International Scholars Journals

International Journal of Virology and Parasitology, Vol. 7 (3), pp. 001-006, March, 2018. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Review

Eyam's Guardian Gene; C282Y, H63D or Delta 32?

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Accepted 20 July, 2017

The little village of Eyam in England's Derbyshire Peak District is well known for its devastating epidemic of plague in 1665. Led by the vicar, William Mompesson, and the puritan minister, Thomas Stanley, the villagers altruistically quarantined themselves for fourteen months to prevent the spread of the infection to neighbouring villages. Up to 80% of the population died, raising the question of what enabled the remaining 20% to survive when surrounded by the dead and dying with a very contagious disease. One possibility is a genetic trait. The most common suggestion is the delta32 mutation of the *CCR5* chemokine-receptor locus on human chromosome 3, but perhaps the previously unconsidered mutations of haemochromatosis (HC) played a significant role. The significance of both mutations is compared.

Keywords: Eyam, Guardian Gene, Delta 32.

INTRODUCTION

The medical visitor to the Eyam village plague museum in Derbyshire will experience grief, great respect and curiosity, grief for the tragedy that overcame the inhabitants, respect for the inhabitants' self-sacrifice to protect neighbouring villages, and curiosity regarding the suggestion that the delta 32 mutation enabled perhaps twenty percent of the inhabitants of Eyam to survive the outbreak of plague in 1665.

The wild type CCR5 protein, a 7-transmembrane G protein–coupled chemokine receptor on the surface of lymphoid cells, enables cellular binding of viruses including the human immunodeficiency and smallpox viruses. The delta32 allele is caused by a 32 base pair deletion in the *CCR5* gene located on chromosome 3 which encodes the CCR5 protein. This deletion results in loss of function of the surface CCR5 receptor protein (Claiborne et al., 1998). The mutated protein is not recognisable by viruses; as a result these viruses can no longer bind to this site and enter the cell causing infection. The delta 32 homozygous person is immune to HIV, and the heterozygote is resistant. (Claiborne et al., 1998).

Survivors in Eyam shared environmental factors and kinship with the victims, raising the possibility that particular recessive gene or genes might have given the survivors a selective advantage. The evidence supporting the delta 32 mutation theory is circumstantial epidemiology, whereas there is more scientific pathophysiological data refuting this concept, raising the previously unconsidered possibility that haemochromatosis was the recessive mutation with the survival benefit.

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Eyam and the plague

Eyam is a picturesque traditional English village in the Derbyshire peak District only ten miles from Sheffield, with a current population just under a thousand. Eyam is forever marked by a devastating outbreak of plague in 1665 in which up to four out of five inhabitants perished. The total number of deaths recorded in the church was 273, a figure that appears accurate given that the rector was present throughout that period. The initial population immediately before the plague of 1665 is debated, with figures between 350 and 887 being suggested, (Clifford 1989). Regardless of this, the minimum death rate was 30%, the maximum 80% and the number of burials was tenfold higher than the yearly average for the village, compared with fivefold in London during the plague outbreak in 1665 (Race 1995). Why some survived is not clearly answered.

History books, novels, poems, songs and opera have been written about this catastrophe, with a tendency to romanticise the event perhaps to the detriment of the facts. A Google search of Eyam Plague Village yields 44,200 hits! The most recent novel about the event is an international 'best-seller' *"The year of wonders"* by the Australian Pulitzer prize winning author Geraldine Brooks. (Brooks 2002).

In 1665, London was experiencing a severe epidemic of plague resulting in many inhabitants choosing alternate safer surroundings to reside. The disease was thought to have been introduced to Eyam from London in a flea-infested bundle of cloth that was delivered to the tailor George Viccars in September 1665. Within four days Viccars was dead, (Clifford 1989), suggesting to the villagers that the plague had arrived.

The Church of England rector, William Mompesson, and the Puritan Minister, Thomas Stanley, led the village's self-imposed quarantine for 14 months, during which up to 80% of the inhabitants died. A one mile exclusion zone around Eyam was declared, a *cordon sanitaire*, such that none should enter or leave. Neighbouring villages brought food and other supplies including medicines to this line, which was otherwise rarely crossed beyond the romantic assignations of one young lady. Emmott Sydall was betrothed to Rowland Torre in a nearby village. They met daily throughout the winter until she adhered to the quarantine in April and sadly succumbed shortly after their last assignation.[3] Mompesson also closed the church and ordered burials to take place as soon as possible near the place of death, usually a home, to reduce gatherings with close contact and to reduce the risk of transmission from the bodies. Services took place in the open with the congregation more dispersed than inside a church (Clifford 1989).

