Egypt 90 Million People Power
Seven Thousands Year Culture
29 Governorates



Recent advances in Molecular Medicine:
Changing the practice of neurology

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As the mysteries of the human genetic code were unraveled, people needed protection so breakthroughs were used to treat and heal, not to isolate and discriminate.

Recent advances in molecular medicine and high throughput screenings, such as array **comparative genomic hybridization (CGH) , exome and whole genome sequencing**, are yielding new regions and new genes of interest in neurogenetic and ASD phenotypes.

Recent studies point to effectiveness of novel treatments that address physiological abnormalities associated with autism spectrum disorder (ASD). We used the emerging technology of exome sequencing to study closely related families that have children with autism spectrum disorder. These children also had a history of seizures or abnormal electrical brain wave activity

Although deficiency of the **branched-chain ketoacid dehydrogenase(BCKDC)**and associated elevations in the BCAAs and their ketoacids have been recognized as the cause of **maple syrup urine disease (MSUD)** for decades, treatment options for this disorder have been limited to dietary interventions.

In recent years, the discovery of improved leucine tolerance after liver transplantation has resulted in a new therapeutic strategy for this disorder. Likewise, targeting the regulation of the **BCKDC** activity may be an alternative potential treatment strategy for **MSUD**.

The regulation of the BCKDC by the branched-chain ketoacid dehydrogenase kinase has also been implicated in a new inborn error of metabolism characterized by autism, intellectual disability and seizures.

Low BCAA may affect neurocognitive phenotypes
Mutations in the genes encoding components of the BCKDC result in increased BCAA levels and MSUD.

In contrast, mutations in the BCKDK, which phosphorylates and activates the BCKDC, have been recently associated with decreased BCAA levels and a phenotype of autism with seizures. The affected patients had lower plasma BCAA levels despite normal protein consumption

Autism

- Initially thought to be a purely psychiatric disorder caused by poor parenting 1950 s
- A genetic disorder caused by unfortunate mingling of two individuals who genes resulted in autism 1970s
- To a complex interaction of genetic susceptibility triggered by an unknown environmental insult-2000

RESEARCH GOALS

- Discover genes that cause autism
- Use those findings to improve the diagnosis and ?treatment

INTERVIEW PROCESS – THREE MEASURES

- **ADI-R (**The Autism Diagnostic Interview – Revised)

- **ADOS (**The Autism Diagnostic Observation Schedule)
  - complementary instrument to the ADI-R
  - **Photographed and videotaped**

- **CARS**
  - **In addition to DSM IV**

Hearing by ABR was normal.
CT brain, Fragile X, Cytogenetics, and CNV analysis were all negative. EEG was abnormal.

Fasting plasma amino acids were essentially normal except for significant reductions in BCAAs
Urine organic acids were normal. Electrolytes and liver function tests were normal.
Lipid profile was normal.

