## Genetic heterogeneity of hereditary stomatocytosis syndromes showing pseudohyperkalemia

Sir,

The hereditary stomatocytoses (HSt) are a group of rare, dominantly inherited hemolytic anemias showing abnormal membrane permeability to the univalent cations $Na^+$ and $K^+$. They are clinically and pathophysiologically heterogenoeous. The group includes *overhydrated HSt* (OHSt), the original condition,[1] dehydrated HSt (DHSt; hereditary xerocytosis),[2] the commonest form, now seen to be heterogenous,[3,4] cryohydrocytosis,[5] in which red cells lyse markedly if stored at low temperatures, and familial pseudohyperkalemia,[6] a non-hemolytic condition in which the red cells show a net loss of K+ at room temperature, manifesting as high plasma [K] levels. The gene responsible for both DHSt and familial pseudohyperkalemia (FP) has been mapped to 16q23-qter.[7,8] In spite of the variability of the presentation, all kindreds that we have mapped so far show linkage to this locus.

Recently, Coles[9] described an unusual form of hereditary stomacytosis associated with very marked pseudohyperkalemia. Temperature studies of the passive leak fluxes of K showed the same *shallow slope* abnormality as seen in classical familial pseudohyperkalemia, but in addition, the fluxes at 37°C were significantly increased; and frank hemolysis, with abnormal intracellular [Na] and [K] levels, was present. Superficial examination might imply that this new condition is simply a more severe, frankly hemolytic, version of 'classical' FP.

We have sought to establish whether the clinical and biochemical heterogeneity of these conditions stems from different mutations of the same gene (allelic heterogeneity) or from mutations in different genes (genetic heterogeneity). Mapping of the DHSt and FP genes[7,9] now allows us to address this problem by means of a linkage analysis. Genomic DNA was obtained from each member of a pedigree described elsewhere.[9] Linkage analysis was performed using 8 microsatellite markers (D16S511, D16S3037, D16S520, D16S498, D16S3074, D16S3026, D16S3121) from the chromosome 16 region where we have previously mapped both DHSt and FP.[7,8] The analysis reveals the absence of segregation between the above mentioned markers and the disease locus, as shown in Table 1. These results indicate that these pseudohyperkalemic variants are genetically heterogenous with a locus on 16q23 and at least one additional locus, which remains to be mapped. Work is in progress to map this new locus and to understand the molecular basis[10] underlying this interesting form of stomatocytosis.

**Table 1. Pairwise LOD scores between FP family and chromosome 16 markers.**

| | Recombinant frequencies | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *0.0* | *0.01* | *0.05* | *0.10* | *0.20* | *0.30* | *0.40* | $Z_{max}$ | $q_{max}$ |
| D16S402 | -Ï | -9.01 | -4.90 | -3.21 | -1.64 | -0.82 | -0.32 | -2.75 | 0.69 |
| D16S511 | -Ï | -9.30 | -5.18 | -3.46 | -1.84 | -0.97 | -0.40 | -5.64 | 0.78 |
| D16S3037 | -Ï | -4.18 | -2.16 | -1.33 | -0.58 | -0.22 | -0.05 | -9.05 | 0.5 |
| D16S520 | -Ï | -10.7 | -5.88 | -3.87 | -1.96 | -0.96 | -0.36 | -1.79 | 0.63 |
| D16S498 | -Ï | -4.26 | -2.23 | -1.40 | -0.63 | -0.27 | -0.07 | -4.44 | 0.53 |
| D16S3074 | -Ï | -7.90 | -4.44 | -2.98 | -1.58 | -0.81 | -0.32 | -2.88 | 0.71 |
| D16S413 | -Ï | -2.51 | -1.18 | -0.67 | -0.25 | -0.08 | -0.01 | -2.48 | 0.50 |
| D16S3026 | -Ï | -10.7 | -5.88 | -3.87 | -1.96 | -0.96 | -0.36 | -1.79 | 0.63 |
| D16S3121 | -Ï | -8.27 | -4.79 | -3.30 | -1.84 | -1.00 | -0.43 | -1.02 | 1.00 |

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### Key words

*Dehydrated hereditary stomatocytosis (DHSt), hemolysis, linkage analysis, pseudohyperkalemia*

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## References

1. Lock SP, Septhon Smith R, Hardistry RM. Stomatocytosis: a hereditary red cell anomaly associated with haemolytic anaemia. Br J Haematol 1961; 7:303-14.
2. Glader BE, Fortier N, Albala MM, Nathan DG. Congenital hemolytic anemia associated with dehydrated erythrocytes and increased potassium loss. N Engl J Med 1974; 291:491-6.
3. Entezami M, Becker R, Menssen HD, Marcinkowski M, Versmold HT. Xerocytosis with concomitant intrauterine ascites: first description and therapeutic approach. Blood 1996; 87:5392-3.
4. Grootenboer S, Schischmanoff PO, Cynober T, et al. A genetic syndrome associating dehydrated hereditary stomatocytosis, pseudohyperkalaemia and peripheral oedema. Br J Haematol 1998; 103:383-6.
5. Miller G, Townes PL, Macwhinney JB. A new congenital hemolytic anaemia with deformed erythrocytes (?"stomatocytes") and remarkable susceptibility of erythrocytes to cold hemolysis in vitro. Pediatrics