Deaths occurred at an alarming pace, with either entire families killed or a rare remaining individual member surviving. The survivors came from both genders and all ages.

- All nine members of the Thorpe family died.
- All seven members of the Talbot family died, except one son who was living away from Eyam at the time.
- Nine members of the Syndall family died, six in fewer than six weeks, leaving a three year old boy, Joseph as the sole survivor.
- John Hancock and his six children died within a few days, leaving his wife as the only survivor.
- The rest of George Viccar's family died except his wife, a survivor in spite of her proximity to other victims. She lost 13 relatives.
- Viccar lodged with a widow, Mrs Cooper. Her two sons and second husband died while she survived.
- Jane Hawksworth was the sole survivor of her family, eventually losing 25 relatives, including her in-laws.
- Mr Morten lost his wife and three children, but survived himself.
- William Mompesson survived but his wife Catherine died and her grave is to be found in the churchyard today.
- Another survivor, Marshall Howe, a miner had a mild illness followed by full recovery, though his wife and son died. Believing himself to be now immune, he became the village grave digger, as many families through age and sickness could not bury their dead. He survived the plague and lived another 32 years. (Clifford 1989; Race 1995; Paul 2012).

Clearly this was a highly contagious virulent disease (Clifford 1989). The above documented model of death suggests mortality in keeping with the 80% estimates. Were these isolated survivors, some 10% of their collective families, just fortuitous, or was there some mechanism reducing their susceptibility to infection? The familial descriptions could suggest a recessive pattern of protection whereby not all families carried the protecting allele and some individuals within families may not be as related as initially thought. Alternatively it may be that survival had multiple genetic and/or environmental influences.

There is no clear bacteriological evidence that *Yersinia pestis* was the causative organism. Some (Duncan and Scott 2005), have suggested an alternative such as unspecified haemorrhagic viral disease.

The facts that the disease continued to spread in winter in the absence of fleas and the rats carrying the disease may not have been present in the environment argue against a Yersinia pestis infection. However Bubonic Plague and the pneumonic variant seem most likely for several reasons.

1) Eyam's plague began in autumn after Vicar received flea infested cloth. Both Bubonic and pneumonic plague outbreaks have previously arisen from human contact with fleas.

2) The pneumonic plague form can be spread from human to human in winter.

3) The physicians in London considered the disease to be the plague.

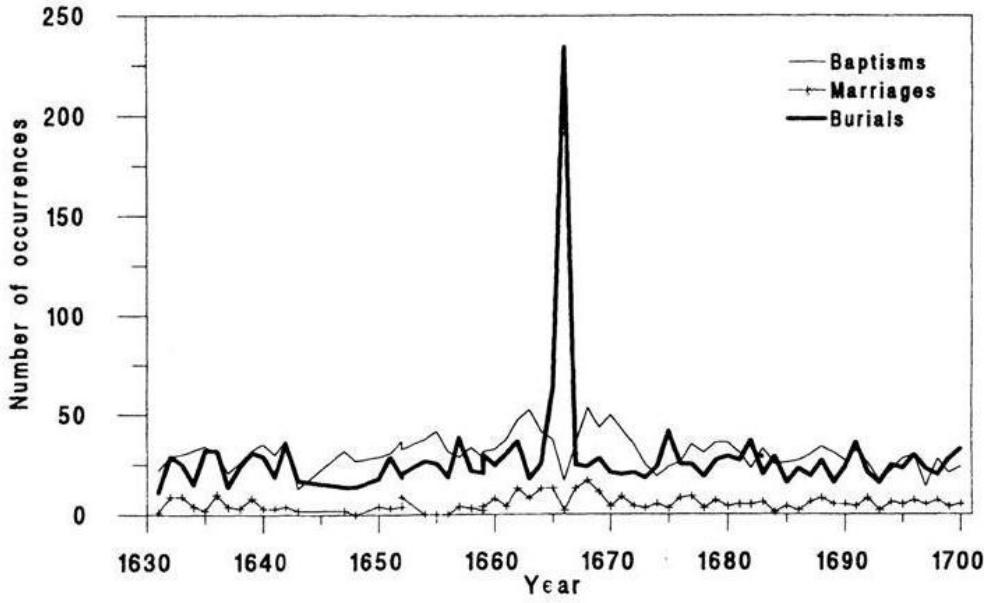

Figure 2 Baptisms, marriages and burials in the parish of Eyam 1631-1700. Five year moving averages

4) The description of Vicar's illness by William Wood (Wood 1842), in the *'The history and antiquities of Eyam'* in 1842, describes the Vicar's final days.

