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Case Report Open Access

Gene Mutation in Tuberous Sclerosis Complex (TSC) and Reversal Treatment of the Mal-formation in Collagens Synthesis: Treatment of Angiofibromas via L-Lysine Therapy

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Abstract

Angiofibromas are malformation of skin caused by genetic disorders and gene mutation. The purpose of this case study is to find a natural and cost effective solution to overcome the formation and proliferation of angiofibroma structures. At the present time there no simple treatment is available for angiofaibromas and the only available techniques involve the use of carbon dioxide and laser. Besides being costly these techniques need repetitive treatments over a long time and no guarantee of permanent removal of these angiofaibromas. They seem to reform in the same location and in some cases may extend to the surrounding area. The manifestation of these, in the facial area, is in addition to the disfiguring of the face cause health problems as a result of bleeding and possible infection. These angiofaibromas with sizes ranging from granules on the micrometer size to larger ones with diameters on the millimeter scale. They are being reddish to brown, depending on the amount of blood vessels and vacuolated blood. Why are angiofibromas developed around the onset of puberty and accompanying hormonal change? What are the physiological changes that occur at this age? Are they associated with skin and protein synthesis and are conducive to the development of angiofibromas?

Because of the fact that these are considered to be genetics the only treatments proposed and performed by medical-doctors only symptomatic treatments. In this paper we propose a treatment of the actual protein synthesis and an enhancement of the production of the proper collagen protein to sharply minimize the formation or may be in some cases totally eradicating the angiofaibromas or in the worst scenario just decrease of blood vacuoles supplement and hence shrinking the fibromas. Results presented here are based on case studies and are very encouraging to perform clinical testing on a larger scale, i.e., a larger group of patients younger than 20 years of age. The limitation of such study is defined under testing patients who do not have any angiofibromas but who are likely to develop angiofibromas. We did not have this kind of sample in the group, which would strengthen the results by providing a baseline on the effectiveness of L-Lysin in inhibiting the initial development of the facial fibromas. In future study this could be compensated for by conducting some tissue culture and monitoring the effect of various amino acids on normal cells vis-à-vis mutated cell lines.

This type of research is very crucial for both researchers and consumers as it emphasizes the concept of simplicity in prevention and treatment. Angiofibromas are also caused as side effects of some seizure medications such as phenytoin and the results are devastating for the patients. We hope that maybe some will benefit from this research by following healthy diets with regard to the inclusion of essential amino acids in a healthy diet especially that of L-Lysine.

Keywords: Gene Mutation; Tuberous Sclerosis Complex; Collagens Synthesis; Treatment of Angiofibromas; L-Lysine Therapy; Angiofibroma Structures; Dietary Supplements; Phenytoin; Skin Lesions

Introduction

Tuberous sclerosis complex (TSC) is a disease that is inherited in an autosomal dominant pattern. The rate of mutation is very high and mutation rate reached the 50 percent mark based on the number of cases being classified as caused by mutation. Patients could express a broad spectrum of manifestations ranging from epilepsy, facial angiofibromas, to mental retardation. Tumors could show up in many organs starting with those under toe or figure nails to the ones that affect kidneys, heart, lungs or the eye retina.

Cortical tubers are most common and so are the giant cell astrocytomas. Spots of hypomeanotic appearance or what is known as the Fitzpatrick patches are used as part of the positive diagnosis of the TSC [1-10].

It is found that TSC is caused by a mutation in either TSC1 or TSC2 genes. Both genes are tumor suppressor genes. TSC1 is located on chromosome 9 and is responsible for the encoding of the protein hamartin. TSC2 is located on chromosome 16 and is responsible for the encoding of tuberin. It is believed that these proteins, although not yet fully understood, may function as tumor suppressors. Tuberin and hamartin seem to play a role in cell growth and differentiation. These proteins are found throughout the body and exhibits interaction with each other [11]. Based on this information it seems that a futuristic treatment of TSC should thus involve a process that bypasses these

dysfunctional genes such as overwhelming the genes into proper production of hemartin and tuberin.

In our study we focus on the possible reversal of the formation of angiofibromas which could also lead to shedding light on the cause of other skin lesions. Ash-leaf spots, shargreen patches, fibrous plaques, periungual fibromas, and confetti lesions.

Abnormal collagen and blood vessel accumulation are the main cause of the fibromas, patches and plaques whilst the defective transfer of melanin results in the formation of ash-leaf spots. Besides laser treatment for minimizing facial angiofibromas, there is also an ongoing research with rapamycin [12].

