Diminutive: exceptionally or notably small (Merriam Webster on-line free dictionary)

Diminutive, interesting word, not one you hear very often in the scientific lexicon. So why my interest in the word diminutive? My interest was piqued by the presence of this word in the title (**Diminutive somatic deletions in the 5q region lead to a phenotype atypical of classical 5q-syndrome**) of a recent report in the journal *Blood (Vlachos et al., 2013)*. The article discusses two patients: patient 1 was diagnosed with Diamond Blackfan anemia and has been receiving transfusion therapy for the past 20 years, whereas patient 2 was diagnosed with myelodysplastic syndrome and received a bone marrow transplant. Both patients had atypical presentation for their respective diagnoses; but given the clinical variability observed in both diseases, atypical presentations don't seem all that atypical anymore.

As any reader of the DBAF Newsletter is aware, there are tremendous efforts underway at numerous sites to identify the genes affected in each and every patient with DBA. These efforts have gone beyond sequencing genes encoding ribosomal protein to sequencing entire genomes. Moreover, since not all patients have mutations that can be identified by DNA sequence analysis, researchers have developed increasingly sophisticated methods to scan whole genomes for increasingly small deletions. Such diminutive deletions are the centerpiece of the report in *Blood*.

The first patient was diagnosed with DBA in 1991 at 5 years of age. The age at which she was diagnosed was unusual, as most patients with DBA are diagnosed in the first year of life. This child, now an adult, was not responsive to steroids and had received monthly transfusions since her diagnosis. Despite being in the DBA Registry for years, the gene affected in this patient was unknown and so investigators continued to hammer away at her genome trying to unlock the secrets held within. It was deletion analysis that ultimately led to that eureka moment. This woman had a relatively small deletion on chromosome 5. Moreover, not all of the cells in her bone marrow contained this deletion, so she was classified as being mosaic, having a mixture of normal and deleted cells.

The natural history of her disease likely began with the deletion arising spontaneously within the progenitor cells in her bone marrow. Cells containing this deletion fail to efficiently differentiate into red blood cells (the reason for this is explained below), so as the percentage of cells harboring this mutation rose high enough in her marrow she eventually became anemic. This mutation which arose spontaneously within a myeloid progenitor cell in the bone marrow is referred to as a somatic mutation, as opposed to a mutation inherited from a parent found in all cell types. The somatic nature of this mutation could explain the delayed presentation of her disease.

Deletions of similar regions of chromosome 5 have been known for some time to cause a form of myelodysplastic syndrome known as 5q- syndrome. This is a rather indolent form of MDS which typically presents in patients in their 60's and is characterized by a refractory macrocytic anemia. Ben Ebert and colleagues showed several years ago now that the refractory anemia observed in 5q- syndrome was caused by the deletion of the ribosomal protein gene, *RPS14 (Ebert et al., 2008)*. The groundwork for this amazing discovery was the work in the DBA field

linking loss of ribosomal protein genes to defects in erythrocyte development. The upshot of all this was that many considered 5q- syndrome an acquired form of DBA.

Given the degree of clinical and molecular overlap between classical DBA and 5q- syndrome one might anticipate that making a differential diagnosis between the two could be a challenge. This challenge is often met by the age of the patient in question and the fact that classical 5q- syndrome can also present with abnormalities in megakaryocytes which give rise to platelets. The fact that patient 1 presented at five years without megakaryocytic abnormalities would tend to swing the diagnostic pendulum towards a late presenting DBA as opposed to an early presenting 5q-. And so the diagnosis stood until the deletion analysis was performed and the deletion on chromosome 5 discovered. The diminutive size of this deletion explains why megakaryocytes were unaffected because although the *RPS14* gene was included in this small deletion, a region of chromosome 5 linked to the megakaryocyte abnormalities in 5q- syndrome was outside of the deleted region, and so her megakaryocytes were normal. Given these findings, the patient's diagnosis was changed from atypical Diamond Blackfan anemia to 5q- syndrome, which was also atypical because of age of onset and the lack of megakaryocyte abnormalities.

This diagnostic distinction is not merely semantic as patients with 5q- syndrome have shown to have favorable responses to the drug lenalidomide. Indeed, when the diagnosis was changed and the patient started on lenalidomide, a favorable response was observed and she been transfusion independent since. Interestingly, the diminutive size of the deletion in this patient raised another important issue. The favorable response to lenalidomide in 5q- patients has been linked to two genes deleted in most patients with 5q- (Wei et al., 2009). Here again, because of the small size of the deletion in patient 1, these genes are not included and so her favorable response to lenalidomide is apparently not a consequence of the loss of these two genes. This result will cause some reconsideration of the potential mechanisms of action of lenalidomide.

Patient 2 was included in this report, not because of the link to DBA but instead because the deletion here is even smaller; in fact, the smallest deletion recorded for 5q- syndrome. This deletion also includes the *RPS14* gene which explains the refractory anemia, but it also raises an important issue when thinking about somatically acquired mutations. This issue is how a cell that has acquired a chromosome deletion that includes a ribosomal protein gene can give rise to progeny cells that increase as a percentage of bone marrow progenitors, a condition referred to as clonal outgrowth. Clonal outgrowth is usually associated with a cell that acquired a mutation that gives it a growth advantage relative to other cells and so with each cell division the clonal cells become an ever increasing percentage of this cell type.

For 5q- syndrome many have felt that while the loss of *RPS14* likely explains the failure of the deletion clone to differentiate along the erythroid lineage, there must be a second gene lost as a consequence of this deletion that provides a growth advantage to the clone. The reasoning behind this view is that the loss of a ribosomal protein gene adversely affects protein synthesis, which in turn adversely affects growth rate. And so, how could a clone with compromised protein synthesis and a reduced growth rate increase as a percentage in a population when it is competing with cells with a normal complement of ribosomes and normal growth rates. The ever decreasing size of deletions identified in patients with 5q- and the increasing number of

DBA patients who are mosaics with mixed populations of progenitor cells in their marrow may cause us to rethink our notion that cells lacking one of two copies of a ribosomal protein gene are at a selective disadvantage. *This wouldn't be the first time that studies on DBA and related areas have revolutionized the way we see the world around us.*

The studies reported here reveal that gene discovery and characterization is still a very active area of DBA research with the potential to have dramatic effects on the quality of life of DBA patients.

Disclosure statement – I am an author on the first two manuscripts cited below

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