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Asymptomatic and Late-Onset Ornithine Transcarbamylase Deficiency Caused by a A208T Mutation: Clinical, Biochemical and DNA Analyses in a Four-Generation Family

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We describe a 4-generation family in which a previously healthy 10-year-old boy died of late-onset ornithine transcarbamylase (OTC) deficiency. Pedigree analysis and allopurinol loading tests in female relatives were not informative. A missense mutation (A208T) in the OTC gene was detected in the deceased patient and in several clinically healthy male and female relatives, the oldest male being 97 years old. OTC deficiency was established in autopsy liver tissue of the propositus and liver biopsy samples of his sister, mother, and a maternal uncle. The males had 4% and 6% residual activity, respectively, the females 58% and 67%, respectively. The observed relation between the mutation and the decreased OTC activity in liver tissue of these subjects suggests that the mutation is a deleterious one. Late-onset, "mild" OTC deficiency can have a fatal or a favorable outcome. The disease can segregate undetected in families. *Am. J. Med. Genet.* 68:236–239, 1997 © 1997 Wiley-Liss, Inc.

KEY WORDS: late-onset ornithine transcarbamylase deficiency; mutation analysis; allopurinol loading test; X-linked inheritance; carrier detection

INTRODUCTION

Ornithine transcarbamylase (OTC) deficiency (McKusick #311250) is an X-linked recessive urea cycle disorder with variable clinical presentation in hemizygous males and heterozygous females [Tuchman, 1992]. The neonatal phenotype, often leading to early death, is caused by complete absence of hepatic OTC activity. It is accompanied by severe hyperammonemia, hypocitrullinemia, and orotic aciduria. Males with a partial (up to 30% of normal) enzyme deficiency generally are free of symptoms but may be at risk during catabolic circumstances and/or high protein intake [Finkelstein et al., 1990]. Onset in these patients has been reported at any age, the highest two being 46 and 58 years [Matsuda et al., 1991], with symptoms characterized by protein avoidance, behavior changes, recurrent vomiting, lethargy, and coma [Drogari and Leonard, 1988]. Females heterozygous for OTC deficiency may be asymptomatic or have similarly variable clinical symptoms which are explained by random X-chromosome inactivation [Batshaw et al., 1986].

To date, approximately 70 mutations and polymorphisms in the OTC gene have been identified [Tuchman et al., 1995b]. Except for very few recurrent mutations such as the R141Q and R277W mutation, most patients with OTC deficiency have unique alterations. Thus far, there is no clear genotype-phenotype correlation [Oppliger et al., 1995].

In this paper we present the results of clinical, biochemical, and molecular genetic investigations performed in a 4-generation family in which a previously healthy boy died of late-onset OTC deficiency at the age of 10 years. It is remarkable that his great-grandfather, who has transmitted the disease, remained asymptomatic until the age of 97 years.

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CLINICAL REPORT

The propositus (IV-5, Fig. 1), a 10-year-old boy, was admitted because of a progressive hepato-encephalopathy in the course of a protracted viral gastroenteritis. Besides unexplained extreme irritability during minor illnesses with fever, noticed from the age of 6 years on, his medical history was uneventful. Physical examination on admission showed a severely ill child with periodical restlessness, dry mucosa, a flushed face, wide pupils with a positive light reflex, alternating periods of bradycardia and tachycardia, slightly diminished deep tendon reflexes, and bilateral Babinski reflexes.

The initial blood ammonium level was 529 $\mu\text{mol/L}$ ($n < 60$). Laboratory studies showed the following abnormal results: glutamine 2,404 $\mu\text{mol/L}$ ($n:596 \pm 66$), citrulline 14 $\mu\text{mol/L}$ ($n:35 \pm 8$), arginine 47 $\mu\text{mol/L}$ ($n:89 \pm 20$), ornithine 31 $\mu\text{mol/L}$ ($n:49 \pm 14$). Urine orotic acid was 760 mmol/mol creatinine ($n: < 2.5$) and orotidine was 2.2 mmol/mol creatinine ($n: < 2.5$).