Earlier human trials have shown that rapamycin (Figure 1) can have serious side effects as it is an immunosuppressant. It is found that rapamycin users are more susceptible to various infections. It has also been linked to hyperlipidemia which can lead to heart diseases. Do these problems counteract the benefit of facial treatment suggested by rapamycin?

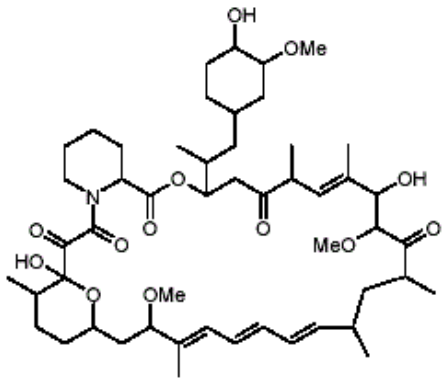

Figure 1: Rapamycin (an immunosuppressant).

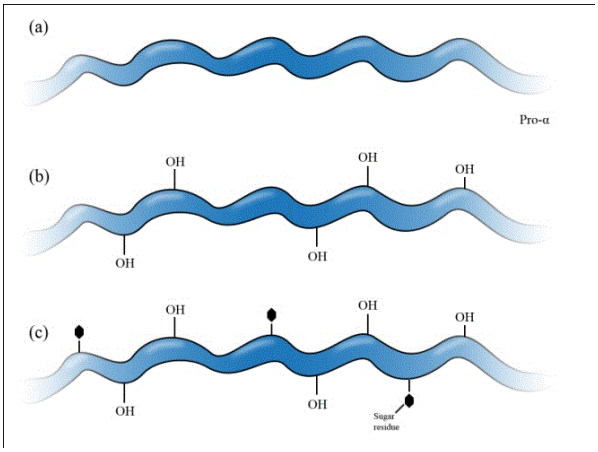

Figure 2: Normal process of hydroxylation of Lysine residues and the later glycosylation as a part of collagen synthesis.

Our research will focus on the aspect and possible treatment of the facial angiofibromas. We will be presenting an analysis of the formation and distribution of the various collagens. While collagens are processed in the endoplasmic reticulum they are found to also undergo post-translational modifications specifically the hydroxylation of certain proline and lysine residues. Glycosylation of some hydroxylysines follows prior to the final folding of the protein into the native triple-helix. Proteins are later exported to the Golgi complex. If mutated polypeptides are accumulated into the endoplasmic reticulum they could be exposed to over hydroxylation or over glycosylation as shown for procollagens I [13,14] (Figure 2-4).

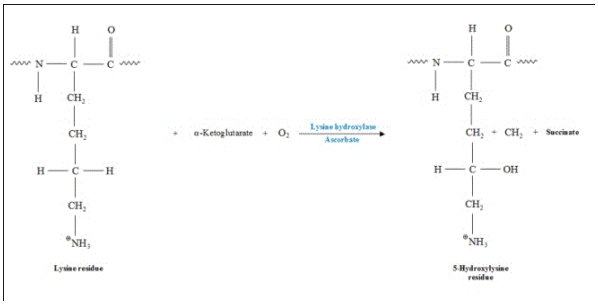

Figure 3: Normal process of hydroxylation of Lysine residues a part of collagen synthesis.

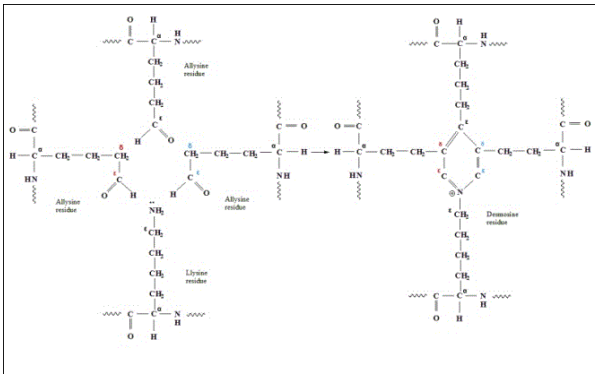

Figure 4: Elastin formation via the cross-linking process. Condensation reaction involves three allysine residues and one lysine producing desmosine.

