Research Protocol

Title:
A Cross-sectional Study Screening for Stickler Syndrome in Children Diagnosed with Perthes Disease

Synopsis
Stickler syndromes are an underdiagnosed group of disorders of type II, IX, and XI collagen associated with retinal detachment, skeletal dysplasia, deafness and cleft palate. The most common phenotypic sub-group is caused by pathogenic variants in the gene encoding type II collagen (*COL2A1*), so-called Type 1 Stickler syndrome.[1] This is especially associated with early retinal detachment, occurring in the 2nd-3rd decade, and has a poor surgical repair rate, with 30% of Stickler syndrome patients suffering sight loss.[2,3] A recent case series has shown a subset of the Type 1 Stickler syndrome population with an historic diagnosis of Legg-Calve-Perthes disease, an avascular necrosis of the hip.[4] Given that the presentation of Perthes disease is typically in the 1st decade of life, this might provide a unique opportunity to screen and identify Stickler syndrome early and offer prophylactic retinopexy to greatly reduce the risk of later retinal detachment and sight loss.[5,6] Thus, this research project proposes to develop a screening tool to identify patients with Stickler syndrome, to utilise it amongst a Perthes disease population, and to offer genetic testing to high-risk patients based on this tool. This will determine whether Stickler syndrome patients are present and can be identified amongst children being assessed for Perthes disease.

Introduction
Stickler syndrome is a genetic disorder of collagen synthesis, with the most common variant (80%) being in *COL2A1*, so-called Type 1 Stickler Syndrome.[1] The overall incidence is estimated to be around 1 in 7500, but it is believed to be widely under-recognised and under-diagnosed. The NHS England Highly Specialised Service for Stickler syndrome in Cambridge University Hospital NHS Trust (CUH) currently sees 70 newly referred families a year from across the UK and Ireland.

The underdiagnosis of Stickler Syndrome is contributed to by poor awareness amongst clinicians, but it is also challenging due to the extreme variation in the Stickler phenotype.[2] Presentation typically includes extreme myopia, hypermobility with associated arthropathy, cleft palate, and hearing loss, but these features are not universal. Palate abnormalities are seen in 45% of patients and hearing loss is present in 52%.[7,8] Whilst there is a classically described genetic facies (flat midface, small jaw, prominent eyes, long philtrum and short nose with anteverted nares), this can be extremely subtle. Many patients can develop early-onset arthritis without diagnosis of a specific connective tissue disorder. Indeed, often patients are only first diagnosed with Stickler syndrome once they have suffered its most life-limiting morbidity: the emergence of retinal detachment typically in the 2nd-3rd decade.

Retinal detachment occurs in up to 78% of type 1 Stickler syndrome patients.[6] Retinal detachment in Stickler syndrome is typically due to Giant Retinal Tear and the surgical repair after this is poor in comparison to spontaneous detachments. This is partly due to the oft delayed presentation in the paediatric population, where children often do not report visual loss in one eye, or not until there is involvement of the second eye. This means surgical repair may only restore limited vision, or worse, be unable to salvage any retinal function. All of the above contributes to up to 30% of Stickler patients losing vision in at least one eye.[3]

However, in contrast to surgical repair, prophylactic treatment has been shown to be highly effective in reducing the risk of visual loss from retinal detachment. Prophylactic cryotherapy techniques developed by our team in Cambridge prevent the initial giant retinal tears occurring and expanding, decreasing the risk of future detachment in Type 1 Stickler syndrome patients by 5-10 fold.[3,5,6,9–11]

Administering this treatment throughout this population can therefore have a huge impact on patient outcomes, but only if there is a significant improvement in the strategies for diagnosing Stickler syndrome.

Currently, Stickler syndrome has no diagnostic criteria, and diagnosis can only be attained definitively through genetic testing. The vast majority of cases show autosomal dominant inheritance so predictive testing of family members is now routinely provided but there is a need to accurately identify new undiagnosed index cases in children prior to avoidable sight loss. One strategy that has been successful in this regard is in the identification of at-risk individuals in other disease categories. For example, Stickler syndrome has been identified as the most common cause of Pierre-Robin sequence (cleft palate, microagnathia, glossoptosis) and children under the Cambridgeshire cleft team will regularly undergo screening for Stickler syndrome.[12] Another strategy may come through screening for common symptoms of Stickler syndrome. At the time of diagnosis most patients have a positive family history for the Stickler phenotype, meaning screening for common traits could identify 'high-risk' individuals.[2] Combining these in order to screen at-risk populations could be hugely successful in identifying index cases, and in conjunction with prophylactic retinopexy, could prevent retinal detachment and associated blindness.

