UNIVERSITÀ DEGLI STUDI DI PARMA

UP-TO-DATE SULLA GENETICA DI SIDS, SIUD E ALTE

F. NONNIS MARZANO

Dipartimento di Biologia Evolutiva e Funzionale
Università di Parma

La Spezia, 26 Novembre 2011
GIORNATA REGIONALE SIDS, SIUD E ALTE 2011

ANNI 2005 - 2011

UNIVERSITA' DEGLI STUDI DI PARMA
Dip. Scienze Ostetriche Ginecologiche e Neonatologia

Dottorato di ricerca in Farmacologia e Tossicologia Sperimentali

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A.O. Ospedale di Circolo e Fondazione Macchi

AZIENDA SANITARIA LOCALE N° 5 "SPEZZINO"
ASL N.5 SPEZZINO

OSPEDALE DI LA SPEZIA
U.O. Neonatologia

UNIVERSITÀ DEGLI STUDI DI MILANO

ISTITUTO DI ANATOMIA PATOLOGICA
Centro di Ricerca "Lino Rossi"

Auckland, Nuova Zelanda (2000) | 1 lavoro

Firenze, Italia (2002) | 4 lavori

Edmonton, Canada (2004) | 4 lavori

Yokohama, Giappone (2006) 12 lavori

Portsmouth, UK (2008) 16 lavori


RICERCA DI BASE
DIAGNOSTICA
PREVENZIONE
GENETICA FORENSE

GENETICS

Crib Death Exoneration Could Usher In New Gene Te

CAMBRIDGE, U.K.—For years, prosecutors in the United Kingdom have applied an unwritten three-strikes-and-you're-out rule to mothers whose babies die in infancy: One unexplained death is tragic but innocent, two is suspicious, and three is murder. This credo, tested in many a court case, led the U.K.'s Crown Prosecution Service to try a pharmacist named Trupti Patel for murder. Over a 4-year span, Patel and her husband, Jay, lost

Vindicated. Jay and Trupti Patel were all smiles outside the courthouse last week after a jury cleared Trupti of wrongdoing. Recent genetic findings appear to have played a decisive role in the verdict.

three babies before the age of 3 months. An open-and-shut case? Far from it. Recent genetic studies that challenge the three-strikes rule were a decisive factor in Patel's stunning acquittal last week in Reading Crown Court.

The ruling could have profound implications for criminal justice. Well-publicized trials in which multiple cases of sudden infant death syndrome (SIDS) led to murder convictions have tended to discredit the idea that SIDS could run in families. In the wake of the Patel ruling, many lawyers and child protection advocates have criticized the eagerness to prosecute cases of multiple unexplained infant deaths. The outcome could lead to more extensive screening of babies for inherited disorders, as well as to genetic testing of mothers accused of killing their babies.

SIDS, sometimes called crib or cot death, is a "diagnosis of exclusion," notes the American Academy of Pediatrics. Doctors assign a death to SIDS only after an autopsy and examination of the baby's environment and medical history reveal no other possibilities. Although the cause or causes of SIDS are unclear, breathing difficulties appear to play a central role. In the last decade, a "Back to Sleep" campaign urging parents to avoid allowing babies to sleep on their stomachs appears to have had major benefits: Since 1991, the number of SIDS cases has fallen by 50% in the United States, although it is still the third leading cause of U.S. infant mortality.

Pathologists have testified that the odds of two or more siblings dying of SIDS are vanishingly small: When factors such as parental smoking or low birth weight, which increase the risk of SIDS, are excluded, coincidence cannot provide a plausible explanation for multiple SIDS deaths. Negligence or child abuse is a far more likely cause, prosecutors argue.

The underpinnings of the three-strikes rule rest largely on a 1977 study by retired U.K. pediatrician and child abuse expert Roy Meadow. He invoked a disorder called "Munchausen syndrome by proxy," in which caregivers in multiple SIDS cases inflict suffering to get attention or sympathy. Meadow has served as an expert witness in several successful prosecutions of multiple unexplained infant deaths, and he testified for the prosecution in the Patel case; he declined to comment for this article.

