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Keshan disease and mitochondrial cardiomyopathy*

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Abstract Keshan disease (KD) is a potentially fatal form of cardiomyopathy (disease of the heart muscle) endemic in certain areas of China. From 1984 to 1986, a national comprehensive scientific investigation on KD in Chuxiong region of Yunnan Province in the southwest China was conducted. The investigation team was composed of epidemiologists, clinic doctors, pathologists, biochemists, biophysicists and specialists in ecological environment. Results of pathological, biochemical and biophysical as well as clinical studies showed: an obvious increase of enlarged and swollen mitochondria with distended crista membranes in myocardium from patients with KD; significant reductions in the activity of oxidative phosphorylation (succinate dehydrogenase, cytochrome oxidase, succinate oxidase, H^+-ATPase) of affected mitochondria; decrease in CoQ, cardiolipin, Se and GSHPx activity, while obvious increase in the Ca^{2+} content. So, it was suggested that mitochondria are the predominant target of the pathogenic factors of KD. Before Chuxiong KD survey only a few cases of mitochondrial cardiomyopathy were studied. During the multidisciplinary scientific investigation on KD in Chuxiong a large amount of samples from KD cases and the positive controls were examined. On the basis of the results obtained it was suggested that KD might be classified as a "Mitochondrial Cardiomyopathy" endemic in China. This is one of the achievements in the three years' survey in Chuxiong and is valuable not only to the deeper understanding of pathogenic mechanism of KD but also to the study of mitochondrial cardiomyopathy in general.

Keshan disease is not a genetic disease, but is closely related to the malnutrition (especially microelement Se deficiency). KD occurs along a low Se belt, and Se supplementation has been effective in prevention of such disease. The incidence of KD has sharply decreased along with the steady raise of living standard and realization of preventive measures. At present, patients of KD are very sparse. In recent years the research on the non-KD mitochondrial cardiomyopathy has progressed rapidly. Given the advances in this aspect a minireview is written to evaluate the classification of KD as a kind of mitochondrial cardiomyopathy.

Keywords: mitochondrial disease, Keshan disease, mitochondrial cardiomyopathy.

1 Mitochondrial disease

Mitochondrion is an important organelle in eukaryotes and can be regarded as the "power plant" within the cell. At mitochondrial inner membrane the final metabolic reactions of cellular respiration after glycolysis and the citric acid cycle proceed. The whole

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process is called "Oxidative Phosphorylation", through which the phosphorylation of ADP to ATP driven by the transfer of electrons to oxygen in the respiratory chain occurs. Oxidative phosphorylation consists of five enzyme complexes: Complex I (NADH: ubiquinone reductase), Complex II (succinate: ubiquinone reductase), Complex III (ubiquinoneH_2: cytochrome c reductase), Complex IV (cytochrome c oxidase) and Complex V (ATP synthase). Complexes I — IV constitute the electron transfer chain that generates a proton gradient across the inner membrane used by complex V to drive ATP synthesis (Fig. 1).

The first case of mitochondrial disease was demonstrated by Luft and his coworkers in 1962 at the Karolinska University in Sweden[1]. A 35-year-old woman presented a clinical picture characterized by profuse perspiration, polydipsia, thinness despite polyphagia and muscular wasting and weakness. However, her thyroid function was normal. Electron microscopic examination of the patient's skeletal muscle revealed an increased amount of mitochondria in certain regions of the skeletal muscle, as well as a vast amount of densely packed cristae. Biochemical studies with patient's isolated skeletal muscle mitochondria showed a loosely coupled state of the oxidative phosphorylation, characterized by a nearly maximal rate of respiration in the absence of phosphate acceptor. It is easier to get sufficient material from skeletal muscles, so more studies were carried out on mitochondrial myopathy than on mitochondrial cardiomyopathy. In recent years the spectrum of mitochondrial diseases has been expanded, including Leber's herediary optic neuropathy (LHON)[2], myoclonic epilepsy[2], neurodegenerative diseases (Parkinson's, Alzheimer's and Huntington's diseases)[3—5], diabetes[4,5] and even cancer[6].

