 oz	34 wk	Yes	Breech	29 d	Yes	Ultrasound: small polycystic kidneys with increased echogenicity	Yes	Yes	Respiratory distress	Situs inversus
A6	4 lbs 2 oz	36 wk	Yes	Breech	20 min	—	—	—	—	—	
A7	4 lbs 2 oz	35 wk	Yes	Breech	2 d	Yes	Ultrasound: bilaterally enlarged kidneys with multiple sized cysts	Yes	Yes	Hemorrhage	Ventricular and atrial septal defects, patent ductus arteriosus
A8	4 lbs 6 oz	28 wk	Yes	Breech	3 h	Yes	Prenatal ultrasound: consistent with polycystic kidneys	—	—	Pulmonary wheezes	
A9	4 lb 5 oz	34-36 wk	Yes	Normal	<6 h	Yes	—	—	—	Respiratory distress	Spina bifida
A10	5 lb 4 oz	34-36 wk	Yes	Breech	<6 h	Yes	Ultrasound: polycystic kidneys	—	Yes	Respiratory distress	
A11	5 lb 9 oz	34-36 wk	Yes	Normal	<6 h	Yes	—	—	—	Respiratory distress	
A12	6 lbs	34-36 wk	Yes	Breech	<6 h	Yes	—	—	—	Lung malformation	

Attending physicians documented cystic kidneys in 11 infants (7 with supporting ultrasound or radiographic evidence), and these were suspected in another (A6) who had 2 affected sibs. It is clear that although the renal aspect of the phenotype was the single consistent feature, other clinical features, although more variable, are sufficiently consistent to suggest a unique syndrome. Pulmonary abnormalities were reported in 11 of 12 infants, ranging from the presence of a single pulmonary lobe to reports of some degree of pulmonary distress in 7 more. Cause of death was associated with respiratory distress in all infants. The bladder was reported as absent in 2 individuals. Normal bladder development was confirmed in 4 individuals, but its presence or absence was not documented in the remaining 6 patients. Several also had cardiac abnormalities, including left ventricular hypertrophy, hypertension, ventricular and atrial septal defects, and 1 with patent ductus arteriosus. Other systemic abnormalities included total situs inversus in 1 case and incomplete closure of the spinal column in another. Low-set ears with some degree of malformation were present in at least 7 infants.

Because of the early lethality of the disorder, few infants had radiographic studies or full physical examinations by a dysmorphologist. Only individual A7, who survived for 2 days, had a complete evaluation and was described as an edematous infant with a short webbed neck and short extremities. The occiput was prominent, and there was a crease across the nasal bridge. Ears were low set with malformed auricles. The nipples were separated by an increased distance. There was clinodactyly of the 5th fingers, elbow dysplasia, joint limitation, and metacarpal and nail hypoplasia, and the palms had a single simian crease.

Molecular Genetics

Genotypes were generated for approximately 50,000 single-nucleotide polymorphisms throughout the genome in 8 of the 12 parental samples (P1, P2, P3, P4, P5, P6, P9, and P10) from which DNA was available at the time and a single affected infant (A8). Analysis of these genome-wide single-nucleotide polymorphism genotypes showed an extended region of homozygosity spanning approximately 24 Mb of chromosome 3 in the single affected case. Examination of