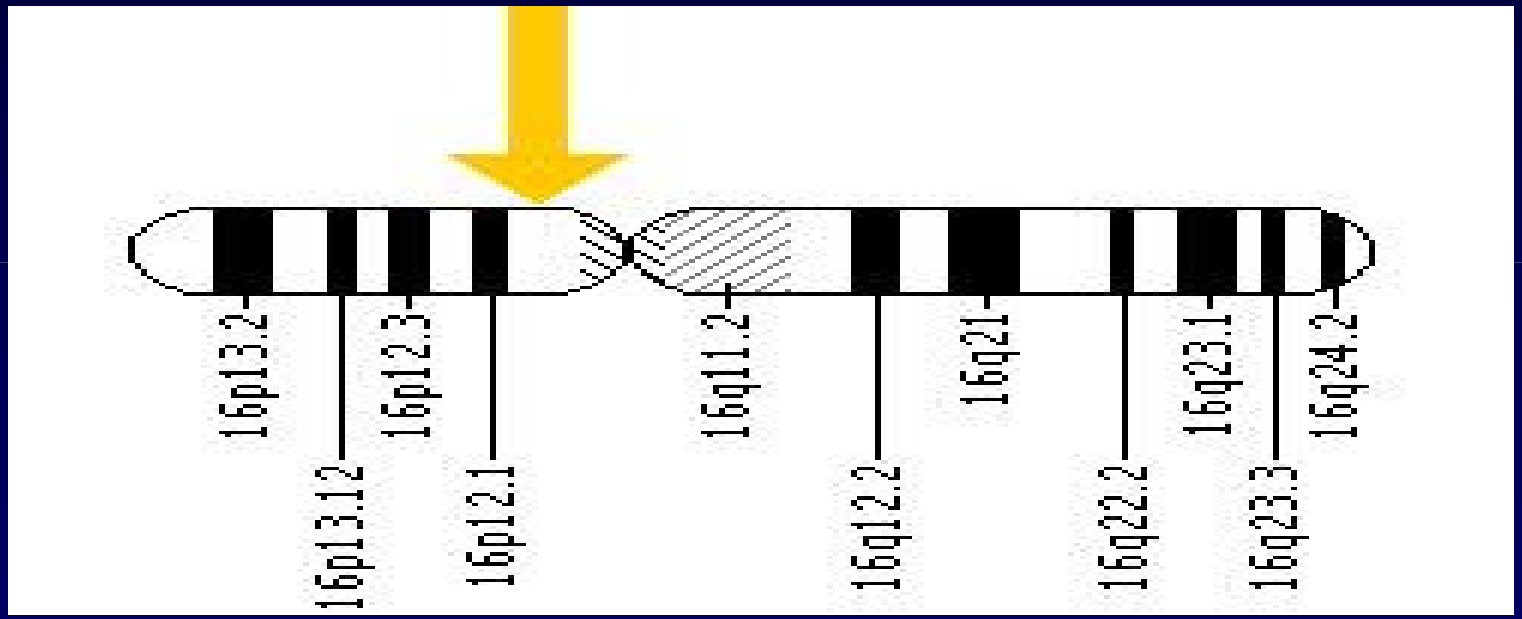
FUNCTION OF BCKDK

- Mutations in BCKDH lead to increased branched chain amino acids.
- BCAA's – essential – important in brain glutamate synthesis
- BCKDK Knockout Mouse
  - BCKDH constitutively on -- significant decrease in BCAA's
  - Neurologic Abnormalities – reduced brain weight, hind limb flexion throughout life, epileptic seizures
  - Reduced growth, reversible with high protein diet

Mice

Experiments were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee at the University of California, San Diego. The mice were fed diets containing 2% or 7% BCAAs (W/W) consisting of a ratio of 2:1:2 (leucine:isoleucine:valine).
The mice were observed twice a day.

Where is the *BCKDK* gene located?
Cytogenetic Location: 16p11.2



NEXT STEPS: MORE FIRMLY ESTABLISHING CAUSAL RELATIONSHIP BETWEEN MUTATIONS AND PHENOTYPE

- Screen matched 500 controls – are these mutations rare in the Egyptian population?
- Extend pedigrees to achieve statistical significance where needed – in case of 3 null mutations, 2 of families LOD's could be increased to 3 or greater.

We found out that the mice displayed a condition very similar to our patients, and also had spontaneous epileptic seizures, just like our patients.

Mice treated with BCAA supplementation displayed improved neurobehavioral symptoms, reinforcing the idea that the approach could work in humans as well.

Human Branched Chain Amino Acid Supplementation:

A powdered branched chain amino acid mixture with a ratio of:
leucine :isoleucine:valine 1:1:1 (Jo Mar Laboratories, Campbell, California, USA) were used to supplement the affected individuals

Lessons learned from therapeutic modulation of branched chain amino acids (BCAA) metabolism in Mendelian inborn errors of metabolism could have significant impact on the treatment of common multifactorial diseases.

We think this work will establish a basis for future screening of all patients with autism and/or epilepsy for this or related genetic mutations, which could be an early predictor of the disease.

Autosomal Recessive (AR) Disorders in the Middle East

- Common AR disorders
- Relatively common AR disorders
- AR disorders that cluster in certain communities
- AR disorders which are limited to 1 or 2 extended families
- New AR disorders

DEFINITION OF THE MIDDLE EAST



Lancet Vol 367, 2006

Aicardi Goutieres syndrome (AGS)

Immune mediated neurodevelopmental disorder caused by mutations in any of six genes (*TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, and ADAR)* all are expressed in AR pattern.