> *"On the second day he grew horribly worse: at intervals, he was delirious, and large swellings began to arise about his neck and groin. On the third day of his illness the plague spot appeared on his breast and he died in horrible agonies the following night."*

Certainly a description compatible with bubonic plague.

Medicines and the Medical Profession

In the absence of an effective cure and the personal risk, doctors appeared less philanthropic than Mompesson and Stanley. Brownlow's family history notes that in London "*the College of Surgeons fled to the country, which did not stop several of its members from writing learned papers about the disease they had been at such pains to avoid"!* (Brownlow 2011).

The College of Physicians remedy was *'Take a great Onion, hollow it, put in a fig rue cut small and a dram of Venice treacle; put it close stuffed in wet paper, and roast it in the embers; apply it hot under the tumour; lay three or four, one after the other; let it lie three hours.'*

The only recorded medical consultation was for a man from nearby Bubnell village who wandered into Eyam with a load of timber in error. He returned home on a very cold and wet day to develop a fever and upper respiratory symptoms, possible symptoms of plague and was isolated in his home. The Earl of Devonshire in his nearby Chatsworth House became aware of this and ordered his physician to see the man, perhaps in case the plague has moved nearer Chatsworth and the Earl should evacuate his family. Such a commission could not be refused; hence the physician requested the unfortunate man to cross the river running through Bubnell village and took the history from the other side across twenty meters of water. With customary physician's skills, plague was excluded, a simple viral infection diagnosed, and the village community reassured, a tribute to the physician's acumen and communication skills. (Clifford 1989).

Richard Meade, FRCP, FRS, and physician to King George II, wrote an account of the Eyam Plague in 1721 from information received from George Mompesson, the son of William, who was only four at the time and not living in Eyam (Mead 1744). Mead noted the origin of the outbreak from a box of material sent to the tailor followed by the death of the tailor and all his family except his wife, the death of between two and three hundred persons, and the isolation organised by Mompesson.

His book starts with the dismissal of many of his colleagues therapies '*how widely most Authors have erred in prescribing a Heap of useless and very often hurtful Medicines* '

Perhaps he had in mind the Eyam village herbalist, Humphrey Merrill who also died (Clifford 1989).

The evidence supporting a role for *DELTA32*

Like HIV, the plague bacterium infects white blood cells. O'Brien hypothesized that the *delta32* allele of CCR5 might

also protect against plague infection, and that the village of Eyam could be a good test case of this hypotheses (Aggarwal and Khushbu 2011). The town has good church records of births, marriages and deaths dating from the 17th century, allowing identification of the casualties and survivors of the plague.

 Analysis of the genes of the present-day descendants of Eyam plague survivors found 14% carried the delta32 mutation, compared with 0% of African controls, providing at least circumstantial epidemiological data that the hypothesis was correct. The number of such carriers aligns closely with the approximate 80% mortality previously discussed.

 Furthermore a study by Claiborne Stephens (Claiborne et al., 1998), estimated that the delta32 mutation, found exclusively in Caucasians, evolved, or became much more common 700 years ago as a result of selection pressure, concordant with the time of the 1346 to 1352 Black Death epidemic.

 Consistent with this conclusion Hummel (Hummel et al., 2005), was able to demonstrate the CCR5-D32 DNA mutation in skeletal remains from graves in Germany and Italy from up to 2900 years old, indicating that this mutation arose long before the 14th century plague pandemic.

 This genetic evidence is supported by *in vitro* studies demonstrating a 30-fold reduced uptake of Yersinia pestis by homozygous delta32 mutation in murine macrophages in vitro compared with the wild type (Elvin et al., 2004).

Whilst, the above supports a possible selective advantage delta32 during plague times, it is also possible that another disease was responsible for selection of delta32 in England near the time of the plague. Given this we can conclude that individuals carrying the delta32 variant of CCR5 were therefore more likely to survive certain, predominantly viral, infections, have children, and, eventually, have descendants living today carrying the delta32 mutation, an extant example of selection pressure.

The evidence refuting a role for delta32

The evidence supporting a role for delta32 in Eyam is circumstantial epidemiology. There is more direct evidence that it is ineffectual against plague.