SNP ID	P1	P2	P3	P4	P5	P6	P9	P10	A8
rs10490863	A B	A B	A A	A B	A B	A B	A B	A B	A A
rs6794333	A B	A B	A A	A B	A B	A B	A B	A B	A A
rs10490862	A B	A B	A A	A B	A B	A B	A B	A B	A A
rs10512873	B B	B B	B B	A A	B B	B B	B B	B A	B B
rs1378807	B A	B A	B B	A A	B A	B A	B A	B B	B B
rs2369832	A A	A A	A A	A A	A A	A A	A B	A A	A A
rs747328	A A	A A	A A	A A	A A	A A	A B	A A	A A
rs1901551	A A	A B	A A	A A	A A	A A	A B	A A	A A
rs2053954	A A	A B	A A	A A	A A	A A	A B	A A	A A
rs1869155	A B	A A	A A	A A	A A	A A	A A	A B	A A
rs10512879	B B	B B	B B	B B	B B	B A	B B	B A	B B
rs2369951	B B	B B	B A	B A	B B	B B	B B	B B	B B
rs6763720	B B	B B	B A	B A	B B	B B	B B	B B	B B
rs4461418	A A	A A	A A	A A	A A	A B	A A	A B	A A
rs1357019	B B	B B	B A	B A	B B	B A	B B	A B	B B
rs10512885	B B	B B	B B	B B	B A	B B	B B	B B	B B
rs10512886	B B	B B	B B	B B	B A	B B	B B	B B	B B
rs10512887	B B	B B	B B	B B	B A	B B	B B	B B	B B
rs10512888	B B	B B	B B	B B	B A	B B	B B	B B	B B
rs7630783	A A	B B	A A	A B	A A	A A	A A	A A	A A
rs9289439	A A	A A	A A	A A	A A	A B	A A	A B	A A
rs10512891	B B	A A	B B	B B	B B	B B	B B	B B	B B
rs1513363	A B	A A	- -	A A	A A	A B	A B	A A	A A
rs1201674	B B	A A	B B	B B	B B	B B	B B	B B	B B

Figure 2. Conserved 14-marker haplotype across the 475-kilobase region delimited by single-nucleotide polymorphisms (SNPs) rs1378807 and rs7630783 in 8 obligate carrier parents and a single affected individual.

haplotypes across this region in the 8 parental samples showed the presence of a single copy of the same conserved haplotype across a 475-kilobase (kb) region, delimited by single-nucleotide polymorphisms rs1378807 and rs7630783 (Fig 2). The 475-kb region encompassed the *NPHP3* gene. Intronic exon-flanking primers were designed to amplify and sequence all 27 exons and associated splice junctions of the *NPHP3* gene. Sequence analysis of the coding regions and associated splice sites of *NPHP3* showed a cytosine to thymine substitution in exon 15 at position 2104 (NM_153240.3: c.2104C→T; numbering is based on +1 being the first nucleotide of the start codon; Fig 3). This variant showed cosegregation consistent with disease status: homozygous in the affected case and heterozygous in all 12 parental samples. This substitution is predicted to produce a nonsense mutation at position 702 of the protein product changing an arginine codon to a stop codon (p.Arg702X). This variant was not present in 100 control chromosomes of European ancestry. Although only a single DNA sample was available to confirm homozygosity of the c.2104C→T variant in an affected case, identification of all parental samples as heterozygous mutation carriers strongly suggests that this variant is respon-

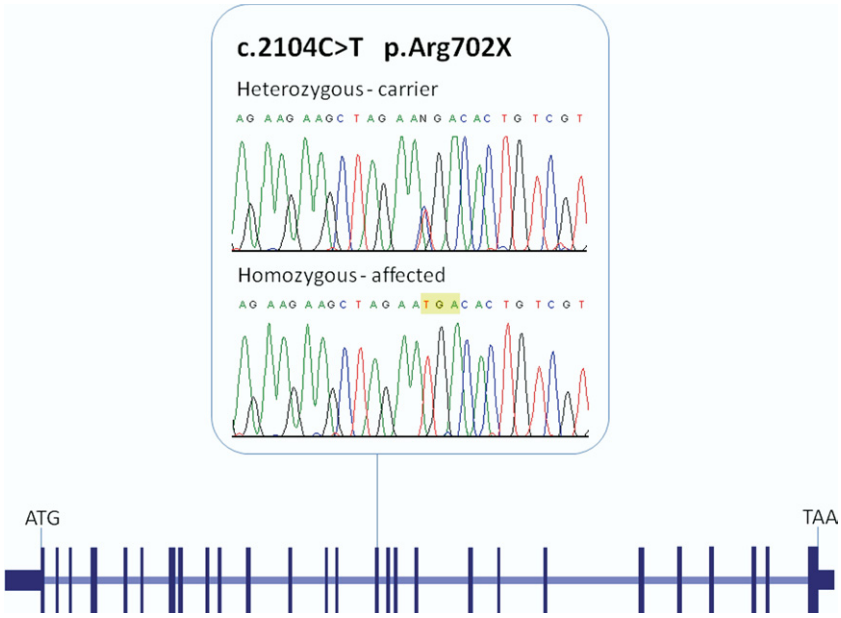

Figure 3. Sequence chromatograms of the nonsense c.2104C→T variant in heterozygous and homozygous individuals and its location in exon 15 of the 27-exon *NPHP3* gene.

sible for the lethal nephronophthisis in this Amish community.