Following the recognition of hyperammonemia, protein intake was stopped immediately and a high-calorie carbohydrate infusion was started. Sodium benzoate (250 mg/kg body weight) was supplemented intravenously during the first two hours followed by 250 mg/kg bodyweight/24 h as a continuous infusion. This was supported by arginine-HCl, lactulose, folic acid and L-carnitine. Despite this treatment, the patient's clinical condition worsened over the next hours, progressing to coma and convulsions. The blood ammonium level was still markedly elevated (530 $\mu\text{mol/L}$). Anti-convulsive drug administration, artificial ventilation and peritoneal dialysis were initiated. Unfortunately, hemodialysis, the most powerful tool for ammonia removal, could not be performed. Plasma

ammonia levels normalised only after 36 hours of treatment, however, without any change in the patient's critical condition. Repeated EEG recordings showed an isoelectric pattern. All treatment was discontinued on the 6th day after admission. One hour after death, autopsy was performed. Cerebral edema, mild patchy bronchopneumonia and diffuse fatty changes of the liver were the main abnormalities.

The diagnosis of OTC deficiency was suspected. This was supported by low plasma citrulline and increased urinary orotic acid. However, the pedigree (Fig. 1) was not informative for a fatal disorder with X-linked recessive inheritance. In order to identify relatives at risk, biochemical investigations and DNA analyses were performed.

MATERIALS AND METHODS

Autopsy liver tissue of the propositus was stored at -70°C until use for enzyme studies. OTC activity was determined according to Kleijer et al. [1984] and expressed as a percentage of the control levels which ranged from 192 to 333 nmol/min/mg protein. The allopurinol loading test was performed as described by Hauser et al. [1990]. The orotic acid and orotidine peak levels represent the mean + 3 S.D., i.e., the maximum value that can be observed in controls [Hauser et al., 1990]. Plasma ammonium and amino acids were analyzed with an amino acid analyzer according to the manufacturer's instructions. Orotic acid and orotidine were measured by high performance liquid chromatography using diode-array UV-detection. Genomic DNA was isolated from peripheral blood leukocytes, and mutation analysis was performed by single-strand conformational polymorphism (SSCP) and direct sequencing [Tsai et al., 1993].

RESULTS

OTC activity in autopsy liver tissue of the propositus was 9 nmol/min/mg protein (4% of OTC activity in controls), confirming the suspected deficiency of this enzyme. SSCP analysis of the exons of the OTC gene showed a band shift of the polymerase chain reaction (PCR) product of exon 6. Direct sequencing of the abnormal DNA fragment identified a A208T mutation in the propositus (data not shown). This mutation causes a substitution of alanine for threonine. Sequencing of all exons, including intron-exon junctions, showed no other mutation. The A208T mutation was detected in the propositus' mother and sister, and in several other relatives (Fig. 1). Four clinically healthy males (ages ranging from 2 to 97 years) were carriers of the mutation.

The allopurinol loading test was performed in the following persons (Fig. 1): the mother (III-3), sister (IV-6), maternal uncle (III-1) of the propositus as well as in II-1, III-2, III-4, IV-3 and IV-9. All 3 males who were tested had abnormal test results, although the variation in the peak levels of orotic acid and orotidine was quite large (Table I). In one female (III-2), urinary orotic acid and orotidine excretion were increased. In three of 4 abnormal allopurinol loading tests, the urinary orotic acid was markedly higher than orotidine.

