



## Making a vampire

Veronika N. Laine

*Netherlands Institute of Ecology (NIOO-KNAW). Wageningen, The Netherlands.*

*Email: veronika.laine@gmail.com*

The modern vampire is often portrayed as a human transformed into a vampire due to a bloodthirsty spirit<sup>1</sup>, demons<sup>2</sup>, viruses and other pathogens<sup>3</sup>, magic or some unknown reason<sup>4</sup>. Neither fiction nor more realistic accounts have shed light on the precise molecular mechanisms of how the transformation happens until the novel trilogy and TV series called *The Strain* (Fig. 1) introduced some ways as to how the transformation could happen. In *The Strain*, parasitic worms carry a virus that causes the vampiric changes to happen through a modification in the expression of genes. This change even creates new organs such as the stinger.

For obvious reasons, no actual experimental studies have been conducted with vampires and so the exact origin and evolution of vampirism remains unknown. A full genome-wide association study or transcriptome

analysis would be preferred to recognize the exact genes behind the vampiric traits, but getting enough samples from vampires will most likely be difficult. Thus, the “candidate gene” approach might be the best method for reaching some conclusions or, if there is enough material, a whole genome sequencing and comparison to human genomes.

In this article I will explore some thoughts on how we could make a vampire in the lab and which part of the genome we would need to alter in order to see the necessary changes. Imagine if genetic engineering would be so advanced that when you tweak little bits of the human genome here and there, you could make whatever traits, even vampiric ones, appear (or disappear) any way you like. Unfortunately, reality is seldom as easy, as it has been shown in movies such as *Gattaca* (Columbia Pictures, 1997), *Splice* (Warner Bros., 2009) and the *X-Men* series (20th Century Fox, 2000–2017), although the genome editing method CRISPR (Cong et al., 2013; Hsu et al., 2014) has lifted genomic modification to a completely new level and has already been used in removing diseases in humans (Ma et al., 2017). Alternatively, what if vampires already existed and we could get our hands on their

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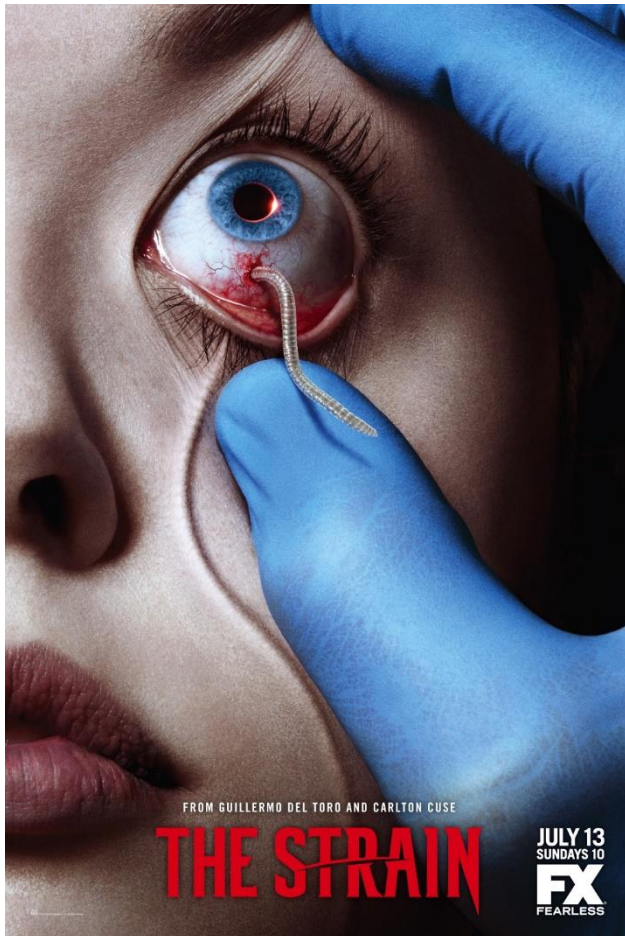
<sup>1</sup> *The Queen of the Damned*, by Anne Rice (1988).

<sup>2</sup> Old folklore; *Buffy the Vampire Slayer* (20th Television, 1997–2003).

