THE HEREDITARY STOMATOCYTOSES

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Sets of rare genetic disorders often split into even rarer subsets when they come to close scrutiny, particularly at the gene level. Meanwhile, such subsets main become close to subsets
that stem from originally distinct entities. Here boundaries are laid out, there they are blurred. Such conflicting trends have marked out the history of stomatocytoses. Today, stomatocytoses are encompassed within a wider group, referred to as the genetic disorders of the passive leak of red cell membrane to monovalent cations, more simply the genetic disorders of the leak.

As for most hereditary hemolytic anemias, stomatocytosis has been coined after a morphological abnormality. Being the first observers of stomatocytosis, Lock *et al* (1961) wrote: … 'The red cells … contain a well demarcated linear unstained mark across their center, instead of the normal circular area; because of this appearance suggests a mouth-like orifice in the cell, we have chosen to use the term 'stomatocytosis' to describe the condition' (Figure 1). It has turned out that the first case involved was an exceptional variety of stomatocytosis, the overhydrated hereditary stomatocytosis, as will be presented below.

General features of stomatocytoses.
Except for familial pseudohyperkalemia, stomatocytoses are hemolytic anemias. The diagnosis relies on the amount of hemoglobin, the reticulocyte count, the red cell indices (a mild macrocytosis is a useful sign and MCHC tells which category of stomatocytosis one is dealing with), bilirubinemia, haptoglobinemia, and an iron status work-up, particularly ferritinemia (for stomatocytoses tend to be iron-loading conditions). Osmotic gradient ektacytometry (Figure 2) is the gold standard for the diagnosis, unfortunately there are so few apparatuses available worlwide that most cases cannot benefit by this technique. SDS-polyacrylamide gel electrophoresis may bring some valuable information. The leak *vs.* temperature curve (Figure 3) provides all sorts of shapes, testament to the fact that the molecular alterations caused by the mutations are multifarious. Evolution is marked by cholelithiasis and secondary hemochromatosis. A major breakthrough, standing for all stomatocytoses presumably, was the realization that splenectomy, beneficial though it may be in many hemolytic anemias, has the major drawback of causing severe, if not lethal, venous thrombo-embolic complications (Stewart *et al*, 1996; Grootenboer *et al*, 2000). The underlying mechanism is unknown. Stomatocytoses have an autosomal dominant mode of transmission. Quite commonly, overhydrated hereditary stomatocytosis arises *de novo*. As far as we know, there are no particular populations in which the incidence of stomatocytoses has

been reported high. As compared to the most common genetic disease of the red cell membrane, hereditary spherocytosis, dehydrated hereditary stomatocytosis is about 10-50-fold less frequent.

Familial pseudohyperkalemia

For the sake of clarity, we will start with familial pseudohyperkalemia (FP) that amounts to a mere genetic trait. It designates a pronounced increase in kalemia *in vitro* when the blood sample is left at room temperature for a couple of hours. FP was first identified in a large Scottish family (Stewart *et al*, 1979) (FP Edinburgh). The leak *vs.* temperature curve is depicted as shallow. The gene of FP Edinburgh was mapped to 16q23-qter (Iolascon *et al*, 1999). In a large Flemish kindred with typical FP (FP Lille), a phenocopy of FP Edinburgh, the trait was found to map to 2q35-36 (Carella *et al*, 2004). It is strange that such a rare trait may further split into two genetic sub-species. This illustrates the fact that the more you analyse a case, the more you tend to define subtypes.

Other cases of FP have been recognized and defined according to the shape of the leak *vs.* the temperature curve: (i) FP Chiswick or Falkirk in which the curve is shouldered, and (ii) FP Cardiff, in which the temperature dependence of the leak shows a 'U-shaped' profile with a minimum at 23°C. The genes mutated in FP Chiswick, Falkirk or Cardiff have not been mapped. We will see that many instances of FP are consistently related to dehydrated hereditary stomatocytosis. FP may also be related to other genetic conditions of the leak (FP Cardiff is related to cryohydrocytosis), which makes its status perplexing altogether.

