# DMT1 (NRAMP2/DCT1) Genetic Variability and Resistance to Recombinant Human Erythropoietin Therapy in Chronic Kidney Disease Patients under Haemodialysis

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The management of anaemia in chronic kidney disease (CKD) was changed by the introduction of recombinant human erythropoietin (rhEPO) therapy, allowing a significant correction of anaemia; however, around 5–10% of the patients show a marked resistance to rhEPO therapy [1, 2]. Several conditions were reported as being associated with rhEPO resistance, but changes in iron homeostasis is one of the most studied causes [3].

CKD patients who do not respond to rhEPO therapy seem to present a 'functional' iron deficiency, characterized by the presence of adequate iron stores as defined by conventional criteria, but apparently with an inability to sufficiently mobilize this iron to adequately support erythropoiesis [4]. We recently found that compared with responder patients, non-responders presented no statistical differences for iron, transferrin saturation and ferritin values, but had a significantly lower haemoglobin concentration and mean cell haemoglobin, as well as significantly higher plasma levels of the soluble transferrin receptor (s-TfR) [4].

Iron uptake from plasma to erythroid precursor cells is regulated by different proteins, namely transferrin receptor, haemochromatosis (HFE) protein and DMT1 (NRAMP2/DCT1). In literature, alterations in the transferrin receptor gene have been associated with HFE [5], and *HFE* gene mutations are associated with a reduction in the amount of rhEPO necessary to support erythropoiesis in haemodialysis patients [6]. However, these 2 proteins are unlikely to be the main causes of the resistance to rhEPO therapy in CKD patients under haemodialysis, as HFE is characterized by increases in serum iron parameters, namely transferrin saturation, not found in non-responder CKD patients. On the other hand, the *DMT1* gene could be a good candidate to underlie the variability of response to rhEPO in CKD patients. Indeed, the few described mutation cases in the *DMT1* gene are associated with an inhibition of intestinal iron absorption and a decrease in erythroid cell precursor iron uptake, resulting in hypochromic and microcytic anaemia [7]. This type of anaemia is similar to that observed in non-responder CKD patients, resulting of a functional iron-depleted erythropoiesis in the presence of adequate serum iron levels [4]. As far as we know, there are no data regarding the association of *DMT1* gene

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**Table 1.** Frequency of studied DMT1 alleles in CKD patients and controls (%)

| | c.309+44C→A | | c.1254T→C | | c.1303T→C | | c.1629–16C→G | |
|---|---|---|---|---|---|---|---|---|
| | C allele | A allele | T allele | C allele | T allele | C allele | C allele | G allele |
| CKD patients (n = 63) | 76.98 | 23.02 | 84.13 | 15.87 | 99.21 | 0.79 | 92.06 | 7.94 |
| rhEPO responders (n = 32) | 73.44 | 26.56 | 82.81 | 17.19 | 100 | 0 | 92.19 | 7.81 |
| rhEPO non-responders (n = 31) | 80.65 | 19.35 | 85.48 | 14.52 | 98.39 | 1.61 | 91.94 | 8.06 |
| Controls (n = 26) | 76.92 | 23.08 | 80.77 | 19.23 | 100 | 0 | 94.23 | 5.77 |

**Table 2.** Haplotype frequencies in CKD patients and controls determined by Arlequin version 3.11 (%)

| | CCTC | ACCC | ACTG |
|---|---|---|---|
| CKD patients (n = 63) | 65.87 | 18.25 | 6.35 |
| rhEPO responders (n = 32) | 64.06 | 21.88 | 7.81 |
| rhEPO non-responders (n = 31) | 67.74 | 14.51 | 4.83 |
| Controls (n = 26) | 71.15 | 5.76 | 0 |

CCTC: c.309+44C; c.1254C; c.1303T; c.1629–16C.
ACCC: c.309+44A; c.1254C; c.1303C; c.1629–16C.
ACTG: c.309+44A; c.1254C; c.1303T; c.1629–16G.

mutations/polymorphisms in patients with 'functional' iron deficiency.