 Firstly the wild type CCR5 receptor is recognised by viruses including smallpox but is not a receptor recognised or utilised by Yersinia pestis. (Mecsas et al., 2004).

 Consistent with this the allele frequency of CCR5-D32 in exhumed victims of the Black Death in Germany is not less than in a historic control group as would be expected if this mutation provided protection from the plague (Hummel et al., 2005).

 Additionally Mescas (Galvani and Slatkin 2003), found no difference in either survival time or bacterial growth in normal wild type and CCR5-deficient mice infected with *Yersinia pestis*, suggesting that the delta32 mutation is unlikely to protect humans against plague unless the disease process is very different in mice compared with humans. Mescas also found that bacterial growth of Y*. pestis* was similar in macrophages from CCR5-deficient animals compared with those from CCR5-expressing mice

 Finally, Galvani (Galvani and Slatkin 2003), studied delta32 allele frequency using a population genetic frequency framework, proposed that infrequent pandemics of plague would not generate sufficient selection pressure to give a allele frequency of the 10% or more found in UK and the Eyam survivors. They postulate that the more common outbreaks of smallpox, a disease with a high mortality in children, is a much more likely driver of selection pressure to give such a high allele frequency.

Plague appears therefore to have not been the selective force which drove a rapid increase in CCR5-D32 gene frequencies in North-West Europe. Smallpox is perhaps a more likely candidate.

The evidence supporting a role for haemochromatosis

Given the above, we propose an alternate hypothesis, namely haemochromatosis, which could have separated survivors from those who passed away from the Eyam plague. Haemochromatosis, well known as the disease of bronze diabetes, is predominately caused in Europeans by two major alleles, C282Y and HD63. The role of H63D is less well understood than C282Y. H63D homozygotes have less iron overload than C282Y homozygotes, though C282Y/H63D compound heterozygotes can develop haemochromatosis and heavy consumers of alcohol have a greater chance of developing cirrhosis if H63D homozygotes (Machado et al., 2009).

1 The prevalence of Haemochromatosis in the Eyam area

Various studies have demonstrated a high prevalence of the two common genetic mutations of haemochromatosis. The frequency of individuals carrying the C282Y allele in the Trent and Sheffield area has been estimated to be between 12.7% and 14% (Chambers et al., 2004; Banypersad et al., 1999; Gleeson et al., 2006). Similarly the H63D mutation has been identified to be carried in 25.9% (Chambers et al., 2004). Thus the carriage frequency of these*s* alleles would be consistent with an approximate 80% mortality and 20% protection from the plague. The event of the plague itself would have thus increased the frequency of the allele, via selection pressure, so that we might expect a higher frequency in the present day population of Eyam. Given this the H63D allele may better represent the allele of interest.

Clearly the two genetic mutations associated with HC, the H63D and C282Y are very common in the Sheffield area. Unfortunately, no figures are available for Eyam

alone, but the C282Y mutation of HC in the area is approximately the same frequency as the delta32 mutation.

2 Infectious disease and the survival benefits of Haemochromatosis

The iron overload found in haemochromatosis is not uniform throughout all the cells of the body and the condition has some survival benefits (Lopes et al., 2013). In haemochromatosis, the hepcidin deficient unregulated iron channel ferroportin (FPN) pumps excessive iron out of the intestinal enterocytes into the body. The same iron channel also pumps iron out of macrophages, such that macrophages in HC are iron depleted.

Many pathogenic bacteria including *Yesinia pestis,* the bacterium causing bubonic plague, are highly dependent on iron for growth, and therefore depend upon iron absorbed from macrophages, so their virulence and reproduction is reduced if macrophages are depleted of iron, (Moalem et al., 2002). Yersinia *pestis* is usually conveyed via the lymphatic system to lymph nodes, particularly in the groin and axilla where painful swollen lymph nodes develop and ultimately burst through the skin, the legendary buboes. This process is reduced in the iron-starved macrophages of people with HC who can better resist the bubonic plague, and survive.

There is considerable evidence to support the concept that iron deficiency is beneficial in promoting resistance to infection (Moalem et al., 2002).

 Men, usually with higher iron levels, appear more susceptible to many infectious diseases than women. (DeWitte 2010), examining skeletons excavated from the East Smithfield Black Death cemetery in London found that men with osteological evidence of physiological stress i.e. concurrent or previous health problems, had a greater death rate than women with the same degree of physiological stress.

