WORKSHOP

Newer Signatures for Evidence Based Evaluation in Strabismus: Imaging and Genetics

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Purpose/Relevance: With newer modalities of investigations available in the form of high-resolution surface-coil orbital imaging for extraocular muscle (EOM) evaluation and genetics in the form of next generation sequencing (NGS) tools to assess the etiology of both, common and special forms of strabismus, it becomes important for the strabismologist to have an overview of the what is available and possible.

Delivery Format: Didactic lecture with open Q/A forum
(a) Imaging as a phenotypic marker in congenital cranial dysinnervation syndrome and muscle heterotopy
(b) Whole exome sequencing (WES) in common forms of strabismus
(c) Case Presentations (Genotype –phenotype correlations)
(d) Question answer forum

Target Audience: Strabismologists

Current Practice: Routine clinical strabismus practices globally do not incorporate these modalities for everyday application. While imaging for strabismus is now an established modality for assessing EOM position as well as function, cost is a major impeding factor. Application of NGS tools in the evaluation of strabismus is a relatively new modality and still in the research domain.

Best Practice: Both imaging and genetics provide customized signatures for the evidence-based diagnosis of any condition including strabismus. Imaging is a very relevant phenotype. With the discovery of newer likely genetic determinants for strabismus, the area of basic research supportive of strabismus has become very exciting.

Content: The workshop will focus on showcasing genotype-phenotype correlations of MRI findings in special forms of strabismus comprising primary cranial neuropathies namely, Duane syndrome, congenital fibrosis of the extraocular muscles, congenital oculomotor palsy, and Möbius syndrome in an essentially Caucasian population as well as review a genetic study of common forms of strabismus, esotropia (ET) and exotropia (XT) in a south Asian population. A detailed pedigree analysis of these south Asian families from northern India along with WES of two informative families from this cohort will be presented. Experimental, bioinformatic and gene-gene interactive network analysis of putatively causal variants segregating with the phenotype would be elaborated. The case specific discussions would comprise 2-3 such examples of genotype-phenotype correlations.

Learning Objective(s) and expected outcomes: At the conclusion of this presentation, the practicing strabismologist gets informed about the latest available tools in the strabismus diagnosis armamentarium. The diagnostic relevance of imaging and genetics will be emphasized. Evidence based highly specific diagnostic signatures for strabismus that has long been in the research domain, have increasing relevance in the everyday practice of strabismology. EOM imaging is already used for diagnosing difficult cases of strabismus and with advances in genetic tools as well as the decrease in cost of many investigations, a
greater plethora of information should soon be available. The practicing strabismologist should be aware of these modalities.

**Proprietary Interest:** None.

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### Imaging as a Phenotypic Marker in Congenital Cranial Dysinnervation Disorders and Extraocular Muscle Heterotopy

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I. The imaging phenotype of extraocular muscle (EOM) dysinnervation

A. Normal motor innervation to EOMs can be imaged by high resolution MRI
   1. Subarachnoid space. Heavily T2 weighted imaging (FIESTA or CISS sequences) in oblique planes parallel to optic chiasm can resolve subarachnoid oculomotor and abducens nerves, and with high field strength, also the trochlear nerve.
   2. Surface coil imaging of the orbit in quasi-coronal image planes can reliably image the motor innervation to the inferior (IR), medial (MR), and lateral rectus (LR), and the inferior oblique (IO) EOMs. Rarely it is possible to resolve innervation to the superior rectus (SR) and superior oblique (SO) EOMs.
   3. Surface coil imaging of the orbit in quasi-coronal image planes can image the optic nerve to quantify size.

B. Hallmarks of normally innervated EOMs.
   1. Normal volume and cross section
   2. Contractility. Volume increases, and maximum cross section shifts posteriorly during contraction; the converse occurs during relaxation.
   3. Motor innervation is complex.
      1. Most EOMs have dual transverse compartments corresponding to differing locations of tendon fiber insertions on the sclera.
      2. Rectus motor nerves bifurcate into major divisions external to the EOM. Each major division then forms a manifold that courses anteriorly along the global surface of the EOM, ultimately entering the belly to terminally arborize only within fibers of the corresponding compartment. Only exceptions:
         a. IR is diffusely innervated by one motor nerve division, and the lateral compartment is selectively innervated by a second division.
         b. SR is diffusely innervated throughout.
      3. SO is innervated in medial (torsional) and lateral (vertical) compartments by separate trochlear nerve divisions.
      4. IO is innervated in two compartments by separate divisions of its main motor nerve.

