Congenital Dyserythropoietic Anemia Type IV with KLF1 E325K Mutation –

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Abstract

Congenital Dyserythropoietic Anemia Type IV (CDA-IV) is still an emerging new disease with approximately 10 cases reported over the past three decades. CDA-IV is known to be caused by a specific mutation in exon 3 of KLF1, an erythroid transcription factor KLF1 with substitution of glutamic acid with lysine at residue 325 (KLF1 E325K). Because of the rarity of this disorder the presenting features are incompletely defined; especially the non-erythroid comorbidities. Here we report a new case, a male child, presenting with fetal hydrops and dysmorphic external genitalia.

Congenital Dyserythropoietic Anemia Type IV with KLF1 E325K Mutation –

a New Case with Dysmorphic Male Genitalia .

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Abbreviations: CDA-IV - Congenital Dyserythropoeitic Anemia Type IV

LSD – lyposomal storage disorder

AML – anti-Mullerian hormone

Abstract:

Congenital Dyserythropoietic Anemia Type IV (CDA-IV) is still an emerging new disease with approximately 10 cases reported over the past three decades. CDA-IV is known to be caused by a specific mutation in exon 3 of KLF1, an erythroid transcription factor KLF1 with substitution of glutamic acid with lysine at residue 325 (KLF1 E325K). Because of the rarity of this disorder the presenting features are incompletely defined; especially the non-erythroid comorbidities. Here we report a new case, a male child, presenting with fetal hydrops and dysmorphic external genitalia.

Introduction:

Congenital Dyserythropoietic Anemia type IV (CDA-IV) is an exceedingly rare disease, with only approximately 10 cases reported over the past 3 decades[1-10]. It is defined by a specific genetic mutation in exon 3 of KLF1 (chr19p13.2), an erythroid transcription factor with substitution of glutamic acid with lysine at residue 325 (KLF1 E325K). The hematologic presentation is variable, but is defined by ineffective erythropoiesis leading to anemia. There are also non-erythroid comorbidities which remain poorly defined[11,12]. We report here a new case of a male child presenting with fetal hydrops and dysmorphic external genitalia.

Case Description:

KC is a firstborn male infant to a Caucasian mother and African-American father. Pre-natal ultrasound at 30 weeks noted cardiomegaly and repeat ultrasound at 35 weeks suggested hydrops fetalis leading to emergency Caesarean section. Born at an outside hospital, the infant was found to have severe anemia, thrombocytopenia, coagulopathy, hepatosplenomegaly and cardiomyopathy compatible with hydrops fetalis. The left testicle was not palpable and he was thought to have a micropenis. Initial laboratory findings are shown in Table 1.

He required a 1 month neonatal intensive care admission during which he underwent an exchange transfusion, multiple transfusions of packed red blood cells, platelets, and fresh frozen plasma. A bone marrow aspirate was thought to be non-diagnostic. A liposomal storage disorder (LSD) was suspected, but enzyme testing for N-acetyl-alpha-glucosaminidase as well as molecular testing for LSD were negative. Newborn metabolic/genetic disease screening was reported as negative.

KC first presented to our institution at 5 months of age. At that time hepatosplenomegaly had resolved, but he still required intermittent transfusions of packed red blood cells. He was noted to have a micropenis, undescended testicle, scrotal tethering and excess suprapubic fat pad. He had been recently transfused, however in the following 4 weeks, hemoglobin dropped to 7.3 g/dl with 7.3% reticulocytes, MCV 89.0 fl, platelets 222 10e9/L, WBC 10.0 10e9/L with 16% nRBC, 46.5% neutrophils, 38.7% lymphocytes, 13.4% monocytes, 0.7% eosinophils and 0.7% basophils. Smear revealed mild myeloid left shift beyond band stage, macrocytic anemia with polychromasia, moderate anisopoikilocytosis with atypical erythrocytes including, spherocytes, ovalocytes and occasional fragmented red cells. Frequent nRBCs were identified (Figure 1a).

