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What does it take to call it a pathogenic mutation?

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In this issue, Graziani et al., report on a 31-year-old male with a history of recurrent respiratory, cutaneous and intestinal infections, associated with sclerosing cholangitis and obstructive pancreatitis (1). Immunological investigations showed low serum IgA and IgM, but normal IgG and an intact ability to produce antibodies to tetanus toxoid and to multiple *S. pneumoniae* serotypes. The proportion of circulating CD19⁺ B lymphocytes declined over time. The patient was originally given the tentative diagnosis of common variable immune deficiency. More recently, a missense mutation was identified in the patient's *BTK* gene, resulting in a Thr316Ala amino acid substitution in the SH2 domain of BTK. Attempts to demonstrate the disease-causing role of the *BTK* mutation have been inconclusive: the authors found that the mutant protein was expressed at normal levels and underwent tyrosine-phosphorylation regularly. Nonetheless, the patient was given a revised diagnosis of atypical X-linked agammaglobulinemia (XLA). The family history, while partially supportive for this diagnosis, was also atypical. Two maternal uncles had died for unknown infections in their first year of life. Furthermore, the mother and two maternal aunts had a history of recurrent respiratory infections, and developed mild to severe pulmonary emphysema. All these three female individuals were found to be heterozygous for the predicted BTK Thr316Ala mutation. Atypical presentations of XLA are being increasingly recognized. In particular, several patients have been identified with borderline or even normal immunoglobulin serum levels (2,3). In some cases, normal antibody response to protein antigens, but reduced response to polysaccharide antigens, was demonstrated (4). From the clinical point of view, atypical cases may initially present beyond the first years of life, and occasionally even in adulthood (5). In any case, the number of circulating B cells is significantly decreased (<2%) even in males with atypical presentations of XLA. Accordingly, enumeration of circulating B cells is considered of utmost importance in the diagnosis of XLA (6). Very recently, Conley et al. have reported on a family in which a missense mutation in the kinase domain of BTK (Tyr418His) resulted in reduced B cell numbers, but absence of clinical symptoms in one of the mutated individuals (7).

This observation, and the family reported by Graziani et al. in this issue, raise the issues of the pathogenicity of some *BTK* mutations, and how far can we go (in terms of clinical phenotype) in the diagnosis of XLA.

In general, the increasing knowledge in the variability of the human genome has led to the reconsideration for the disease-causing activity of some previously identified nucleotide changes. In this regard, although polymorphisms are defined as variations in the DNA sequence that are observed in at least 1% of the general population, it is obvious that variations with a lower frequency are not of necessity pathogenetic. In particular, rare variants may occur that do not affect substantially expression and function of the gene product. Accordingly, the fact that a specific nucleotide change observed in one affected individual is not detected in 100 chromosomes from unrelated subjects of the same ethnic group is not sufficient to rule out a benign, non disease-causing, effect for that specific change.

Bioinformatics may provide valuable information (8). Several databases have been compiled that collect information on variations within the sequence of genes, and their relationship to disease. The Human Genome Variation Society maintains a comprehensive list of locus-specific databases. Resources such as Ensembl and dbSNP represent important repositories of genome polymorphisms. Definition of the potential impact of single nucleotide variations that result in amino acid changes is based on a variety of arguments, including analysis of the evolutionary conservation of the amino acid residue, and of the structural similarities of differences between the wild-type and the mutated amino acid. Using these and other considerations, a powerful software such as SIFT (Sorting Intolerant from Tolerant), has found that as many as 25% of the single nucleotide polymorphisms listed in dbSNP are likely to affect protein function (9).

Then, what is actually required to define the disease-causing role of novel mutations that result in single amino acid substitution? And, in particular, is the case reported by Graziani et al. really a case of XLA, although atypical?

While a rigorous analysis of the literature (including search for previous reports of the same change in either affected or in healthy individuals) remains necessary, it should be coupled with a similarly strenuous query using currently available human genome databases. In this regard, the Internet may be as important as the collection of publications available at the National Library of Medicine. It is interesting to observe that with one exception (Ala230Val substitution in the SH3 domain) (10), no *BTK* gene polymorphisms have been identified that modify the amino acid sequence of the protein without contributing to immune deficiency.

However, even more important is the attempt to validate or rule-out the disease-causing role of nucleotide changes by means of independent assays, and functional analysis in particular. In the case reported, Graziani et al. have analysed expression of the mutant BTK protein, and its ability to undergo tyrosine phosphorylation at position 551. Both assays gave normal results. However, additional testing could be carried out, such as analysis of calcium flux following B-cell receptor cross-linking, using the patient's B cells, or upon transfection of the mutant protein in the BTK-negative chicken DT-40 cell line (7).

Moreover, the possibility should be considered that other modifying genetic factors contribute to the variability of the clinical severity of XLA (5, 11). In particular, variations in genes that encode for B-cell-intrinsic proteins involved in intracellular signaling might

compensate, at least partially, for BTK deficiency. However, polymorphisms in Tec, another tyrosine kinase that might substitute for BTK, were not found to impact on the immunological and clinical phenotype in patients with *BTK* mutations (12).

Overall, much more than classical presentations, atypical putative cases of human genetic disorders may warrant sophisticated and often laborious and expensive investigations. At the end, there may not be sufficient reward for the investigator, especially if the nucleotide change turns out to be functionally neutral. The need to define the boundaries of human diseases may thus collide with the priorities on the investigator's side. Perhaps, advances in technology will help solve this issue, by facilitating and expediting lower cost analysis of the functional effects of variations in the human genome. By now, cases such as that described by Graziani et al. should be treated with caution and considered as unresolved. At the same time, the case of the asymptomatic 58-year-old male with a *BTK* mutation reported by Conley et al. (7), indicates that the presence of a *BTK* mutation associated with very low proportion of circulating B cells (ie, the current criteria to make a definitive diagnosis of XLA) are not sufficient to warrant aggressive treatment in all cases.

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