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Commentary

Supercentenarians and transthyretin amyloidosis: The next frontier of human life extension

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Transthyretin (TTR) Amyloidosis is a hereditary, systemic disease caused by a mutation in the Transthyretin gene located on human chromosome 18 (18q12.1). The TTR protein is synthesized primarily in the liver and is normally a carrier for thyroid hormones and Retinol Binding Proteins (RBP). There are two other thyroid-transport proteins in addition to TTR: Thyroid Binding Globulin (TBG) and Albumin; this redundancy suggests the essential importance of thyroxin to maintain basal metabolic rate and body temperature within tight limits. As far as TTR's four-subunit structure is concerned, the TTR protein is a 55-kilo Dalton homotetramer. It is also synthesized in the choroid plexus, and the retinal pigment epithelium for secretion into the cerebral spinal fluid and the eye, respectively. Each monomer is a carefully folded 127-amino-acid polypeptide chain rich in beta-pleated-sheet structures. Association of two monomers via their edge beta-strands forms an extended beta "sandwich." Further association of two of these dimers in a face-to-face fashion produces the native homotetrameric structure and thereby establishes two thyroxine binding-sites per tetramer. This dimer–dimer interface, comprising the two thyroxin-binding sites, is the weaker dimer–dimer interface, so this is the portion that comes apart first in a process of tetramer dissociation, following a continuing chemical reaction of dissociation/re-association.

Any of several different mutations (or SNP's) in the TTR gene may result in misfolded, sticky amyloid proteins that aggregate into long fibers that can accumulate on the interior surface of blood vessels and throughout our tissues. These fibers damage body organs, such as the peripheral nerves and the heart, resulting in intractable peripheral sensory neuropathy, autonomic neuropathy, and cardiomyopathy. Three disease conditions have been designated as follows: (1) Senile Systemic Amyloidosis (SSA); (2) Familial Amyloid Polyneuropathy (FAP); and (3) Familial Amyloid Cardiomyopathy (FAC). The first condition, as the name implies, affects geriatric patients and is relatively commonplace, while the more rare familial forms are the result of one of several inherited mutations that cause the individual TTR units to become unstable or more susceptible to unraveling and misfolding. The latter two diagnoses affect young children and are typically fatal before the age of 40 without a liver transplant. Some European families with an autosomal dominant mutation tend to cluster in Portugal or in Northern Sweden. We can distinguish at least 80 different SNP's [see http://www.hotthyroidology.com/editorial_131.html] that contribute to a familiar form [see http://www.omim.org], so it should not be surprising that there could be a wide variety of more-or-less unstable TTR forms that are inherited randomly or occur sporadically in the general population that don't appear as a non-fatal cardiomyopathy until much older ages and could result in a cause-of-death diagnosis of Congestive Heart Failure (CHF) at extreme age. This is by analogy with an APO-E SNP that contributes to a susceptibility to (but does not guarantee) Alzheimer's disease as a cause of death, unless, of course, one were to escape from all other forms of death, so that this particular form might then reveal itself. In the future, when full genome sequences become commonplace at a price of less than $1000 each and we have hundreds of thousands of such sequences in a searchable computer data base, we will be able to associate CHF as a cause-of-death on death certificates with a particular configuration of TTR SNP's that occur randomly in the general population. Therefore, we believe that the investigation of the prevalence of SNP's for TTR amyloidosis in the general population will have important implications for public health for all of us in the future.

In connection with the genetic basis for SSA, epigenetic changes (methyl groups which decorate our DNA) that naturally occur with chronological age in all our somatic cells, sometimes referred to as *epigenetic drift*, may also be implicated in the tendency to suffer SSA at extreme ages because these methyl groups regulate gene-expression profiles. Thus, simply knowing one's full genome would not be sufficient to fully predict one's health status or prognosis for longevity at any particular age at least unless one's average epigenetic pattern is also measured annually in the lab on a continuing routine basis (a saliva sample is needed).

Patients with a diagnosis of FAP have a mean life expectancy of nine to eleven years from symptom onset and the only present treatment option is liver transplantation; as a result there is a significant need for new therapies to treat patients who possess a mutation in the TTR gene. SSA, on the other hand, is a disease that has been well known to anatomical pathologists conducting autopsies of older

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patients for decades. But it was normally described as an "incidental finding" rather than as a "primary cause of death." Although this diagnosis is not immediately recognized by the public when compared with heart disease, cancer, or stroke, for supercentenarians (who escape from the commonplace causes of death due to their extreme longevity), it can then be cited as a true "cause of death."

