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Hasan F. Cakmakli, *Ankara University*  
Rosa J. Torres, *La Paz University Hospital*  
Araceli Menendez, *La Paz University Hospital*  
Gul Yalcin-Cakmakli, *Hacettepe University*  
[Christopher Porter](#), *Emory University*  
Juan Garcia Puig, *La Paz University Hospital*  
[Hyder Jinnah](#), *Emory University*

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## Macrocytic Anemia In Lesch-Nyhan Disease and its Variants

Hasan F. Cakmakli<sup>1,\*</sup>, Rosa J. Torres<sup>2,3,\*</sup>, Araceli Menendez<sup>4</sup>, Gul Yalcin-Cakmakli<sup>5</sup>, Christopher C. Porter<sup>6,7</sup>, Juan Garcia Puig<sup>4</sup>, and H.A. Jinnah<sup>8</sup>

<sup>1</sup>Department of Pediatric Hematology and Oncology, Faculty of Medicine, Ankara University, Ankara, Turkey

<sup>2</sup>Foundation for Biomedical Research, La Paz University Hospital (FIBHULP), IdiPaz, Madrid, Spain

<sup>3</sup>Center for Biomedical Network Research on Rare Diseases (CIBERER), ISCIII, Spain

<sup>4</sup>Department of Internal Medicine, Metabolic-Vascular Unit, La Paz University Hospital, IdiPaz, Madrid, Spain

<sup>5</sup>Department of Neurology, Faculty of Medicine, Hacettepe University, Ankara, Turkey

<sup>6</sup>Department of Pediatrics, Emory University School of Medicine, Atlanta GA, USA

<sup>7</sup>Aflac Cancer and Blood Disorders Center, Children's Healthcare of Atlanta, Atlanta GA, USA

<sup>8</sup>Departments of Neurology, Human Genetics & Pediatrics, Emory University School of Medicine, Atlanta GA, USA

### Abstract

**Purpose**—Lesch-Nyhan disease (LND) is an inherited metabolic disorder characterized by overproduction of uric acid and neurobehavioral abnormalities. The purpose of this study is to describe macrocytic erythrocytes as another common aspect of the phenotype.

**Methods**—Results of 257 complete blood counts (CBC) from a total of 65 patients over a 23-year period were collected from two reference centers where many patients are seen regularly.

**Results**—Macrocytic erythrocytes occurred in 81–92% of subjects with LND or its neurological variants. After excluding cases with iron deficiency because it might pseudo-normalize erythrocyte volumes, macrocytosis occurred in 97% of subjects. Macrocytic erythrocytes were sometimes accompanied by mild anemia, and rarely by severe anemia.

**Conclusions**—These results establish macrocytic erythrocytes as very common aspect of the clinical phenotype of LND and its neurological variants. Macrocytosis is so characteristic that its absence should prompt suspicion for some secondary process, such as iron deficiency. Because macrocytosis is uncommon in normal children, it can also be used as a clue for early diagnosis in

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Correspondence: H. A. Jinnah, M.D., Ph.D., Professor, Departments of Neurology, Human Genetics & Pediatrics, Emory University, Suite 6300 Woodruff Memorial Building, 101 Woodruff Circle, Atlanta GA, 30322, Phone: 404-727-9107, Fax: 404-712-8576, [hjinnah@emory.edu](mailto:hjinnah@emory.edu).

\*These authors contributed equally to this work.

children with neurodevelopmental delay. Better recognition of this characteristic feature of the disorder will also help to prevent unnecessary diagnostic testing and unnecessary attempts to treat it with folate or B12 supplements.

### Keywords

Lesch-Nyhan disease; HPRT1; hypoxanthine-guanine phosphoribosyltransferase; macrocytic anemia; megaloblastic anemia

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## INTRODUCTION

Lesch Nyhan Disease (LND) is an inherited metabolic disorder caused by deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGprt). This enzyme plays a role in the purine salvage pathway, in which hypoxanthine and guanine are recycled into purine nucleotides. Individuals with LND have a well-described clinical phenotype that includes overproduction of uric acid, behavioral abnormalities, and neurological impairment.<sup>1-3</sup> In addition to the classic phenotype, there are attenuated variants with overproduction of uric acid, but the neurobehavioral features are milder or even absent.<sup>3-5</sup> Some reports of LND and its variants occasionally also mentioned another feature of the phenotype, macrocytic anemia.<sup>6,7</sup> However, this problem is not widely recognized because it has received very little attention in the literature. There is no information on its frequency, severity, or clinical significance.