A recent uncontrolled, observational case series at CUH was reported due to the association of Stickler syndrome with an historic diagnosis of Legg-Calve-Perthes disease.[4] Perthes disease is an avascular necrosis of the hip, with an incidence of 1 in 10,000 but poorly defined aetiology and pathophysiology.[13,14] Amongst this reported case series of 19 patients, all had *COL2A1* variants (type 1 Stickler syndrome), and there were 18 cases of retinal detachment with 7 patients experiencing sight loss in one eye and one patient now blind. This case series mirrors other recent reports of *COL2A1* variants associated with Perthes disease without associations being demonstrated at a population level.[15,16] However, no study up to this point has tested a screening tool to identify patients at high risk of Stickler syndrome amongst the Perthes disease population, nor genetically tested those at a higher risk to confirm a diagnosis.

This project will aim to achieve both, thus potentially allowing early diagnosis of Stickler syndrome and provision of preventative treatment for retinal detachment, arthritis, and other comorbidities of Stickler syndrome. It may also create a model that could be followed in the diagnosis of Stickler syndrome in other associated comorbidities, or indeed the diagnosis of other rare diseases that may be associated with Perthes disease.

Aims and Objectives

The overall aim of the study is to improve the early diagnosis of Stickler syndrome patients, specifically by investigating the observational association between historic Perthes disease diagnosis and Type 1 Stickler syndrome.

The specific objectives are:

1. To ratify a screening tool that can identify children with a diagnosis of Stickler syndrome from their medical and family history.
2. To utilise the screening tool in a population of paediatric patients with newly diagnosed Perthes disease to assess for Stickler syndrome amongst this population.

Secondary goals include formally estimating the incidence of Perthes disease amongst the Stickler syndrome patient population and estimating the incidence of other comorbidities dependent on Stickler syndrome genotype. There will also be analysis of the screening tool after use to propose alterations that will improve the identification of Stickler syndrome patients in the future.

1. To ratify a screening tool that can identify children with a diagnosis of Stickler syndrome from their medical and family history.

A screening tool was suggested as part of the case series identifying historic diagnosis of Perthes disease amongst Type 1 Stickler syndrome patients.[4] This screening tool encompasses symptoms patients may have experienced in early life, as well as important elements of family history, which is positive in 80% of new diagnoses of Stickler syndrome.[2] However it is currently based on anecdotal impressions of the most important factors in Stickler Syndrome and has not yet been tested in Stickler syndrome patients for its sensitivity, nor validated for its specificity by comparing the scores of the general paediatric population.

Ophthalmic	Retinal detachment	4
	Myopia (short-sight) *before* the age of 10 years	2
	Family history of retinal detachment: single relative	2
	Family history of retinal detachment: more than 1 relative	4
Orofacial	History of cleft palate repair	3
	High arch palate or bifid uvula on examination	2
	Family history of cleft palate repair	2
Auditory	Sensorineural loss	2
	Family history of deafness	1
Musculo-skeletal	Spinal abnormalities (flattened vertebrae, end plate changes, scoliosis, kyphosis) Short stature	2
	Hypermobility (Beighton score>6)	1
Ancillary questionnaire for patients with Legg-Calvé-Perthes' disease. Stickler syndrome should be excluded in all patients who score 4 or above.		

Table 1 – proposed screening tool for Stickler syndrome amongst Perthes disease patients, as per Wang et al. (2023). Originally proposed that a score of ≥4 should be considered 'high-risk'.

Thus, the first aim of this study would be to evidence the validity of the screening tool and its scores. This would require an initial literature review of the signs and symptoms of Stickler syndrome, to ensure that weighting of the individual scores represents the prominence of these risk factors amongst the population. However, most epidemiological data on the comorbidities associated with Stickler syndrome does not have matching data on patient genotypes, meaning that the current data would not distinguish type 1 Stickler syndrome from other iterations. Thus, in addition to a review of the literature, the Stickler Syndrome UK charity (SSUK) patient membership survey will also be used. This will allow data on the Stickler phenotype to be collected from previously genotyped patients. This will more precisely advise on the most predominant comorbidities present in Type 1 Stickler Syndrome, and in the wider Stickler syndrome cohort. Our initial focus will be on the specific comorbidities listed in Wang et al.'s proposed screening tool, plus any others identified in the literature review as being high occurrence. Further data collection to give a wider estimate of the comorbidities across the population would be done separately in conjunction with SSUK. Additionally, paediatric patients without Perthes disease identified in the above survey that would also be used later to ratify the screening tool will be excluded from this development to avoid bias. If this data was not sufficient to estimate incidence of the above comorbidities in type 1 Stickler syndrome, then data on Perthes and Stickler will also be sampled from UK Biobank, as genotyping data would also be available here.