Dehydrated hereditary stomatocytosis

Dehydrated hereditary stomatocytosis (DHSt) is the most frequent form of hereditary stomatocytosis.

Dehydrated hereditary stomatocytosis alone

Its first description, assessing the red cell dehydrated state, is owned to Glader *et al* (1974). DHSt usually has a mild presentation. Anemia is well or fully compensated. Ektacytometry shows an increase of the osmotic resistance and cell dehydration (Figure 2). The leak *vs.* temperature curve is referred to as shallow. It is not uncommon that DHSt be discovered behind one of its complication, such as iron overload. The gene was mapped to 16q23-qter in a large Irish kindred (Carella *et al*, 1998). Further studies narrowed down the region of interest (16q24-qter) in a large French kindred, and showed that this locus failed to account for all DHSt cases (Beaurain *et al*, 2007, and Beaurain *et al*, unpublished data).

The pleiotropic syndrome: DHSt-pseudohyperkalemia-perinatal effusions

Entezami *et al* first observed the association of DHSt and perinatal effusions (1996). Later on (Grootenboer *et al* 1998; Grootenboer *et al* 2000), it was shown that a number of DHSt cases are associated with perinatal effusions, and/or pseudohyperkalemia.

The perinatal effusions vary from a mere ascites, which only sonography can detect, to life-threatening hydrops fetalis. They are not caused by anemia, which is not pronounced enough to account for edema, but coexist with it, stemming from the same cause yet to be determined. They may be chylous. The most severe cases demand drainage of the effusions. Remarkably, the effusions, which may disappear prior to birth, recede spontaneously a few weeks or months following birth for never to reappear anyhow. Pseudohyperkalemia is the third element of the triad.

We have mentioned that some DHSt cases map to 16q, as FP Edinburgh. We assume that pseudohyperkalemia might be a truncated form of the pleiotropic syndrome, and that the same gene is involved. One may hypothesize, likewise that the non-16q cases of DHSt would map to 2q, as FP Lille, mirroring the situation in 16q. No one has been having so far the possibility to test this hypothesis, so small in size are most of the known DHSt kindreds.

Hereditary cryohydrocytosis with stomatin

Hereditary cryohydrocytosis with stomatin (CHC) resembles DHSt in some points. It has also been coined as cold sensitive stomatocytosis. It may be associated with pseudohyperkalemia. However no fluid effusions have ever been reported. More to the point, the leak *vs.* temperature curve is quite steep down to 20°C and then soars up again, hence the prefix of 'cryo'. The protein stomatin (see below) is present. At least six different families have been identified in the UK. Dramatically, two mutations, named Hemel and Hurstpierpoint, were found in the *SLC4A1* gene, which encodes the anion exchanger (Bruce *et al*, 2005)

At this point, it ought to be mentioned that some cases first diagnosed as hereditary spherocytosis (HS) have been found atypical as far as the leak *vs.* temperature curve is concerned: 'shallow slope' (mutation Blackburn) (Figure 3), 'low temperature leak' (mutation Horam), with a resumption of the leak at low temperatures. Indeed, the cases were later dismissed as HS by the presence of mutations in the anion exchanger again. (Note that these mutations are 'qualitative', and not 'quantitative', that is, are not associated with a reduction in band 3, as is so commonly found in typical HS.) It thus appears that there is a continuum between the genetic disorders of the leak and HS. This illustrates the fact that advanced phenotype investigation using sophisticated tests and, the more so, genetic elucidation of the responsible genes may question the traditional, morphology-based classification of diseases.

Overhydrated hereditary stomatocytosis

Overhydrated hereditary stomatocytosis (OHSt) is an exceptional form of stomatocytosis, although it was the first hereditary stomatocytosis to be described (Lock *et al*, 1961). Often enough, OHSt stems from *de novo* mutations. Ektacytometry shows a reduction in osmotic fragility and a pronounced overhydration. The leak is 'torrential' and the leak *vs.* temperature curve is steep enough, though monotonic. One spectacular fact has misled many scientists, that is, the reduction, or even the absence of the protein stomatin. This protein, however, was dismissed as causal because its gene, the *EPB72* gene, carries no particular changes in OHSt (Fricke *et al*, 2003) and because mice with *EPB72* gene inactivation show no stomatocytosis (Zhu *et al*, 1999). In OHSt, stomatin seems more and more to be the innocent victim of a wide trafficking disarray, yet to be defined. It has been recently shown that stomatin shifts the function of Glut1, as a glucose transporter, to that of dehydroascorbic acid in humans, which are unable to synthesize vitamin C (Montel-Hagen *et al*, 2008). It turned out that this shift was reverted in OHSt.