In the current study, we summarize longitudinal data from two referral centers where many patients with LND or its variants are evaluated. We also review the world's literature on macrocytosis and/or anemia in LND and its variants. Together the results show that macrocytosis, with or without anemia, is a nearly universal feature of LND and its neurological variants. In fact, macrocytosis appears to be so common in this population that its absence should raise concern for some secondary process such as iron deficiency. Because macrocytosis is uncommon in normal children, it can also be used as a clue for the early detection of LND in children with neurodevelopmental delay of unknown cause. The findings are valuable for raising awareness of this common finding in this disorder, for preventing unnecessary diagnostic testing, and for reducing unnecessary vitamin supplementation.

## METHODS

### Review of patient records

**Subjects**—After obtaining approval from the local ethics boards, records of subjects diagnosed with LND or its variants were reviewed from two referral centers, La Paz University Hospital in Madrid (Spain) and Emory University in Atlanta (USA). All subjects gave written informed consent or verbal assent. All subjects were diagnosed according to currently accepted criteria.<sup>2,4</sup> Because HGprt deficiency is inherited as an X-linked recessive disorder, all cases were males. According to prior convention regarding the classification of the spectrum of HGprt deficiency, subjects were divided into three groups. They included classic LND, HGprt-related neurological dysfunction (HND), and HGprt-related

hyperuricemia (HRH). For LND the clinical criteria included overproduction of uric acid, self-injurious behavior, neuromotor impairments, and often some degree of intellectual disability. The diagnosis was confirmed by detection of a mutation in the *HPRT1* gene and/or reduced HGPrt enzyme activity in fibroblasts or blood cells. The clinical criteria for HND were the same as those for LND, except HND subjects lacked self-injurious behavior. All HND cases had varying degrees of neuromotor impairment, with scores  $\geq 5$  on the Burke-Fahn-Marsden dystonia rating scale. The criteria for HRH were the same as those for HND, except that clinically relevant neuromotor abnormalities were clinically insignificant, with scores  $<5$  on the Burke-Fahn-Marsden dystonia rating scale. All subjects who had at least one CBC were included in the summary statistics, except for 2 who had transfusions that might have influenced CBC results. These two cases are presented separately.

**Hematological studies**—For each subject, we recorded phenotypic subgroup and age at time of each complete blood count (CBC). When multiple CBCs were collected over time, we evaluated each CBC, but used the most recently collected values for the summary tables. CBC parameters recorded included mean corpuscular volume (MCV), hemoglobin (Hb), and red blood cell (RBC) count. Many CBC parameters vary significantly according to age. Macrocytosis is typically defined by red blood cells (RBC) with an abnormally large mean corpuscular volume (MCV) of  $>100\text{fL}$ .<sup>8</sup> However, the value is lower in children.<sup>9</sup> For MCV in children, previously published normative curves were used to define normal age-adjusted values, and any values above the 97<sup>th</sup> percentile were considered abnormal.<sup>9</sup> For ages above 20, the upper limit of normal was defined according to tradition as 100 fL. A subject was considered to have macrocytosis if there was at least one abnormal CBC.

Macrocytosis can occur without anemia. Anemia typically is defined by either low hemoglobin (Hb) or low hematocrit (Hct), which again can vary by age.<sup>10</sup> For Hb and RBC counts, we used the normal hematological values commonly used in daily practice for children.<sup>11</sup> Iron deficiency was defined as low ferritin ( $<12\ \mu\text{g/L}$  for children  $<5$  years old and  $<15\ \mu\text{g/L}$  for  $>5$  years old) or low transferrin saturation ( $<16\%$ , for all ages).<sup>12</sup>

Where available, we also recorded results for reticulocyte count, vitamin B12, folic acid, erythrocyte folic acid, haptoglobin, thyroid function tests, direct and indirect bilirubin, erythropoietin levels, serum iron and iron binding capacity, and lactate dehydrogenase (LDH). We also recorded results of bone marrow biopsy, if available.

**Data analysis**—Descriptive statistics are provided in the form of average values  $\pm$  standard deviation. All statistical analyses were performed using SPSS 20. Independent samples t-tests were used for comparing groups. Correlations between MCV and RBC counts were calculated using the Pearson test. Graphs demonstrating age-specific red blood cell indices were prepared using Sigma-Plot version 10.

## Review of published literature

**Selection of reports**—The PubMed database was used to perform a comprehensive search for all prior English-language reports addressing macrocytosis and/or anemia in LND and its variants. The following search terms were used: Lesch-Nyhan or Kelley-Seegmiller, in various combinations with macrocytosis, megaloblastosis, an(a)emia, macrocytic anemia,

mean corpuscular volume or MCV, and complete blood count or CBC. Other reports were found through the bibliographies of these papers. We also screened individual case reports and case series for reports of hematological studies, because these search terms were often omitted from the key word list or abstract.

