# Erythrocyte Carbonic Anhydrase I: Inherited Deficiency in Humans

Abstract. *The virtually complete absence of erythrocyte carbonic anhydrase I is reported in three members of a family from the Greek island of Icaria. Two members with moderately reduced levels are believed to be heterozygous for the deficiency. There are no obvious hematological or renal consequences of the severe deficiency state.*

Carbonic anhydrases in mammalian erythrocytes catalyze the rapid hydration of metabolic $CO_2$ from the tissues and the dehydration of $HCO_3^-$ in the lungs, and also function in the transfer and accumulation of $H^+$ and $HCO_3^-$ in secretory cells (*1*). Two isozymes of carbonic anhydrase, carbonic anhydrase I (CA I) and carbonic anhydrase II (CA II), which appear to be the products of two closely linked genes, are present in human erythrocytes (*2, 3*). In normal adult Caucasians, the mean CA I and CA II concentrations as measured by radioimmunoassay (*4*) are, respectively, 11.57 ± 2.26 and 1.83 ± 0.26 μg per milligram of hemoglobin (Hb), and there is some evidence that the normal variation in the concentrations of these isozymes is under genetic control (*5, 6*). Inherited deficiencies of red cell CA I have been described in the chinchilla (*7*) and pigtail macaque (*2, 8*), in which the levels of CA I in mature red cells have been reduced to trace amounts in individuals homozygous for the deficiency gene. However, as yet no deficiency of this magnitude has been reported in humans after the examination of some 23,000 individual hemolysates from Negro, Caucasian, Oceanian, Mongoloid, and Amerindian populations (*5*).

Here we report on an inherited deficiency of red cell CA I in three members of a kindred of Greek origin. The pedigree is depicted in Fig. 1, and the red cell CA I and CA II levels and electrophoretic patterns of the five tested individuals are given in Fig. 2. Greatly reduced concentrations of red cell CA I (0.6 ng/mg Hb) were found in the healthy 15-year-old proband as well as her 14-year-old brother (0.7 ng/mg Hb) and 63-year-old maternal grandmother (0.8 ng/mg Hb). Since the CA I levels of the parents of the two deficient children were, respectively, 4.32 and 5.96 μg/mg Hb, which are about one-half the normal mean levels of CA I, it was assumed that they were heterozygous for the deficiency gene and that the CA I–deficient individuals were homozygous. The concentrations of the other isozyme, CA II, were all within normal limits (Fig. 1). Although consanguinity could not be demonstrated, the kindred originated from the small Greek Aegean island of Icaria, whose population has been reported to be about 9500 (1971 census).

All of the tested individuals (I-3, II-3,4, and III-1,2) appeared healthy with no stigmata of thalassemia, and their hemoglobins A, $A_2$, and F values were normal. In addition, their hematocrits, reticulocyte and white cell counts, and mean cell volumes were also within normal limits. Since it is known that red cell CA I levels may be considerably suppressed in hyperthyroidism (*5*), plasma thyroxine (T4) levels were measured in the proband (III-1) and her brother (III-2); they were found to be, respectively, 6.6 and 7.7 μg/dl, which are within normal limits. An inherited variant of human CA I with decreased specific activity has been reported in which the presumed homozygotes are unable to acidify their urine and develop renal tubular acidosis (*9*). In the present study, no alteration in the renal acidification process was observed when metabolic acidosis was induced in the proband's brother by ammonium chloride ingestion (*10*). Osborne *et al.* (*11*) observed that pigtail macaques with inherited deficiencies of red cell CA I were also able to acidify their urine after the induction of acidosis by ammonium chloride.

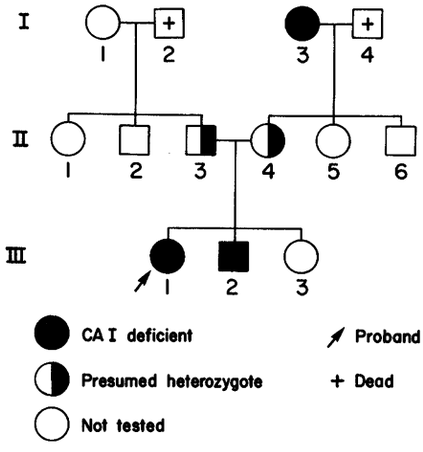


Fig. 1. Pedigree showing inheritance of red cell CA I deficiency.

It is of interest to compare the human red cell CA I deficiency reported here with similar inherited CA I deficiencies in the chinchilla and pigtail macaque. The most notable difference is the effect on the levels of the other isozyme, CA II. In the CA I–deficient macaques, CA II concentrations are reduced about 60 percent (*2, 8*), whereas there appears to be very little if any reduction in the concentrations of CA II in the CA I–deficient humans (Fig. 1) and chinchillas (*7*). Thus, the marked *cis*-polar suppression of CA II in the macaque CA I deficiency (*2*) was not observed in the human and chinchilla deficiencies. If the mutations responsible for these red cell CA I deficiencies reside in the structural genes for CA I, then their positions in the CA I genes seem to differentially affect the expression of the closely linked CA II genes.

