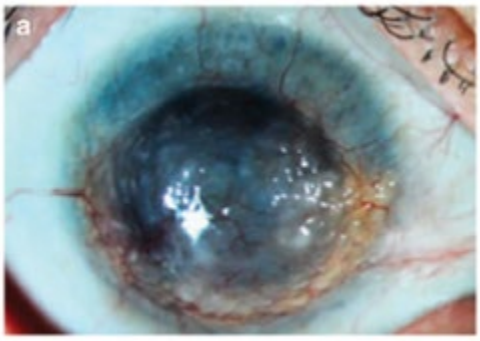
Questions

1. Genomic coordinates of variant. **1p32.1 [1]. The gene is found on the complement strand, between 56994778 and 58700091 [5].**
2. Transcript level coordinates of variant if within a coding region
    1. 5'-UTR, 3', intron, exon, or splice site as examples **Narrative**
    2. If exonic, type of variant (SNP of CNV) and resultant changes in protein or protein level. **Narrative**
3. If variant is in non-coding region and involved in transcriptional regulation, describe proposed pathway. **N/A**
4. Molecular mechanism of variant involved in the causing disease.
    1. Protein, transcript, and/or cellular processes disrupted **Narrative**
5. Transmission pattern. **Autosomal recessive**
6. Phenotypic characterization of the disease. **Narrative**
7. Description of genomic/epigenomic/cytogenetic/transcriptomic/proteomic characterization/detection of disease (how is the genetic cause determined). **Narrative**
8. Flow chart for determination of the genetic cause at the clinical level (i.e. phenomic upon first presentation to clinic to confirmation of genetic disorder).
9. A three-generation pedigree with at least 15 family members.
10. A narrative describing the pedigree.

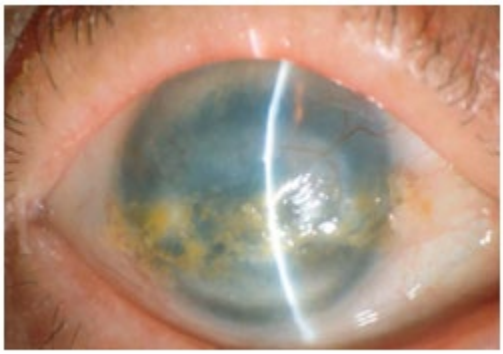
Narrative / Additional Information

The tumor-associated calcium signal transducer 2 (*TACSTD2*) gene is found on chromosome 1, location p32.1. There are four variants know within this gene which causes Gelatinous drop-like dystrophy (GDLD). The four identified variants that lead to expression of GDLD are p.Gln118Ter (Q118X), c.632delA, p.Gln207Ter (Q207X) and p.Ser170Ter (S170X). Of these four, the most commonly observed is p.Gln118Ter (Q118X).

Chromosome 1p32.1 is found on the short arm of chromosome 1 in a region consisting only of exons (no introns) (Figure 1, Figure 2)[2]. The p.Gln118Ter (Q118X) variant consists of a C>T nonsense mutation, replacing the glutamine codon at position 118 with a stop codon, resulting in a truncated protein[1]. This truncated protein can result in abnormal functioning protein, but more commonly, this is a loss of function mutation.



Jongkhajornpong



Tsujikawa

Sakura is a 25 year old female who presents to the ophthalmologist with primary complaints of increased watering eyes over the past 6 months, decreased vision in both eyes, periodic redness in both eyes, and a slight change in color in the eyes. The first evaluation completed upon arrival to the office of the ophthalmologist is the 20 feet Snellen acuity chart[3]. This shows reduced vision since Sakura's last visit. Upon examination via slit lamp, the Ophthalmologist notices mulberry-like appearance (multiple small nodular lesions on corneal surface) in both eyes. The ophthalmologist suspects GDLD and asks the patient for a family history of eye disorders. The patient reported that her paternal grandfather (also mother's uncle) went blind in his late 30s due to corneal amyloidosis and her great-aunt was blind from the age of 12, but she did not know the cause. All Sakura remembers about her great-aunt is that her eyes looked cloudy and bumpy. Sakura also mentioned that her parents were first cousins and married in Japan before immigrating to the United States. Once settled in the US, they began their family. Sakura is the first born child, with a younger brother (Minato, age 13) and a younger sister (Ema, age 8). So far, their vision is fine.