The tool would also be retrospectively compared to the data collected for the case series of 19 patients, plus any others found to have an historic diagnosis of Perthes disease. These would be identified either from the population under the care of CUH (and thus consented via their clinical care team) or via the SSUK patient membership survey.

Once scoring for the screening tool is formally agreed, then it will be tested in an appropriate population. The screening tool should be able to identify the vast majority of Stickler patients as 'high-risk', but also have a high specificity to filter out patients in the general population. To verify this, the screening tool would be tested in patients with Type 1 Stickler syndrome that do not have a diagnosis of Perthes disease and are in the age range of Perthes disease presentation (4-10 years). This would require a selection of paediatric patients from our Stickler syndrome database at CUH to be contacted, with parents consented and asked details relevant to the screening tool.

This cohort would then be compared to a random sample from the general paediatric clinic population in the same age range, with patients excluded if they have an underlying connective tissue disorder, Perthes diagnosis, or if are being assessed for either. This population will be convenient to access and being a generally well population will have a low risk of biasing the cut-off scores of the screening tool. They will be recruited through the patient pool in attendance at CUH general paediatric clinics.

To quantify any statistically significant difference between the mean scores of the Stickler paediatric cohort and the general paediatric cohort, a two-tailed t-test will be performed using the scores of the two groups. Given that Stickler syndrome is a rare disease, only a large effect size would be useful in future screening to detect the small proportions of a patient population that could have underlying Stickler syndrome. Therefore, in order to detect a large effect size ($d = 0.8$), with an α error probability of 5% and power of 80%, 52 patients would be required.[17] In addition, the sensitivity and specificity of the current cut off score for 'high-risk' patients would be analysed to see if this could be further optimised. If in the process of the ratification of this screening tool any other signs or symptoms are identified as specific to Stickler syndrome patients, they will be considered for addition to future iterations of the screening tool, and will be analysed alongside the rest of the individual components of the screening tool at the end of the study.

Through the above processes, a screening tool for Stickler syndrome will be ratified in the paediatric population, allowing use in the Perthes disease population. Additionally, the secondary aim of collecting data on the comorbidities associated with different Stickler syndrome genotypes will be achieved through the SSUK patient membership survey.

2. To utilise the screening tool in a population of paediatric patients with newly diagnosed Perthes disease to assess for Stickler syndrome amongst this population.

The second aim of this project is to identify any Stickler syndrome patients in a population of Perthes disease patients using the screening tool to aid selection. We have collaborated with the paediatric orthopaedic team based in Alder Hey Children's Hospital, Liverpool, who have a large cohort of Perthes disease patients under their care. This population will have the screening tool applied to them and will have a blood test collected for Stickler syndrome genetic testing. Data will be collected across 1-2 years, with an expectation of 100-200 patients. Their screening tool results will be divided into 'high risk' and 'low risk' for Stickler syndrome as above, and compared to their genetic results. This will allow us to assess both if there is Stickler syndrome present in the Perthes disease population, and if our screening tool could identify it. The identification of any patients positive for Stickler syndrome would be considered a justification for future exploration of the screening tool, given that Stickler syndrome is a rare disease that should not be present in this population.

Type 1 Stickler syndrome accounts for over 80% of diagnoses, and most commonly is due to the introduction of premature termination codons into the reading frame of *COL2A1* mRNA, resulting in haploinsufficiency of type II collagen. In our case series and in the literature, we have only identified a link between Type 1 Stickler syndrome, *COL2A1*, and Perthes disease, and thus this will be the only gene tested via long range PCR. The Genomics England Stickler syndrome panel includes *COL2A1* but also *COL11A1, COL11A2, COL9A1, COL9A2, COL9A3, BMP4, VCAN, GZF1, LOXL3, and LRP2*, diagnosing all known forms of Stickler syndrome as well as conditions with significant overlap in clinical phenotype (eg Wagner and Larsen syndromes); however none of these have been linked with Perthes and none have yet been shown to benefit from prophylactic retinopexy to prevent retinal detachment. Thus, only *COL2A1* will be tested, reducing the cost and storage associated with a larger genetic test, limiting the risk of VUS discovery, and adhering to the need for an accepted treatment as per the Wilson criteria for screening tools. The intron coding sequence of *COL2A1* will also be analysed by our team for more subtle variants causing Type 1 Stickler syndrome.