1965; 35:906-15.
6. Stewart GW, Corrall RJ, Fyffe JA, Stockdill G, Strong JA. Familial pseudohyperkalaemia. A new syndrome. Lancet 1979; 28:175-7.
7. Carella M, Stewart G, Ajetunmobi JF, et al. A mapping of dehydrated hereditary stomatocytosis (hereditary xerocytosis) locus to chromosome 16 (16q23-qter) by genome wide search. Am J Human Genet 1998; 63:810-6.
8. Iolascon A, Stewart G, Ajentoumbi JF, et al. Hereditary xerocytosis and pseudohyperkaliemia map to the same locus. Blood 1999; 93:3120-3.
9. Coles S, Ho M, Chetty M, Nicolaou A, Stewart G. A variant of Hereditary stomatocytosis with marked pseudohyperkalaemia. Br J Haematol 1999 (in press).
10. Iolascon A, Miraglia del Giudice E, Perrotta S, Alloisio N, Morle L, Delaunay J. Hereditary spherocytosis: from clinical to molecular defects. Haematologica 1998; 83:240-57.

## *In vitro* drug-induced cytotoxicity predicts clinical response to high-dose chlorambucil in B-cell chronic lymphocytic leukemia

Sir,

Chlorambucil (CLB) can be considered the golden standard of front-line therapy for B-cell chronic lymphocytic leukemia (CLL).[1] In spite of its long use, the best schedule of CLB administration has not yet been established. We have demonstrated the clinical advantage of a high-dose continuous CLB schedule (HD-CLB) over standard-dose intermittent CLB[2] and the CHOP regimen.[3] In addition, according to the results of an interim analysis of the ongoing randomized EORTC study, the efficacy of HD-CLB is apparently not inferior to that of fludarabine,[4] thus confirming that we can now two efficient therapeutic options for front-line therapy of CLL.

We recenlty demonstrated that *in vitro* drug-sensitivity to fludarabine is associated with *in vivo* response.[5] This study was aimed at analyzing whether the ability of CLB to induce *in vitro* cytotoxicity in fresh isolated CLL cells might be related to the *in vivo* outcome of patients treated with the HD-CLB schedule.

Twenty-nine previously untreated CLL patients entered this study. Clinico-hematologic data, CLB-$LD_{50}$ value and the *in vivo* clinical response to high-dose CLB are reported in Table 1. Criteria for treatment, high-dose CLB schedules and definitions of response have been described elsewhere.[2-4] The drug-induced effect on cell viability was assessed by a non-clonogenic 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT) assay that has been repeatedly used to test *in vitro* chemosensitivity of CLL cells to CLB.[7,8] Cells were continuously exposed to the drug for 4 days. Median CLB-$LD_{50}$ value was 25, 16 and 58 µg/mL in patients obtaining complete (CR), partial (PR) and no response (NR), respectively (p=ns for Kruskall Wallis test).

Rai and Binet stage, TTM score and age (Table 2) did not affect the response rate (CR+PR). However, a significantly higher number of responses was observed in males and in those patients showing *in vitro* CLB-$LD_{50}$ values below 26 µg/mL. In spite of the relatively small number of cases, the relevance of this parameter was confirmed by multivariate analysis. The short median follow-up time hampers a statistically reliable analysis of survival. However, since response to therapy is a parameter well known to be associated with longer survival,[9] it is reasonable to expect that *in vitro* resistance to CLB coulde be an early indicator of an overall unfavorable outcome of patients treated with this alkylating agent.

In conclusion, in our experience the *in vitro* MTT assay is a useful tool for predicting *in vivo* treatment failure to HD-CLB in CLL. Thus, from the results obtained in cytotoxicity tests induced by different

**Table 1. Main clinico-hematological data, CLB-$LD_{50}$ value and clinical response to high-dose CLB.**

| | |
|---|---|
| Sex, male/female | 13/16 |
| Age, mean ± sem | 69.9 ± 2.5 |
| Rai stage | |
| 0 | 1 |
| I | 4 |
| II | 9 |
| III | 11 |
| IV | 4 |
| Binet stage | |
| A | 7 |
| B | 11 |
| C | 11 |
| TTM score, mean ± sem | 12.4 ± 0.72 |
| ≤ 9 | 5 |
| > 9 | 24 |
| CLB-$LD_{50}$ µg/mL (mean ± sem) | 47.1 ± 7.2 |
| Clinical response | |
| CR | 16 |
| PR | 6 |
| NR | 7 |

**Table 2. Univariate and logistic multivariate regression analysis of prognostic variables for clinical response to HD-CLB.**

| *Variable* | *CR+PR* | *NR* | *Uni-* variate p* | *Multi-° p* |
|---|---|---|---|---|
| Rai stage (0-II v III-IV) | 11 v 11 | 3 v 4 | 1.0 | --- |
| Binet stage (A-B v C) | 15 v 7 | 3 v 4 | 0.3 | --- |
| TTM score (≤ 11.4 v > 11.4) | 11 v 11 | 5 v 2 | 0.4 | --- |
| Age, years (≤ 74 v > 74) | 11 v 11 | 4 v 3 | 1.0 | --- |
| Sex (male v female) | 13 v 9 | 0 v 7 | 0.008 | ns |
| CLB-$LD_{50}$, µg/mL (≤ 26 v > 26) | 14 v 8 | 1 v 6 | 0.035 | 0.05 |

**Fisher exact test (2-tail); °Logistic regression analysis.*