There are known cases where one amino acid substitution is better tolerated in some proteins than in others. For example a glycine substitution in fibrillar collagens leads to the production of structurally altered polypeptides, whilst a glycine substitution in collagen XVII leads to a milder alteration in protein function. A glycine substitution in Col15 domain seems to interfere with the structural formation of the triple-helix and with ectodomain shedding of the collagen XVII. These structural changes then are the lead cause of skin blistering [14,15].

Case Study

The possible causes of this mal-synthesis or deposition of collagens are believed to be one or a combination of:

Genetic code mutation where the code of the amino acid lysine is mutated to possibly glutamine or a STOP code

Defective hydroxylation process of lysine or proline

Decreased efficiency of the use of available essential amino acids such as Lysine

Any of the three listed possibilities or a combination of them will lead one to speculate that if the level of Lysine is dramatically increased, it will cause a reverse of the damaged skin and eliminate new formation of angiofibromas.

An anatomical examination reveals that angiofibromas are tissue-free except for the very fine skin where more than 90% constitutes blood vacuoles [16]. The membranous-skin structure is seemingly caused by a higher plasticity of the skin as a result of protein malformation. If one causes modifications to decrease the elasticity of this skin then it will cause a skin tightening and decrease the size of the blood vacuoles and eventually the angofibromas will turn into skin lesions similar to the shagreen patches except for they are smaller in size. This works very well with the smaller angiofibromas. More testing is needed to be conducted to study the effect on other types of skin lesions including hypopigmented macules, shagreen patches and ungula fibromas.

Treatment and dosage

This amino acid therapy is built upon the very "raw materials" that build and rebuild the connective tissue destroyed as a result of the mutated protein synthesis process. Lysine treatment results in healing and rejuvenating the tissue-angiofaibromas. The demand for effective treatment that is also natural is more of a miracle. Laser treatment against angiofaibroma deals only with symptoms [17]. Additionally, laser is costly and requires many sessions and yet could not be used on tumors that have blood vessels. L-lysine is an amino acid that cannot be synthesized in the body and is usually ingested through consumption of high protein foods. Children with TSC, in general, have problems with dental ability to consume foods that require chewing thus is very likely to have deficiency in L-lysine.

The subjects are two females 17 years of age both exhibit moderate manifestation of TSC with mild facial angiofibromas in one case (A) and the second case has medium to severe facial angiofibromas (B) (Figures 5A-5C).

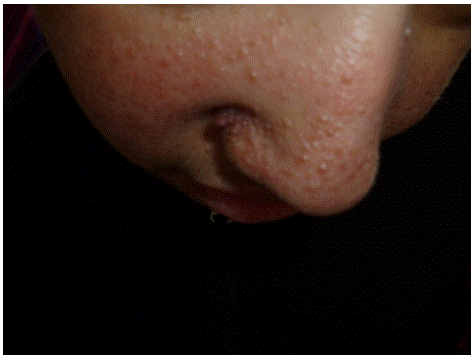

Figure 5A: Mild case of angiofibromas with very few blood vessels; nearly disappearing after 32-week administration of 1000 mg L-Lysine per day with 1500 mg dosages for the first 4-weeks of treatment. The results of the treatment are best in mild cases with regard to reversal of the angiofibromas structures.

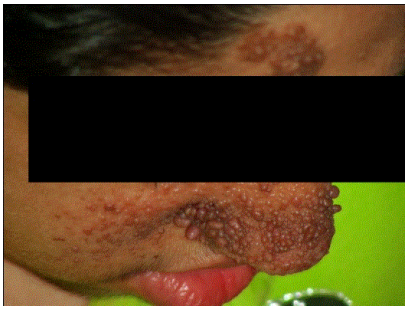

Figure 5B: Severe case of angiofibromas diminishing in size after a 4-week administration of 1500 mg L-Lysine per day. This is a 50% decrease in number and size of angiofibromas with inhibition of any new formation of angiofibromas.

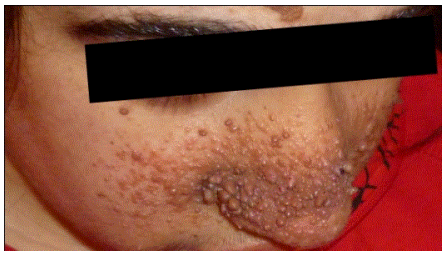

Figure 5C: Very slow progress is noted after 32-week of treatment in addition to the important fact that no new formation is noticed.

A dosage of 500 mg of Lysine was administered orally three times daily. Dosage can be up to 30 mg/kg or up to 3000 mg per day (average dose is at one gram/day).