C. Hallmarks of abnormal EOM innervation
   1. Atrophy. Hypo-innervated EOMs are ways hypoplastic. Denervated EOMs develop atrophy within 5 weeks.
   2. Reduced or absent contractility.
   3. These features do NOT depend upon insertion of the EOM on the globe. Even a “lost” EOM will exhibit contractile behavior.
4. Abnormal motor nerves:
   a. Hypoplastic or absent congenitally.
   b. Atrophic due to compressive neuropathy or neurosurgical sectioning.
   c. Hyperplastic with gadodiamide contrast enhancement on T1 imaging in setting of
      Schwannoma, an intrinsic glial tumor of the nerve.
   d. Misdirected to abnormal target muscle.

D. Typical findings in congenital cranial dysinnervation disorders
   1. Sporadic Duane syndrome
      a. Abducens nerve is hypoplastic or absent
      b. When abducens nerve is present, inferior compartment of LR is innervated by a motor
         branch in the inferior division of the oculomotor nerve, and the abducens nerve
         innervates only the superior or both LR compartments.
      c. When abducens nerve is absent, the medial rectus motor branch innervates the entire
         LR.
      d. Optic nerve typically normal.
   2. Dominant Duane syndrome DURS2 (activating mutation of α2-chimerin, \textit{CHN1} MIM 604356)
      a. unilateral or bilateral abduction limitation, or both abduction and adduction
      b. palpebral fissure narrowing and globe retraction in adduction.
      c. Orbital motor nerves were typically small, with the abducens nerve often
         nondetectable.
      d. LR muscles often structurally abnormal
      e. Often oculomotor nerve (CN3) innervation from vertical rectus EOMs leading to A or
         V patterns of strabismus in three cases.
      f. Occasional oculomotor nerve SO, SR, and levator hypoplasia.
      g. Only the MR, IR, and IO EOMs spared.
   3. Duane radial ray syndrome (Okihiro syndrome caused by mutations in transcription factor
      SALL4).
      a. radial ray abnormalities including thumb, radial artery, radial bone, and pectoral
         muscle hypoplasia.
      b. Unilateral or bilateral.
      c. Limitation of both abduction and adduction or only abduction.
      d. lid fissure narrowing and retraction in adduction.
      e. Intraorbital and intracranial abducens nerves small to absent, particularly ipsilateral to
         abduction deficiency.
      f. Normal oculomotor and optic nerves.
      g. Oculomotor nerve branch sometimes innervates the LR.
      h. EOMs and pulleys were structurally normal in most subjects.
   4. Horizontal gaze palsy with progressive scoliosis [HGPPS (OMIM) 607313]
      a. Absence of conjugate horizontal eye movement and severe scoliosis
      b. Abnormal flattening of the basis pontis and hypoplasia in the pontine tegmentum
      c. medulla appeared abnormally butterfly-like, with anterior flattening and an unusual
         midline cleft
      d. Normal EOMs.
   5. Classical congenital fibrosis of extraocular muscles due to kinesin mutation KIF21A (classic
      CFEOM1 and some cases of CFEOM3). At least 6 known single nucleotide mutations.
      a. severe bilateral blepharoptosis, limited supraduction, and variable ophthalmoplegia.
      b. Profound hypoplasia of levator palpebrae superioris and superior rectus.
      c. Small or absent orbital motor nerves, especially oculomotor nerve.
      d. EOMs exhibited variable atrophy and an abnormally bright T1 signal.
      e. Frequently A-pattern strabismus, with misinnervation of the lateral rectus muscle by an
         oculomotor nerve branch similar to DURS2 Duane syndrome.
      f. Rectus pulley locations generally normal.
      g. Subclinical but statistically significant optic nerve hypoplasia.
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II. Heterotopy (malpositioning) of rectus pulleys causes incomitant strabismus phenotypes.