In the Patel case, the defense challenged the basis of the three-strikes rule, arguing that genetics, not coincidence, lies behind the tragic deaths. Suggestions of a genetic link came from Patel's grandmother, who told the jury that five of her own children died—including three before the age of 6 weeks of unexplained causes—in the 1940s in Gujarat, India. The prosecution did not offer evidence to the contrary.

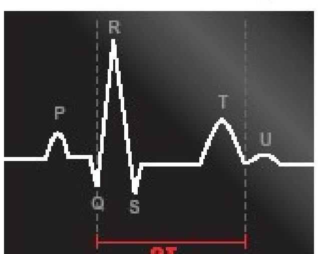
But it was the scientific testimony that provided the real fireworks. M clinical geneticist at St. Geo London, testified that an auto inheritance pattern with "vari could explain the Patel famil fant deaths. He suggested t mitochondrial respiratory ch set of conditions in which n clear and mitochondrial DN metabolism—and long QT s arrhythmia known for striki athletes and linked to mutati transport genes. Patton estim miss about 30% of long QT identifiable by electrocardiog

Doctors had tested Patel's for problems with heart rhyth 10 days before her death and malities. However, biochemic the infants provided some su tion of a mitochondrial disor based defense was robust e reasonable doubt in the mi which cleared Patel after barely 90 minutes.

Other findings not aired could explain some SIDS cas an inability to metabolize fa masquerade as SIDS. And linked to the IL-10 gene: Ba ticular allele have an exagger sponse to common infections ogist David Drucker of th Manchester, U.K. Inherite combine synergistically, he s such as parental smoking or s to trigger harmful fluid build

The bottom line, says P pediatrician at the University testified in the Patel case, is should routinely take and DNA samples and screen fo orders and heart arrythmias. rector of the National Cent orders Research in Bethesda an expert on SIDS diagnosis are multiple genetic risk fa just as for any other conditio

The Patel case has given proposal last month from t pendent Review of Corone panels conduct inquiries in they arise. In the United Sta genetic explanations for mul is "just beginning to cha widely accepted," Hunt says the explosion of genetic i need to take a fresh look at



Telltale electrocardiogram. A sometimes fatal heart arrhythmia called long QT syndrome may underlie some SIDS cases.

Quinn Eastman has just comple in the Cambridge, U.K., office of

Relazione di consulenza ostetrica e medico-legale nel procedimento penale n 5572/07.

- Procura della Repubblica presso il Tribunale di [redacted]

- Dr.ssa [redacted]

- Proc. n 5572/07.

- Incarico del 9 luglio 2008.

[redacted] 20 gennaio 2009

Dott. [redacted]

Dott. [redacted]

The Sudden Infant Death Syndrome Gene: Does It Exist?

Siri H. Opdal, PhD, and Torleiv O. Rognum, MD

ABSTRACT. ***Background.*** Sudden infant death syndrome (SIDS) is in a difficult position between the legal and medical systems. In the United Kingdom, prosecutors have for years applied the simple rule that 1 unexpected death in a family is a tragedy, 2 are suspicious, and 3 are murder. However, it seems that the pendulum has now swung to the opposite extreme; mutations or polymorphisms with unclear biological significance are accepted in court as possible causes of death. This development makes research on genetic predisposing factors for SIDS increasingly important, from the standpoint of the legal protection of infants. The genetic component of sudden infant death can be divided into 2 categories, ie (1) mutations that give rise to genetic disorders that constitute the cause of death by themselves and (2) polymorphisms that might predispose infants to death in critical situations. Distinguishing between these 2 categories is essential, and cases in which a mutation causing a lethal genetic disorder is identified should be diagnosed not as SIDS but as explained death.