**Hematologic studies**—Reports including information about hemoglobin and MCV were included, even when results only included subjective interpretations, such as “microcytic” or “macrocytic”. A total of 15 articles and 2 post-graduate theses encompassing 23 different subjects with LND or its variants were found with at least some comment on hematological parameters (Supplementary Table S1). Publications referring to the same cases were combined to avoid redundant data from the same case.

## RESULTS

### Review of patient records

**Subjects and CBCs**—We retrieved records for 33 subjects from Spain and 32 subjects from USA, who had at least one CBC (Table 1). The Spanish subjects included 19 LND, 9 HND and 5 HRH. The American subjects included 20 LND, 10 HND and 2 HRH. Altogether, there were 204 CBCs from Spain, and 53 CBCs from America collected over 23 years. The greater number of CBCs from Spain reflects a regional practice difference. Hematology studies are routinely collected at every clinic visit in Spain, because the Mediterranean diet predisposes to iron deficiency anemia, especially in developmentally disabled children.

**Macrocytosis**—Considering all subjects from Spain and USA combined, macrocytosis was evident in at least one CBC for 92.1% (35/38) of LND patients and 83.3% (15/18) of HND patients. The majority of these patients had persistent macrocytosis on multiple CBCs taken over several years, although an occasional CBC fell within the normal range. Overall, macrocytosis was seen for 84.6% (159/188) of the total CBCs for LND and 81.1% (43/53) for HND (Figure 1A,B). Macrocytosis was not seen in any of 16 CBCs for any of the 7 HRH patients. Only one LND patient and one HND patient with thalassemia trait had microcytosis.

Although CBC results for infants were not available, macrocytosis could be detected as early as 6 months of age. There were 16 patients with LND below 4 years of age, and 14 had macrocytosis on at least one CBC. There were 2 HND patients in this age group, and both had macrocytic CBCs. Thus, macrocytosis typically was a very early finding in LND and HRND. In some subjects, macrocytosis appeared to worsen with age, although this observation was difficult to confirm because of the changes in normative values over age combined with the paucity of multiple samples from the same subjects over time.

**Anemia**—Considering all subjects from Spain and USA combined, 42.1% (16/38) of LND cases had Hb values below normal at least once during the period evaluated (Figure 2A,B). Among the total 189 CBCs during this period, only 28 revealed anemia. For the HND group, 38.9% (7/18) cases had anemia at least once. Among the total of 53 CBCs in this group, Hb was low in 14. Only 1 of 7 HRH cases had anemia.

The total RBC count was below normal at least once for 57.9% (22/38) of LND cases, 83.3% (15/18) of HND cases, and 14.3% (1/7) of HRH cases (Figure 2C,D). The total RBC count was below normal for 41.8% (74/177) of CBCs in the LND group, 79.6% (39/49) of CBCs in the HND group, and 6.2% (1/16) of the HRH group. There was a strong negative correlation between RBC counts and MCV values ( $r=0.795$ ,  $p<0.05$ , Figure 3). Therefore, Hct values were less prominently affected (not shown).

**Iron status**—Among subjects with LND and HND, nutritional status is sometimes impaired because of difficulties with swallowing, and approximately 30% receive enteral nutrition.<sup>2</sup> We therefore paid close attention to laboratory findings related to nutritional deficiencies that could influence CBC parameters. The most common among these is iron deficiency.

For the Spanish cases, 191 of 204 CBCs were accompanied by simultaneous iron studies, because iron deficiency is common among populations that follow a Mediterranean diet. For the American cases, iron studies typically were conducted only after the discovery of anemia, so CBCs simultaneous with iron studies were not available. Therefore, the influence of iron status was evaluated only for the Spanish cases. Longitudinal analysis showed that 84.2% (16/19) in LND group, and 11.1% (1/9) in HND group experienced iron deficiency at least once. None of the HRH subjects had evidence for iron deficiency. Taking all of the CBCs with iron parameters into consideration revealed that CBCs were collected during an iron-deficient period for 57 of 145 CBCs in the LND group and 1 of 32 CBCs in the HND group.

Because iron deficiency causes microcytosis and might falsely normalize macrocytosis, we further examined the interaction between macrocytosis and iron deficiency. Of 157 CBCs from the Spanish group, only 23 had normocytic values. After excluding CBCs taken during iron-deficient periods ( $n=60$ ) and CBCs taken without simultaneous iron parameters ( $n=12$ ), 96.6% (85/88) of the CBCs were macrocytic (Figure 1C,D). Only 3 CBCs collected during iron-sufficient periods were normocytic.