In addition, due to the likelihood of a very small number of patients being positive for Stickler Syndrome, further analysis of the individual elements of the screening tool would only be done as part of the discussion to advise on future changes to scoring in the screening tool, and not to confirm the utility of each individual element of the tool in predicting Stickler Syndrome in this research. This will avoid biasing the tool towards specific individual cases post-analysis.

All patients tested genetically for Stickler syndrome during the course of this study will be informed of the outcome of that test. As a diagnosis of Stickler syndrome has implications for the future health of that individual and their family, any patients with genetic testing positive for Stickler syndrome will be referred to the Highly Specialised Service for confirmation of diagnosis and further management, including the offer of prophylactic retinopexy for those with Type 1 Stickler syndrome. This will be included in the patient information sheet, with the GP contacted to arrange an NHS referral to the Highly Specialised Service for Stickler syndrome. Patients will also be contacted directly and provided an information sheet about Stickler syndrome in order to explain the diagnosis and next steps. The paediatric orthopaedic team in Liverpool will also be contacted as a positive diagnosis may change the management of their Perthes disease.

Schedule of Assessments

A rough schedule of assessments for patient information is included below.

Stage	Activity	Schedule
Development of Screening Tool	Contact of Stickler patients with Perthes disease. SSUK patient membership survey.	Feb 24 – Apr 25 (ongoing data collection for wider Stickler demographics alongside Stickler Syndrome UK charity)
Test of Screening Tool	Application of screening tool to Stickler syndrome and paediatric patients	Mar 25 – May 25
Application of Screening Tool	Screening tool applied to paediatric Perthes disease population	Apr 25 – May 26
	Blood collection and genetic testing of paediatric Perthes disease population	Apr 25 – Jun 26

People
The principal investigator responsible for this project will be the applicant Dr Robert Smyth, ST3 Paediatric Trainee in East of England and Fellow-Commoner in Medicine at Trinity Hall College, University of Cambridge. Robert has shown an aptitude and dedication to research already during his short career and has received a Addenbrookes Charitable Trust Clinical Research Fellow post and a Cambridge Trust Scholarship to fund this project. The applicant will dedicate 2 days per week over 2 years to the research programme.

The project will also be overseen by Mr Martin Snead, Consultant Ophthalmic Surgeon at Addenbrooke's Hospital and a Director of Research at The University of Cambridge, and Dr Peter Bale, Consultant Rheumatologist at Addenbrooke's Hospital. Mr Snead has a proven track record of research in the field of vitreoretinal disorders, inherited vitreoretinopathies and retinal detachment repair with research grants from The Iris Fund for Prevention of Blindness, The Stanley Thomas Johnson Foundation, the Stickler Syndrome Support Group, The Guide Dogs For The Blind Association, The Isaac Newton Trust and The London Law Trust. Dr Bale has a unique insight into the complexity of paediatric Stickler syndrome and Perthes disease, running the paediatric rheumatology portion of the Highly Specialised Service for Stickler Syndrome.

Professor Daniel Perry will provide key oversight of the Perthes disease patient cohort in Liverpool. Prof Perry is an orthopaedic surgeon with an extensive background of clinical trials, research and publications on Perthes disease in his time at the University of Liverpool. He is currently undertaking innovative research into the conservative versus surgical management of Perthes disease in conjunction with the National Institute for Health and Care Research. Professor Christian Hedrich will also support the recruitment and collection of bloods from the paediatric population in this research. Prof Hedrich is a paediatric rheumatologist with a special interest in cytokine dysregulation in autoimmune/inflammatory disease, and since 2020 has served as Head of the Department of Women's and Children's Health at the University of Liverpool.

The subjects of this study will include current Stickler patients with historic diagnoses of Perthes disease, a paediatric sample already diagnosed with Stickler syndrome, a paediatric sample from a general paediatric clinic, and a population of paediatric patients diagnosed with Perthes disease. Stickler patients will be recruited from the cohort under the care of the Highly Specialised Service for Stickler Syndrome, and the use of their electronic records will allow any patients with an historic diagnosis of Perthes disease to be identified by their clinical care team at CUH and consented for further contact by the research team. The clinical care team will also identify and consent paediatric patients and their parents as above. Paediatric patients for the control group will be recruited directly from general paediatric clinics with parental consent. Finally, the paediatric population with Perthes disease that will be used to test the screening tool will be recruited from the Perthes population under Alder Hey hospital, Liverpool, by their clinical care team, with the survey and bloods collected at clinic appointments to reduce barriers to participation.