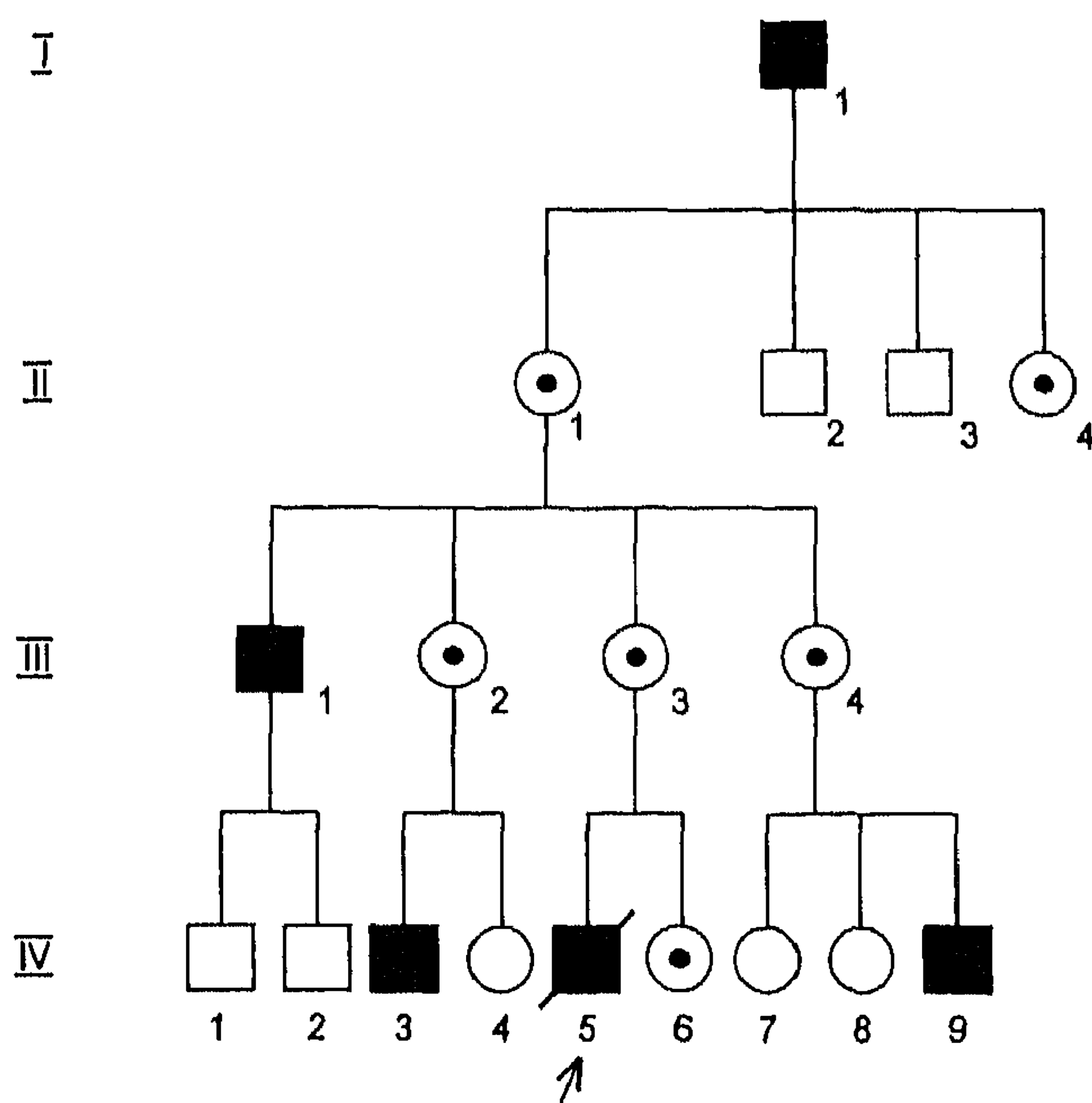


Fig. 1. Pedigree of a four-generation family with OTC deficiency caused by a A208T mutation. ○, Female heterozygous for the A208T mutation; ■, male hemizygous for the A208T mutation.

TABLE I. Results of the Allopurinol Loading Test, Plasma Glutamine and Liver OTC Activity in Carriers of the A208T Mutation

Person	Gender	Age (yr)	Allopurinol loading test		Liver OTC activity (nmol/min/mg protein)	Plasma glutamine (μ mol/L)
			Orotic acid peak level (mmol/mol creatinine)	Orotidine peak level		
I-1	m	97		np ^a	np	np
II-1	f	66	3.7	4.4	np	np
III-1	m	41	110	18.0	16	706
III-2	f	40	142	13.3	np	np
III-3	f	35	8.2	3.3	170	611
III-4	f	32	4.1	6.2	np	np
IV-3	m	14	19.2	1.8	np	710
IV-5	m	10		np	9	2,404
IV-6	f	8	3.7	3.8	144	584
IV-9	m	2	8.6	14	np	588
Controls			14.4 ^c	9.1 ^c	250 ^b	596 \pm 66 ^d

^anp, not performed.

^bMean control OTC activity. The control levels ranged from 192 to 333 nmol/min/mg protein (n = 4).

^cThis value represents the mean + 3 S.D., i.e., the maximum value that can be observed in controls [Hauser et al., 1990].

^dArmstrong and Stae [1973].