**Other hematologic parameters**—Table 2 summarizes results of additional hematological studies. For the Spanish LND subjects, reticulocyte counts and vitamin B12 levels were normal in all cases where they were tested. Folate acid levels were low for 6 of 18 patients at least once. In the HND group 3 of 9 subjects had mild vitamin B12 deficiency and 2 had low folic acid levels. In the HRH group, one subject had normal vitamin B12 and folic acid. Folic acid supplements did not correct macrocytosis for any case where it was given.

In the American group, all subjects for whom testing was conducted had normal vitamin B12, folic acid, and reticulocyte counts. Homocysteine and methylmalonic acid levels were normal for all 3 subjects for whom they were collected. Erythrocyte folic acid levels were normal for all 6 subjects for whom this was evaluated.

**Severe anemia**—There were two cases with severe anemia. One Spanish LND case was followed for 18 years and all CBCs showed extreme macrocytosis, reaching 137 fL at 23

years of age. Hb levels were low from 10 years of age, with the lowest being 7.2 g/dL and resulting blood transfusion. He also had borderline leucopenia ( $3500/\text{mm}^3$ ). An extensive evaluation revealed normal vitamin B12, serum and erythrocyte folate levels, haptoglobin and iron parameters. The peripheral smear showed anisocytosis, poikilocytosis, tear-drop cells, schistocytes, elliptocytes, dimorphic platelets, and micro and macrocytosis at the same time. With epoetin alpha treatment, anemia improved and Hb levels increased, but not higher than 10 g/dL. After withdrawal of this treatment, he again required blood transfusion. Reintroducing epoetin alpha again increased Hb levels.

A second case with severe anemia from America had HND. He also had macrocytosis from an early age, with Hb values as low as 6.4 gr/dL, low reticulocyte counts, and mild thrombocytopenia (platelet count  $80.000\text{--}100.000/\text{mm}^3$ ). Vitamin B12, folic acid, and iron parameters were normal. Erythropoietin level was elevated (2096 mU/mL; normal range, 7.3–27.7). He had 6–7 blood transfusions starting from 50 years of age. The bone marrow was normocellular with megaloblastoid hyperplasia in erythroid lineages without any sign of myelodysplastic syndrome. His workup revealed no sign of blood loss via the gastrointestinal system, and ultrasound did not reveal any sign of hypersplenism. However, he had chronic renal failure, which may cause anemia of chronic disease. A younger brother who also had HND with the same *HPRT1* mutation, had normal renal function with no signs of anemia or thrombocytopenia.

Both of these cases with severe anemia had multiple blood transfusions. Despite extensive workups, a cause for anemia could not be found. There was never any suggestion of immunodeficiency, in terms of frequent or long-lasting infections. These two patients resemble some cases of *HGPrt* deficiency with severe macrocytic anemia in the prior literature, although details of the workup of these other cases were not available.<sup>13,14</sup>

## Review of published literature

**Case reports that included CBC**—A comprehensive literature review revealed 23 reported cases of LND or its variants with at least one CBC (Supplementary Table S1). These cases ranged from 3 weeks to 43 years of age. There were 17 cases with LND, and 16 had macrocytosis in at least one CBC (94.1%). Only 6 of these cases had anemia, which was usually mild. There were 5 cases with HND, and all were reported to have macrocytosis without anemia (100%). Vitamin B12 was tested for 8 cases, and all were normal. Folic acid was tested for 9 cases, and 2 were considered low. Bone marrow examinations were performed for 4 LND and 3 HND cases. All were reported to show erythroid megaloblastosis, although details were not provided.

There was only 1 patient with HRH, who had normocytic anemia.<sup>15</sup> However, this patient also had chronic renal failure, which is associated with microcytic or normocytic anemia and may therefore falsely normalize macrocytosis.

Additional descriptions of hematologic studies were found in two graduate thesis reports from Nijmegen University.<sup>16,17</sup> These two reports included 61 cases of *HGPrt* deficiency, with CBC results for 32. Among these cases, 11 had severe anemia, sometimes leading to blood transfusion. Of these cases with severe anemia, 7 were reported to be megaloblastic, 2

were normocytic, 1 was microcytic, and 1 undetermined. One of the 7 cases with megaloblastic anemia was reported to respond to folic acid and another to adenine.

The long-term natural history of LND was described for another series of 10 cases in Japan.<sup>18</sup> This article included a schematic figure showing macrocytic anemia beginning as early as the first year of life, and continuing through adulthood. However, no CBC data were included. Overall, the prior literature confirms that macrocytosis, sometimes with anemia, is common in HGprt deficiency.