It is a mendelian inflammatory disease most typically characterised by microcephaly, spasticity, dystonia, psychomotor retardation, and, in about 35%of cases, childhood death.

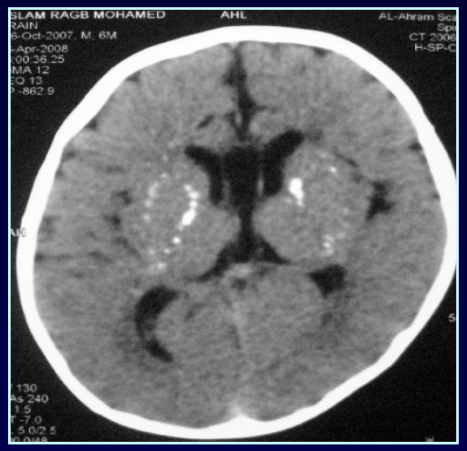
Type	Gene	Locus
AGS1	*TREX1*	3p21.31
AGS2	RANASES26	13q14.3
AGS3	*RNASEH2C*	11q13.1
AGS4	*RNASEH2A*	19p13.2
AGS5	*SAMHD1*	20q11.23
AGS6	*ADAR*	1q21.3

A recently identified 7th gene IFIH1 (also called *MDA5*) presented in heterozygous form (AD).

Raised concentrations of interferon α in CSF and serum of patients with AGS which is highly consistent diagnostic marker of early disease and has fundamental insights into the pathogenesis of AGS.

A subgroup of infants with AGS present at birth with abnormal neurologic findings, hepatosplenomegaly, elevated liver enzymes, and thrombocytopenia, a picture highly suggestive of congenital infection.

