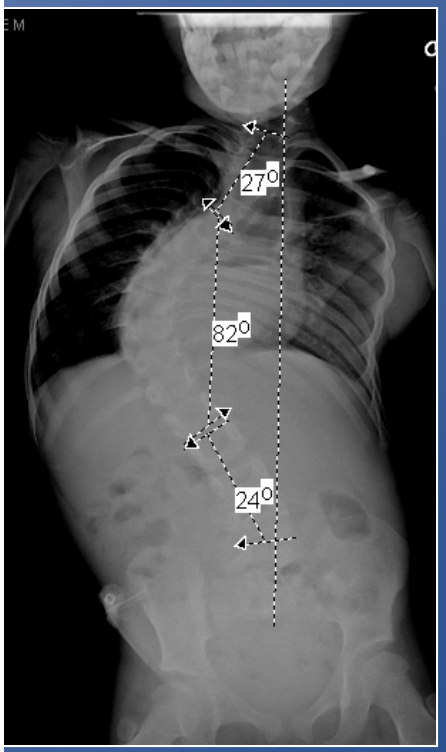
International Congress on Early Onset Scoliosis
and Growing Spine 2009
Istanbul



GENETICS OF EARLY ONSET SCOLIOSIS

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Adolescent Idiopathic Scoliosis

- Genetic factors established
  - Wynne Davies – dominant or multiple gene
  - Robin & Cohen – autosomal or multiple gene
  - Cowell – sex-linked dominant with variable expressivity and incomplete penetrance
- Miller – linkage analysis est. candidate genes
  - 202 families
  - Regions on chromosomes 6, 9, 16, 17, 19

Adolescent Idiopathic Scoliosis

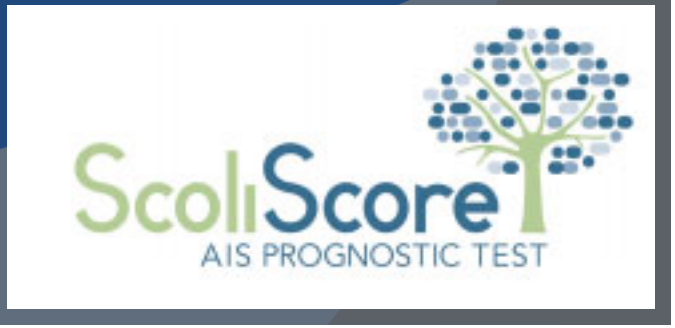
- Complex genetic disorder
  - High prevalence and extreme variability
- Specific mode of genetic inheritance is not known

Adolescent Idiopathic Scoliosis

- Increased in family members
- Twin studies
- Ogilvie and Ward
- “Founder effect” study from Utah valley population
- Genome wide association study

Adolescent Idiopathic Scoliosis Prognostic Genetic Testing

- Recently developed
- Saliva sample
- Based on 6500 research samples
- 53 genetic markers with predictive value
- Field testing completed
- Commercially available



axial BIOTECH™ | ScoliScore AIS PROGNOSTIC TEST

2749 East Parleys Way, Suite 200, Salt Lake City, UT 84109-9921
Toll Free: (877) 294-2598 | Tel: (801) 984-9098 | Fax: (801) 984-9099 | www.axialbiotech.com

For Sample Use Only DO NOT MAIL

AIS Prognostic Assay Test Result Form

PATIENT INFORMATION

Patient Name	JANE SAMPLE	Specimen Barcode	SCO-5395-02306_9876
Medical Record #		Gender	Female
Cobb Angle	14		
Physician Fax	801-994-1000	Date of Birth	14 - Jul - 2009
Ethnicity	Caucasian		
Physician Name	Dr Clinical Trial	Collection Date	14 - Jul - 2009
Physician Address	2749 E. Parleys Way Suite 200 Salt Lake City UT 84109	Receive Date	14 - Jul - 2009
		Report Date	22 - Jul - 2009 10:20 AM

ASSAY DESCRIPTION

ScoliScore AIS Prognostic Assay is preformed on DNA extracted saliva. A multiplex PCR reaction and Taqman detection are used to determine the genotype for a panel of 53 single nucleotide polymorphisms (SNP's). The ScoliScore AIS Progression Score ranges from 1 - 200, is calculated using marker weighting factors and a simple accumulation algorithm.