<sup>3</sup> *Daybreakers* (Lionsgate, 2010); the *Underworld* film series (Lakeshore Entertainment, 2003–2016); *The Strain* (20th Television, 2014–2017).

<sup>4</sup> *Dracula*, by Bram Stoker (1897); *The Vampire Diaries* (Warner Bros., 2009–2017); *The Twilight Saga* (Summit Entertainment, 2008–2012); *True Blood* (HBO Enterprises, 2008–2014).

genome sequence? Which genes would be affected by the transformation? Intriguingly, there are real life examples of species and conditions that could be thought of as vampiric and we can find potential candidate genes for vampirism from these traits. These “vampire building blocks” could then be used in constructing a lab vampire (at least hypothetically).



**Figure 1.** Promotional poster of *The Strain* TV series, directed by Guillermo del Toro and Chuck Hogan. Image retrieved from: IMP Awards (<http://www.impawards.com/>).

The myth of vampires has been around for thousands of years and the descriptions of vampirism vary between times and cultures.

The vampires we know today date back to the 17th century and they have been covered by every platform in our popular culture. A good summary of the evolution of vampire myths can be found in Harris (2001).

The exact way in which humans transform into vampires depends on the source of the story you are reading and it often remains a mystery. In the extensive study of the science of vampirism, Dr. Pecos and Dr. Lomax (2001–2017) from the late Federal Vampire & Zombie Agency (FVZA) suspected that it is a human vampirism virus (HVV) that causes the transformation. The origin of the virus is suspected to be the vampire bats and their fleas, which sounds very plausible since bats are known to be carriers of many diseases such as SARS, ebola and rabies (Biek et al., 2006; Smith & Wang, 2013), and it was also suggested in the movies *Daybreakers* and the *Underworld* series. Furthermore, rabies has been suggested to be the actual origin of the modern vampire myth (Gomez-Alonso, 1998).

In this article, I will present real life examples of vampiric traits and hypothesize possible molecular mechanisms and candidate genes that could be mutated after the transformation. I will concentrate on the following three vampiric traits that are common to many descriptions of vampires:

1. Hematophagy (that is, feeding on blood)
2. Immortality
3. Sunlight avoidance

### HEMATOPHAGY

For many people, bloodsucking is the first vampiric trait that comes to mind. Blood is a nutritious fluid tissue, full of proteins and lipids

and it is easy to consume. In nature, blood consumption has evolved in several unrelated species throughout the animal kingdom. Among invertebrates, leeches, mosquitos and fleas are the best known examples, and some fish (lampreys) are also known to feed on blood. There are several bird species that practice hematophagy, such as the oxpeckers, hooded mockingbirds and vampire finches. Among mammals, the best known hematophagic species are the vampire bats.

Several changes in the genome are needed in order for animals to survive exclusively on blood. One of the key features is to prevent the victim's blood from coagulating while feeding. In vampire bats the plasminogen activator (PA) genes have gone through gene duplication, domain loss and sequence evolution (Tellgren-Roth et al., 2009). These genes are expressed in the saliva glands of vampire bats and the proteins they produce help to process the blood of birds and mammals. In humans, these genes protect against heart attacks by producing proteins that clear the blood vessels by degrading blood clots. The hairy-legged vampire bat's (*Diphylla ecaudata*) PA genes resemble the PA genes of the closely related non-blood feeding bat species. These bats feed on the blood of birds and it seems that the activation of PA in saliva glands is enough to keep the bird blood flowing. However, in the two bat species that feed on mammal blood, common vampire bats (*Desmodus rotundus*) and white-winged vampire bats (*Diaemus youngi*, which also feed on birds), the PA genes have gone through more extensive modifications in order to better tackle the natural inhibitors of PA proteins in mammal

blood. A transcriptome and proteome study of common vampire bats found additional genes expressed in the salivary glands (Francischetti et al., 2013). Furthermore, by comparing vampire bats and leeches to non-blood feeding species, Phillips & Baker (2015) found additional genes related to blood feeding, such as ectonucleoside triphosphate diphosphohydrolase-1 (*ENTPD1*), which has not before been linked to secretory expression. They also suggest that alternative splicing of genes has been an important mechanism for these species to rapidly evolve to feeding on blood.