In this context, we studied 63 CKD patients under haemodialysis (36 males, 27 females; age 62.1 ± 15.7 years) and rhEPO treatment (10 with darbopoietin-α and 53 with epoetin), including 32 responders and 31 non-responders to rhEPO therapy [8]. The rhEPO maintenance dose for responders was 89.65 ± 57.62 U/kg/week and for non-responders 572.99 ± 193.84 U/kg/week. Patients with autoimmune disease, malignancy, haematological disorders and acute or chronic infection were excluded. Intravenous iron supplementation (iron sucrose) was based on the European Best Practice Guidelines for the Management of Anemia in CKD patients under haemodialysis [8]. No statistically significant difference was found in total iron supplement given during the last year, between responder and non-responder patients. We also studied a control group with 26 healthy volunteers, presenting normal haematological and biochemical values, with no history of renal or inflammatory diseases, and, as far as possible, age and gender matched with the CKD patients (8 males, 17 females; mean age 47.81 ± 14.69). All patients and controls gave their informed consent to participate in this study.

In all participants (patients and controls), haematological counts were performed (Sysmex K1000; Sysmex, Hamburg, Germany) as well as the evaluation of serum iron levels (Randox Laboratories Ltd., UK), ferritin (Randox Laboratories Ltd.) and transferrin (Randox Laboratories Ltd.) and of plasma s-TfR (human sTfR immunoassay, R&D Systems, Minneapolis, Minn., USA). Genotype of the c.309+44C→A, c.1254T→C, c.1303T→C and c.1629–16C→G *DMT1* polymorphisms/mutations was performed by restriction fragment length analysis, using the conditions previously described [9], with minor modifications. Mutation nomenclature was used according to the recommendations of the Human Genome Variation Society (2005; http://www.hgvs.org/mutnomen).

The frequencies of the *DMT1* gene polymorphisms/mutations tested are shown in table 1. No statistically significant differences were found in allele frequency between the controls and CKD patients, nor when comparing the 2 groups of patients. These frequencies were similar to those previously described in literature [9].

Estimations of the haplotype frequencies were performed using the program Arlequin version 3.11 [10]. This program estimates the maximum likelihood haplotype frequencies from observed data using an expectation-maximization algorithm for multilocus genotype data when the genetic phase is unknown. The most frequent haplotypes found were: CCTC (c.309+44C, c.1254C, c.1303T, c.1629–16C), ACCC (c.309+44A, c.1254C, c.1303C, c.1629–16C) and ACTG (c.309+44A, c.1254C, c.1303T, c.1629–16G) (table 2). No statistically significant differences were found in the frequencies of the CCTC and ACCC haplotypes between controls and CKD patients, nor between responders and non-responder patients. The frequencies of these 2 haplotypes are very similar to those found in other populations [9]. However, the ACTG haplotype was statistically more frequent in CKD patients than in the control group ($p < 0.05$); nevertheless, no statistical differences were found between

responder and non-responder CKD patients. The frequency of this ACTG haplotype in CKD patients was also very similar to that found in literature; however, in the control group, the prevalence of this haplotype is significantly lower than that found in other previously described control populations [9]. These differences can result from the small sample size of our control group.

When we compared rhEPO doses, haematological data and iron status (iron, ferritin and transferrin saturation) of CKD patients under haemodialysis by haplotype, no statistically significant differences were found (data not shown), suggesting that *DMT1* gene haplotypes are not associated with changes in haematological data and iron status in CKD patients, nor in rhEPO doses required to achieve target haemoglobin levels.

CKD patients under haemodialysis are treated with intravenous iron; thus, the main factors limiting iron availability for erythropoiesis could be a deficiency in iron uptake to erythroblast and/or iron trapping into macrophages. The latter is associated with a failure in recycling/export mainly due to a decreased activity of the iron export ferroportin-1 (related to increased hepcidin expression associated with the inflammatory process found in haemodialysis patients). As DMT1 protein is related to both mechanisms, it is reasonable to hypothesize that mutations/polymorphisms in this gene may decrease the iron availability for erythroblast iron metabolism and haem biosynthesis in CKD non-responder patients to rhEPO therapy, even in the presence of adequate serum iron levels. However, our study failed to demonstrate a link between mutations/polymorphisms in the *DMT1* gene and resistance to rhEPO therapy. Moreover, no influence of *DMT1* gene mutations/polymorphisms on rhEPO doses, haematological data and iron status was demonstrated in our CKD patients under haemodialysis. Further studies are warranted, namely using a higher number of CKD patients, full screening of mutations in *DMT1* gene and/or other genes related to iron metabolism, to clarify the mechanism underlying rhEPO resistance.


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