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# Neuroacanthocytosis: Observations, Theories and Perspectives on the Origin and Significance of Acanthocytes

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## Abstract

The presence of acanthocytes in the blood is characteristic of patients suffering from neuroacanthocytosis (NA). Recent studies have described abnormal phosphorylation of the proteins involved in connecting the membrane and cytoskeleton in patient-derived erythrocytes. The involvement of lipids in the underlying signaling pathways and recent reports on *in vitro* disease-associated lipid alterations support renewed research into lipid composition, signal transduction, and metabolism in patient erythrocytes. In addition to morphology, changes in membrane organization affect erythrocyte function and survival. Patient erythrocytes may have a decreased ability to deform, and this may contribute to accelerated erythrocyte removal and a decreased oxygen supply, especially in vulnerable brain regions. The presently available data indicate that acanthocytes are likely to originate in the bone marrow, making erythropoiesis an obvious new focus in NA research. Moreover, new, detailed morphological observations indicate that acanthocytes may be the tip of the iceberg with regard to misshapen erythrocytes in the circulation of patients with NA.

A systematic assessment of patient erythrocyte morphology, deformability, oxygen delivery, and metabolism will be instrumental in determining the putative contribution of erythrocyte function to NA clinical symptoms.

**Keywords:** Acanthocytosis, erythrocytes, membrane, neurodegeneration, signaling

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## Neuroacanthocytosis: an introduction

Patients suffering from a neuroacanthocytosis (NA) syndrome experience neurological symptoms due to basal ganglia degeneration, and their blood contains acanthocytes with thorn-like protrusions. Genes mutated in patients with chorea-acanthocytosis (ChAc), McLeod syndrome (MLS), and Huntington's disease-like 2 (HDL-2) have been identified, but neither the functions of the proteins they encode nor the pathophysiologic processes caused by these mutations are well understood. There is also limited knowledge regarding the causes of neurodegeneration with brain iron accumulation (NBIA) such as pantothenate kinase-associated neurodegeneration (PKAN), where the functions of the affected gene products center around lipid modification and metabolism.<sup>1,2</sup> Given the paucity of relevant phenotypes in currently available cellular and animal models, research

into the molecular causes of acanthocyte formation and consequences could be performed using easily accessible erythrocytes. This feasible approach could identify mechanisms underlying neurodegenerative processes. Here, we present an inventory of the actual knowledge and current hypotheses, as well as some of our ideas on the most fruitful approaches for unraveling the molecular mechanisms behind acanthocyte formation and subsequent neurodegeneration.

## Acanthocyte generation

Acanthocytosis is associated with NA syndromes, but the presence and number of acanthocytes are inconsistent. An overview of the published data suggests that considerable acanthocytosis is associated with MLS, but it is much more variable in ChAc, HDL-2, and PKAN.<sup>1,2</sup> Anecdotal evidence indicates that the fraction of acanthocytes varies in individual patients over time, without a clear association

with clinical symptoms (G. Irvine, personal communication). This seems to exclude the possibility that the transition of possibly susceptible erythrocytes with a normal, discocyte morphology to an acanthocyte is secondary to mechanical stress due to involuntary movements or seizures. This view is supported by the inability to (bio)chemically induce acanthocyte formation *in vitro* and by the normal deformability of both acanthocytes and discocytes of NA patients in a capillary-mimicking system.<sup>3</sup> In addition, detailed microscopic analyses have shown that methodological aspects cannot be underestimated in distinguishing between acanthocytes and erythrocytes with an acanthocyte-like morphology.<sup>3,4</sup> The issue remains unresolved given the complexity of clinical symptoms in patients with NA and the likely but largely unknown effects of many drugs on erythrocyte membrane characteristics.<sup>5</sup> This matter may be even more complex because acanthocytes may not be the only erythrocytes with an aberrant shape in patients with NA or their relatives.<sup>3</sup> Thus, a meaningful interpretation of acanthocytosis data is hampered by the lack of a systematic inventory of the numbers of acanthocytes and other misshapen erythrocytes in patients and nonaffected family members, as well as changes in these numbers over time.