## Discussion

These studies show that macrocytic erythrocytes, with or without anemia, are very commonly observed in routine CBCs from subjects with LND and its neurological variants. Nutritional deficiencies were also common in this patient population, particularly iron. When iron deficiency was excluded, macrocytosis was nearly universal. Because data were obtained from two expert centers and a comprehensive review of the literature, the findings are not likely to be restricted to specific populations. Furthermore, data were collected over a wide age range, making findings relevant for adults and children of all ages. Macrocytosis was absent in subjects with overproduction of uric acid but no neurobehavioral abnormalities (HRH). Overt anemia was less common across the whole spectrum of HGprt deficiency. When anemia occurred, it was typically mild. Although severe anemia may occur, it appears to be uncommon.

**Clinical Relevance of Macrocytosis**—Macrocytosis is typically defined by an abnormally large MCV of >100fL. However, this value applies only to adults over 20 years of age. There is a steady increase in the MCV from 6 months of age to 20 years (Figure 1), but the normal limits are often reported by hematology laboratories in discontinuous age blocks. The lower limit is 84 fL up to 1 year of age, 85 fL up to 3 years of age, 87 fL up to 7 years of age, 90 fL up to 11 years of age, 94 fL up to 16 years of age, and 100 fL after the age 20.<sup>9</sup> Because these discontinuous age blocks do not follow the more continual graded changes with age, macrocytosis is easy to overlook in routine CBC reports.

Macrocytosis is most commonly caused by deficiency of vitamin B12 or folic acid, hypothyroidism, or liver disease. It can also be caused by certain drugs such as alcohol, valproic acid, phenytoin, isoniazide, trimethoprim/sulfamethazole, atovaquone/proguanil, antiretroviral drugs, methotrexate, and some chemotherapy drugs (purine antimetabolites and hydroxyurea).<sup>19</sup> Less common etiologies include reticulocytosis, leukemia, myelodysplastic or myeloproliferative syndrome, and bone marrow failure syndromes. Extreme macrocytosis (MCV>130 fL) has been reported rarely in association with vitamin B12 deficiency, folate deficiency, antiretroviral therapy for HIV, hemolytic anemia and hydroxyurea treatment.<sup>20</sup> The detection of macrocytosis or anemia in a CBC in a patient with LND often leads to tests to rule out more commonly recognized causes. The results from the current study argue that these tests may not be necessary in most cases. In fact, attempts to treat macrocytosis with vitamin B12 or folate in the absence of overt deficiency do not appear to reverse it. The knowledge that macrocytosis is a common feature of these

disorders may be useful to reduce unnecessary testing or ineffective vitamin supplements in this population.

Macrocytosis is evident from the earliest ages evaluated in LND and its neurological variants. Conditions with macrocytosis in infancy combined with neurodevelopmental delay include B12 deficiency, Down syndrome,<sup>21</sup> and mutations *KLF1*<sup>322</sup> or *ZFYVE20*.<sup>23</sup> The current study suggests that HGprt deficiency should be added to the list of possible causes when unexpected macrocytosis is found in a routine CBC from a child with neurodevelopmental disability. In fact, macrocytosis may provide one of the earliest clues for the diagnosis of LND in a patient with unexplained neurodevelopmental delay, because CBCs are routinely performed at all ages. By itself, macrocytosis lacks specificity and sensitivity to serve as a definitive diagnostic tool, but alerts the clinician to the need for more specific testing for HGprt. In this regard, macrocytosis is similar to uric acid, which is included in many standard clinical chemistry reports, and can provide an early clue for HGprt deficiency. In fact, the coincidental findings of macrocytic erythrocytes along elevated uric acid produces a very high level of suspicion, although this suspicion must still be confirmed by tests more specific to HGprt, such as enzyme assay or *HPRT1* gene testing.

**Relevance of nutritional deficiencies**—Macrocytosis appears to be so common in LND and HND that its absence should prompt consideration of factors that may falsely normalize it, most notably iron-deficiency anemia and less likely thalassemia, chronic diseases, lead intoxication and others. Conversely a baseline of macrocytosis may mask the diagnosis of iron deficiency. Thirteen out of 15 subjects with iron deficiency in the current series would have been missed using the standard screening tests of Hb or MCV typically recommended for developing children. For diagnosing iron deficiency among patients with LND or HND, an expanded laboratory panel should be used, including a CBC, reticulocyte count, serum iron, TIBC, and serum ferritin.

