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Juvenile cobalamin deficiency in individuals of African ancestry is caused by a founder mutation in the intrinsic factor gene *GIF*

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In the Western World, potentially fatal megaloblastic anaemia in children is usually caused by lack of Cbl known as Juvenile Cobalamin Deficiency (JCD), which leads to hematological and neurological disturbances. The classic cause of JCD is malabsorption, the inability of the small intestine to absorb the essential molecule from dietary sources. Consensus is lacking on which laboratory tests are the best for explaining the etiology of JCD due to the myriad of potential causes (Carmel *et al*, 2003). Underlying mechanisms vary in different parts of the world and in different socioeconomic groups (Gräsbeck 2006). However, patients may go improperly diagnosed for long periods because the serial exclusion testing for various causes can take months. Consequently, developmental delay is potentially common but easily overlooked in young children. Two main forms of inherited JCD exist: Imerslund-Gräsbeck syndrome (IGS; OMIM261100; Imerslund 1960; Gräsbeck *et al*, 1960) and Intrinsic factor Deficiency (IFD; OMIM261000; Katz *et al*, 1972). In IGS, deleterious mutations in either *CUBN* (Aminoff *et al*, 1999) or *AMN* (Tanner *et al*, 2003) cause malabsorption of Cbl. In IFD, mutations in the *GIF* gene itself are causative (Yassin *et al*, 2004; Tanner *et al*, 2005). All three genes can be sequenced in search of mutations in genomic DNA (Tanner *et al*, 2004, 2005). However, the screening task is daunting given that *CUBN* has 67 exons and *AMN* is very GC-rich, complicating the PCR-based analysis. To our knowledge commercial screening is not offered and research based testing is not widely available. Especially for under-served socioeconomic groups, affordable genetic testing may be years away. During our ongoing genetic studies of JCD, we have encountered four patients of West-African ancestry. Three were from the USA and one was referred to us from the UK. Sequencing of *GIF* revealed that all alleles with African

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roots carried the same c.183_186delGAAT frameshift mutation (M61fs), suggesting either a founder event or mutational recurrence. We undertook the present study to clarify this question.

Genomic DNA samples were isolated from peripheral blood after informed consent by standard phenol-chloroform-ethanol-precipitation. Families 8 (Tanner *et al*, 2005) and 55 (Yassin *et al*, 2004) were described previously and are of West African decent. Family 33 is Jamaican with African ancestry living in the UK and family 39 is from the US East Coast, with the father reporting African-Native American and the mother reporting European ancestry. All four patients were given Cbl replacement therapy by parenteral Cbl injection and no longer show any clinical symptoms. Control DNA samples from 93 African-American and 93 Caucasian with no evidence of JCD were collected from the metropolitan Columbus area under a separate IRB approved protocol. For mutation analysis, we amplified *GIF* exon 2 from genomic DNA by PCR and analyzed it as previously described (Tanner *et al*, 2005). Genotyping was performed with the three microsatellite markers *GIF* M1 (~28 kb proximal of *GIF*), *GIF* M3b (intron 7), and *GIF* M4 (~2kb distal), and analyzed using fluorescent-labeled primers as described (Tanner *et al*, 2004). PCR conditions and primer sequences are available upon request.

To elucidate if the haplotype carrying the c.183_186delGAAT mutation is an ancestral founder event, we genotyped the three flanking markers *GIF* M1, *GIF* M3b, and *GIF* M4 in the four families. Based on a parsimonious analysis requiring the least number of recombinations and implying linkage disequilibrium across this 40 kb region, we inferred the disease haplotype as *180-M61fs-235-168*. This ancestral haplotype was found unaltered in three out of seven parents and can be explained in the remaining four by one recombination each (Fig 1). Naturally, this assumption is not taking into account the true haplotype frequencies in Africans. Thus, we studied DNA samples from 93 African-American (AA) and 93 European-American (EA) controls. After excluding the presence of the mutation in all controls, we constructed definitive haplotypes based on homozygosity and then possible haplotypes based on the observed definitive haplotypes, again using the parsimony principle. Through the resulting haplotype frequencies (Supplemental Table I), we found that none of the 93 EA control samples had the ancestral haplotype *180-235-168* on which the mutation probably arose, while only two of the 93 AA control samples carried *180-235-168* (~1%). Allele *180* of marker *GIF* M1 was found among 14/186 (7.5%) alleles in the AA controls, and was not detected in the EA controls (Supplemental Table I). Likewise, allele *168* of marker *GIF* M4 was seen in 10/186 AA alleles (5.4%) with no occurrence in EA. This allele was found in strong linkage disequilibrium with M61fs in the JCD parents with six out of seven carrying it (Fig 1). Because the ancestral haplotype *180–235-168* existed only in AA and is quite rare amongst them (~1%), we concluded that the c.183_186delGAAT mutation very likely represents a founder event for JCD patients of the Sub-Saharan West-African lineage. This mutation was never detected in ~120 cases of JCD other than these 4 cases of African ancestry. Moreover, we have not encountered any AA patient so far that had any other JCD mutation, although the numbers are still small (n=4).