There was no known family history of a hemolytic disorder. Diagnostic work-up revealed Hemoglobin electrophoresis of 68.2% Hemoglobin A, 2.1% Hemoglobin A2 and 29.7% Hemoglobin F. Quantitative measurements of glucose 6-phosphatase and pyruvate kinase enzyme activities were normal, as was RBC Band

3 protein reduction for hereditary spherocytosis. Because of the frequent transfusions, and thus possible inaccuracy of the previous tests, Clinical Exome Sequence Analysis was performed through GeneDx (https://www.genedx.com; Gaithersburg, PA; USA). KP was found to be heterozygous for a pathogenic variant in the KLF1 gene (c.973 G>A; p.E325K). This was a de novo mutation and established the diagnosis of congenital dyserythropoeitic anemia (CDA) type 4. He remains transfusion dependent and on iron chelation therapy and has developed hemolytic facies and splenomegaly.

Discussion:

Clinical diagnosis of KLF1 E325K associated Congenital Dyserythropoietic anemia – (CDA -IV) remains complex and CDA-IV as a cause of non-immune fetal hydrops is under appreciated. This and the Detroit case underscore this point.[4,13] Fetal hydrops was suspected in both cases. Blood group incompatibility was excluded by clinical testing. The child described here was noted to have severe anemia with high MCV - a smear review was not available but the extraordinarily high white cell count suggested the presence of a leukoerythroblastic picture. Post transfusion blood examination shows certain hall marks of the aberrant erythroid maturation and block in the fetal to beta globin transition by KLF1 E325K- ie persistent high fetal hemoglobin, lack of CD44 surface expression red cells (Figure 1b) and the presence of increased nRBC, some with binuclearity. The latter are indicative of the well identified enucleation defect associated with the KLF1 E325K mutation.[3,14] Documentation of other features such as the presence of embryonic hemoglobin require isoelectric focusing or complex proteomics. Search for Colton a-/b- caused by aquaporin1 (AQP1) deficiency and In(Lu) blood group (Lutheran blood group/ BCAM deficiency caused by lack of CD44 on red cell membrane) are likewise complex and are not readily available. However, CD44 surface marker testing by flowcytometry is feasible and could be incorporated in to the testing of non-immune hydrops cases with erythroblastosis fetalis as it discriminates between native and transfused red cells.[3,15]

Non-hematologic comorbidities in CDA-4 have not received sufficient focus. The hemolytic anemia appears to be more severe in males and in addition two thirds of affected males showed genital dysmorphology including complete sex reversal in the Detroit case.[3,15] Two had no genital dysplasia described.[5,7] Our present case, a 46 XY male has micropenis, undescended left testis with scrotal tethering. An immediate alternative hormonal cause is not evident. Retrospective evaluation of clinical exome sequencing did not identify a pathogenic germline mutation nor insertions/deletions in genes reported to cause disorders of sex differentiation (with the exception of CYP21A2 which has poor coverage by exome sequencing). In fact, the only pathogenic mutation identified on clinical exome sequencing was the E325K mutation in KLF1, a transcription factor regulating erythroid differentiation and maturation. Endocrine evaluation at 37 months of age in the present child showed the following laboratory values- total testosterone: <2.5 ng/dl (<2.5-10); androstenedione: <10 ng/dl (0-22); dihydroepiandrostenedione <20ng/dl (0-67); 17-hydroxyprogesterone: 11ng/dl (0-90); FSH 0.6mlU/ml (0.9-12.0); anti-Mullerian hormone (AMH): >960ng/ml - (Males at birth 32.7-262.9); inhibin B: 306.8 pg/ml (42-268). Elevated levels of AMH have not been previously reported but suggest an adaptive response to an as yet unidentified early gonadal dysgenesis event.

Identification and reporting of additional cases of CDA-IV particularly those in 46 XY males will be helpful in fully delineating the non-erythroid off-target effects of the KLF1 E325K mutant transcription factor. Progress toward this goal has also been made in the recent development of a human cellular model of CDA-IV which may allow for future analysis of the disordered biological processes associated with this rare molecular defect.[16]

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