The study of supercentenarians has a long history in the field of demography and dates back to the 1870s with the work of William John Thoms. As medical researchers became increasingly interested in studying the medical conditions associated with our longest-lived humans, the initial focus was on determining whether extreme age claims were verifiable or not.

A wide variety of suspicious claims were subsequently investigated and shown to be simple age exaggerations or fraudulent attempts to avoid military service or gain respect in the local community. Because the lifespan of the *Homo sapiens* species is categorically determined by the length of life of the single longest-lived human being at any point in history (by definition), most demographers focused on attempting to define the ostensible statistical limits of the human lifespan. Madam Jeanne-Louise Calment of France, who died in 1997 at age 122 years 164 days, is the current *Guinness Book of World Records* documented holder of this record for our own species. In 1997, the Calment case first raised excitement about pushing the frontiers of human longevity, but recent gains in human longevity at the highest ages have either flattened or "plateaued." Curiously, despite impressive gains in average life expectancy, no one has come close to breaking the "Calment Limit" during the last 17 years (first set by her on October 17, 1995), so she still remains a true "outlier." Therefore, although it is not mathematically impossible, it has been suggested that this "Calment Limit" may stand the test of time, unless medical science were to discover a true intervention into the aging process itself, in which case "all bets are off."

The Supercentenarian Research Foundation (SRF) was formed in October 2004 with the expressed mission of focusing on the biomedical aspects of *quasi-supercentenarians* (this is defined as women 108 and older and men 105 and older, in an attempt to equilibrate the observed disparity in the genders, as it is well known that there are more than ten times as many females as males 110 years or older, for reasons that are not presently understood). Emerging SRF autopsy data continue to strongly suggest that supercentenarians die of amyloidosis-related causes (the primary cause of death in ~70% with the balance due to aspiration pneumonia). It is still too early to draw definitive conclusions; however, these early results suggest that we need to take a second look at implicating amyloidosis as one of the major components of the aging process.

As stated above, *amyloidosis* is defined as "a disorder in which insoluble protein fibers systematically infiltrate multiple tissues and organs, progressively impairing their function." It occurs when native or mutant polypeptides misfold and aggregate as fibrils. Amyloidosis can take several forms: *primary* (or idiopathic) amyloidosis is seen as localized to certain organs (such as the liver or the heart) and not associated with other diseases except for multiple myeloma, while *secondary* amyloidosis is linked to chronic disease. In addition, as stated earlier, some types of amyloidosis are found to be hereditary. Essentially, amyloidosis causes death through a clogging of blood vessels: just as a drain pipe in an old house eventually becomes blocked, impeding the flow of water. So the buildup of long chains of extra-cellular amyloid proteins eventually choke off the function of vital organs (such as the heart and liver), ultimately leading to death. Just as plaque build-up in coronary arteries can lead to Myocardial Infarction (an MI), so the buildup of sticky amyloid fibers (or 'lardaceous' tissue) leads to the failure of the affected organs.

Physicians have distinguished at least 27 different types of proteins associated with amyloid formation. The most commonly-known type, *beta amyloid* (Aβ or A-beta) is a polypeptide of [39–43] amino acids which appears as the primary constituent of plaques in the brains of patients with Alzheimer's disease. Other common types include fibrils derived from monoclonal immunoglobulin light chains (such as *kappa* or *lambda*) caused by Multiple Myeloma (a cancer of the plasma cells in the blood), which can be distinguished by immunohistochemical staining following Congo-Red staining.