AGS1 linked to *TREX1 gene*



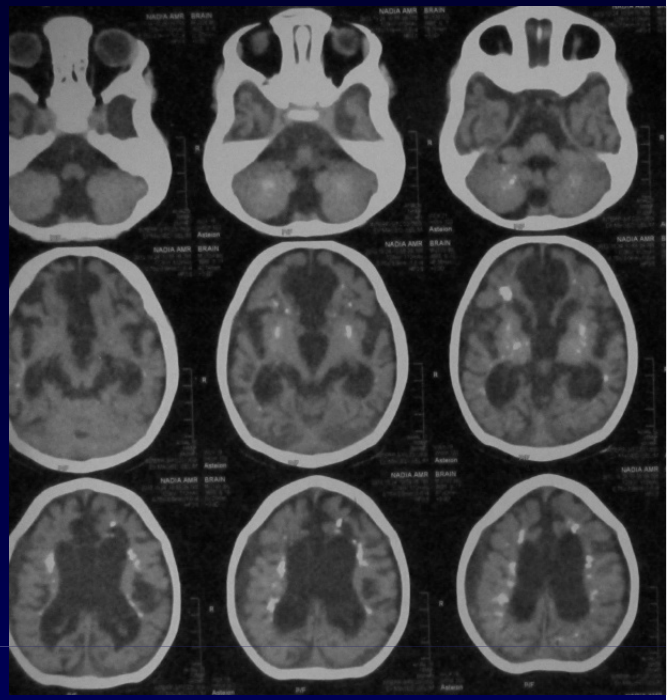






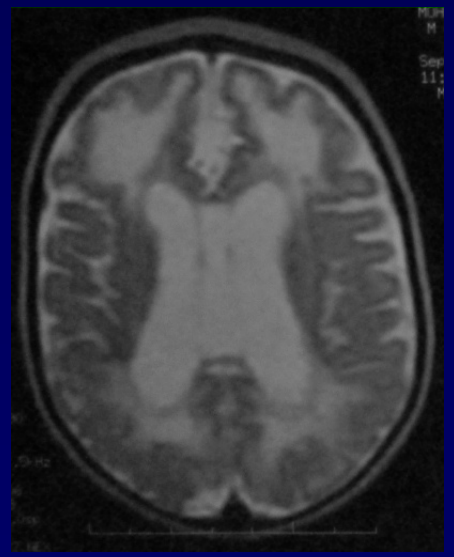
AGS linked to *RNASEH 2A*





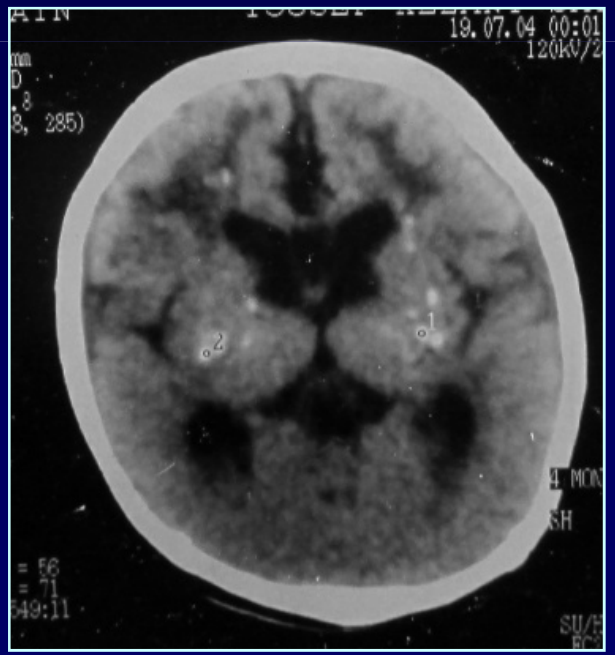
Chilblains

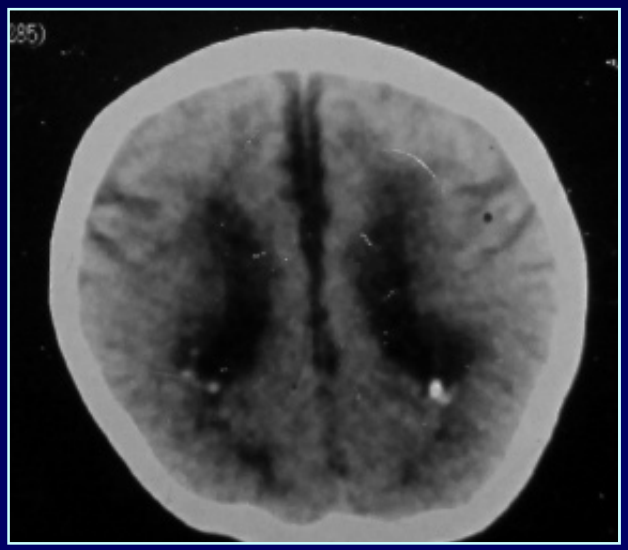




AGS3 linked to *RNASEH2C*







Band like calcification(BLC) or pseudoTORCH syndrome

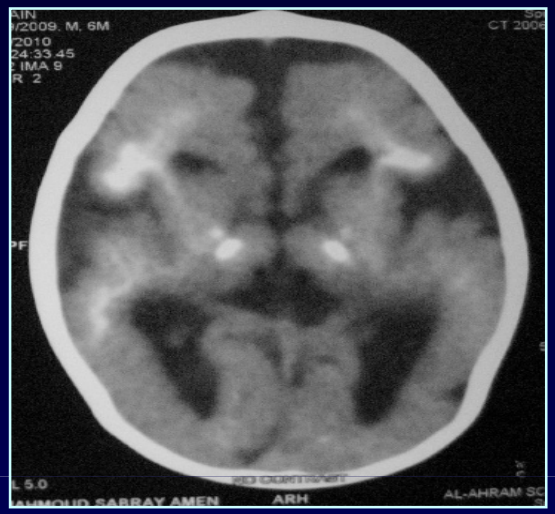
Band-like brain calcification (BLC) or pseudo TORCH syndrome is a rare AR with distinctive clinical and neuroimaging.

Severe microcephaly, early onset seizures, profound developmental delay together with band-like calcification in brain, simplified gyral pattern and polymicorgyria are the hallmark of the syndrome.