ASSAY RESULTS

SNP ID #	GENOTYPE	SNP ID #	GENOTYPE	SNP ID #	GENOTYPE	SNP ID #	GENOTYPE	SNP ID #	GENOTYPE
RS10000472	AA	RS10004901	CT	RS10168146	AA	RS10493083	CT	RS10787096	CG
RS10794280	CC	RS10798036	GG	RS11083276	TT	RS11747787	AC	RS12474952	TC
RS12618119	AA	RS1265566	TT	RS132898	AA	RS1349887	GG	RS136187	AC
RS1437480	GG	RS1558729	AC	RS16865244	TT	RS16902899	TT	RS16909285	GG
RS16945692	GA	RS16968878	CT	RS17021437	GG	RS17044552	GG	RS17165447	CC
RS17210350	AA	RS17635546	CC	RS17719756	CC	RS1991127	TC	RS2045904	CC
RS2209158	AA	RS239794	TC	RS2449539	TT	RS2700910	AA	RS2976514	GG
RS448013	GG	RS4661748	TG	RS4724981	AA	RS4765072	TT	RS4782809	GC
RS500243	GC	RS6414345	TT	RS6420139	CC	RS6528028	GG	RS6691909	AG
RS6693477	TC	RS6798946	CC	RS6952104	CT	RS7613792	CG	RS7840870	CA
RS8093693	GC	RS831653	AA	RS9945359	GG				

SCOLISCORE AIS PROGRESSION SCORE = 25

ASSAY INTERPRETATION

This score, based on testing the patient's genotypes for the panel of risk factors described above, predicts a 99 percent probability that this patient's idiopathic scoliosis will not progress to a severe curve (Cobb angle greater than 40 degrees during skeletal growth, or greater than 50 degrees at skeletal maturity). This result may allow you to decrease the frequency or extent of serial radiographs; however, when developing a management plan for this patient, ScoliScore™ results must be interpreted in the context of all other clinical and diagnostic information for this patient. If significant curve progression does occur, consider evaluating the patient for myopathies, syndromes, and other spinal lesions that may cause scoliosis.

Note: Use or interpretation of ScoliScore™ for purposes or patients outside the intended use population has not been validated.

ASSAY ACCURACY

NPV: 99%
(Negative Predictive Value)

Although rare, genotyping errors can occur due to misincorporation of DNA bases by the enzyme used to perform the test, sample misidentification, sample contamination or general laboratory errors.

Laboratory Director: Kenneth Ward, MD Kenneth Ward M.D.

This Laboratory Developed Test was developed and its performance characteristics determined by Axial Biotech Laboratories. The laboratory is regulated under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical testing and has established and verified the test's accuracy. This test has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not neccessary. These results are adjunctive to an ordering physician's diagnosis.