Genetic Alterations That May Cause Sudden Infant Death. Deficiencies in fatty acid metabolism have been extensively studied in cases of SIDS, and by far the most well-investigated mutation is the A985G mutation in the medium-chain acyl-CoA dehydrogenase (MCAD) gene, which is the most prevalent mutation causing MCAD deficiency. How
investigated ha
chemical profil
disorders in a n
portance of inv
other than MCA
may cause sudd
novel polymorphisms have been found when key proteins involved in the regulation of blood glucose levels are investigated in cases of SIDS. The long QT syndrome (LQTS) is another inherited condition proposed as the cause of death in some cases of sudden infant death. The LQTS is caused by mutations in genes encoding cardiac ion channels, and mutations in the genes ***KVLQT1*** and ***SCNA5*** have been identified in cases initially diagnosed as SIDS, in addition to several polymorphisms in these 2 genes and in the ***HERG*** gene. In addition, genetic risk factors for thrombosis were investigated in a small number of SIDS cases; the study concluded that venous thrombosis is not a major cause of sudden infant death.

Gene Polymorphisms That May Predispose Infants to Sudden Infant Death Under Certain Circumstances. Many SIDS victims have an activated immune system, which may indicate that they are vulnerable to simple infections. One reason for such vulnerability may be partial deletions of the complement component 4 gene. In cases of SIDS, an association between slight infections before death and partial deletions of the complement component 4 gene has been identified, which may indicate that this combination represents increased risk of sudden infant death. There have been a few studies investigating HLA-DR genotypes and SIDS, but no association has been demonstrated. The most common polymorphisms in the interleukin-10 (IL-10) gene promoter have been investigated in SIDS cases, and the ***ATA/ATA*** genotype has been reported to be associated with both SIDS and infectious death. The findings may indicate that, in a given situation, an infant with an unfavorable IL-10 genotype may exhibit aberrant IL-10 production, and they confirm the assumption that genes involved in the immune system are of importance with respect to sudden unexpected infant death. Another gene that has been investigated is the serotonin transporter gene, and an association between the long alleles of this gene and SIDS has been demonstrated. Serotonin influences a broad range of physiologic systems, as well as the interactions between the immune and nervous systems, and findings of decreased serotonergic binding in parts of the brainstem, together with the findings in the serotonin

ages between common polymorphisms in these genes and SIDS. A number of human diseases are attributable to mutations in mitochondrial DNA (mtDNA), and there are several reasons to think that mtDNA mutations also are involved in SIDS. Both a higher substitution frequency and a different substitution pattern in the ***HVR-I*** region of mtDNA have been reported in SIDS cases, compared with control cases. A number of coding region mtDNA mutations have also been reported, but many are found only in 1 or a few SIDS cases, and, to date, no predominant mtDNA mutation has been found to be associated with SIDS.

Conclusions. All mutations giving rise to metabolic disorders known to be associated with life-threatening events are possible candidates for genes involved in cases of sudden infant death, either as a cause of death or as a predisposing factor. It is necessary to distinguish between lethal mutations leading to diseases such as MCAD and LQTS, and polymorphisms (for instance, in the IL-10 gene and mtDNA) that are normal gene variants but might be suboptimal in critical situations and thus predispose infants to sudden infant death. It is unlikely that one mutation or polymorphism is the predisposing factor in all SIDS cases. However, it is likely that there are "SIDS genes" operating as a polygenic inheritance predisposing infants to sudden infant death, in combi-