Cryohydrocytosis, stomatin-deficient, with mental retardation, seizures, cataracts, and massive hepatosplenomegaly

This new syndrome, whose hematological side resembles OHSt, has been described in only two patients, one in San Francisco, USA, the other one in Montpellier, France. As in CHC, the leak *vs.* temperature curve is biphasic, first descending and then soaring up again below

20-25°C. Besides the hematological symptoms, there were mental retardation, seizures, cataracts and massive hepatosplenomegaly (Fricke *et al*, 2004).

Future Directions

Future developments are dramatically dependent on the elucidation of the different genes involved. Their search is the greatest urgency of all. Sequencing genes more or less systematically in a region defined by linkage analysis is time consuming and may be exposed to errors due to genetic heterogeneity. Generally speaking, one needs some supplementary hint, for example a cytogenetic event or protein alteration, however slim and subtle. Once the mutations are elucidated, numerous vistas of research throw open, tending to elucidate the structure-function relationship within proteins through the disturbances brought about by the mutations.

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Table I. The hereditary disorders of the cation passive leak, including mostly the hereditary stomatocytosis: a bird's-eye view.

| Types	Subtypes	OMIM
Familial pseudohyperkalemia	Edinburgh,	%177720
	Lille	%609153
Dehydrated hereditary stomatocytosis	Isolated	%194380
	Within a pleiotropic syndrome	#603528
Hereditary cryohydrocytosis		[a])
Overhydrated hereditary stomatocytosis	Isolated	%185000[b]
	Associated with neurological manifestations	608885

[a] Curiously enough, hereditary cryohydrocytosis has no entry on its own in the OMIM database.