### The possible role of membrane-cytoskeleton interactions

It is not clear why erythrocytes and basal ganglia neurons are specifically affected in NA. Erythrocyte morphology depends largely on the ankyrin and junctional complexes that link the plasma membrane to the spectrin-actin cytoskeleton.<sup>6,7</sup> Phosphorylation regulates the composition of these complexes and thereby influences the strength and type of cytoskeleton linkages.<sup>8</sup> The proteins involved in erythrocyte membrane-cytoskeleton interactions are expressed in neurons. Interestingly, specific ankyrin-R splice variants<sup>9</sup> and  $\beta$ -adducin<sup>10</sup> are only expressed in brain tissue and erythrocytes. Disturbances in connectivity between the cytoskeleton and plasma membrane often have pathologic consequences for erythrocytes<sup>6,7</sup> and may lead to neuronal death.<sup>11</sup> In addition, membrane-cytoskeleton interactions and activities are regulated by lipids in both cell types. For example, the cytoskeleton proteins  $\beta$ -spectrin and protein 4.1 bind to both ankyrin and the phosphoinositide PtdIns(4,5)P<sub>2</sub> through a pleckstrin homology (PH) domain or FERM domain, respectively, and this may affect erythrocyte morphology and neuronal glutamate transporter activity.<sup>12–15</sup>

### Membrane lipid involvement

Numerous studies have focused on defining the composition and/or organization of the acanthocyte membrane. Because the main known cause of acanthocytosis is the effect of betalipoproteinemia-associated lipid metabolism disturbance,<sup>16</sup> research into lipid abnormalities is an obvious focus. In general, this approach has not generated any data that clearly indicate altered lipid composition in the erythrocyte membrane as a pivotal factor in NA. Phospholipid analysis has not revealed any consistent differences in the relative amounts of the main phospholipid classes or membrane cholesterol in erythrocytes from NA

patients compared with those of control donors.<sup>17,18</sup> This statement was corroborated by a detailed semi-quantitative analysis of the erythrocyte membrane fatty acid composition.<sup>19</sup> Currently available sensitive and specific mass spectrometric analysis methods to generate lipidomic data have not yet been applied to this field. However, quantitative analyses may not reveal local alterations in membrane composition (e.g., microdomain distribution and composition). In view of the known role of lipid organization in membrane behavior (see above) and the likely involvement of NBIA gene products in lipid metabolism,<sup>2</sup> minor changes in lipid composition may turn out to be instrumental for the acanthocytic shape. This supposition is supported by recent findings in both yeast and human cells, showing that knockout of *VPS13A* (the gene that encodes chorein, the protein affected in ChAc patients) leads to a decrease in the phosphoinositide PtdIns4P.<sup>20,21</sup> In addition, heterogeneity in the erythrocyte population, in particular the percentage of clearly identifiable acanthocytes,<sup>3</sup> may have obscured the presence of cell morphology-specific alterations in lipid organization. This suggests that it may be worthwhile to reinvestigate this issue using presently available cell separation methods and lipidomic analysis technologies. Together with the known possibility of influencing membrane lipid composition—and possibly neurodegeneration—by diet interventions,<sup>22,23</sup> molecular identification of a lipid metabolism flaw in erythrocytes would constitute an interesting starting point for the development of novel treatments.

### Proteins involved in acanthocyte formation

Protein staining of the membrane fractions of erythrocytes from patients with NA has failed to reveal any conspicuous aberrations. However, immunochemical analyses showed consistently abnormal patterns using antibodies against band 3.<sup>19,24</sup> Subsequent data suggested that these patterns arise from acanthocytosis-associated degradation that especially affect the N-terminal, cytoplasmic domain of band 3.<sup>25</sup> It has to be noted that this approach (i.e., an investigation of acanthocytosis-associated alterations using specific antibodies) was mainly triggered by the description of altered band 3 structure and function in acanthocytosis,<sup>26</sup> and this has not been systematically assessed for other erythrocyte membrane proteins. Recent data from proteomic inventories have triggered other approaches. These inventories of erythrocyte membrane fractions from patients with ChAc, MLS, and HDL-2 have revealed both acanthocytosis-associated quantitative changes in membrane protein composition revolving around band 3 and data suggesting that these changes are caused by alterations in phosphorylation-mediated protein-protein interactions.<sup>25,27</sup> These observations led to findings suggesting that alterations in signaling pathways associated with altered membrane recruitment and/or abnormal activity of the Src-family kinase (SFK) Lyn are primarily responsible for decreased band 3-mediated binding of the cytoskeleton to the lipid bilayer in ChAc patients.<sup>27</sup> A relationship between the absence of chorein, altered cytoskeleton organization, and protein phosphorylation was confirmed in a report that phosphorylation of the PI3K subunit p85 was decreased in erythrocytes from ChAc