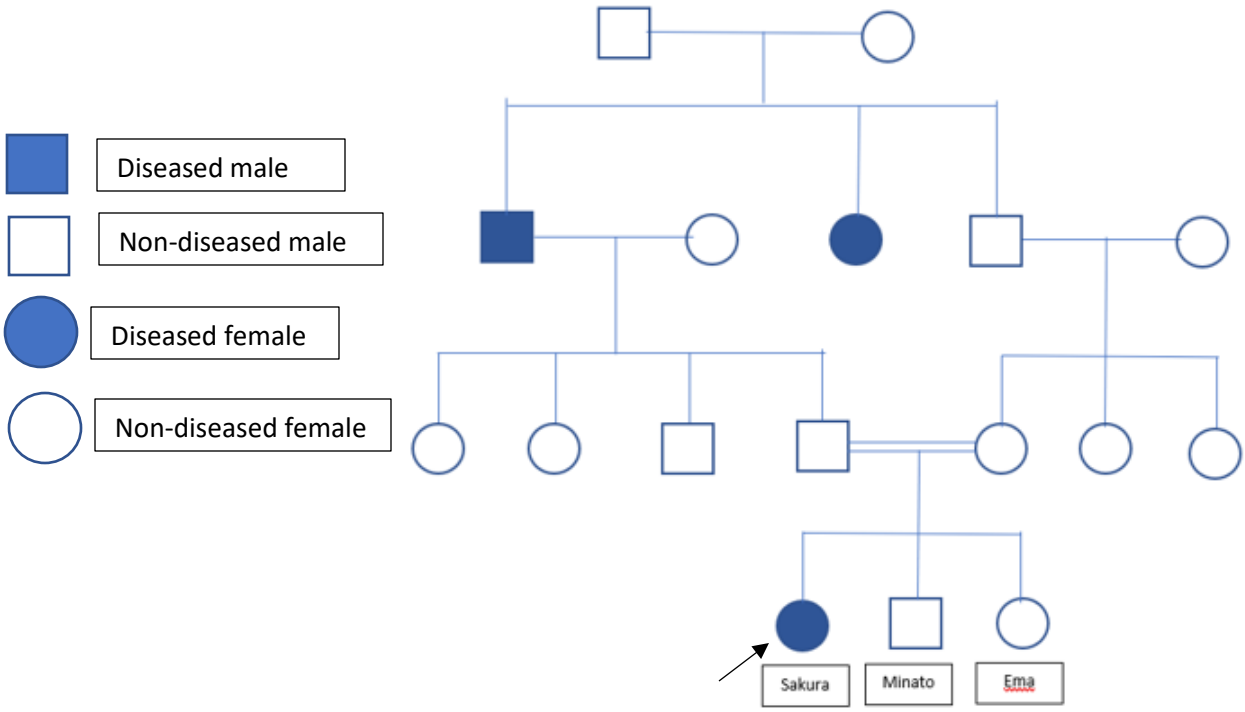


Figure 1. Pedigree going back four generations to identify the inheritance pattern of Gelatinous drop-like corneal dystrophy in Sakura's family. The pedigree supports an autosomal recessive inheritance pattern. Because of the consanguineous relationship of Sakura's mother and father, penetrance of the autosomal recessive disease is shown.

Considering the family history of consanguinity between her parents and two cases of blindness in the pedigree following an autosomal recessive disease, the ophthalmologist decides to continue on the path of investigating GDLD and orders genetic testing. The ophthalmologist connects Sakura and her family to a genetic counselor.

The genetic counselor collects a sample of blood from Sakura and amplifies via PCR then performs direct Sanger sequencing for only the *TACSTD2* gene. The results of the sequencing confirms the hypothesis of both the ophthalmologist and genetic counselor. Sakura has the p.Gln118Ter (Q118X) variant on chromosome 1, in the region coding for the *TACSTD2* protein.

