The Structural Effect of the E148Q *MEFV* Mutation on the Pyrin Protein: A Study Using a Quantum Chemistry Model

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ABSTRACT: **Background:** Familial Mediterranean fever (FMF) is a recessively inherited disease with a variety of clinical presentations. The disease is associated with mutations in the FMF gene (*MEFV*), which encodes for the pyrin protein. The role of the E148Q pyrin mutation in the FMF phenotype remains inconclusive, and some authors even view it as a disease-insignificant polymorphism. The calculated change imposed by this mutation on pyrin structure may help to understand the role of this mutation

Objectives: To calculate the relative electrochemical effect of the E148Q mutation on the structure of pyrin protein.

Methods: The electronic properties of the wild-type pyrin molecule and its common mutated forms were computed for the full-length molecule and its segments, encoded by exons 2 and 10, using the HyperChem 7.5 program with one of the molecular mechanical methods (MM+). The change in the structure of the molecule, expressed as a change in energy gain, conferred by the mutations was determined.

Results: The E148Q mutation caused deviation from the wild-type pyrin segment encoded by exon 2 by 1.15% and from the whole pyrin molecule by 0.75%, which was comparable to the R202Q mutation and less than the M694V mutation which caused a deviation from the wild-type structure of the whole pyrin molecule by 1.5%.

Conclusions: A quantum chemistry-based model suggests that the structural effect of the E148Q mutation is indeed low but not zero.

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KEY WORDS: E148Q mutation, *MEFV*, pyrin, quantum chemistry model, familial Mediterranean fever

Familial Mediterranean fever is an inherited disorder that is relatively common in Israel. The disease prevails also among Turks, Armenians, Sephardic Jews, and Middle Eastern Arabs, but is rare in other populations. It is characterized by recurrent episodes of fever, accompanied by abdominal, chest or joint pain [1]. The development of AA renal amyloidosis is the most severe complication of the disease, and prior to the colchicine treatment era was a major cause of morbidity and mortality. FMF is caused by mutations in the FMF gene *(MEFV)*, which is composed of 10 exons and encodes pyrin, a protein of 781 amino acids [2]. More than 180 sequence changes have been identified: 70 with known clinical effect and more than 110 with minimal, or without influence on the phenotype. Most mutations are extremely rare [3].

The association between the disease and many FMF gene mutations, such as M694V, M694I and V726A, has been clearly established. However, controversy exists regarding the role of some amino acid substitutions, particularly for E148Q, where glutamine (Q) substitutes for glutamic acid (E). Initially, this sequence variation was described as a disease-causing mutation with low penetrance and mild symptoms, but in more recent studies some investigators found a similar frequency of E148Q among patients and controls and therefore suggested that it is no more than a benign polymorphism [4,5]. Supporting this view is the high prevalence (more than 25%) of the E148Q mutation and the contrasting low rate of FMF in populations in the Far East (Japan, China).

This uncertainty led us to examine the role of E148Q change in FMF, using a chemical rather than clinical or genetic approach, by calculating the relative electrochemical effect of the E148Q mutation on the structure of pyrin protein. Molecular modeling has become a well-established discipline in pharmacological and biological research. It has created new research paths in studies of the molecular basis of the different biological interactions, for instance between drugs and molecules of the living organism [6]. Advances in computer hardware and software are reflected in the rising numbers of medical chemistry publications containing molecular modeling analyses.

MATERIALS AND METHODS

The primary structure of pyrin was obtained from the PubMed site [7]. The computer calculations were made with the HyperChem 7.5 software, using one of the molecular

FMF = familial Mediterranean fever

mechanical methods, the MM+ method, which was developed primarily for the investigation of organic molecules [8]. While the MM+ method is less accurate than other methods of quantum chemistry (ab initio or semi-empirical methods), it is useful as a computational tool for comparing between the electrochemical properties of homological molecules. It computes the electrical power of the molecule, following energy-derived optimization of its three-dimensional conformation. The computer-determined energy of the molecule in its 3D structure is then compared to the energy of the molecule, while in its secondary structure, which is determined by the arithmetic sum of the energies conferred by the amino acids (each amino acid has its own energy value) and the peptic bonds (minus 5 kcal/mol for each bond). The ratio between the energies (3D/2D) implies the energy gain acquired by the tertiary structure of the molecule. In general, mutations are expected to vary the 3D configuration of the studied molecule. This is reflected in a change of the energy gain of the mutated protein, compared to the energy gain computed for the wild-type protein. In this study we determined the effect of the E148Q change on the energy gain of the pyrin molecule.

Figure 1. [A] The presumed 3D α-helix structure of wild-type pyrin calculated by the MM+ method. **[B]** The projected image of the highest occupied molecular orbital (HOMO) of wild-type pyrin (180° rotation of A). The HOMO is the molecular moiety (colored areas) that could act as an electron donor since it is in the outermost layer containing electrons.