patients. This was associated with decreased phosphorylation and activation of its substrates PAK1 and Rac1 and an apparent decrease in actin polymerization.<sup>28,29</sup>

### NA pathways

Recent years have witnessed a transition in research into NA erythrocytes from a hypothesis-driven to a phenomenological approach. The latter was almost immediately followed by investigation into the signaling pathways regulating (abnormal) protein-protein interactions in acanthocytes. This suggests that the field is influenced by the growing awareness that regulation of erythrocyte structure and function is intimately interwoven with organismal homeostasis. The emerging question is: how can the current knowledge regarding regulation of erythrocyte shape contribute to insight into the mechanisms leading to the acanthocyte shape and possibly to altered erythrocyte function? An overview of the current knowledge regarding the causes of aberrant erythrocyte morphology in genetic membranopathies reveals many molecular and structural details regarding the interaction between integral and cytoskeletal proteins. Unfortunately, little is known about the underlying signaling pathways.<sup>6,7</sup> Upon comparing the current knowledge on acanthocytosis with that of specific erythrocyte membranopathies, we have to conclude that the theory that disturbance of band 3-cytoskeleton interactions induces acanthocytosis lacks sufficient heuristic value. Indeed, there is one band 3 mutation that is associated with acanthocytosis,<sup>26</sup> but numerous others lack acanthocytes as a prominent feature. Moreover, the blood of individuals with mutations in other proteins of membrane-cytoskeleton complexes contains many more spherocytes or elliptocytes than acanthocytes.<sup>6,7</sup> In addition, altered phosphorylation of band 3 involving Lyn and related protein kinases induces changes in band 3 organization that have not been described in acanthocytes.<sup>8,30</sup> Finally, attempts to generate acanthocytes *in vitro* by disturbance of vertical and/or horizontal protein-protein interactions have not been successful. For example, disturbance of the phosphorylation status confirmed previous data that the transition from a discocyte to an echinocytic shape can be induced by increased band 3 phosphorylation,<sup>8,30</sup> but the often postulated transition from an echinocytic to a clearly identifiable acanthocytic shape has not been published. This implies that the presence of acanthocytes is a highly specific phenomenon in patients with NA. This is the more surprising given that the known genetic causes of NA involve proteins with very diverse functions, none of which are presently known to be specific or critically important for mature erythrocytes.

### Autophagy

An elegant model combines the various molecular details of NA into a hypothesis that centers on autophagy dysfunction.<sup>31</sup> Indeed, flaws in autophagy have been reported in several neurodegenerative diseases<sup>11,32</sup> and may influence erythrocyte differentiation during erythropoiesis.<sup>33</sup> Active class I PI3K is known to inhibit autophagy,<sup>34,35</sup> so a decrease in its activity as described in chorein-deficient cells would be expected to increase autophagy. However, such

decreased activity may also constitute a compensatory mechanism, as has been postulated to explain the late onset of Huntington's disease.<sup>36,37</sup> Abnormal Lyn activity in ChAc erythrocytes may constitute another counteracting mechanism as Lyn activity may promote autophagy.<sup>38</sup> The phosphoinositides PtdIns4P and PtdIns(4,5)P<sub>2</sub> regulate membrane-cytoskeleton interactions (see above) and are emerging as important players in the autophagy process.<sup>39</sup> The development of tools that allow on-demand lipid and kinase manipulation is needed to investigate their influence on erythrocyte shape.

