

## Hidden chaperonopathies: Alerting physicians and pathologists on the possibility that uncharacteristic, baffling clinical features in otherwise known diseases may be due to failure of the chaperoning system

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### Summary

Inherited metabolic syndromes caused by enzymatic deficiencies are typically detected through newborn screening. In most cases, a mutation in the gene encoding the defective enzyme is the pathogenic factor. However there are cases, the frequency of which is currently unknown but it can be assumed to be considerable, in which a defect in the chaperoning system contributes to disease development. The chaperoning system is responsible for the correct folding of enzymes and for maintaining their functional configuration and assembly but when a chaperone is defective the pertinent enzyme is totally or partially inactive. It is now known that in some metabolic syndromes the enzymopathy is caused by chaperone deficiency; it is in fact a genetic chaperonopathy with the gene encoding the enzyme being normal but its product is abnormal due to improper chaperoning. Likewise, cases of autoinflammation caused by mutation of the gene encoding the anti-inflammation protein pyrin can be complicated by a concomitant mutation in the gene encoding the ER chaperone TRAP1. This is an example of diseases considered

monogenic that can in fact be sometimes digenic: a chaperonopathy acts as a second pathogenic factor, making the clinical-pathological picture very severe. These chaperonopathies can be classified as hidden because they are overshadowed by a clinical-pathological picture characteristic of a known condition typically not linked to a failure of the chaperone system. Physicians and pathologists should be on the alert toward diagnosing chaperonopathies because patients will greatly benefit by proper treatment, including chaperonotherapy.

### Introduction

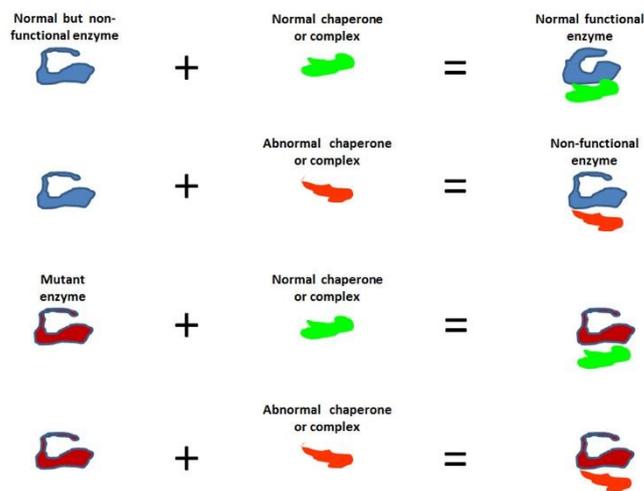
Molecular chaperones, the main components of the chaperoning (chaperone) system, are typically cytoprotective but, if abnormal, they can cause diseases, the chaperonopathies (Macario and Conway de Macario, 2005; Macario et al., 2013; The Chaperonopathies Website). Many chaperonopathies are unseen, i.e., undiagnosed, simply because physicians and pathologists are unaware of their existence since they are not yet taught in Medical schools. In addition, some chaperonopathies are ignored because the clinical picture and laboratory analysis results are

for the most part typical of a known disease traditionally not associated with failure of the chaperone system. However, in many of these cases, a defective chaperone may contribute to the pathogenic mechanism in addition to the pathogenic role of another (the known cause) non-chaperone molecule, which is also defective. These conditions may be said to be complicated by hidden chaperonopathies and have been found, for example, in patients with autoinflammation or with metabolic diseases. The latter are typically associated with failure of a protein enzyme due to mutation. The consequences of the enzyme deficiency and the detection of a mutation in the gene encoding the enzyme, usually overshadow the underlying chaperonopathy. When the clinical and pathological features do not completely match the typical manifestations of the enzymopathy and/or an unexpected resistance to an established treatment known to be efficacious is observed, the assumption can be made that another etiopathogenic factor is involved. This factor may turn out to be a defective chaperone, a chaperonopathy. In these situations, the abnormal chaperone may be said to lie down, hidden as it were eluding detection unless the clinician thinks of it and proceeds to investigate its presence.

## Enzymopathies

The clinical picture of an enzymopathy is characteristically associated with a defect in the affected enzyme molecule and this defect is typically the consequence of a mutation that changes the structure-function of the enzyme. However, a similar or identical clinical picture may be caused by a defect of the chaperone, or chaperone complex, whose tasks are to ensure that the enzyme reaches its native conformation after synthesis and to maintain it throughout the cell's life. This kind of chaperone-related enzymopathy can occur in two varieties: in one the enzyme is normal but cannot function correctly because of a failure of the pertinent chaperone or chaperone complex and in another,

both, the enzyme and the pertinent chaperone are structurally-functionally defective. These possibilities are schematically outlined in Figure 1.



**Figure 1. Schematics of the role of the chaperoning system in enzyme biology and pathology to illustrate the pathogenic impact of defective chaperones.** **Top row:** a normal, newly synthesized, immature enzyme requires the participation of the chaperoning system to integrate with its non-protein co-factor and to achieve its native, functional configuration, including a quaternary structure if pertinent. **Second row:** if a component of the chaperoning system required by the enzyme to achieve functionality is defective, e.g., a mutation in a chaperone that severely impairs or abolish its function, an enzyme deficiency will occur. **Third row:** Typical enzymopathy caused by a mutation of the enzyme itself that cannot function even in the presence of a normal chaperoning system. **Fourth row:** Compound enzymopathy in which the enzyme and its corresponding chaperone are both defective.