Mitochondria, a kind of semi-autonomous organelles, contain their own DNA (mtDNA). Human mtDNA is a circular double stranded molecule. Being about 16.6 kb long, it is much smaller than most nuclear genes. mtDNA encodes 22 transfer and two ribosomal RNAs and for 13 peptides (7, complex I; 1, complex III; 3,complex IV, 2, complex V (see Fig. 1)). It has been known that over 80 different polypeptides

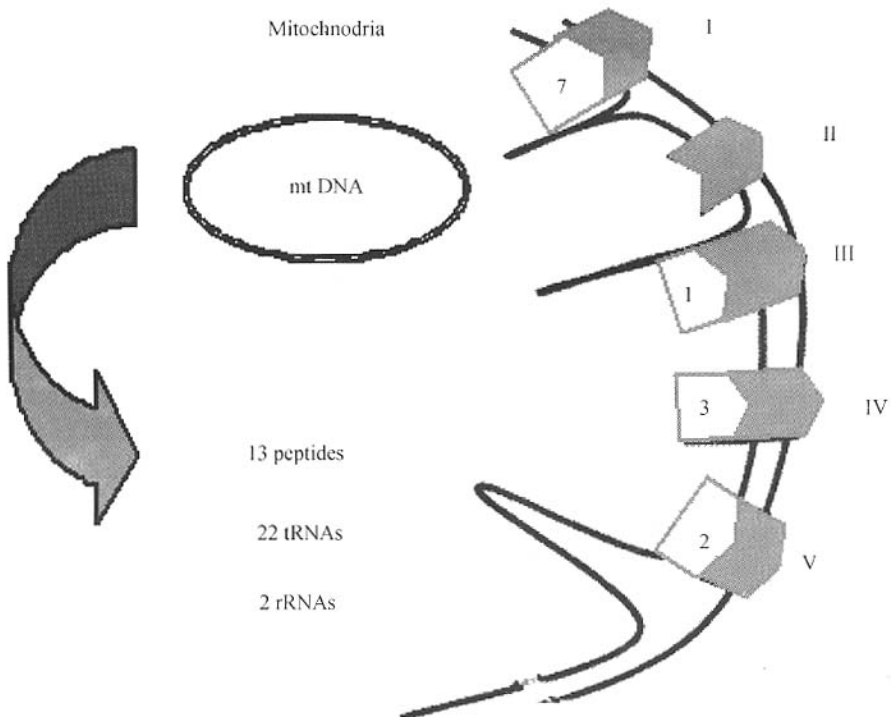

Fig. 1. Human mitochondrial DNA (mt DNA) encodes for 13 peptides of the oxidative phosphorylation system of mitochondria. 7, Complex I; 1, complex III; 3, complex IV; 2, complex V. tRNA, transfer RNA; rRNA, ribosome RNA.

interact on the mitochondrial membrane to form the respiratory chain. The vast majority of subunits are synthesized within the cytosol from nuclear gene transcripts but 13 essential subunits are encoded by the mtDNA.

Mitochondrial synthesis and function require approximately 1000 polypeptides, 13 of which are encoded by mtDNA, and the rest by nuclear DNA, in other words, some 99% of human mitochondrial proteins are encoded in the nucleus; all the proteins and other molecules required to build mitochondria are synthesized in the cytoplasm, then imported into the organelle.