Hereditary forms of amyloidosis are autosomal-dominant diseases characterized by the deposition of a variety of proteins that infiltrate all the organs of the body, including the lungs, the heart, the kidneys, the lymph nodes, and so on, leading to hypertension, congestive heart failure, interstitial fibrosis of the lungs, polyneuropathy, and other untoward conditions leading to death at a relatively young age. The most common form of SSA is the TTR homotetrameric carrier protein (consisting of four identical subunits stuck together like ping-pong balls with a pocket or 'dimple' in the center). This pocket facilitates the transport of two Thyroxin (T_4) hormone molecules in the blood stream from the thyroid gland (where it's made) to all the cells of the body (essential for maintaining mitochondrial function and normal body temperature). It can also carry Retinol (Vitamin A). The subscript after the letter "T" refers to the number of Iodine atoms in the hormone (either 3 or 4). The designation rT3 refers to a form of Thyroxin called "reverse T3" which allows for a reserve pool of Throxine to become available when needed. As explained above, mutations in the TTR gene may cause the tetramer to become unstable triggering the formation of fibrils. However, even the wild-type TTR (without mutations) can cause (non-familial) amyloidosis in older patients, such as supercentenarians, who have lived long enough to reveal an underlying relentless pathological process which manifests clinical symptoms. As the TTR subunits become unstable over time, they tend to break apart. When a single unit misfolds, the monomer can no longer reaggregate into its native tetrameric form, and given its sticky nature to itself, it forms fibrils (Peterson et al., 1998). While amyloid has long been considered untreatable and fatal by the medical community; in fact, this concept suggests a straight-forward solution: would not a substance that breaks up or dissolves the accumulation of amyloid fibers help to restore organ function? After all, if amyloid is an impending, indirect cause of death, should not its removal or breakup lead to a longer life? The preliminary diagnostic technique has been to use 'Congo Red' stain, since the 1920s, making its diagnosis relatively easy… however, this test is occasionally overlooked. While some researchers have concluded that SSA is a "disorder of elderly men," four of the six supercentenarians confirmed to have SSA were females. Since women tend to live longer than men by an average of [5–7] years in developed nations, it may be that, rather than women not getting SSA, there is a delayed onset of the condition. Thus, any protective effects of the female gender against amyloidosis may be temporary, not permanent. Therefore, it seems that further research is warranted before any conclusions regarding SSA and gender can be drawn.

In Japan, the Aichi study of 19 Japanese centenarians (including 17 women), "there was a high incidence of amyloid angiopathy (16 of 19 cases)." This means that amyloid buildup was present in a significant majority of cases, including females. Again, the ages of the patients [100–116] and the relative lack of this problem in younger patients, particularly female, suggest that there was a correlation between extreme age and amyloidosis. It is still not clear whether amyloidosis is an integral part of the aging process among the oldest-old, and whether it is treatable. The medical coverage of amyloidosis remains sparse. The publication *Amyloidosis: Cause and Manifestation of Senile Deterioration* by Schwartz (Schwartz, 1970) speaks to amyloidosis being the cause of senile deterioration. This is interesting given that amyloidosis has been largely ignored for many decades and that proponents as prominent as Rudolph Virchow have suggested that amyloidosis was an important aspect of the aging process.

This 1970 book is almost a lone voice in the wilderness. In the dedication, Dr. Schwartz noted that "since 1927, reports were ignored and the correctness of his observations doubted or denied." Schwartz

noted that the term was coined by Rudolph Virchow, who thought that the "infiltrating material has some essential chemical resemblance to a starch present in plants" (amyllum). Schwartz then juxtaposes his own conclusion that amyloidosis is a condition that "cannot be overestimated in human and animal pathology." A contrary opinion from *The Journal of the American Medical Association* (1967) concluded that "amyloid disease will never enjoy more than casual clinical importance" and "needs no further confirmation." Against that laissez-faire attitude, Schwartz warned "amyloid degeneration in industrialized countries is most important because it is the most frequent morphologically recognizable, significant disease in human pathology. It seems that no one who lives long enough escapes it." Such words seem especially prescient, in that this disease has re-emerged as a significant cause of death in the supercentenarian population.

If Schwartz was a lone voice in the 1970s, it seems that whoever is an expert on amyloidosis now recognizes its significance. In the 1990s we find some rehabilitation of the significance of amyloidosis (although it was not a headline newsmaker). Sir Mark Pepys (Pepys, 1995) asserted that "clinically-significant amyloidosis is not rare" and that "amyloid deposits usually persist and accumulate, leading inexorably to organ failure and death." As of the early 1990s, there was still no treatment. Perhaps there was no treatment yet because there wasn't a focused effort to investigate the problem. Pepys recognized that amyloidosis is a major complication and is involved in everything from renal failure to Alzheimer's disease, denoted as the "fifth-most-common cause of death in the Western world."