In summary, we identified a specific *GIF* mutation to be responsible for all JCD cases of West-African origin so far. The strong association of a particular mutation with a medically under-served minority could be used to their immediate benefit. It allows for quick and easy genetic testing in a disease that is notoriously difficult to diagnose but easy to treat, once the correct diagnosis is made. We believe that one simple PCR-based test in suspected cases of JCD in patients of West-African origin should be considered early during diagnosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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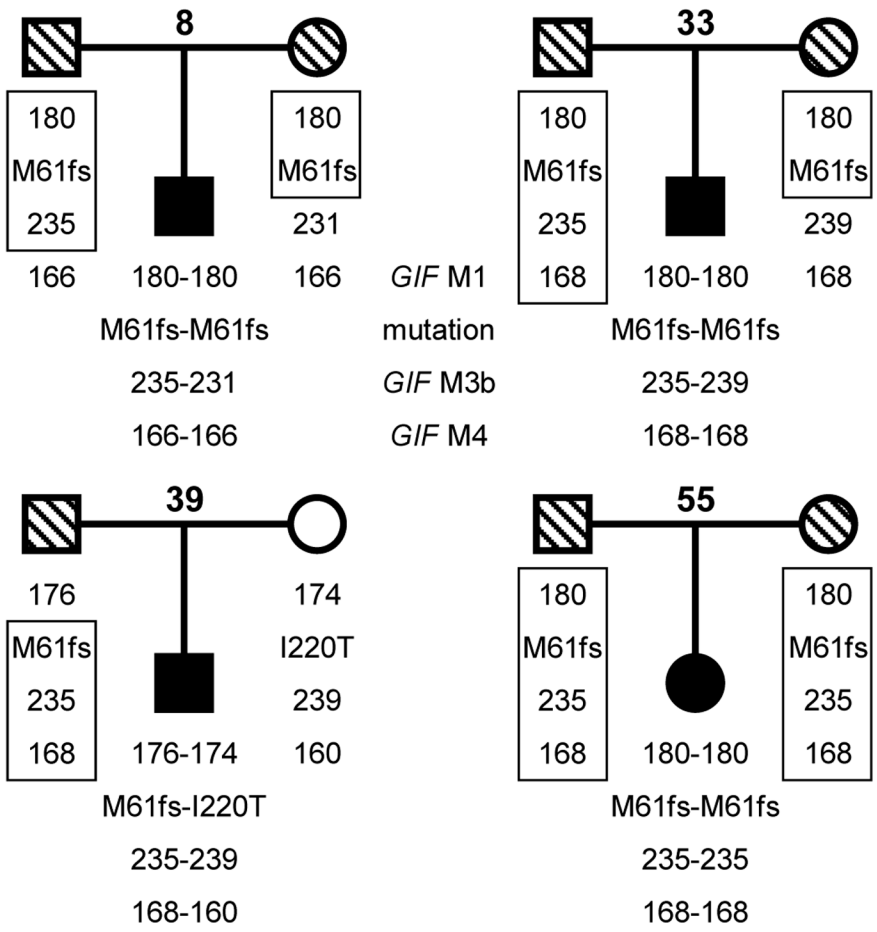

Fig 1.

Pedigrees of four patients affected by intrinsic factor deficiency (IFD, filled symbols) due to the c.183_186delGAAT frameshift mutation (M61fs) in the intrinsic factor gene, *GIF*. Haplotyping with markers *GIF* M1, *GIF* M3b, and *GIF* M4 flanking mutation M61fs revealed that haplotype *180-M61fs-235-168* was shared intact among three parents, while a partial haplotype was seen in the four remaining parents (boxes). Parents with African ancestry are diagonally striped, while the mother in family 39 (unfilled circle) had European ancestry and contributed missense mutation I220T.