The results were normal in the other tested individuals (Table I). Plasma glutamine, another marker of urea cycle disorders, was normal in all asymptomatic relatives (Table I). In III-1, the ammonia level increased from 44 μ M to 204 μ M (n:<60) after a protein-rich meal. During this episode of hyperammonemia, no neurological or other symptoms were observed. There was a persistently low level of plasma citrulline. Liver biopsy was performed in the mother (III-3), sister (IV-6) and maternal uncle (III-1) of the patient showing 170, 144 and 16 nmol/min/mg protein OTC activity, respectively (Table I). None of the heterozygous females (II-1, II-4, III-2, III-3, III-4 and IV-6, Fig. 1) ever had experienced clinical symptoms associated with OTC deficiency.

DISCUSSION

OTC deficiency is an X-linked recessive disorder with a variable clinical phenotype. The dramatic clinical presentation in male infants is well-known. In patients with a partial enzyme deficiency, the age of onset may vary. The clinical symptoms include bizarre behavior [DiMugno et al., 1986; Spada et al., 1994] and neurological abnormalities [Drogari and Leonard, 1988]. Matsuda et al. [1991] have shown that late-onset OTC deficiency is a potentially lethal disorder. On the other hand, our family shows that symptom-free survival up to a very advanced age is also possible.

According to Pelet et al. [1990] the prior minimum risk of a mother of an affected male being a carrier is 84%. Recently, Tuchman et al. [1995a] suggested that this risk is at least 90%. Thus, carrier detection is important when OTC deficiency is first diagnosed in a family. Carrier detection can be performed by *in vivo* testing (acute and chronic protein loading tests, allopurinol loading test), enzyme analysis in liver biopsy samples and molecular genetic investigations. With respect to the allopurinol test, Hauser et al. [1990] reported that less than 5% of the obligate heterozygotes

did have normal results. However, in the present family, the allopurinol test was negative in 4 of the 5 females tested, indicating that this test should not be used to identify female carriers in families with late-onset OTC deficiency.

In our family, 3 of 4 abnormal allopurinol loading tests showed a prevalence of urinary orotic acid over orotidine. This contrasts with the findings of Hauser et al. [1990], who suggested that orotidine is a better marker than orotic acid on allopurinol testing. The individual activities of pyrimidine nucleoside phosphorylase, catalyzing the conversion of orotidine to orotic acid, may influence the ratios of these two substances. Plasma glutamine is considered to be a marker of urea cycle disorders, as it is formed from glutamate and excess ammonia. In the present family, all tested asymptomatic subjects had normal glutamine levels. From that we conclude that this analyte has no discriminative power in our family.

In families where the mutation is known, SSCP analysis of the OTC gene or direct sequencing are the methods of choice for carrier detection [Tsai et al., 1993]. We detected a A208T missense mutation in the proband. Sequencing of all exons, including intron-exon junctions, showed no other mutation. The A208T mutation was present in several other relatives (Fig. 1). In 3 of them, liver OTC activity was measured (Table I). The observed decreased OTC activity in two females and the low OTC activity in the proband and his maternal uncle seems to confirm the deleterious nature of the mutation. The alanine at position 208 is evolutionary conserved among mammalian OTC sequences and in more than 20 species [Tuchman et al., 1995b]. It is therefore highly suggestive that the alteration is not neutral. However, future expression studies of mutant cDNA are required to establish firmly the relationship between the mutation and the enzyme deficiency.

The OTC activity was not measured in the great-grandfather of the proband. He was clinically

healthy at age 97 years. In the propositus and his uncle the OTC activity was 4% and 6%, respectively. The propositus died, while his uncle never has had major health problems. Apparently, factors other than in vitro OTC activity play a role in the severity of symptoms, but this is not well understood [Finkelstein et al., 1990].

To date, about 70 different mutations (including polymorphisms) have been identified in the OTC gene of patients with OTC deficiency [Tuchman et al., 1995b]. Most individuals with deficient enzyme activity have unique mutations. The A208T mutation was described previously in a large Polish pedigree with late-onset OTC deficiency [Bakker et al., 1995]. The mutation originated from the great-grandfather of the propositus who died at the age of 59 years without evidence of episodes of hyperammonemia.

In conclusion, we think that OTC deficiency can segregate undetected in families. When first diagnosed in a family, carrier detection is an important aspect of genetic counselling. No relative can be reassured until molecular genetic and biochemical investigations have been performed. In the reported 4-generation family, late-onset OTC deficiency was caused by a missense (A208T) mutation. The disease can have a favorable as well as a fatal outcome. Consequently, it is important to identify individuals at risk.

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