Iron deficiency typically causes microcytic anemia and may falsely normalize MCV values for patients with LND and HND. Iron deficiency is very common in chronically debilitated patients, most frequently due to nutritional deficiencies caused by difficulties in solid food consumption. In a study of patients with cerebral palsy, hypochromic anemia was found in 33% and iron deficiency in 38%.<sup>24</sup> In patients on liquid diets, anemia was found in 87%. In our study, 84% (16/19) of LND patients showed iron deficiency, and this high rate is most probably related to feeding problems with subsequent nutritional deficiencies. The lower prevalence of iron deficiency in HND group where feeding is less affected (11%; 1/9) supports this hypothesis. In view of these observations, patients with LND and HND should have routine iron studies (serum iron, serum iron binding capacity, transferrin saturation, ferritin), especially if there are known feeding difficulties. The CBC alone is insufficient for detecting iron deficiency, because baseline macrocytosis will obscure detection of more typical microcytic anemia.

**Biological mechanisms for macrocytosis**—Our main goal was to call attention to clinical factors relating to macrocytosis and anemia in LND, so the underlying biological mechanisms were not evaluated. However, it is interesting to speculate on these mechanisms. Only a few prior studies have addressed this issue. One study evaluated hemoglobin turnover

by assessing the incorporation of [<sup>15</sup>N]delta-aminolavulinic acid or [<sup>15</sup>N]glycine into bilirubin in a single subject with LND.<sup>25</sup> The authors concluded that macrocytic anemia was caused by ineffective erythropoiesis and increased turnover of erythrocytes, although the molecular mechanisms were not elucidated.

Another study evaluated the growth of bone marrow cells from 2 patients with LND and 1 with HND.<sup>26</sup> Bone marrow samples from all 3 cases generated fewer colonies that grew smaller compared with normal individuals. The mechanisms for poor growth were not determined. The authors noted that purines in bone marrow cells are heavily dependent on recycling via HGPrt because of an inherently low capacity for de novo purine synthesis,<sup>27</sup> and they speculated that HGPrt-deficient marrow cells grew poorly because they were starved for purine nucleotides.<sup>7,26</sup> Others have suggested that the microtubular cytoskeleton might be disrupted due to GTP deficiency, leading to abnormally shaped erythrocytes.<sup>28</sup>

Several studies have reported purine nucleotide measurements in LND erythrocytes, but a comprehensive review disclosed no consistent changes.<sup>29</sup> Some studies reported decreases in guanine nucleotides with normal adenine nucleotides, while other studies reported changes in adenine nucleotides with no changes in guanine nucleotides. Thus purine nucleotide deficiency has not been empirically established as a cause for macrocytosis. Even if we accept the idea that purine nucleotide deficiency may occur, the mechanisms by which such deficiency could cause macrocytosis remain to be determined.

Macrocytosis appears to be unique to erythroid lineages, because cells in other tissues such as brain neurons or skin fibroblasts are not abnormally large. Macrocytic anemia does not appear to reflect a global failure of the bone marrow, because leukocytes or platelet numbers are usually normal, and recurrent infections or excessive bleeding are not common. However, marrow specimens from one LND case showed macromyelocytes implying potential involvement of the myeloid series as well.<sup>26</sup>

Cell size is normally very precisely regulated, reflecting a tight balance between cell division and increases in cell mass.<sup>30</sup> In erythroblasts, a size-sensing mechanism may trigger cell division.<sup>31</sup> It seems feasible that purine nucleotide deficiency slows DNA replication more than it slows the growth of other cellular constituents. If this is the case, the lag in cell division with respect to increases in cell mass may result in abnormally large cells. In this regard, vitamin B12 and folate deficiency are thought to cause macrocytic anemia by reducing purine nucleotides for DNA synthesis.<sup>32</sup> Further, chemotherapy agents (purine antimetabolites and hydroxyurea) that cause macrocytic anemia also reduce intracellular purine nucleotides. These observations suggest that HGPrt deficiency may lead to purine nucleotide deficiency in bone marrow cells. If this is the case, then it could be possible to treat macrocytic anemia through the administration of purine precursors that bypass HGPrt.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