[b] This numbering ('%') is erroneous since it is based on the location of the *EPB72* gene (9q34.1) that encodes stomatin.
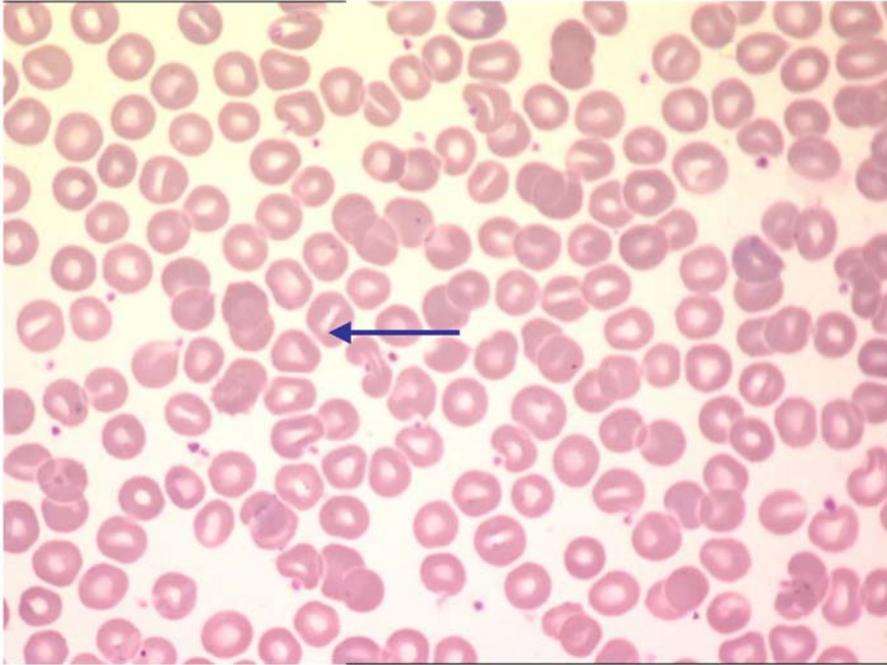
Figure 1. Blood smears.
One can see numerous and well formed stomatocytes, with a groove instead of the circular depression in the centre of the red cells. These are overhydraded stomatocytes (unpublished). The arrow points to a typical stomatocyte.
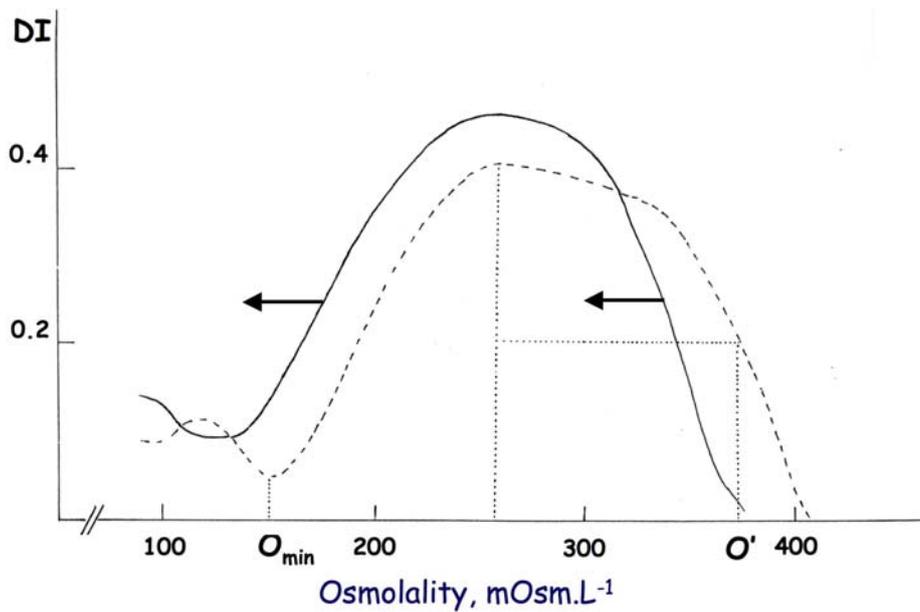
Figure 2. Osmoctic gradient osmotic curve.
Osmotic gradient ektacytometry is an unambiguous way to determine the type of stomatocytosis. In a normal person (dotted line) the maximum deformability index [DI_{max}: normal: 0.41-0.53 (arbitrary units)] is the maximum value of the deformability index (DI). The 'hypo-osmotic point' (O_{min}: normal values: 143-163 mOsm/L) is the osmolality at which the deformability index reaches a minimum in the hypotonic region; it is the same as the osmolality at which 50% of the erythrocytes hemolyse in a standard osmotic resistance test. The 'hyper-osmotic point' (O': normal: 325-375 mOsm/L) is the osmolality in the hypertonic region (right leg of the curve) at which the deformability index reaches half its maximum value). In the patient shown (full line), the left shift of the bell shape curve assesses the diagnosis of dehydrated hereditary stomatocytosis.
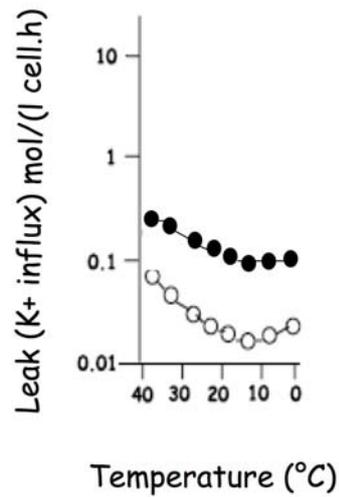
Figure 3. The variations of the leak as a function of the temperature.
Open circles: control. Full circles: patient (redrawn from Bruce *et al*, 2005). The initial diagnosis was "hereditary spherocytosis", however the shallow slope or the leak *vs.* temperature curve led to reconsider the diagnosis. The underlying mutation, mutation Blackburn, was found out in the *SLC4A1* gene (Bruce *et al*, 2005).