Patient Public Involvement
The premise and design of this research project was presented to patients at the annual Stickler Syndrome UK charity conference 2024, with patients particularly approving of the musculoskeletal focus of the project and aim to achieve earlier diagnosis. The musculoskeletal element of Stickler syndrome is an area of research that has been less well explored, with retinal detachment typically the most popular topic of study. There was also a general assent that emphasis on early diagnosis in the paediatric population would be beneficial to future patients and families.

As part of this work with SSUK, we will utilise the SSUK patient membership survey to collect further data on Perthes disease and other comorbidities sorted by Stickler syndrome genotype. This consists

of patients affected by Stickler syndrome across the country who have volunteered to provide their time and information for further study, and is advertised via SSUK's newsletters and social media platforms. Thus, by working with the charity, who are currently planning and administering this survey, we can access a larger population of willing participants and provide research that continues to align with the community's desires and values.

Findings of this research will also be disseminated through the charity via their newsletters, website and conferences. If the study identifies Stickler syndrome patients amongst the Perthes disease population, then this can also be added to the charity's website. We have a number of patients self-referrals to our specialist service, and expanding the known associations of Stickler syndrome in this way will be beneficial for identifying further index cases.

Ethical Considerations
As the majority of patients involved in this study will be paediatric patients, careful consent and discussion with parents must be done prior to study enrolment. The screening tool will deliberately be designed to be minimally invasive and easy-to-use, reducing the burden on the healthcare professional administering it as well as for the parent and child providing answers. We will also offer it in multiple formats to allow it to be most suited to the family.

Furthermore, as this research involves genetic testing, patients and parents must be informed exactly what is being tested, and the implications of a positive diagnosis of this autosomal dominant condition for the individual and their family. To reduce the risk of patients withdrawing, patients will have blood collected upfront for genetic testing. We would blind patients to their survey results at the time of testing and would ask them to agree to this principle at the start of the study. We will also coordinate genetic blood tests with other research blood tests they will be receiving, reducing the burden on patient and parents in terms of travel and time.

All patients tested genetically for Stickler syndrome during the course of this study will be informed of the outcome of that test. As a diagnosis of Stickler syndrome has implications for the future health of that individual and their family, any patients with genetic testing positive for Stickler syndrome will be referred to the Highly Specialised Service for confirmation of diagnosis and further management, including the offer of prophylactic retinopexy for those with Type 1 Stickler syndrome. This will be included in the patient information sheet, with the GP contacted to arrange an NHS referral to the Highly Specialised Service for Stickler syndrome. Patients will also be contacted directly and provided an information sheet about Stickler syndrome in order to explain the diagnosis and next steps. The paediatric orthopaedic team in Liverpool will also be contacted as a positive diagnosis may change the management of their Perthes disease.

Environment
The Highly Specialised Service for Stickler syndrome at Addenbrooke's Hospital CUH is a national referral service for patients from the UK and Ireland with Stickler syndrome. It encompasses the vitreoretinal service, a regional and national tertiary referral service for inherited vitreoretinopathies, and tertiary paediatric and adult rheumatology services. It is an NHS service, free at point of use.

The Alder Hey Children's Hospital, Liverpool, provides a leading regional paediatric orthopaedic service for the management of Perthes disease.

The genomics lab used will be the Stratified Medicine Core Laboratory Next Generation Sequencing Hub (SMCL NGS Hub). This lab is housed in the East GLH and works within the University of Cambridge and CUH. This will provide high quality genetic diagnoses. However, as it is not one of the seven hubs that make up the national genomic laboratory service, any patients positive for Stickler

syndrome will have their genetic tests repeated through the East GLH on referral to the Highly Specialised Service.

Initial data collection would use EPIC, the IT system in CUH that has details on all patients under the Highly Specialised Service for Stickler syndrome. Patients would be identified as eligible for the study by their clinical care team, and initially contacted and consented for the research by the same team. Additionally, the use of the Stickler syndrome patient membership survey will allow further access to volunteers from the Stickler community, who have already agreed to provide their information for research purposes. All patient data stored for analysis will be stored securely and encrypted to maintain confidentiality, and will be anonymised at time of publication.