CLIA Number: 46D1077919

ScoliScore™

TEST RESULT OF 25

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RISK OF PROGRESSION CHART

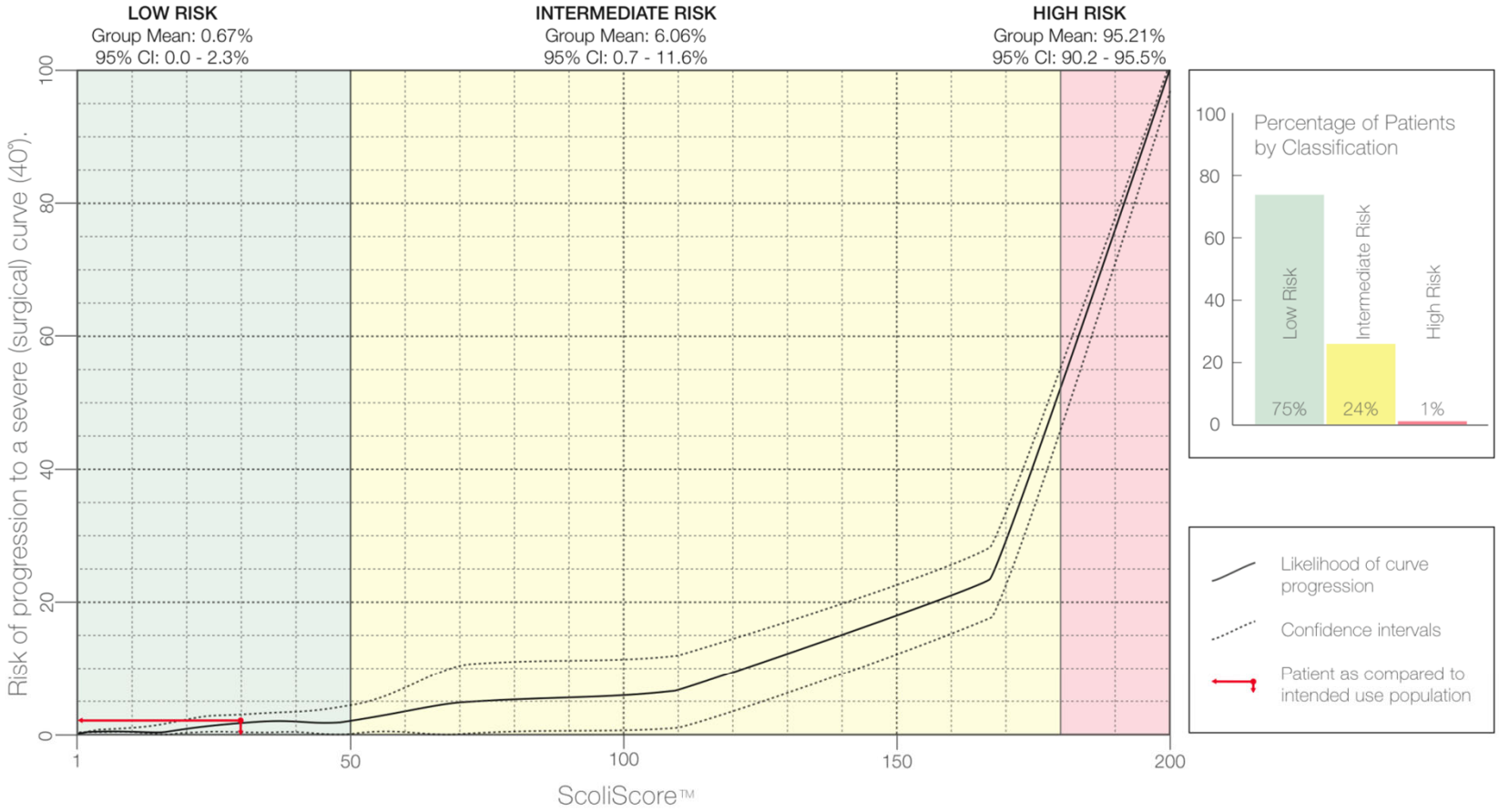


Table 1: The risk of progression curve was developed from 1.083 validation subjects extrapolated to an intended use population based on 4% [1,2] of the samples being defined as severe (Cobb Angle > 40 degrees in skeletally immature subjects and >50 degrees in skeletally mature subjects).

1 Asher, M.A. & Burton, D.C. Adolescent idiopathic scoliosis: natural history and long term treatment effects. Scoliosis 1, 2 (2006).
2 Lonstein, J.E. Screening for spinal deformities in Minnesota schools. Clin Orthop Relat Res, 33-42 (1977).