In addition to blood coagulation, the vampire bats needed to overcome the bitter taste of blood. Bitterness in nature often means that the substance is poisonous and should be avoided. However, in all of the three vampire bat species there is a greater percentage of non-functioning DNA in the bitter taste receptor genes than in other bat species. These results suggest that these genes have been relaxed from selective constraint in vampire bats, which has led to a reduction of bitter taste function (Hong & Zhao, 2014).

Lastly, the problem with consuming blood is the ratio between amount of nutrition needed and the liquid consumed. A typical vampire bat can consume half of its weight in blood in one feeding. The blood is then rapidly processed and the excess liquids are urinated within two minutes of feeding in order for the bat to take flight. Conceivably, the same effect would not be very convenient for vampires. If the vampire weighed for example 70 kg, it would need to consume 35 kg of blood in one feeding and urinate the excess liquid almost immediately,

because the bladder can only hold about half litre of liquids. Furthermore, humans have about 5 kg of blood on average, so vampires would need to suck dry about seven people per night and urinate between victims, something that has not been discussed or shown in vampire stories, except in *The Strain*, where vampires defecate the blood while drinking. To compensate for the low intake of nutrients, vampires might slow down their metabolism and go to a hibernation mode and thus avoid the need to suck several litres of blood in one go. It would also enable fasting through hard times. In many stories, vampires have managed to survive without blood for days (see below).

### **IMMORTALITY**

Vampires are often regarded as undead; they are dead but behave like living beings, which in turn gives them eternal “life”. In this paper, I am not going to discuss whether vampires have a heartbeat or if they breathe (for that we would need actual vampire specimens); I will instead concentrate on how actual immortality could be achieved by giving real life examples.

First, we need to define what immortality is. The concept of biological immortality means that there is no mortality from senescence, which is biological aging. This of course means that the organism is not truly immortal, it can die through injury or disease. Vampires are often presented as highly resilient beings who can survive disease and injuries, but there are things that still kill them, like sunlight, a wooden stake through the heart, fire or beheading.

What is then the ultimate cause of senescence? It is still unclear how the process of senescence happens exactly, since it is a very complex phenomenon. This subject is under heavy research, especially in regard to how we could slow down or even reverse aging (de Keizer, 2017; see movies *Self/less* [Focus Features, 2015] and *Mr. Nobody* [Wild Bunch, 2009] for further thoughts). The research has been concentrating on gene expression changes, chemical and DNA damage, and telomere shortening. Telomeres are repetitive regions at the end of chromosomes. Every time cells divide, the ends of the chromosomes are progressively clipped in the replication process. Because the repetitive sequences in the telomeres are not protein coding, the clipping does not affect cell functions. When the telomeres are gone after a certain number of divisions, the cells stop dividing (Hornsby, 2007). However, cells have ways of replenishing the telomeres with an enzyme called “telomerase reverse transcriptase”. The drawback is that the majority of adult somatic (that is, non-reproductive) cells do not express telomerase, but it can be found for example in embryonic stem cells, male sperm cells, epidermal cells and in most cancer cells. In vampires, this enzyme might be active also in the adult somatic cells but this might pose an increased cancer risk. However, vampires might have ways to avoid cancer, as discussed below.

The way senescence happens is not universal; there are species where aging is negligible or cannot even be detected. There are two well-known examples of truly immortal species, the immortal jellyfish (*Turritopsis dohrnii*) and the animals from the *Hydra* genus.

The immortal jellyfish, originally from the Caribbean Sea and now spread around the world, can use the process known as transdifferentiation to rejuvenate itself from its sexually mature free-swimming medusa form to sessile polyp form when the conditions turn harsh for the animal. When conditions are suitable again, the immortal jellyfish again transforms to its medusa form. This cycle can in theory continue forever, making the species immortal in the biological sense. However, this does not save the jellyfish from predators and diseases. The immortal jellyfish also appeared in the TV series *Blacklist* (Sony Pictures Television, 2013–present), where its cells were injected into humans in order to generate immortality. In the real world, science is not that advanced yet and it is also highly unlikely that it would be this easy to achieve immortality.