The historic reappraising of amyloidosis in the 1990s was explained most directly, however, by Cohen and Skinner (1993), who noted that "in the 1920s, [amyloidosis] was then considered an extremely rare degenerative condition," but that "it has become apparent, however, that amyloidosis is far more common than had been thought, that it is often of great significance, and that it is associated with an extraordinarily wide variety of diseases." Thus, it can be unequivocally stated that what first seemed a new discovery in 2004 (that amyloidosis was a major factor in the human aging process) was in fact a re-discovery of an idea that had some favor in the 19th- and early 20th-century, but had largely been bypassed as the push toward antibiotics and modern cures led to its near-abandonment. Ironically, it is only after the gains made in other fields have slowed that the patients now demand that the problem of amyloidosis no longer be ignored; with longevity gains made in other aspects of human aging (such as atherosclerosis and hypertension), the law of diminishing returns suggests that more improvements can be made in areas largely unexplored or untreated. Certainly, amyloidosis, long an apparent but overlooked cause of death in extreme old age (and in a few younger individuals with a genetic propensity for amyloid buildup) is now a major problem on the frontier of extending human lifespan. For those who have claimed that 'eliminating cancer and heart disease would only add about ten years to the human lifespan' because people would die of something else, it seems increasingly likely that the 'something else' could well be amyloidosis.

Is amyloidosis a part of the aging process, or is it merely one more chronic disease that can be treated? Will treating amyloidosis lead to increases in human lifespan? Both first-generation and second-generation drugs, such as Diltiazem, Verapamil, Celastrol, 4-PDA, taurine-conjugated ursodeoxycholic acid, and CHPHC, are under development for the management of the disease (Coelho et al., 2008; Balch et al., 2008). It seems to us that these questions may lead us to the next frontier in the extension of human lifespan. At the very least, the recognition that amyloidosis is a common and treatable condition in the oldest old should lead supercentenarians to having a better quality of life in the future, a further confirmation of what has been called the "Compression of Morbidity" (Aldwin and Gilmer, 2004; Hamosh et al., 2004; Olshansky et al., 1990; Quintas et al., 2001; Tribe and Silver, 1969; Fries, 1980).

Conflict of interest statement

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References

Aldwin, C.N., Gilmer, D.F., 2004. Health, Illness, and Optimal Aging: Biological and Psychosocial Perspectives. Thousand Oaks, California, Sage Publications.
Balch, W.E., Morimoto, R.I., Dillin, A., Kelley, J.W., 2008. Adapting proteostasis for disease intervention. Science 319, 916–919.
Coelho, T., Ericzon, B.G., Falk, R., Grogan, D.R., Ikeda, S.I., Maurer, M., Plante-Bordeneuve, V., Suhr, O.B., Trigo, P., 2008. A Physician's Guide to Transthyretin Amyloidosis. FoldRx Pharmaceuticals, Inc., Cambridge, MA.
Cohen, A.S., Skinner, M., 1993. Amyloidosis of the Liver. In: Schiff, Leon, Schiff, Eugene (Eds.), Diseases of the Liver. J. B. Lipincott, Philadelphia, p. 1465.
Fries, J.F., 1980. Aging, natural death, and the compression of morbidity. N. Engl. J. Med. 303, 130–135.
Hamosh, A., Scott, A., Amberger, J., Bocchini, C., McKusick, V., 2004. Online Mendelian Inheritance in Man (OMIM), A Knowledge Base of Human Genes and Genetic Disorders.
Olshansky, S.J., Carnes, B.A., Cassel, C., 1990. In search of Methuselah: estimating the upper limit of human longevity. Science 250, 634–640.
Pepys, M., 1995. Amyloidosis, In: Frank, M.M., et al. (Ed.), Samter's Immunologic Diseases, 5th Ed. Little, Brown and Co., Boston, pp. 637–655.
Peterson, S.A., Klabunde, T., Lashuel, H.A., Purkey, H., Sacchettini, J.C., Kelly, J.W., 1998. Inhibiting transthyretin conformational changes that lead to amyloid fibril formation. Proc. Natl. Acad. Sci. U. S. A. 95, 12956–12960.
Quintas, A., Vaz, D.C., Cardoso, I., Saraiva, M.J.M., 2001. Tetramer dissociation and monomer partial unfolding precedes protofibril formation in amyloidogenic transthyretin variants. J. Biol. Chem. 276, 27207–27213.
Schwartz, P., 1970. Amyloidosis: Cause and Manifestation of Senile Deterioration. Charles C. Thomas, Springfield, Illinois.
Tribe, C.R., Silver, J.R. (Eds.), 1969. Renal Failure in Paraplegia. Pittman Medical Pub. Co, Ltd., London, p. 54.