Indemnity
Cambridge University Hospitals NHS Foundation Trust, as a member of the NHS Clinical Negligence Scheme for Trusts, will accept full financial liability for harm caused to participants in the study caused through the negligence of its employees and honorary contract holders. There are no specific arrangements for compensation should a participant be harmed through participation in the study, but no-one has acted negligently.

The University of Cambridge will arrange insurance for negligent harm caused as a result of protocol design and for non-negligent harm arising through participation in the study.

Investigators:
Dr Robert Smyth, Principal Investigator, MPhil Student and Paediatric ST3
Mr Martin Snead, Chief Investigator, Director of Research UoC, Consultant Ophthalmic Surgeon CUH
Dr Peter Bale, Academic Supervisor, Consultant Paediatric Rheumatologist CUH
Prof Daniel Perry, NIHR Research Professor, University of Liverpool, Consultant Paediatric Orthopaedic Surgeon Alder Hey Hospital
Prof Christian Hedrich, Professor of Child Health, University of Liverpool, Consultant Paediatric Rheumatologist, Alder Hey Hospital
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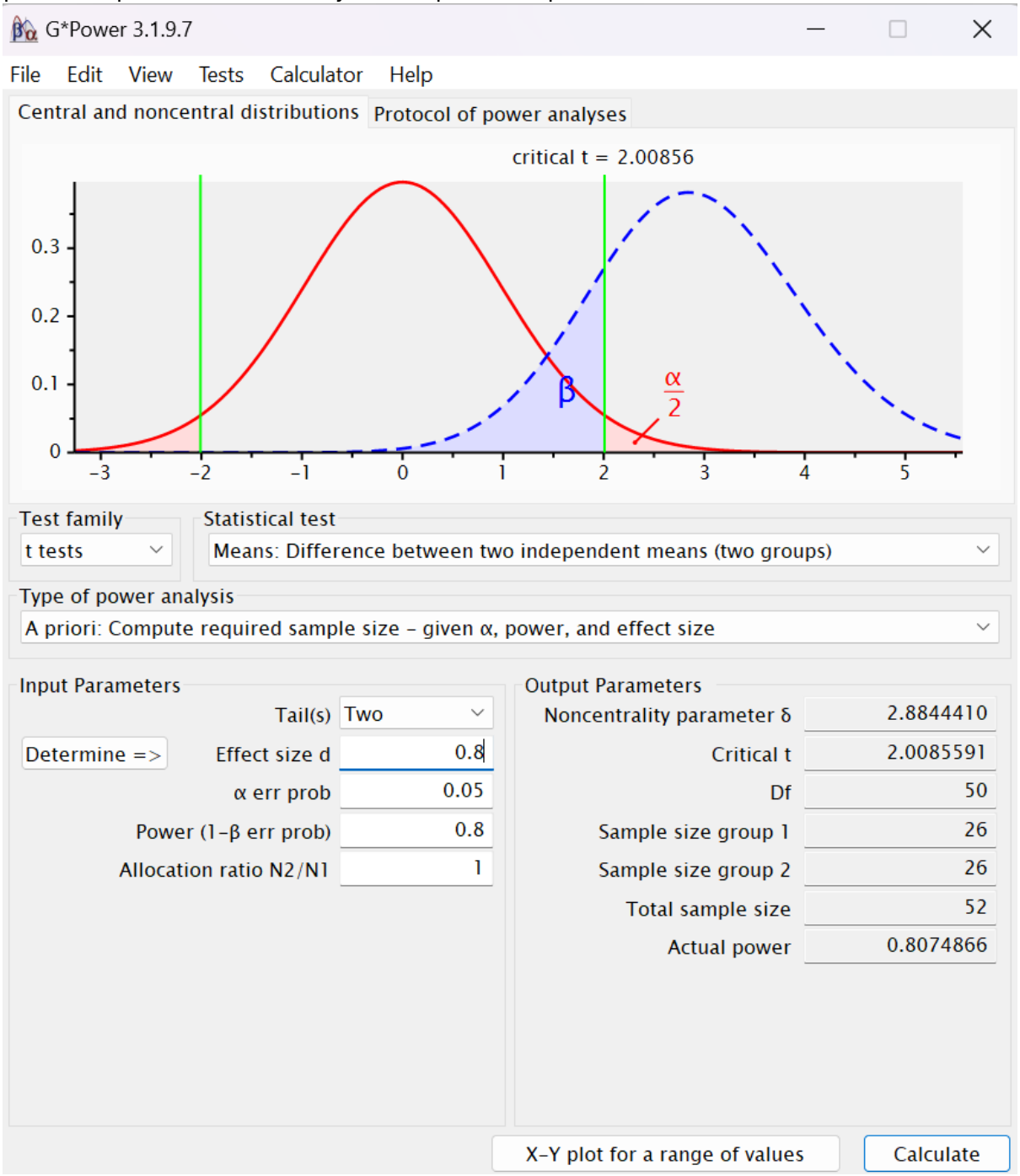
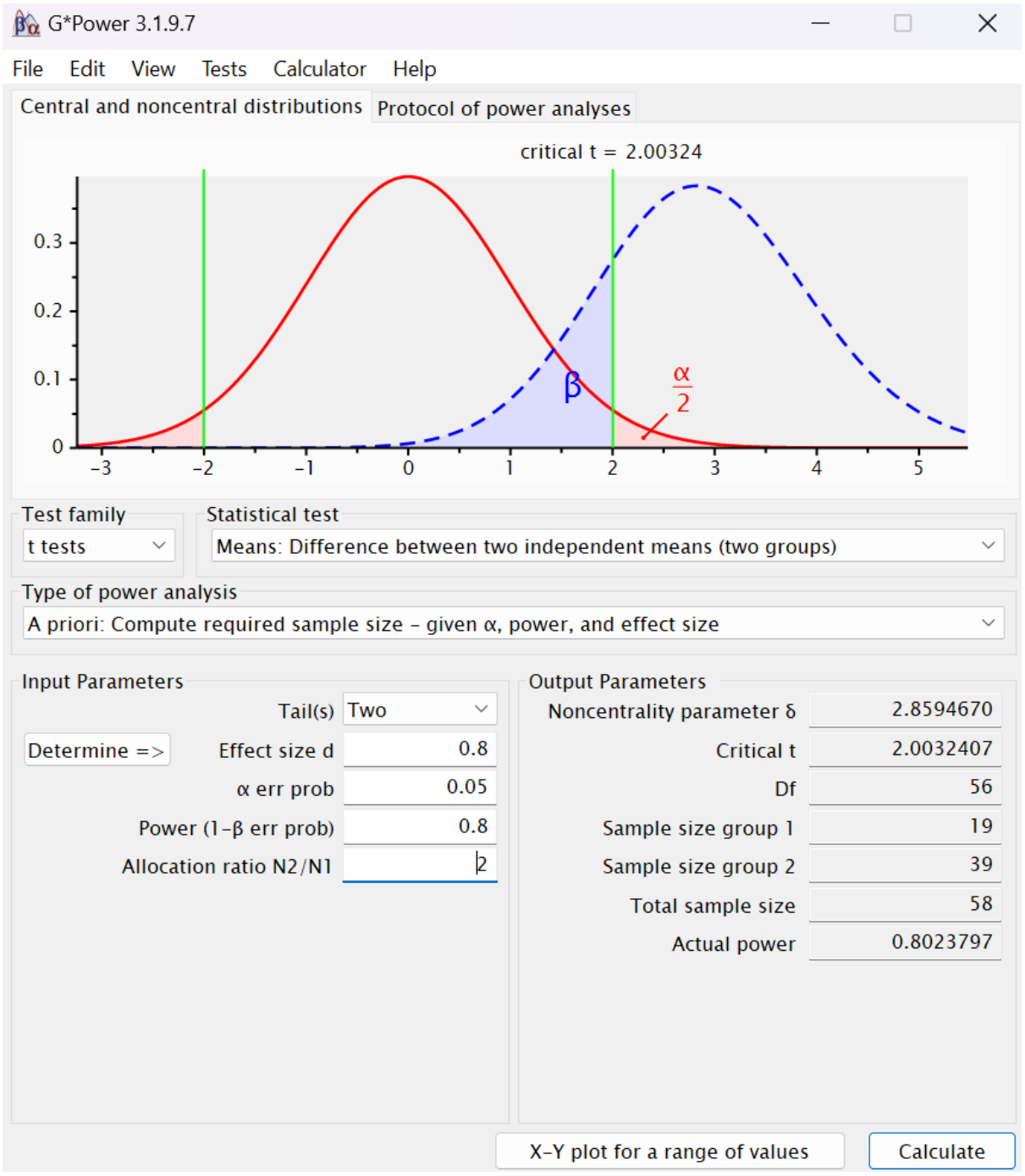
References