Unlike nuclear genes, which are wrapped in protective histones, mitochondrial genes are vulnerable to be attacked from highly reactive superoxides, which are generated during electron transfer along the respiratory chain. In mammals, the mutation rate of mitochondrial genes is 10 to 20 times higher than that of the nuclear genes[4]. The determination of the sequence of human mtDNA was accomplished by Sanger's Lab in 1981[7]. The molecular age of mitochondrial diseases began in 1988, with the report of the first pathogenic mutations in mtDNA, large-scale single deletions in patients with mitochondrial myopathies[8] and a point mutation in the gene encoding subunit ND4 of complex I in a family with Leber's herediary optic neuropathy (LHON)[9]. Since then, "mtDNA fever" has appeared to search for new mtDNA pathogenic mutations[10]. Over 100 point mutations of mtDNA associated with human diseases have been reported in protein coding genes, tRNAs, and rRNAs[11]. But the link between genotype and phenotype in mitochondrial diseases is rather complex[5]. The same phenotype can be caused by several different mutations, and the same mutation can result in multiple phenotypes[5]. Besides, human mitochondrial diseases encompass mutations of mtDNA and nuclear DNA as well as harmful factors-induced defects[4,5]. Several diseases have now been shown to be due to mutations in nuclear genes encoding mitochondrial proteins. In recent years the research on this aspect has progressed rapidly, but much still remains to be learnt and elucidated.

2 Keshan disease and mitochondrial cardiomyopathy

Keshan disease (KD) is a potentially fatal form of cardiomyopathy endemic in certain areas of China. Its clinical features are acute and chronic episodes of heart disorder characterized by hard breath, arrhythmia, ECG changes and even cardiogenic shock. In 1935 there was a violent break-out of this disease in Keshan County, Heilongjiang Province, northeast China, and the disease was named after this county. Most of the susceptible population were children and women of breeding age in rural areas, covering 14 provinces and autonomous regions. The incidence varied from year to year: more than 8000 people were affected in each of the three peak years (1959, 1964 and 1970). Four types of KD were found: acute, subacute, chronic, and latent. The basic pathological change was multiple areas of myocardial necrosis and myocardial scar formation. The incidence of KD has sharply decreased after 1980 along with the steady raise of living standard and realization of preventive measures.

From 1984 to 1986, a national comprehensive scientific investigation on KD was conducted in Chuxiong Region of Yunnan Province in the southwest China, where the average annual morbidity was 9.93/100,000[12] between 1974 and 1983. The comprehensive scientific team was composed of epidemiologists, clinic doctors, pathologists, biochemists, biophysicists and specialists in ecological environment.

One of the research projects of our scientific survey focused on the pathological changes of mitochondria in myocardium of patients with KD. Before this, the abnormality of morphology and respiratory enzymatic activities of heart mitochondria from patients with KD had been reported by Chinese scientists[13,14]. In the course of Chuxiong's survey, large-scale investigations were performed in this aspect, and results were obtained as follows:

(1) Pathological studies[15]

Fine structural investigation disclosed an obvious increase of enlarged and swollen mitochondria with distended crista membranes. It might be a consequence of a functional defect with compensatory hyperplasia of mitochondria, which seems to occur at

the earlier stages of pathogenesis of KD. An abnormal amount of moderately electron dense amorphous inclusions were observed in matrix under an electron microscope. Using X-ray microanalysis and exposure to protein digestion reagent it was demonstrated that these inclusions are not $Ca_3(PO_4)_2$, but are probably proteinaceous in nature.

Enzyme-histochemical analysis showed that the enzymatic activity of succinate dehydrogenase and cytochrome oxidase of heart mitochondria from patients with KD were both decreased.

(2) Biochemical and biophysical studies[16—18]

Biochemical analysis demonstrated significant reductions in the activity of oxidative phorphorylation (succinate dehydrogenase, cytochrome oxidase, succinate oxidase, H^+-ATPase) of affected mitochondria. A decrease in CoQ and cardiolipin content has also been demonstrated. Affected mitochondria had markedly decreased the selenium content and enzymatic activity of Se-content enzyme — glutathione peroxidase(GSHPx), while the Ca^{2+} content was obviously increased. It was interesting to note that the lipid fluidity of the affected mitochondria was obviously lower than that of control.

(3) Clinic studies[19]

It was reported that coenzyme Q10 could improve the cardiac function in patients with KD.

All the samples were myocardial tissues (left ventrile) isolated at postmortem. Autopsies were performed within 5 h of death and the tissue was stored in liquid N_2 before use. The experimental samples were from 19 patients with Keshan disease (11 subacute, 8 chronic) in Chuxiong Region. The control samples were collected from healthy victims of accidents in non-endemic areas also within 5 h after death and treated with the same procedure.