DISCUSSION

The case series of 12 affected individuals clearly shows the severity of this inherited disorder in the Old Order Amish. Because of the early lethality of the disease, detailed clinical investigations were not performed in all cases. However, from the clinical information available, it is clear that beyond the renal element of the phenotype, there are subtle variations in the disease expression.

Mutations in *NPHP3* were originally described in a cohort of young adults with progressive renal failure.[3] However, it subsequently has been shown that mutations with a more severe effect on the NPHP3 protein lead to a more severe phenotype.[4] The phenotypic presentation of the affected cases in the present study is severe, leading to death within the first few weeks of life in all cases, consistent with identification of the predicted protein truncating mutation (c.2104C→T, p.Arg702X) cosegregating with the disease status. Homozygosity for 2 other mutations with similar detrimental effect on the NPHP3 protein recently have been identified in individuals with features similar to those presented in the present study. Homozygosity of a deletion of the invariant AG at the intron/exon splice acceptor site (NM_153240.3:c.2694–1_2del) and a nonsense mutation (NM_153240.3:c.1729C→T [p.Arg577X]) were recently reported in 2 affected females from a Turkish kindred and 2 Cameroonian males, respectively.[4] All were reported to have enlarged multicystic dysplastic kidneys and died in the neonatal period. Low volumes of amniotic fluid were a consistent feature. Three had various cardiac defects, and respiratory insufficiency was reported in 2. A similar perinatal death of an individual of Swiss origin also was reported by Bergmann et al.[4] The individual was a compound heterozygote for mutations in *NPHP3*, a missense mutation (NM_153240.3:c.2918G→A [p.Arg973Gln]) inherited paternally, and a nonsense mutation (NM_153240.3:c.3340C→T [p.Gln1114X]) inherited maternally. An affected sib carrying the same 2 sequence variants in *NPHP3* survived the neonatal period and underwent combined liver and kidney transplantation at age 3 years, which prolonged life until 17 years of age. The predicted truncating effect of the c.3340C→T (p.Gln1114X) mutation is similar to the predicted effect of the previously reported c.2694–1_2del and c.1729C→T (p.Arg577X) mutations and the c.2104C→T (p.Arg702X) mutation identified in the present study. However, the less severe effect of the missense c.2918G→A (p.Arg973Gln) on the pre-

dicted protein is more consistent with those identified in patients with less severe phenotypes and generally later onset of renal disease.[3,4]

The pattern of both embryonic-onset severe renal disease and less severe early adult-onset disease is mirrored by findings in mouse studies of *Nphp3*. The naturally occurring polycystic kidney (pcy) phenotype in the mouse is a later onset polycystic renal disease caused by a homozygous missense mutation (c.1841T→G [p.Ile614Ser]) in *Nphp3*.[3] Conversely, recently generated homozygous *Nphp3*-deficient mice show a more severe phenotype leading to embryonic lethality; investigation of the *Nphp3*-deficient embryos showed that they displayed a wide and variable spectrum of patterning defects, including randomization of left-right body asymmetry and complex cardiac defects.[4] The pattern of genotype-phenotype correlation in both humans and mice suggests that the complete loss of function of NPHP3 underlies the observed embryonic malformations, whereas less severe mutations of *NPHP3* have a less pronounced effect on embryonic kidney development, but affect renal functioning in later life.

In addition to the clear genotype-phenotype correlation of severity of mutation with severity of disease expression, the present study highlights the subtle variations in phenotypic expression in a cohort of patients with the same fundamental genetic insult. Interestingly, many of the additional differentially expressed phenotypic features presented in this study have also been observed in other reported cases of human and mouse *NPHP3* mutations, including cardiac abnormalities and situs inversus totalis. It remains to be elucidated how similar and, as seen in the present study, even identical mutations of this gene lead to these different phenotypic manifestations. Although several of the observed additional phenotypic elements may represent the direct effect of oligohydramnios during development, others may be the result of additional genetic or environmental modifying factors. The future study of this disease, particularly in this Amish deme for whom the disease is at a greater frequency than in the general population, will offer the opportunity to identify these potential modifiers that may have a role in the differential phenotypic expression of this disorder.

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