Within the first week of treatment, angiofibromas was noticed to start shrinking in size and the smallest ones became part of the facial skin but not as smooth, more like pumps.

By the end of the third week of administering lysine orally, a 20% decrease in sizes of the angiofibromas was observed. After 16 weeks, the patient with mild angiofibromas shows a 90% disappearance of the fibromas and no formation of new ones. For severe angiofibromas, progress of treatment seems slow, however, angiofibromas shrunk in size, no new formations of fibromas, and the area with smaller sizes were cleared. The treatment continues to work even after the 16 week-period of treatments but at slow pace. This is due to massive build-up of blood vessels in these tumors.

Discussion

The decrease in size of the angiofibromas took place during the initial phase of treatment or the first three weeks. During the next two phases the improvements were slow and by the end of the fourth week the angiofibromas seem to lose the vesicular structures and turned into mere rough structures leveling with the normal skin. By the end of week six, most of them took the normal color of the skin with very few remain brown. At this stage all the small angiofibromas are only visible when inspected very closely.

The genetic code for lysine is (AAA) or (AAG); the code for Glutamine is (CAA) or (CAG); the STOP code is (UAA) or (UAG). This close similarity in the genetic code provides a possible explanation for either a STOP code or a deletion of Lysine and its replacement by a Glutamine. An insertion of a STOP or deletion of a Lysine will result in the disruption of the triple helix of collagen. Healthy skin is known to contain a larger percentage of collagen III. Synthesis of collagen III requires a high percentage of lysine and proline as well as a later hydroxylation to convert lysine into hydroxyl lysine. Wild type collagen III contains 25% of hydroxyl proline and hydroxyl lysine, there are no genetic codes for these and hydroxylation takes place after synthesis of the protein (Figure 6).

HBB Sequence in Normal Adult Hemoglobin (Hb A):

Nucleotide	CTG	ACT	CCT	GAG	GAG	AAG	TCT
Amino Acid	Leu	Thr	Pro	Glu	Glu	Lys	Ser
	|			|			|
	3			6			9

HBB Sequence in Mutant Adult Hemoglobin (Hb S):

Nucleotide	CTG	ACT	CCT	GTG	GAG	AAG	TCT
Amino Acid	Leu	Thr	Pro	Val	Glu	Lys	Ser
	|			|			|
	3			6			9

Figure 6: Example of mutation in one genetic amino acid code and the effect on protein structure such is the case of hemoglobin sickling.

Normal Collagens Synthesis:

Nucleotide	xxx	AAA	xxx
or	xxx	AAG	xxx
Amino Acids	aa	Lys	aa

Mutated Collagens Synthesis:

Nucleotide	xxx	CAA	xxx
or	xxx	CAG	xxx
Amino Acids	aa	Gln	aa

Mutated(2) Collagens Synthesis:

Nucleotide	xxx	UAA	xxx	
or	xxx	UAG	xxx	
UAA or UAG } STOP Code				
Amino Acids	aa	STOP	aa	(Termination of Chain)

Figure 7: Proposed mutation in case of collagens and the possible replacement of L-Lysine by L-Glutamine or termination of the chain.

It is important to recall here the case of sickle anemia where residue six of glutamic acid (code: GAG) in normal hemoglobin is replaced by valine (code: GTG) in sickle hemoglobin.

In theory if mutation takes place in the synthesis of collagens then a possible sketch of results could be as shown in Figure 7.

Figure 8 represent the structures of both L-lysine an L-glutamine which they differ at one end, glutamine has an amide functional group where it's replaced with amino group in lysine. If lysine is replaced by glutamine as demonstrated in Figure 7 then this would affects the protein structure and property. Glutamine, a highly-polarized-side-chain, keeps the folded part of the protein outside and is able to cross-link via hydrogen bonding of the side chain which resulted in skin abnormality. The normal process of hydroxylation of what should have been a lysine residue is also disrupted; hydroxylation of lysine is crucial for the later attachment of the carbohydrate to the collagen structure. The structure of collagen is important in the skin as it forms loosely woven fibers that can expand in all directions. Collagen is a glycoprotein and this is the unique function of the hydroxyl group of hydroxyl lysine as it serves as the point of attachment of the carbohydrate.