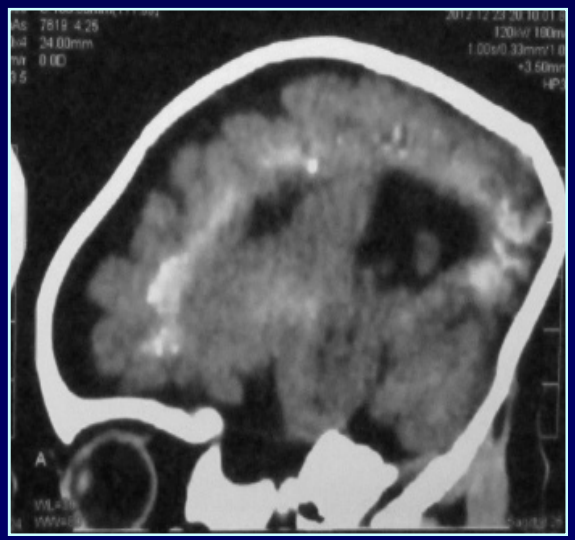
In 2010 O'Driscoll et al. attributed BLC to homozygous mutation in occludin (OCLN) gene through a description of 5 families from a worldwide series including an Egyptian one.

Band like calcification(BLC) or Pseudo-TORCH syndrome







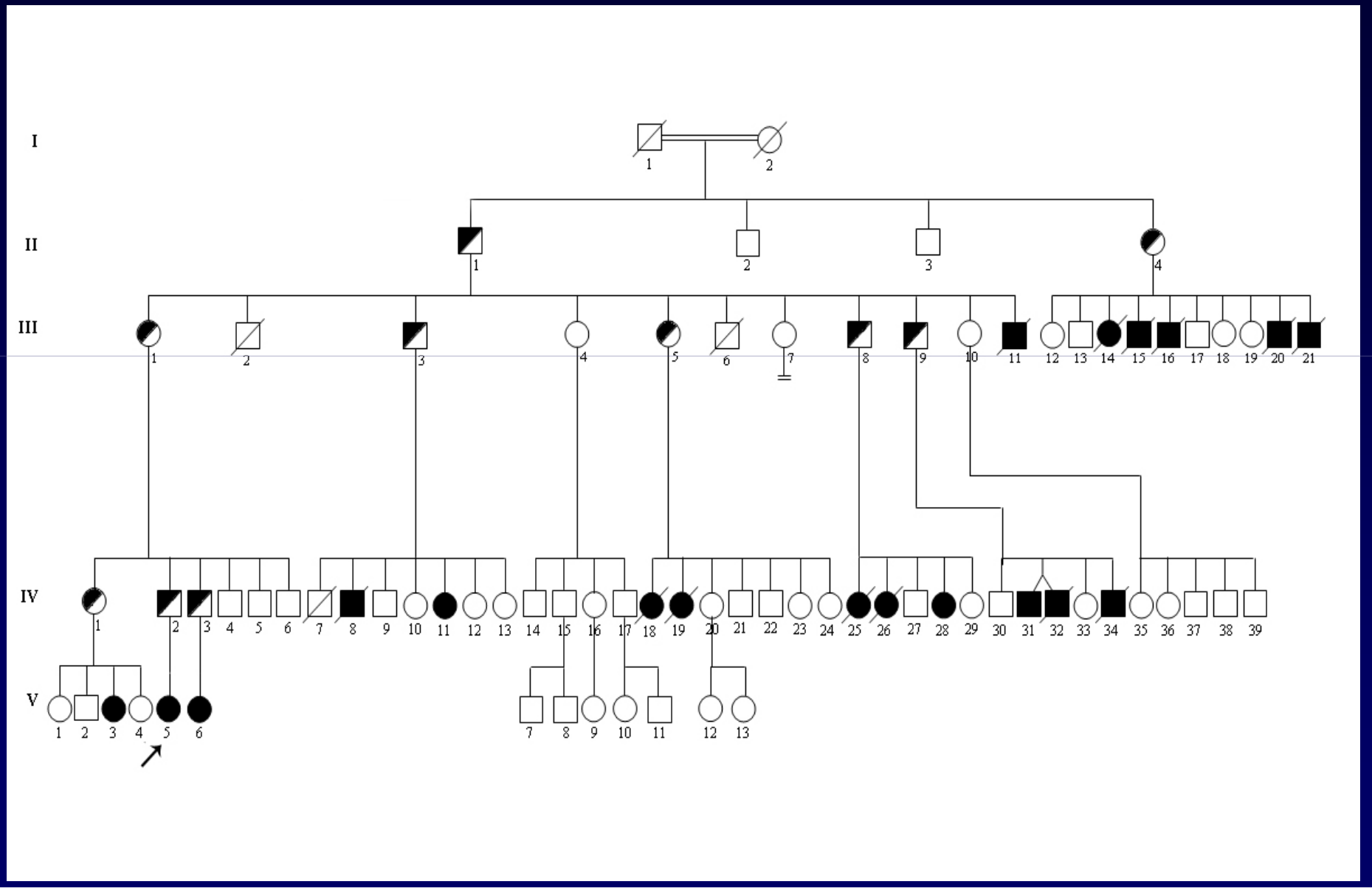




Familial Intellectual Disability: Extended Phenotype in Four Generations Due to 1;16 Translocation and Duplication 16p13

This study correlate genotype/phenotype in unbalanced 1;16 translocation to emphasize the role of proper genetic testing in prevention of intellectual disability in offspring of the balanced carriers using **FISH technique** and **Array CGH**

Pedigree analysis in five generations of this family showed nineteen affected patients (only 6 are alive) and eleven obligate carriers.




IV:11
IV:28
IV:11
IV:31
IV:34
IV:34
V:3
V:5
V:6

Intellectual disability, hypertelorism, cupped ears, everted upper lip and limb anomalies could be the presenting clinical features of 16p13 duplication syndrome.

while deep set eyes could be due to 1p terminal deletion. Prevention of recurrence of intellectual disability in this family can be achieved through carrier detection and prenatal genetic diagnosis.

FISH for the proband showed that this patient had only one copy of 1p subtelomere whereas the 1q probe (red) hybridize to both homologues . The 16p probe (green) hybridize to three chromosomes, two 16 and one chrom 1p(arrow), the red signals are 16q. To detect the copy number of some genes close to 1p and 16p subtelomeres, we used locus specific identifier (LSI) for 1p36 and 16p13.3. We found no deletion in 1p36, the deletion involved only 1p subtelomere, and there were three copies of Rubinstein Taybi locus 2 on 16p and 1 on 1p .