Idiopathic Early Onset Scoliosis

- Onset before age 4 (JIP James)
- Unlike AIS, limited evidence that IEOS has familial clustering
  - 3% parents/sibs (Wynne-Davies)
  - Family cohort (van Rhijn)
  - Wynne-Davies has suggested genetic predisposition that can be triggered
    - "on or off" by environmental factors

Idiopathic Early Onset Scoliosis

- Unlike AIS, IEOS has no consistent symptoms or segregation
  - Associated with plagiocephaly, but less commonly MR, DDH
  - Many different entities
    - "Sturdy" phenotype
    - "Syndromic" phenotype

Idiopathic Early Onset Scoliosis

- Axial Biotech, Inc. data (Ogilvie): 298 patients with known outcome (prog to surgery)
  - Using AIS 53 SNP panel of AIS markers, 47% had a low risk score
  - i.e. not useful in predicting progression
  - Hardy-Weinberg equilibrium: in a stable population, allele freq does not change
    - This suggests that many IEOS patients are new mutations, unlike AIS

Idiopathic Early Onset Scoliosis

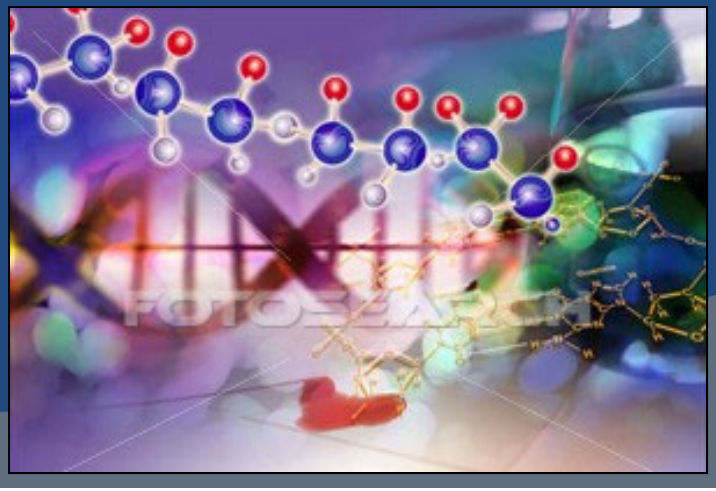
- Genotyping IEOS patients may identify common pathways to etiology of multiple spinal deformities
  - Ca+ channel blockers or axonal pathways
- More IEOS patient DNA is needed to determine markers common to AIS or if new GWAS is necessary
  - Family studies to bank DNA
  - Two to three generation pedigrees
    - ? Familial occurrence
  - Intermountain region genetic database
    - Salt Lake City, UT

Idiopathic Early Onset Scoliosis

- Genome wide association study (GWAS)
- Linkage analysis, less money, need parents
- Candidate gene identification:
  - is the trait assoc with the gene?
- New mutation vs. environmental factors
- Transmission Disequilibrium Testing (TDT)

Congenital Scoliosis

- Environmental factors
  - Low oxygen tension (Ingalls and Curley, Rivard)
  - Chemicals (valproic acid, boric acid)
  - Folic acid deficiency