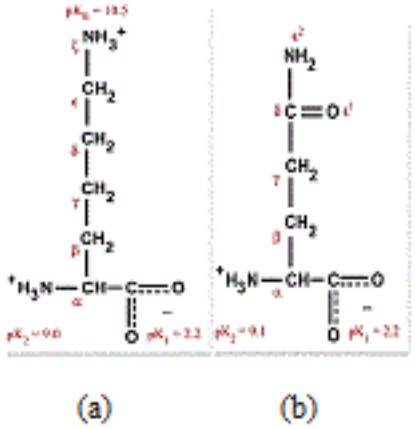

Figure 8: Chemical structure of (a) L-lysine and (b) L-glutamine.

In summary, if lysine (a) is replaced by glutamine (b) in a mutated collagen then at least two crucial structures are destroyed; the hydroxylation of the lysine and the failure to attach the ca;rbohydrate group via the hydroxyl group of hydroxyl lysine. At this point it is important to recall that the deficiency of vitamin C leads to failure of hydroxylation of lysine. When hydroxyl lysine is not synthesized then collagen is not assembled properly. In this case, skin and blood vessels become very weak leading to skin lesions and bleeding gums. In this case of vitamin C deficiency the process of gum bleeding is reversed via intake of vitamin C.

A similar logic would lead us to think of reversing skin lesions by increasing intake of the essential amino acid lysine. At this point we would like to call this as a pseudo-vitamin C deficiency.

Another possible scenario would have to be the percentage of available lysine affects the availability of collagen III. This in turn will affect the composition of the different collagens and results into mal-formation of skin structure. By reaching high levels of lysine one is able to reverse this process and restore the correct percentage of the different collagens and namely collagen III, thus restoring normal facial skin structure.

This is very different than the use of laser in treatment as laser is both costly and in-effective as it does not resolve the problem at the roots, i.e. production of normal tissue.

The laser conventional treatment does not cure the tissue but rather removes the abnormal tissue and does not stop it from growing back.

The amino acid therapy is not known to doctors and neither is researched by pharmaceutical companies. This is caused by the fact that the primary source of information is the pharmaceutical and medical device industry, which has no incentive to publicize or research non-patentable inexpensive natural remedies or cures!

We propose, in this paper, a cheap and non-patentable natural remedies and cures which in fact represent a major threat to many profitable seekers especially to pharmaceutical industry.

Lysine treatment is a safe, inexpensive approach to the prevention of angiofibromas formulation. Supplementation with adequate doses of Lysine (1000-2000 mg) could prevent the formation of these fibromas and the reversal of newly formed fibromas to a 70% reduction in size and quantity.

Lysine is also found to improve one's concentration and assist in tissue repair. Lysine ameliorates TSC symptoms of facial angiofibromas and possible autism. This is without side effects and unlike the drug Sirolimus (marketed under the name Rapamune) which is an immuonosuppressant [18].

Further research findings involve the auto immune disease associated with the skin and resulting in skin scaling. The process of skin scaling could be interfered with at the level of skin synthesis and reversal of skin lesions. However the patient would need to continue on supplementing the diet with increased levels of Lysine for longer time as the repair is only on the protein synthesis level and not on the genetic level.

Other effects of lysine that must be monitored are those affecting the wellbeing and mental health such as levels of lysine and arginine in CSF in patients with Parkinson's disease and in cases of Alzheimer's dementia (AD). The formation of plaques and tangles are histological hallmarks of AD. These structures are also hallmarks in cases of TSC. One should further research the possibility of treating such structures existing in the grey matter of the brain. It's possible that lysine could be an effective treatment to retard the progression of AD. This strategy is directed at reducing or eliminating the production of amyloidal plaques or even eliminating plaques already produced. These plaques are common amongst elderly with AD and also patients with TSC.

Our future work will involve testing on cancer cells as there is evidence of possible effect on cancer cells rate of growth [19-21]. Others have studied many other aspects of L-Lysine effect and found great health benefits to a balanced intake of L-Lysine. This available research is worthy of analyzing in order to find new natural cures for related ailments [19,22-27].

Since L-Lysine is natural and will not fall under FDA testing or regulations then to best benefit from these scientific finding leads us to recommend that children who are diagnosed with TSC must just like all children should adhere to a balanced diet including the adequate intake of essential amino acids and specifically the essential amino acid L-Lysine. This is very crucial as the efficiency of L-Lysine is better in cases where angiofibromas are in the initial stages of formation. The reversal of the skin to revert to normal conditions is much difficult once blood vessels are formed. The same applies for laser surgery procedures of removing the angiofibromas.

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The authors declare that they have no conflict of interest.

All ethical procedures were followed in dealing with the TSC patients.

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