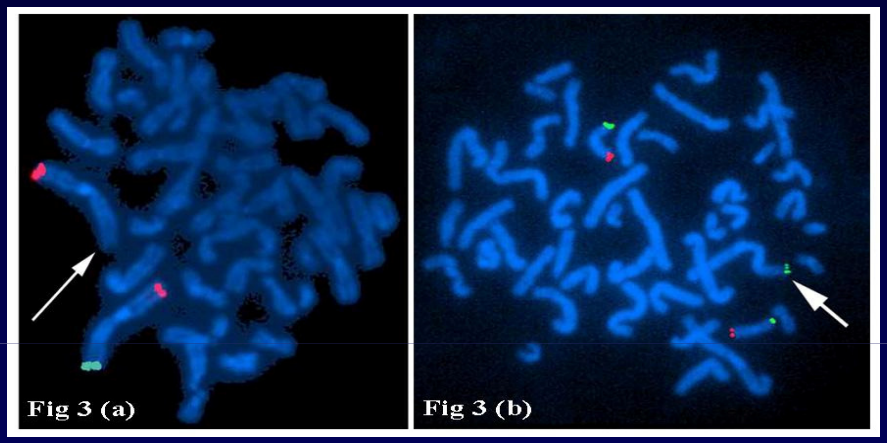
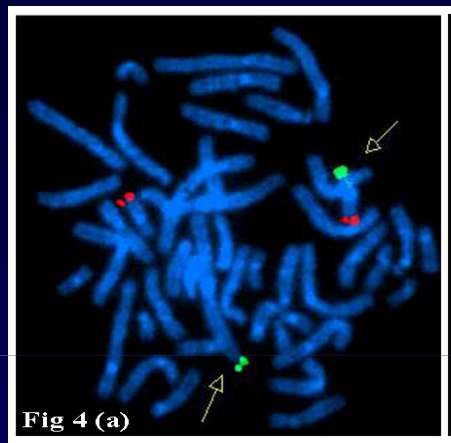
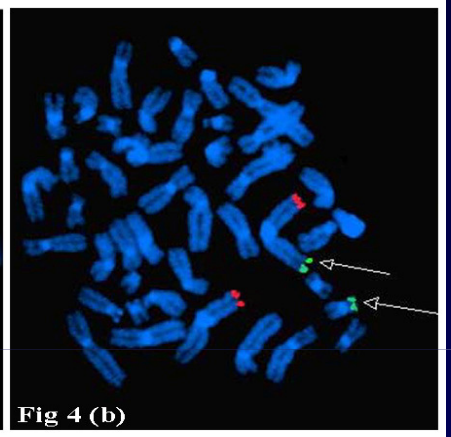
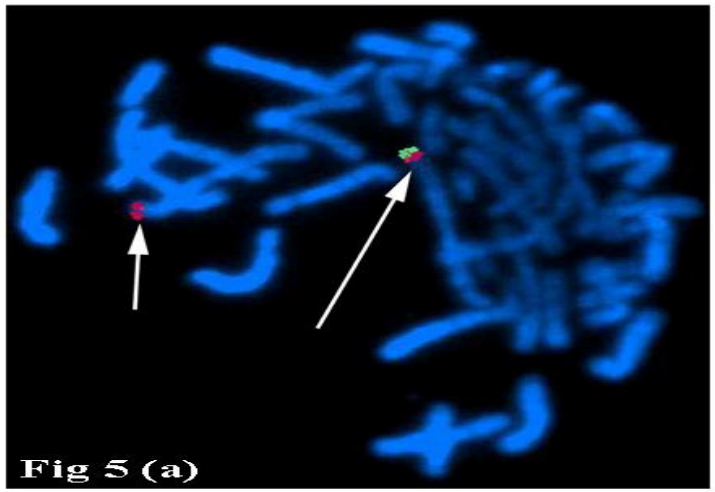
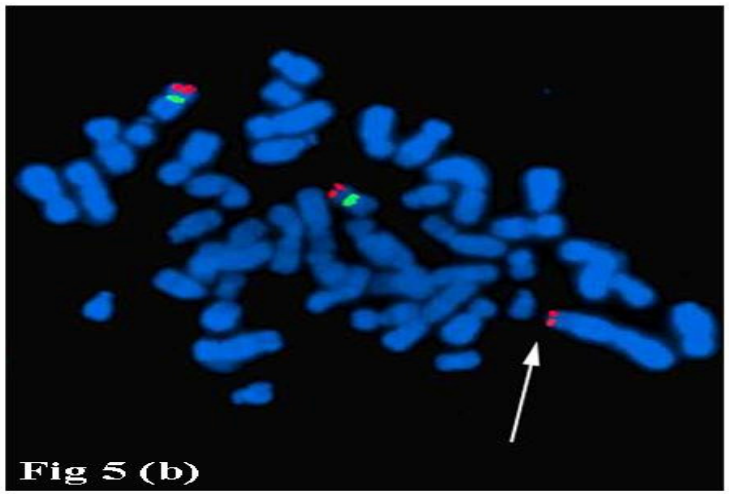

Fig 3 (a)

Fig 3 (b)


Fig 4 (a)


Fig 4 (b)


Fig 5 (a)


Fig 5 (b)

In conclusion, a balanced cryptic subtelomeric translocation segregated five generations in this family.

FISH was the only way to detect balanced carriers .

The identification of the t(1;16 translocation) in this large pedigree highlights the importance of retrospective studies of cryptic subtelomeric rearrangement in patients with familial ID.

Those carriers with balanced translocation should be offered **prenatal or pre-implantation FISH testing** for chromosomes 1p and 16p sub telomeres to prevent recurrence of intellectual disability.


Thank you