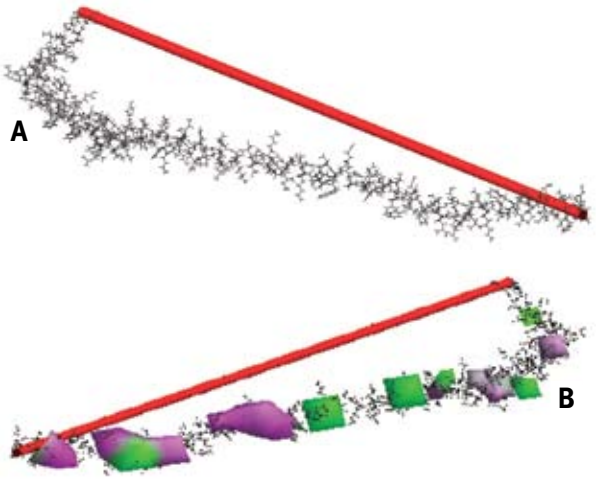

Table 1. Energy gain by the presumed 3D structure of exon 2

	Wild type	E148Q	R202Q
∑ of amino acid energies (kcal/mol)	642.54	635.03	627.41
Energy of peptide bonds (kcal/mol)	-1050	-1050	-1050
Secondary structure (arithmetic energy) (kcal/mol)	-407.46	-414.97	-422.60
Tertiary structure (computed energy) (kcal/mol)	-1413.29	-1422.12	-1426.26
Energy gain	3.47	3.43	3.38
Deviation index (% change from wild type)		1.15	2.59

Table 2. Energy gain by the presumed 3D structure of full-length pyrin

	Wild type	E148Q	R202Q	M694V
∑ of amino acid energies (kcal/mol)	2515.30	2507.79	2500.16	2491.55
Energy of peptide bonds (kcal/mol)	-3900	-3900	-3900	-3900
Secondary structure (arithmetic energy) (kcal/mol)	-1384.70	-1392.21	-1399.84	-1408.45
Tertiary structure (computed energy) (kcal/mol)	-5518.67	-5517.68	-5536.88	-5534.51
Energetic gain	3.99	3.96	3.96	3.93
Deviation index (% change from wild type)		0.75	0.75	1.5

RESULTS

Figure 1 shows a computerized alpha helix model of the wild-type pyrin molecule in its 3D form [A], and notes the highest occupied molecular orbital zones, accounting for the intermolecular interactions that determine the 3D molecule configuration [B]. Of note, the most electron-condensed areas in the HOMO structure are located in the segment encoded by exon 10, which forms the hot spot for the most known pyrin mutations, and therefore might have a detrimental effect on the stability of the molecule.

Table 1 shows the effect of the E148Q mutation on exon 2 configuration. While the energy gain of the 3D structure in the exon 2 of wild-type pyrin is 3.47, the E148Q mutation causes less energy gain, deviating from the wild-type energy gain by 1.15%. Of note, the effect of the E148Q on the electrochemical properties of exon 2 is less than that of the R202Q change considered by most authors to be a polymorphic change.

When compared to the effect of clinically more "severe" mutations, the E148Q mutation seems to have a minor structural impact. The deviation index of exon 10 of pyrin caused by the M694V mutation is 4.64%, by the M680I mutation 4.15%, and by the M694I mutation 4.06%. A less "severe" mutation, for example V726A, affects the molecular structure to a lesser extent with a deviation index of 0.41%, suggesting that the impact of E148Q is nevertheless in the range of mild mutations.

Finally, when the effect of the E148Q mutation is determined for the whole pyrin molecule [Table 2], it seems to cause a mild change in electrochemical energy, almost comparable to that of the R202Q mutation. Of note, even the effect of the M694V mutation seems to be reduced in the context of the whole molecule, when compared to its effect in exon 10 alone (deviation index 1.5% vs. 4.64%)

3D = three-dimensional
HOMO = highest occupied molecular orbital

DISCUSSION

Using a computerized electrochemical model of pyrin, it was possible to determine the structure-modulating effect of various mutations, as reflected by the electrical properties of the molecule. According to this model the impact of the E148Q on the structure of pyrin is indeed low but not zero.

This study supplements the previous debate on the role of the E148Q change with new arguments that support a borderline effect. It concurs with the only mild increase in the rate of this mutation in patients (compared to healthy population), and with the high prevalence of individuals who are homozygous to this change yet experience no clinical symptoms of FMF. Although the clinical consequences are mild when on one or two alleles, this mutation becomes clinically important when it appears as a complex allele (usually combined with the V726A mutation on the same chromosome), or when it shares a genotype with M694V mutation in the other chromosome.

The validity of our results stems mainly from the positive correlation between the electrical and the clinical influence of the examined four mutations, although functional implications of structural changes are of restricted value. The limitation of the study is that it ignores the effect of the fluid interphase and the electrical interactions of pyrin with other molecules in its vicinity. However, the impact of this limitation is minimal due to the homology of the compared molecules

In conclusion, based on the present study it appears that the E148Q variation is associated with a mild effect on the structure and perhaps the function of the pyrin.

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