Hydras have been under more research than the immortal jellyfish. Hydras are simple freshwater animals (also cnidarians, like the immortal jellyfish) whose cells can continually divide and not undergo senescence. One gene, “Forkhead box O” (*FOXO*) has been extensively studied in hydras (and also in other species, like the nematode *Caenorhabditis elegans*, mice and humans) (Boehm et al., 2012; Martins et al., 2016). In hydras, this gene is the main player behind the renewal of the cells. In other species, this gene has been linked to aging and longevity in many studies. In an essay by Schaible & Sussman (2013), the authors suggested that during the evolution of the *FOXO* gene, its function changed from *Hydra*’s life span extending role to many other pathways related to maintenance, which

altered the gene’s rejuvenating functions in multicellular eukaryotes such as humans. Thus it might be that in vampires this gene (or actually all the *FOXO* genes – mammals have four of these genes) have retained the original function of *FOXOs*.

In the mammalian world, naked mole rats (*Heterocephalus glaber*) and Brandt’s bats (*Myotis brandtii*) are exceptionally long-lived compared to other small sized mammals. Naked mole rats are known for some very peculiar characteristics. They can survive anoxic conditions, they have delayed ageing and live up to 32 years, and the species is highly resistant to cancer, among other things, making them a very interesting species for scientists to study. In studies of the longevity and cancer resistance of this species, scientists found that a gene called *INK4*, which is the most frequently mutated gene in human cancer, produced a new product through alternative splicing. This protein isoform (that is, protein variant), called pALT(*INK4a/b*), prevented the mutated cells from clustering together and thus made the naked mole rats more resilient to cancer (Tian et al., 2015). In another study by the same group, extremely high-molecular-mass hyaluronic acid was found in naked mole rat fibroblasts (the most common cells in the connective tissue of animals). The molecular weight was over five times larger than that of human or mouse hyaluronic acid. It was speculated that a higher concentration of hyaluronic acid evolved to keep the skin elastic in underground tunnels. In addition to skin elasticity, long hyaluronic acid molecules wrap around cells tightly, preventing tumor cells from replicating (Tian et al., 2013). Whole

genome sequencing revealed additional genes that could be linked to longevity in this species (Kim et al., 2011).

Brandt's bats are known to live for over 40 years, making it the most long-lived mammal of its size. In the whole genome study of the species, Seim et al. (2013) suggested that a combination of different adaptive characteristics such as hibernation, low reproductive rate, cave roosting and an altered growth hormone/insulin-like growth factor 1 axis could extend the Brandt's bat's lifespan. Furthermore, *FOXO1* gene was expressed in high levels in Brandt's bat suggesting a possible role also in the longevity of this species. Hibernation in general has been linked to survival of different species allowing them to withstand extreme conditions (Turbill et al., 2011; Wu & Storey, 2016). The molecular difference between hibernators and non-hibernators seems to be in gene regulation rather than a difference in the DNA sequence itself. Differential expression was detected in the genes that were involved in metabolic pathways, feeding behavior, and circadian rhythms (Faherty et al., 2016). Hibernation or some other kind of dormant state seems to be present in vampires as well, helping them to get through tough times. In the *Vampire Chronicles* by Anne Rice, the vampires go to a hibernation-related state to cope with changing times. In the *Underworld* movies, two of the elders are kept in hibernation while a third reigns over the vampires. The reign goes in cycles, each of elders having their turn over the vampires and slave lycans. This cycle has social reasons, but it also gives rest for the elders from their immortal life.

## SUNLIGHT AVOIDANCE

Vampires are creatures of the night and sunlight is often regarded as deadly to them; in many occasions, they burst into flames whenever in contact with sunlight. It is an adverse trait for vampires and most probably emerged through pleiotropism. Pleiotropism is a phenomenon where one gene affects two or more unrelated traits (Paaby & Rockman, 2013). Mutations in genes causing immortality or blood consumption could also cause death by sunlight (antagonistic pleiotropy). Real life examples of bursting into flames due to sunlight are obviously not found, but sun can cause problems to people with certain conditions. Sunlight can cause severe allergic reactions, people can suffer from blood disease called porphyria, or have a rare recessive genetic disorder called "xeroderma pigmentosum".