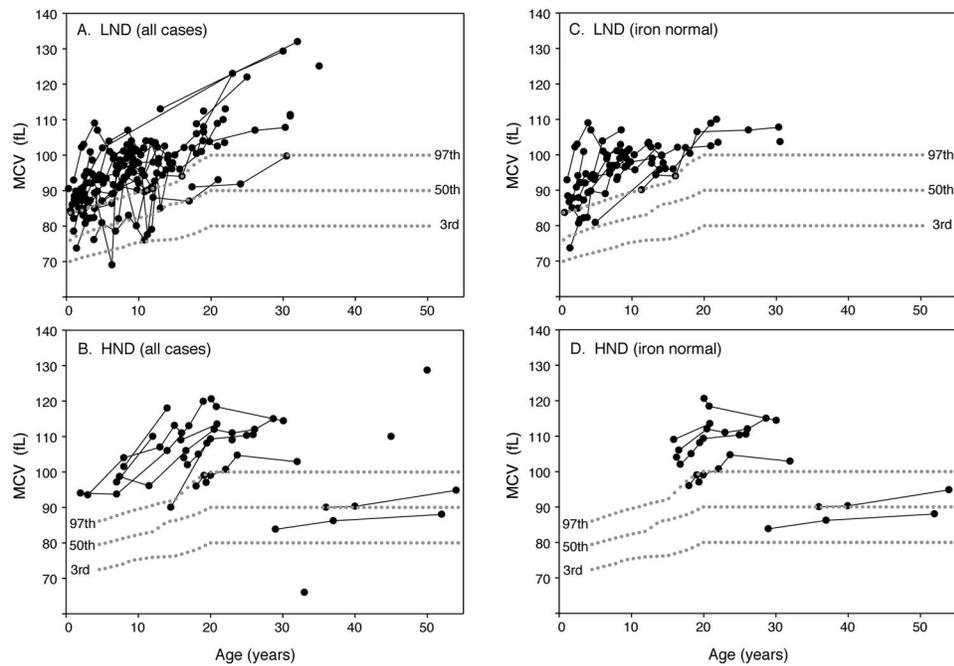
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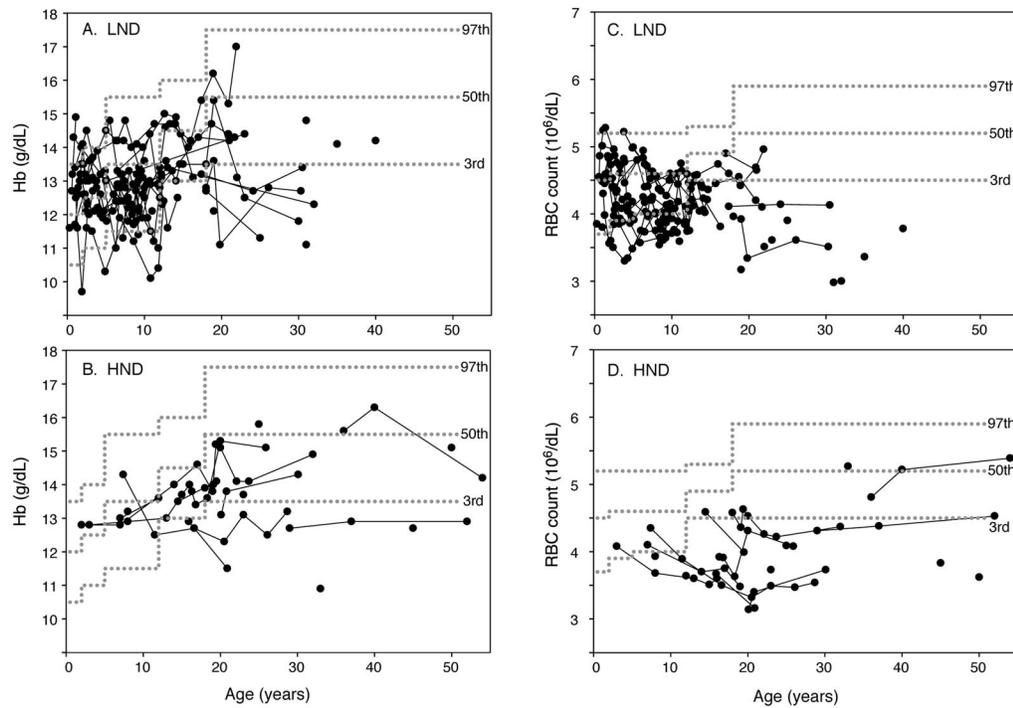
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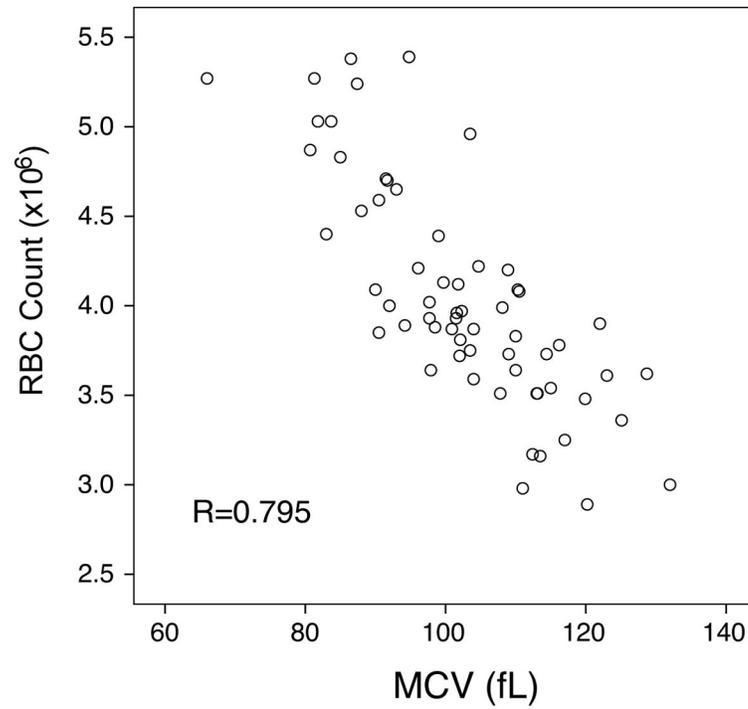