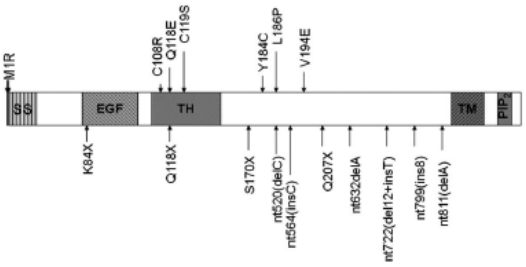
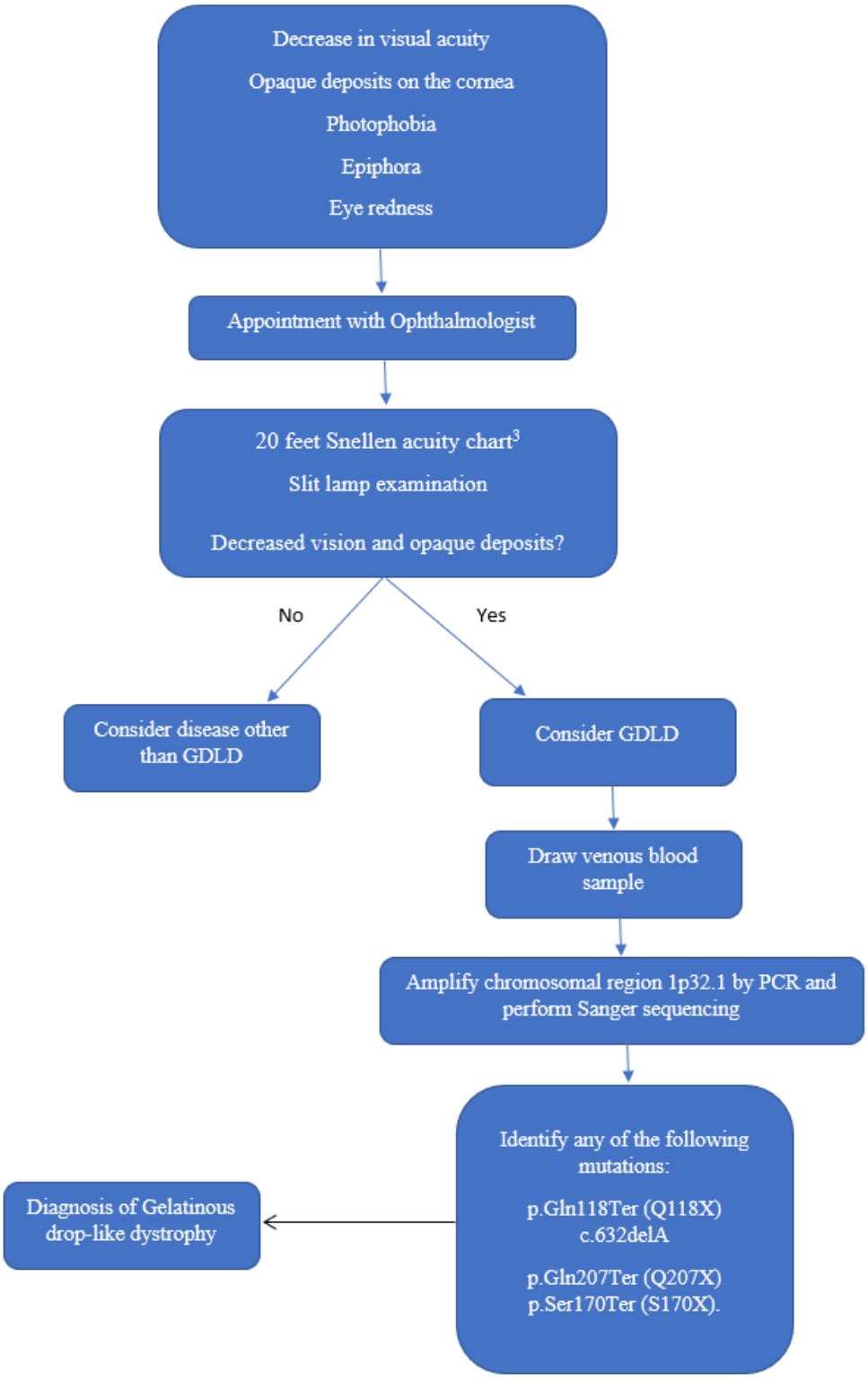


FIGURE 4. Schematic representation of the structure of TACSTD2. EGF, epidermal growth factor–like repeat; PIP2, phosphatidylinositol 4,5-bisphosphate–binding sequence; SS, signal sequence; TH, thyroglobulin repeat; TM, transmembrane domain. Arrows indicate the locations of the reported mutations.