There is some evidence that the CA I deficiency condition in the pigtail macaque may be due to a mutation in the structural gene for CA I rather than in a

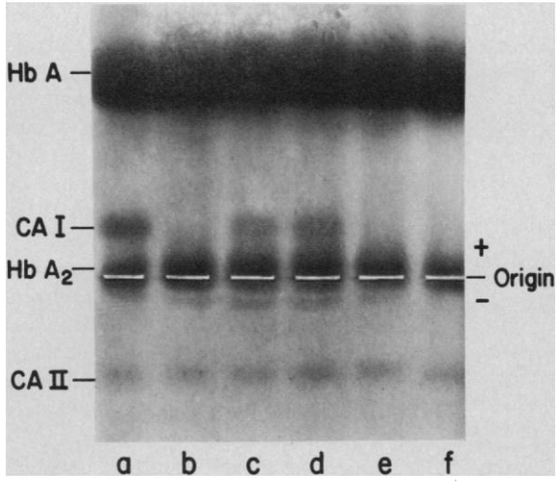


Fig. 2. Protein-stained electrophoretic patterns of red cell CA I and CA II of individuals represented in Fig. 1: (a) normal control, (b) III-1, (c) II-3, (d) II-4, (e) III-2, and (f) I-3. Vertical starch gel electrophoresis was carried out at 4°C in 10 m*M* sodium borate buffer, *p*H 8.6, for 21 hours at 8 volt/cm. Gels were stained with 0.4 percent nigrosine in methanol, acetic acid, and water (5:1:5). The CA I and CA II values (micrograms per milligram of hemoglobin) were, respectively: (a) 12.30 and 2.01, (b) 0.00006 and 2.06, (c) 5.96 and 2.14, (d) 4.32 and 2.18, (e) 0.0007 and 1.73, and (f) 0.0008 and 1.81. The values were determined by radioimmunoassay (*4*).

control gene. This is based on the observation that when marrow cells were tested for the presence of CA I by a single-cell immunofluorescence method (*12*), moderately high levels of fluorescence were detected in a number of unidentified cells (*13*). This finding suggests that a variant CA I may be synthesized in red cell precursor cells, but that it, or its messenger RNA, is degraded at some point during red cell maturation. It is possible that a similar mechanism is responsible for the human CA I deficiency.

The role of CA I in mature mammalian erythrocytes remains unclear. Not only may the levels of this isozyme be greatly suppressed as the result of a mutation, but CA I is also characteristically absent from peripheral red cells (although present in other tissues) of ruminants such as the ox (*14*) and the sheep (*15*) and felids such as the domestic cat (*16*). As yet, a similar reduction in the levels of CA II has not been reported in mammalian red cells. It would thus appear that CA II has a more important role than CA I in the mature red cell. It has even been suggested that CA I may not function in the hydration of $CO_2$ in mammalian red cells because concentrations of chloride and bicarbonate equivalent to those found in normal human red cells have been observed to greatly inhibit this reaction in vitro (*17*).

If the CA I variant reported here is found to be due to a defect in the structural gene for CA I, we propose that it be designated CA I Icaria.

It is worth noting that another rare genetic mutation, resulting in an elongated alpha globin chain and the formation of hemoglobin Icaria, has been reported in a family from the same sparsely populated Greek island (*18*).

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## Calcium-Dependent Depression of a Late Outward Current in Snail Neurons

Abstract. *Neuron cell bodies of* Helix pomatia *were voltage-clamped with a 300-millisecond depolarizing test pulse (pulse II) delivered 1 second after a depolarizing conditioning pulse (pulse I). The outward current, measured 200 milliseconds after the onset of pulse II, exhibited a strong depression that was dependent on the presence of pulse I. The maximum depression of the pulse II outward current occurred when pulse I voltages lay in the range over which calcium influx is inferred to be greatest; depression of the pulse II current subsided as pulse I potentials approached the putative calcium equilibrium potential. In the presence of extracellular [ethylenebis(oxyethylenenitrilo)]tetraacetic acid (EGTA) or D600, the intensity of the pulse II current became largely independent of pulse I, approaching the values of maximal depression seen in normal Ringer solution. On the other hand, lowering the intracellular pH with extracellular carbon dioxide–carbonate buffer had no measurable effect on the outward currents. Other experiments showed that it is primarily the calcium-dependent, outward-current hump of the N-shaped late current-voltage curve that is depressed by presentation of the conditioning pulse. It was concluded that distinct from an early potassium-activating role, calcium entering during a depolarization leads, during a subsequent depolarization, to a depression of the calcium-activated potassium system that persists for many seconds.*

Depolarizations of nerve cell membranes are accompanied by a voltage- and time-dependent delayed flow of outward current carried by potassium ions. During normal nervous function this outward current plays an important role in repolarizing the membrane from a depolarized state. Potassium activation has been implicated in the repolarization of nerve, muscle, and cardiac action potentials and in the regulation of pacemaker potentials (*1–4*). In a number of membranes the delayed outward current shows a pronounced decay with time during maintained depolarization. In neurons of the snail, depression of outward current is also apparent in the second of two identical pulses separated by an interval of up to several seconds (*5–8*). Measurement of potassium activity during such experiments has shown that a major reason for such outward current depression is an actual drop in $K^+$ efflux during the second of two identical depolarizing pulses, although a facilitating partial inward current also appears to participate (*6, 8*).

The experiments reported here indicate that a large component of the depression of $K^+$ efflux depends on a prior influx of $Ca^{2+}$. The presence of a slow calcium system has been demonstrated by voltage clamp experiments on snail neurons for small depolarizations (*9*) and has been inferred for larger depolarizations in which the massive $K^+$ efflux obscures the smaller inward current (*8–11*). An inward calcium current during a depolarizing pulse is essential for activation of a component of the outward potassium current, $I_{K(Ca)}$ (*12, 13*). We present evidence here that distinct from the mediation of a calcium-coupled potassium current, there develops a depression or desensitization of the calcium-activated potassium conductance, $g_{K(Ca)}$, which lasts many seconds. This depression is causally related to a prior influx of $Ca^{2+}$ and appears to play a role in the normal functioning of some excitable membranes.

The soma of a bursting pacemaker neuron in the right parietal ganglion of *Helix pomatia* was exposed, impaled with capillary electrodes filled with 3*M* KCl (2 to 10 megohms), and voltage-clamped, and the membrane current was measured locally from a portion of the