"Sun allergy" is an umbrella term for a number of conditions where rash and blisters occur on skin that has been exposed to sunlight. Some people have a hereditary type of sun allergy, such as hereditary polymorphous light eruption, others a non-heritable type, such as solar urticaria. In some cases, symptoms only occur when triggered by another factor, such as certain medications or skin exposure to certain plants. The allergic reaction to sunlight occurs in the same way as in any other allergic reaction, although it is still not clear what the triggering component is. Somehow, the immune system recognizes the sun-altered skin as foreign to the body, which in turn activates the immune defences against it. If vampires suffer from sun allergy, could strong antihistamines and a high sun protection factor

sunscreen help them survive under the sunlight, in the same way as people with sun allergies? As death is a very severe reaction to sunlight, it is likely that vampires do not suffer from a sun allergy but from something more serious.

Porphyria, a group of blood diseases, have been suggested as a possible explanation for vampire myths but these ideas have been rejected in later papers (Winkler & Anderson, 1990). However, the mechanism behind porphyria could still shed light on why sunlight would be poisonous for modern vampires. In the cutaneous forms of porphyria where the skin is mostly affected, sunlight can cause pain, blisters or open sores to the patients. The disease is often hereditary due to a mutation in one of the genes that make the heme molecule (a component of hemoglobin, the red pigment in our blood): *ALAD*, *ALAS2*, *CPOX*, *FECH*, *HMBS*, *PPOX*, *UROD*, or *UROS* (Badminton & Elder, 2005). These genes could also be suitable candidates for vampire sunlight avoidance.

There is an even more severe sunlight sensitivity illness, the rare hereditary condition called “xeroderma pigmentosum” (XP). In extreme cases, the patients need to avoid all exposure to sunlight as it can cause severe sunburn with redness and blistering. If not protected from the sun, people with XP have a high risk of developing skin cancer. XP patients’ eyes are also very sensitive to sunlight and some of the patients have neurological problems such as seizures and hearing loss. The condition is caused by mutations in the genes that repair DNA damage. This causes a deficiency in DNA repair after ultraviolet damage to cells, which in turn accumulates

abnormalities to the DNA causing the cell to become cancerous or die. In most of XP cases, mutations occur in these four nucleotide excision repair related genes: *POLH*, *XPA*, *XPC* or *ERCC2* (Schubert et al., 2014). In addition to porphyria genes, these are also potential candidates for vampires’ adverse reactions to sunlight.