A. A pulley out of place will cause the EOM to pull in the wrong direction.
B. Heterotopic pulleys can be diagnosed by appropriate orbital MRI or computed tomography (CT).
C. Computer simulations of the mechanical actions of the EOMs show a close correspondence between pulley heterotopy and patterns of incomitant strabismus in individual patients.
D. Some common patterns of heterotopy and their clinical manifestations:
   1. A pattern
      a. superior location of lateral relative to medial rectus pulley in one or both orbits, so that the medial acts as a relative depression in adduction.
      b. or lateral location of inferior relative to superior rectus pulley in one or both orbits, so that the inferior rectus acts as an abductor in depression.
      c. both kinds of heterotopy will manifest over-depression in adduction and be confused clinically with superior oblique overaction.
      d. Lateral canthus is typically superior to the medial canthus.
   2. V pattern
      a. inferior location of lateral relative to medial rectus pulley in one or both orbits, so that the medial rectus acts as a relative elevator in adduction.
      b. or medial location of inferior relative to superior rectus pulley in one or both orbits, so that the inferior rectus acts as an adductor in depression.
      c. both kinds of heterotopy will manifest over-elevation in adduction and be confused clinically with inferior oblique overaction.
      d. Lateral canthus is occasionally inferior to the medial canthus.
   4. Myopic strabismus fixus – large angle esotropia and ipsilateral hypotropia associated with high axial myopia. The lateral rectus pulley slips under the globe, converting the lateral rectus from an abductor to a depressor.

E. Heterotopies are often associated with dystopia of the palpebral canthi or facial asymmetry as often seen in craniosynostosis.
F. However, it is impossible to characterize pulley heterotopies by clinical examination alone; orbital imaging is required to do this. Imaging is generally required to distinguish pulley heterotopy from oblique muscle dysfunction.
G. Evidence from patients with superior oblique palsy suggests that pulley heterotopy is the cause of strabismus, rather than the effect.

References


**FDA Disclosure:** Improved surface coils not approved by FDA were employed for some imaging studies.

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**Whole exome sequencing in common forms of strabismus**

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**Research Synopsis**

Primary concomitant strabismus (PCS), implying misalignment of the eyes, is an important childhood morbidity comprising esotropia (ET) and exotropia (XT). It exists as sporadic or familial forms, latter suggestive of a genetic basis. Only three chromosomal loci have been linked to PCS but they do not explain all tested families. Identification of additional genetic determinants for this condition using next generation sequencing approach was the aim of this doctoral study. Recruitment of informative PCS families was the first objective. 39 families thus recruited comprised of 18 ET, 18 XT and 03 with both, with more vertical transmission seen in XT (Chaudhuri et al, Strabismus, 2017), making it the choice for further studies.

Identification of known/novel variant(s) causal/associated with XT was the second objective and two informative families were selected for whole exome sequencing (WES). In the first family with the presumptive autosomal dominant inheritance pattern, all five affected and three unaffected members were sequenced and three rare heterozygous missense variants, one each in *EPHA2*, *NUP160* and *STIP1* segregating with the phenotype were identified. Based on available literature and interaction network analysis done in this study, *EPHA2*(1p36.13) emerged as a strong candidate for XT in this first report. Of note, Sanger sequencing of all 17 exons of *EPHA2* in an independent PCS cohort identified two
additional rare variants. In the other family (presented as a case), with an unclear inheritance pattern, WES data of three affected and three unaffected members did not identify segregating variant(s). On revisiting clinical details and considering only one arm of the family, with likely autosomal recessive inheritance with two unaffected and two affected siblings showing foveal hypoplasia and nystagmus, a homozygous stopgain mutation, segregated with the phenotype. These promising findings encourage further discovery genomics, trans-ethnic replication studies and functional validation.

**Research Impact and Significance**

At the initiation of this study, it was hypothesized that familial forms of PCS, could be analysed by next generation sequencing (NGS), in particular, WES, to identify putatively causal genes for PCS. Further variant screening in a familial or sporadic cohort could be useful to identify other putatively causal variants. In the absence of any known gene for PCS and the relative low cost of NGS, this rare variant identification approach by NGS was hypothesized as being relevant in aiding understanding of mechanisms underlying PCS development, thus enabling translational applications of management related to maintenance of functional binocular vision in this childhood population, in the future.

**References:**

1. Chaudhuri Z, John A, Aneja S, Thelma BK. Identification of a novel putative variant in the *EPHA2* gene on chromosome 1p in a family with exotropia by whole exome sequencing. Oral presentation at the ARVO 2017; Program No 3439; Strabismus: Basic and Clinical Session on May 9, 2017 (ARVO 2017 Annual Program Abstracts)


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