 Moalem noted that the death rate from an outbreak of Bubonic plague in St Botulph's parish in London in 1625 was twice as high in men aged fifteen to forty-four as in women of the same age (Moalem et al., 2002)**.**

 Murray (Murray et al., 1978). evaluated the response to iron replacement therapy in a randomised trial with 137 nomadic Somali people with iron deficiency. Over the next thirty days seven infections occurred among the sixty-seven in the placebo group, but thirty-six infections occurred in the seventy-one treated with iron including MTB, *Salmonella typhimurium*, brucellosis and malaria, suggesting better immunity in the iron depleted state.

 Similarly the growth of MTB has been shown to be reduced by 50% in patient with HH, as the absorption of iron from macrophages is reduced by 90%. Similarly the growth of Salmonella typhimurium can be enhanced in vitro by the addition of hepcidin (Lopes et al., 2013).

 Yersinia species are similarly highly dependent on iron, and grow in iron-enriched host fluids as well as in macrophages. Excess iron can impair the immune system and T-cell response with increased CD8 and reduced CD4 cell counts, causing a diminished antigen respons (Ashrafian 2003).

 Hfe knockout mice have reduced inflammatory enterocolitis compared to the wild-type mice when infected with *Salmonella typhimurium*, and the macrophages from Hfe knock-out mice when also infected with *Salmonella typhimurium* had reduced secretion of tumour necrosis factor and interleukin 6 cytokines, thus reducing the counter-productive overwhelming and potentially fatal cytokine storm generated by infections such as the plague (Wang et al., 2008).

 Peripheral blood mononuclear cells from patients homozygous for the C282Y mutation have reduced cell surface expression of MHC Class I molecules and lower stability MHC-b2 microglobulin-peptide complexes, (De Almeida et al., 2005). The significance of reduced beta2 microglobulin (β2m) expression on C282Y leucocytes remains unclear. There are conflicting results as to the function of β2m and free unbound HLAs (28, 29). One possible option is that reduction but not absence of the β2m could modulate cytokine signalling which may be beneficial. In support of this genes encoding various interleukins, and proteins within the TNFa and NF-KB pathways, have been linked to loci associated with plague resistance (Tollenaere et al., 2013).

Given the above, the high prevalence of C282Y mutation in Europe could be attributed to the 'Black Death' of 1347, when a pandemic of plague swept across Europe with a 30-40% mortality rate, leaving a considerably increased proportion of people amongst the survivors. Three quarters of a millennium later, the highest prevalence of the HFE mutation in Europe is found today in Belgium, France, England and the rest of Northern Europe where the highest mortality rates from the plague occurred.

The role of the H63D mutation in infectious diseases is less well understood. Once H63D was not found to be associated with severe iron overload, it attracted less research interest and projects. H63D homozygotes have non-significantly reduced levels of hepcidin (Nelson et al., 2012), perhaps implying iron depleted macrophages. Increased levels of the surface cytokine, Monocyte Chemoattractant Protein-1 have been reported in H63D homozygotes, suggesting an abnormal response to infection (Lawless et al., 2007).

Other genetic forms of resistance

The known genes, encoded proteins and pathways of the immune system number in the tens of thousands, providing a huge number of genetic alternatives to the immune response. For example the millennia of vigorous East–West travel in Europe, with exchange of culture, trade and DNA, led to a wide genetic biodiversity of histo-compatibility antigens, the genetic key to immunological

defence against viruses. The pre-Columbian North Americans had 64 times less HLA biodiversity than that of the Europeans, and succumbed to European viruses not previously encountered (Stride 2011).

For example there is evidence that HLA-DRB1 heterozygosity protects against hepatitis C and reduces progression to end-stage liver disease. There may have been any number of other unknown genetic factors promoting resistance and survival amongst the fortunate members of the Eyam community.

CONCLUSION

The prevalence of the CCR5 delta mutation in Eyam is more likely to be a survival benefit from some infection other than plague; smallpox is the most likely candidate.

The C282Y and H63D mutations causing HC are equally or more common in the Eyam area than delta32, and there is a known scientific basis for the resistance to Yersinia in subjects with haemochromatosis due to the C282Y mutation. The immune-modulating role of H63D is unclear, but given the allele frequency is twice that of either C282Y or delta32 which have known survival benefits, suggesting a greater selection pressure, it may represent the allele of interest with as yet unknown even greater survival benefits.

The survivors in Eyam are more likely to have survived thanks to haemochromatosis than delta32.

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