It was clearly shown that the results obtained from the three aspects of study on the myocardial mitochondrial changes of patients with KD were highly consistent with each other. So, it was suggested that mitochondria are the predominant target of the pathogenic factors of Keshan disease and Keshan disease may be classified as a "Mitochondrial Cardiomyopathy" endemic in China[20].

Before Chuxiong KD scientific survey (1984—1986) the study on the mitochondrial cardiomyopathy was rather sparse. In 1982 Grantzow *et al*.[21] found an extreme increase of often abnormally structured and enlarged mitochondria in the heart muscle cells from a 21-month old infant girl at postmortem. Bogousslavsky *et al*.[22] reported in the same year a case of ragged-red fiber myopathy with abnormal mitochondria also present in the heart. In both cases only morphological studies were carried out. During the multidisciplinary scientific investigation on KD in Chuxiong Region a large amount of samples from KD cases and the positive controls were examined. On the basis of the results obtained it was suggested that KD might be classified as a form of 'mitochondrial cardiomypathy' endemic in China. This is one of the achievements in the three years' survey in Chuxiong and is valuable not only to the deeper understanding of pathogenic mechanism of KD, but also to the study of mitochondrial cardiomyopathy in general.

After the accomplishment of the scientific investigation in Chuxiong in 1986, the incidence rate of KD was further strikly decreased. Of course we are very glad about this, but on the other hand, the research on the pathogenic mechanism became quite difficult due to the limited number of patients with KD and unsuccessful establishment of an ideal animal model.

In 1988 two laboratories [8,9] reported separately that pathogenic mutations in mtDNA were associated with various forms of mitochondrial disease. Since then the study of mitochondrial disease, including non-KD mitochondrial cardiomyopathy, has been strikingly strengthened[4,5,10,23,24]. However, as mentioned above, it would be problematical if mtDNA mutation was suggested as a basic feature of mitochondrial disease[5,23,25]. The mitochondrial genome is highly polymorphic and not all base changes, therefore, will be pathological. To complicate this further, individual mutations can cause very different phenotypes. Given the defective respiratory chain or oxidative phorphorylation is a common feature of mitochondrial cardiomyopathy, Marin-Garcia and Goldenthal[24] suggested in 1997 that "mitochondria cardiomyopathy can be defined as an oxidative phosphorylation disease characterized by abnormal cardiac mitochondria either in number, structure or function". These criteria for defining "mitochondrial cardiomyopathy" coincided with that we suggested in 1986 that Keshan disease be

classified as a form of "Mitochondrial Cardiomyopathy"[17,20].

Keshan disease is not a genetic disease, but is closely related to the malnutrition (especially microelement Se deficiency). KD occurs along a belt where the Se content of soils and food is low[26] and Se supplementation has been shown to be effective in preventing such a disease[27,28]. This conclusion was further confirmed through Chuxiong scientific investigation[29]. It has been generally accepted that a major Se function involves glutathione peroxidase (GSHPx) in maintaining the integrity of biomembranes and in preventing oxidative damage by removing superoxides. And superoxides are formed along with the electron transfer in the respiratory chain of mitochondria. Mitochondrial dysfunction enhances the generation of superoxides, resulting in damage of mitochondrial membranes and mtDNA. Moreover, *in vitro* experiments have shown that Se could stabilize directly the membrane and membrane skeleton of human erythrocytes[30,31]. So, it is likely that the abnormalities of the structure and function of myocardial mitochondria from patients with KD may be brought about as a consequence of Se deficiency[16]. However, it was also reported that Se deficiency is a necessary but not sufficient factor required for pathogenesis of Keshan disease[32]. In the 1960s Chinese scientists suggested that Coxsackie viruses might be involved in KD[32]. Though further studies on this aspect have been carried out in several labs in recent years[33,34], the pathogenic factors of KD are not well understood yet. Now, patients of KD are rare, so the establishment of an ideal animal model of KD has become the most urgent for further study on both the pathogenic factors and pathogenic mechanism of KD.

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