Flow chart showing appropriate steps to follow in order to conclude GDLD.

The genetic counselor suggests to test Minato and Ema to identify their genotype in relation to the TACSTD2 gene prior to reproduction, if they chose to reproduce. If their partner is non-Japanese, the chance of the individual being heterozygous for the allele is slim, but testing is also suggested for future partners. If the parents of Minato and Ema decide it best to investigate their phenotype now, that is fine, but unfortunately there is not much that can be done to stop the prevention of the disease. The genetic counselor suggests to eat a balanced diet, protect eyes from UV rays, and have regular eye exams to check for ocular pressure. Genetic testing at this point, considering their age, would be mostly informative. If the parents of Minato and Ema decide that it is best to wait, Minato and/or Ema may start to show symptoms within just a few years.

The tumor-associated calcium signal transducer 2 protein is involved in tight junctions in epithelial barriers by binding to claudin 1 and 7. When these tight junctions are lost, spaces develop in the cornea, increasing corneal epithelia permeability. This corneal separation leads to increased vascularization and accumulation of acellular eosinophilic deposits. These deposits increase over time and cause decreased visual acuity, leading to loss of vision.

The complaints of Sakura are concerning because these are typical phenotypes of GDLD.The symptoms can vary among affected individuals within a family, and also between ethnicities. However, the amyloid deposits and decreased vision are maintained throughout. Sakura's paternal grandfather (also mother's uncle) and great-aunt (aunt of both mother and father) had blindness. No one in the next generation had any eye disorders, i.e. blindness or loss of vision. The "skipped generation" is indicative of an autosomal recessive inheritance pattern.

In regards to the genetic sequencing, the ophthalmologist knows what gene to focus on, but the specific mutation should be identified. The different mutations can be point mutations or deletions. Possible treatment could be carried out via CRISPR, so it may be beneficial to know the specific variant.

Minato and Ema do not need to get tested immediately, however it may be beneficial. *TACSTD2* is a gene rich in CpG and has 22 SNPs. Presence of the CpG islands may be indicative of epigenetic regulation and therefore expression of the gene[3]. The suggestions for regular eye exams, a healthy diet, and eye protection are all measures that can be taken to preserve the eyes and delay the onset slightly. Exposure to UV light may be a risk factor[1]. Unfortunately, prevention of the disease is not discussed. The prevalence of GDLD is highest in Japanese populations, however this also affects Indian, Chinese, Tunsinian, Turksih, and Iranian families[1].

References

[1] Kaza, H., Barik, M. R., Reddy, M. M., Mittal, R., & Das, S. (2016). Gelatinous drop-like corneal dystrophy: A review. *Br J Ophthalmol,* 10-15. doi:10.1136/bjophthalmol-2016-309555

[2] Tsujikawa, M., MD, PhD. (2012). Gelatinous Drop–Like Corneal Dystrophy. *Cornea, 31*, S37-S40.

[3] Alehabib, E., Jamshidi, J., Ghaedr, H., Emamalizadeh, B., Andarva, M., Daftarian, N., . . . Darvish, H. (2017). Novel Mutations in TACSTD2 Gene in Families with Gelatinous Drop-like Corneal Dystrophy (GDLD). *Int J Mol Cell Med, 6*(4), 204-211. doi:10..22088//BUMS..6..4..204

[4] Jongkhajornpong, P., Lekanont, K., Ueta, M., Kitazawa, K., Kawasaki, S., & Kinoshita, S. (2015). Novel TACSTD2 mutation in gelatinous drop-like corneal dystrophy. *Human Genome Variation,* 1-4. doi:10.1038/hgv.2015.47

[5] https://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=DetailsSearch&Term=4070