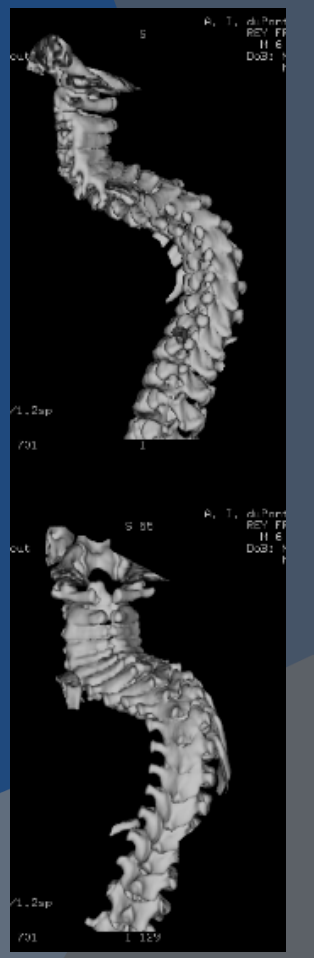


Congenital Scoliosis

- Genetic factors
  - Congenital scoliosis reported in family cohorts
    - Increased risk for NTD and mult. vertebral anomalies in families with multiple defects (Wynne-Davies)
    - 4% increased risk for sibs (Connor)
    - Monzygotic twins (Akbarnia)
  - Notch family of genes
    - Regulate cell fate determination and embryonic patterning in animals
    - Notch 1: coordinates process of somatogenesis (Conlon)

Congenital Scoliosis

- Interference with segmentation
- Genetic mutations in humans:
  - Spondylocostal dysostosis (AR and AD)
    - Generalized vertebral anomalies, rib fusions, absent ribs, kyphoscoliosis
  - Spondylothoracic dysplasia (AR)
    - Lethal phenotype, short trunk, sym rib fusions
  - Alagille syndrome
    - Multiorgan disorder assoc with vertebral anomalies, liver & heart prob

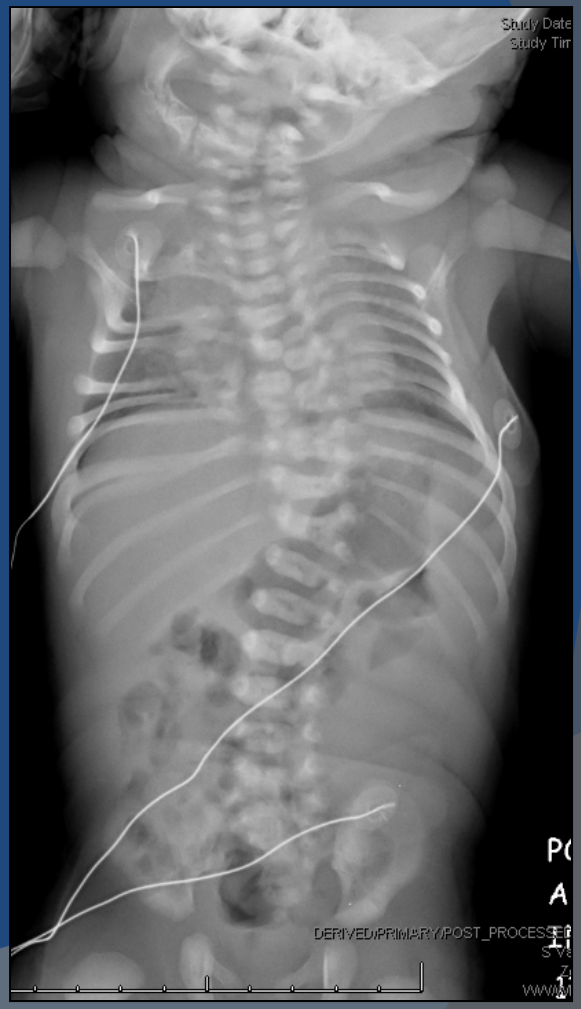


Congenital Scoliosis

- Homeobox or Hox genes
  - Early differentiation of axial and appendicular skeleton
  - Regulate segmentation by activation/repression of DNA
  - Low oxygen tension may modulate expression
    - sonic hedgehog or homeobox genes
    - involved in vertebral segmentation (Loder)

Syndromic Early Onset Scoliosis

- Marfan
  - Gene encoding fibrillin-1 (15)
  - Autosomal dominant
  - 25% incidence of spont mutations
  - New mutations (Sponseller)
- Larsen
  - AD, some sporadic
- Arthrogryposis
  - Heterogeneous, sporadic
- Goldenhaar
- Jeune



Genetics of Early Onset Scoliosis

- Variable patterns
- Different phenotypes
- Unlike AIS
- Sporadic mutations

- Linkage analysis
- Genome wide association study

Thank you

Nemours

Alfred I. duPont Hospital for Children

Nemours Children's Clinic