### Defective erythrocyte function and brain hypoperfusion

Most erythrocyte malformations have clinical consequences through decreased cell survival and secondary pathology,<sup>6,7</sup> but this is much less conspicuous in patients with NA. Clinical descriptions of patients with NA concentrate on neurological and neuromuscular symptoms and are sparse in indications of hemolysis or anemia. However, decreased erythrocyte deformability and increased erythrocyte loss, as implied by splenomegaly, may be more common than suggested by the few descriptions in patients with McLeod disease.<sup>1,40</sup> Recent *in vitro* findings showing decreased passage of acanthocytes and other erythrocytes from PKAN patients through a spleen-mimicking device point toward a general phenomenon.<sup>3</sup> A reduction in haptoglobin, a marker for hemolysis, has been reported in several patients.<sup>1,40</sup> This suggests that acanthocytes have a decreased capacity for extensive deformability.

Decreased deformability is associated with anemia, and even subclinical deformability can hamper oxygen delivery. The striatum, which is the part of the basal ganglia most severely afflicted in patients with NA, is also highly sensitive to hypoperfusion.<sup>41</sup> Oxygen deprivation leads to excessive glutamate release in the brain and subsequent overactivation of N-methyl-D-aspartate (NMDA)-receptors and cell death. Various reports have shown that NMDA-receptor activity is increased by Src family kinases.<sup>42</sup> Moreover, the activity of these kinases is increased under hypoxic circumstances, providing a possible explanation for abnormal Lyn activation in ChAc patients. Medium spiny neurons, the most afflicted cells in NA, are more susceptible than striatal interneurons to excitotoxic damage,<sup>43</sup> and the selective loss of specific striatal neuronal subpopulations in a mouse model of Huntington's disease correlates with motor symptoms similar to those observed in NA patients.<sup>44</sup>

Insufficient erythrocyte deformability, aggravated by hypoxic circumstances, may exacerbate hypoperfusion. Recent perfusion and metabolic imaging studies have shown impairment of brain perfusion in patients with sickle cell anemia,<sup>45</sup> which may contribute to the recently observed deficiencies in cognitive functioning in sickle cell patients.<sup>46</sup> Hypoperfusion has also been described in a NA patient using various imaging methods,<sup>47</sup> underscoring the validity of investigating microvascular blood flow in the brain of patients with NA. Thus, hypoperfusion caused by erythrocyte dysfunction may provide a partial explanation for neuropathologic changes in patients with NA.

The where and when of acanthocyte generation have been largely neglected. Do acanthocytes evolve from normal, susceptible discocytes in circulation, or are they derived from bone marrow? Also, could NA-associated disturbance of neuronal homeostasis in the central nervous system affect erythropoiesis as suggested by reports on the involvement of nerve cells in the regulation of hematopoietic stem cell kinetics?<sup>248,49</sup> The answers to these questions have implications for future research strategies: if acanthocytes are already formed in the bone marrow, investigation of the underlying mechanisms is likely to yield more insight, especially if we focus on genes currently known to be mutated in NA. If acanthocytes are formed in circulation, identifying the factors that induce this transition and its molecular and/or functional consequences may be more effective. From this perspective, an overview of the currently available—albeit circumstantial—evidence indicates that the acanthocytic shape may already be present upon appearance in the circulation. 1) There are no data suggesting that the acanthocytic shape increases with erythrocyte age in patients with NA. On the contrary: the percentage of acanthocytes in control donors is highest in cell fractions that contain the youngest erythrocytes.<sup>25</sup> 2) There is no known association between the extent of acanthocytosis and the severity or nature of neuromuscular symptoms. 3) It has not been possible to induce acanthocytosis *in vitro* or to generate discocytes from patient-derived acanthocytes. 4) An increased amount of fetal hemoglobin in acanthocyte-enriched cell fractions indicates disturbed erythropoiesis in patients with NA.<sup>3</sup>

### Conclusion

The present overview of the available data supports the conclusion that erythrocyte-centered and acanthocyte-inspired research approaches remain instrumental to developing a better understanding of NA pathophysiology. Important goals include: 1) the effect of NA-associated mechanisms on erythrocyte structure and function, 2) a detailed comparative study of the characteristics of relevant signaling pathways in erythrocytes from patients with various forms of acanthocytosis other than ChAc, and 3) the effect of acanthocytosis on oxygen delivery.

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