**Figure 1.**

Erythrocyte volumes as a function of age. Mean corpuscular volume (MCV) values for LND are shown in the top panels (A,C) while those for HND are in the bottom panels (B,D). All results are shown in the left panels (A,B) while results from patients with simultaneous normal iron studies to exclude iron deficiency are shown in the right panels (C,D). Each circle represents an individual CBC result. Circles that are connected with a line indicate multiple values for the same patient. Age-adjusted normal MCV values are shown as dotted lines at the 3<sup>rd</sup>, 50<sup>th</sup>, and 97<sup>th</sup> percentiles.



**Figure 2.** Total hemoglobin and erythrocyte numbers as a function of age. Results for subjects with LND are shown in the top panels (A,C) while those for HND are in the bottom panels (B,D). Hemoglobin (Hb) values are shown in the left panels (A,B), while erythrocyte numbers (RBC count) are shown in the right panels (C,D). Each circle represents an individual CBC result. Circles that are connected with a line indicate multiple values for the same patient. Age-adjusted normal values for Hb and RBC counts are shown as dotted lines at the 3<sup>rd</sup>, 50<sup>th</sup>, and 97<sup>th</sup> percentiles.



**Figure 3.** Correlation of erythrocyte counts vs erythrocyte volumes. Erythrocyte numbers (RBC count) were plotted against corresponding mean corpuscular volumes (MCV) for all cases.

## Hematology Results from America and Spain

Table 1

Country of origin	LND		HND		HRH	
	USA	Spain	USA	Spain	USA	Spain
Number of cases	20	19	9	9	2	5
Mean age (range)	18.8 (0.3–40)	14.0 (1.1–30.5)	29.9 (8–67)	31.0 (19.5–54)	36 (27–45)	16.0 (11.5–20)
Total number of CBCs	31	156	19	34	2	14
CBCs with iron parameters	0	145	0	32	0	14

The last visit with CBC was used to calculate the mean age of the subjects. Abbreviations: CBC, complete blood count; LND, Lesch-Nyhan disease; HND, complete blood count; HRH, HGprt-related neurological dysfunction; HRH, HGprt-related hyperuricemia.

**Table 2**

## Other hematology studies

	<b>LND</b>	<b>HND</b>	<b>HRH</b>
Vitamin B12 (211–911 pg/mL)	762±384 n=24	504±487 n=11	535 n=1
Folic acid (3.1–17.5 ng/mL)	10.8±6.5 n=21	10.9±5.9 n=12	6.4 n=1
LDH (200–400 U/L)	272.2±75.9 n=14	241±32.9 n=6	332.7±99.1 n=3
TSH (0.55–4.68 µIU/mL)	2.7±1.2 n=12	2.4±0.9 n=8	2.4 n=1
Free T4 (0.89–1.72 ng/dL)	1.2±0.2 n=12	1.1±0.1 n=8	1.2 n=1
Haptoglobin (30–200 µg/dL)	125±73 n=11	124±53 n=6	NRA
Iron (20–150 µg/dL)	65.3±29.5 n=19	108.6±38.5 n=9	85.2±11.8 n=5
Ferritin (<5yrs: <12 µg/L) (>5yrs: <15 µg/L)	64.1±68.2 n=19	187.8±122.3 n=9	60.8±22.8 n=5
TFS (>16%)	18.7±8.9 n=19	36.6±14.0 n=9	21.6±5.0 n=5
Reticulocytes (0.5–2.0%)	0.96±0.52 (n=9)	1.25±0.26 (n=3)	NRA

This table includes all available results from all cases from Spain and USA combined. The numbers of data points varied according to measure and patient group, and are shown underneath each result. Normal reference ranges for each measure are shown in parentheses. Abbreviations: LDH, lactate dehydrogenase; LND, Lesch-Nyhan disease; HND, HGprt-related neurological dysfunction; HRH, HGprt-related hyperuricemia; NRA, no result available; T4, thyroid hormone; TFS, transferrin saturation; TSH, thyroid stimulating hormone