1. Mortier, G. *Stickler Syndrome*. *GeneReviews®* (2023).
2. Stickler, G. B., Hughes, W. & Houchin, P. Clinical features of hereditary progressive arthro-ophthalmopathy (Stickler syndrome): A survey. *Genetics in Medicine* **3**, 192–196 (2001).
3. Alexander, P. & Snead, M. P. Prevention of Blindness in Stickler Syndrome. *Genes (Basel)* **13**, (2022).
4. Wang, A. *et al*. Legg-Calve-Perthes' disease: An opportunity to prevent blindness? *Archives of Disease in Childhood* vol. 108 789–791 Preprint at https://doi.org/10.1136/archdischild-2022-325059 (2023).
5. Fincham, G. S. *et al*. Prevention of retinal detachment in stickler syndrome: The cambridge prophylactic cryotherapy protocol. *Ophthalmology* **121**, 1588–1597 (2014).
6. Alexander, P. *et al*. Cambridge Prophylactic Protocol, Retinal Detachment, and Stickler Syndrome. *New England Journal of Medicine* **388**, 1337–1339 (2023).
7. Zimmermann, J. *et al*. Stickler Syndrome: Airway Complications in a Case Series of 502 Patients. *Anesth Analg* **132**, 202–209 (2021).
8. Acke, F. R. E. & De Leenheer, E. M. R. Hearing Loss in Stickler Syndrome: An Update. *Genes* vol. 13 Preprint at https://doi.org/10.3390/genes13091571 (2022).
9. Naravane, A. V., Belin, P. J., Pierce, B. & Quiram, P. A. Risk and Prevention of Retinal Detachments in Patients with Stickler Syndrome. *Ophthalmic Surg Lasers Imaging Retina* **53**, 7–11 (2022).
10. Morris, R. E. & Kuhn, F. Retinal Detachments in Stickler Syndrome. *Ophthalmic Surgery Lasers and Imaging Retina* vol. 53 522–523 Preprint at https://doi.org/10.3928/23258160-20220815-03 (2022).
11. Carroll, C., Papaioannou, D., Rees, A. & Kaltenthaler, E. The clinical effectiveness and safety of prophylactic retinal interventions to reduce the risk of retinal detachment and subsequent vision loss in adults and children with Stickler syndrome: a systematic review. *Health Technol Assess (Rockv)* **15**, 1–68 (2011).
12. Izumi, K., Konczal, L. L., Mitchell, A. L. & Jones, M. C. Underlying Genetic Diagnosis of Pierre Robin Sequence: Retrospective Chart Review at Two Children's Hospitals and a Systematic Literature Review. *J Pediatr* **160**, 645-650.e2 (2012).
13. Perry, D. C. Unravelling the enigma of Perthes disease. *Ann R Coll Surg Engl* **95**, 311–316 (2013).
14. Hall, A. J., Barker, D. J., Dangerfield, P. H. & Taylor, J. F. Perthes' disease of the hip in Liverpool. *BMJ* **287**, 1757–1759 (1983).
15. Kenet, G. *et al*. Perthes' disease and the search for genetic associations. *J Bone Joint Surg Br* **90-B**, 1507–1511 (2008).
16. Lindblad, M., Bladh, M., Björnsson-Hallgren, H., Sydsjö, G. & Johansson, T. No correlation to collagen synthesis disorders in patients with Perthes' disease: a nationwide Swedish register study of 3488 patients. *BMC Musculoskelet Disord* **25**, 42 (2024).
17. Faul, F., Erdfelder, E., Buchner, A. & Lang, A.-G. Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. *Behav Res Methods* **41**, 1149–1160 (2009).

Appendix A

G*Power calculations for two-tailed t-test comparing the mean screening tool score in Stickler syndrome paediatric patients versus general paediatric patients. Two examples are included, with the first assuming equal sample sizes and the second assuming twice as many general paediatric patients as Stickler syndrome paediatric patients.

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Means: Difference between two independent means (two groups)
Type of power analysis
A priori: Compute required sample size – given α, power, and effect size
Input Parameters
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Determine =>
Effect size d 0.8
α err prob 0.05
Power (1−β err prob) 0.8
Allocation ratio N2/N1 2
Output Parameters
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Sample size group 2 39
Total sample size 58
Actual power 0.8023797
X-Y plot for a range of values
Calculate