It can be seen that the enzyme's failure is the dominant common factor in the clinico-pathological picture, but the underlying mechanisms are different.

Thus, a proper approach to the understanding, diagnosis, and treatment of metabolic diseases caused by enzymopathies must consider the chaperoning system in addition to the enzymes. Each enzyme usually interacts with non-protein co-factors to form the enzymatic complex that is functionally active. Enzymes depend for correct

folding, integration with co-factors, and functionality on one or more members of the chaperoning system. Therefore, it is no surprise that failure of a member of the chaperoning system may cause an enzymopathy.

### **The chaperoning (chaperone) system and chaperonopathies**

The chaperoning (chaperone) system of an organism is constituted of the entire set of chaperones, co-chaperones, and chaperone co-factors, and their interactors and receptors (Macario and Conway de Macario, 2019). It is a complex system with multiple components, whose exact composition may vary in the various cell types, but it is always central in protein homeostasis and, consequently, it is essential for the maintenance of the correct structure and functionality of enzymes. Therefore, failure of a component of the chaperone system, i.e., a chaperonopathy, in any given cell type may lead to failure of an enzyme, i.e., an enzymopathy.

### **Hidden chaperonopathies**

Examples of hidden chaperonopathies are varied but we will here consider only a couple, which are illustrative.

#### *Inborn errors of metabolism*

Inborn errors of metabolism are metabolic disorders associated with enzymopathies caused by pathogenic mutations in the abnormal enzymes. A common example, phenylketonuria (PKU), is usually detected through newborn screening and is characterized by hyperphenylalaninemia (HPA). The majority of cases with HPA harbor mutations in the gene encoding the enzyme phenylalanine hydroxylase (PAH), but about 2% of patients have tetrahydrobiopterin (BH4) deficiency and show also neurotransmitter (dopamine and serotonin) abnormalities. The typical clinical manifestations are progressive neurodevelopmental delay, dystonia, and a unique profile of neurotransmitter deficiencies. However, there

are patients with these signs and symptoms but do not have PAH mutations or BH4 deficiency (Anikster et al., 2017; Blau et al., 2018; Veenma et al., 2018; Feng et al. 2019). In these cases, the pathogenic mutations occurred in the gene encoding the co-chaperone DnaJC12. This protein is a member of the chaperoning system known to interact with other members of this system to assist nascent polypeptides, e.g., a “young” enzyme, in their maturation toward a fully functional protein (an enzyme in its native conformation). For instance, DnaJC12 participates in the maturation of phenylalanine, tyrosine, and neuronal tryptophan hydroxylases. These enzymes are all seriously affected when DnaJC12 is defective and their enzymatic activity is greatly reduced or totally lost, which leads to HPA and neurotransmitter deficiency.

In summary patients with DnaJC12 pathogenic mutations present hyperphenylalaninemia accompanied by global developmental delay, movement disorder, cognitive dysfunction, and autism spectrum disorder.

#### *Autoinflammation*

A hidden chaperonopathy has also been implicated in the syndrome of autoinflammation. This syndrome is typically associated with the malfunctioning of pyrin (also known as marenostriin) coded by the MEFV1 (Mediterranean fever) gene. Pyrin plays a critical role in maintaining the level of inflammation in the body within the normal range but if mutated autoinflammation soars causing disease. The protein TRAP1 (tumor necrosis factor receptor associated protein 1), a chaperone that resides in the ER (endoplasmic reticulum), was found mutated and contributing to the pathogenesis in three patients carrying the MEFV p.S208C mutation, which is considered the typical genetic abnormality causing this disease (Standing et al., 2019) These three patients showed very severe clinical manifestations. Initially, the excessive severity of the disease in these patients, far greater than in the typical cases of MEFV p.S208C mutation, suggested that there was an additional pathogenic factor. The fact that another,

unrelated, patient with autoinflammation carried a mutation of TRAP1 but no mutation of MEFV was identified, alerted the investigators to the possibility that the three very severe cases of the disease might carry not only the expected mutation in the MEFV gene but also a mutation in the TRAP1 gene. Therefore, they proceeded to search for the TRAP1 mutation in the DNA of the three patients and found it. TRAP1 failure causes severe cellular stress with increased levels of the pro-inflammatory cytokine IL-18.

## Conclusions and perspective for the future

The main conclusions are that: a) in some enzymopathies, the gene encoding the enzyme is normal but the latter is inactive because of lack of proper support by the chaperone in charge of its folding and assembly; and b) a disease known to be monogenic may in some patients be digenic with a mutant chaperone being involved in the pathogenic mechanism. With this idea in mind, physicians and pathologists ought to be able to look for, and diagnose, many chaperonopathies previously ignored or misdiagnosed. This new diagnostic attitude and clinical alertness will certainly improve knowledge of pathogenic mechanisms and histopathological lesions as well as enlarge the number of patients benefiting from adequate treatment that should include chaperonotherapy (Macario and Conway de Macario, 2020).

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