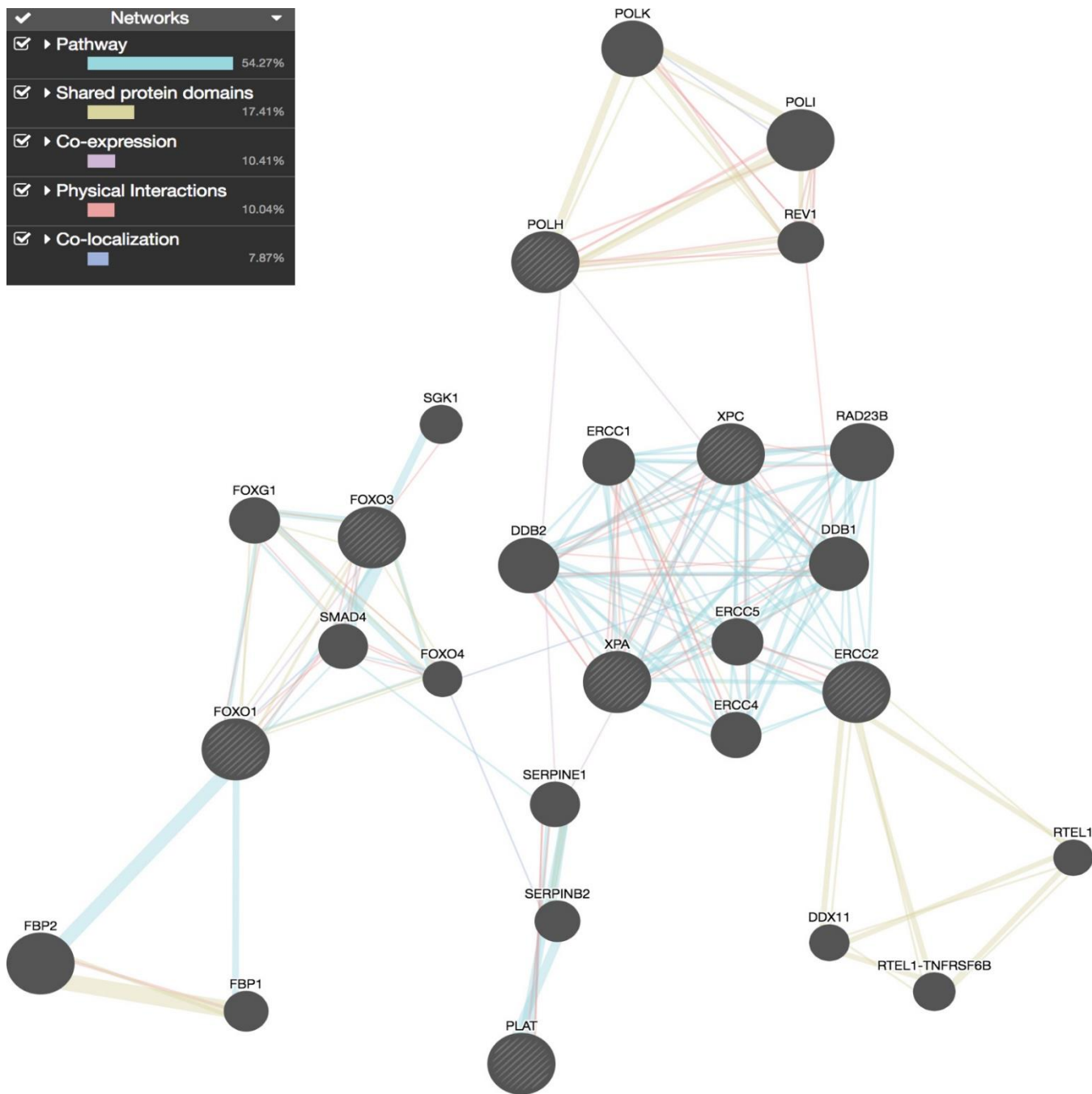
## CONCLUSIONS

Obviously, the transformation from human to vampire would affect many genes, some of the changes being bigger than others, which makes the genetic modification of human to vampire even more difficult. From the real life examples, the PA (blood coagulation) and FOXO (immortality) genes seem to be strong candidates. Furthermore, it is also possible to find more suitable genes to test and to investigate interactions between hematophagy, immortality and sun avoidance genes by using network analysis such as Genemania (Ward-Farley et al., 2010). For example, when inserting the human ortholog (roughly put, the equivalent gene) of bat PA gene, the plasminogen activator, tissue type (*PLAT*), the FOXO genes *FOXO1* and *FOXO3*, and the four XP genes, *POLH*, *XPA*, *XPC* and *ERCC2* to Genemania, it is possible to see how the genes are linked and what additional genes might be involved (Fig. 2).

In many of the traits mentioned above, we assumed that mutations in these candidate genes would be the cause of the vampiric traits. However, mutations are not the only possible cause. Epigenetic changes are functional changes in the genome that do not involve modifications in the DNA. Such mechanisms

are, for example, DNA methylation and histone modification. External or environmental effects can cause DNA methylation and change gene expression. In vampires, both mutations and epigenetics could be possible players, causing

changes and vampiric traits. Furthermore, if vampirism is caused by a virus or a parasite, we need to take into consideration the possible ways the pathogen could affect the human cells, which is a topic of its own.



**Figure 2.** Gene interaction network of the genes PLAT, FOXO1, FOXO3, POLH, XPA, XPC and ERCC2 done with Genemania. Showing 20 related genes with 27 total genes and 207 total links. Input genes are indicated with stripes.



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## ABOUT THE AUTHOR

Dr. **Veronika Laine** is a molecular biologist working currently with the great tit and she is especially interested in behavior, genes, pleiotropism, bats, kittens and vampires, especially Eric Northman. She plays too much video games.