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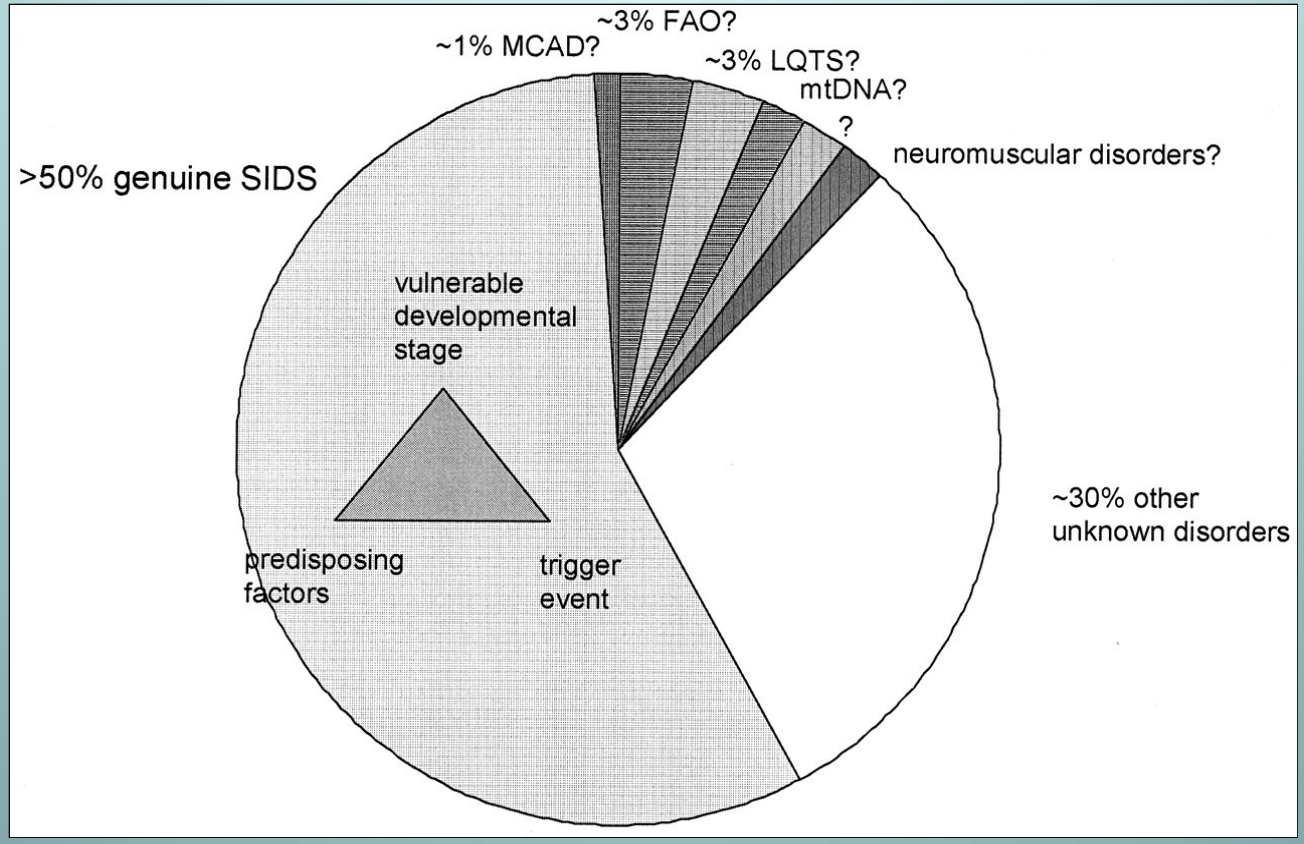
SIDS: Esiste un gene specifico?



PEDIATRICS®

DIAGNOSI ISTOPATOLOGICA

10 “TRUE” SIDS

DIAGNOSI MOLECOLARE

1 Sindrome Klinefelter

2 Sindromi QT Lungo (SCN5A, KCNQ1)

GENETICA e SIDS

Opdal e Rognum (2004); Hunt (2005)

1. MUTAZIONI

↓

MALATTIE GENETICHE

↓

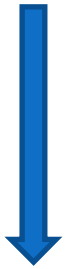
MORTE

2. POLIMORFISMI

↓

PREDISPOSIZIONE - INTERAZIONE CON AMBIENTE

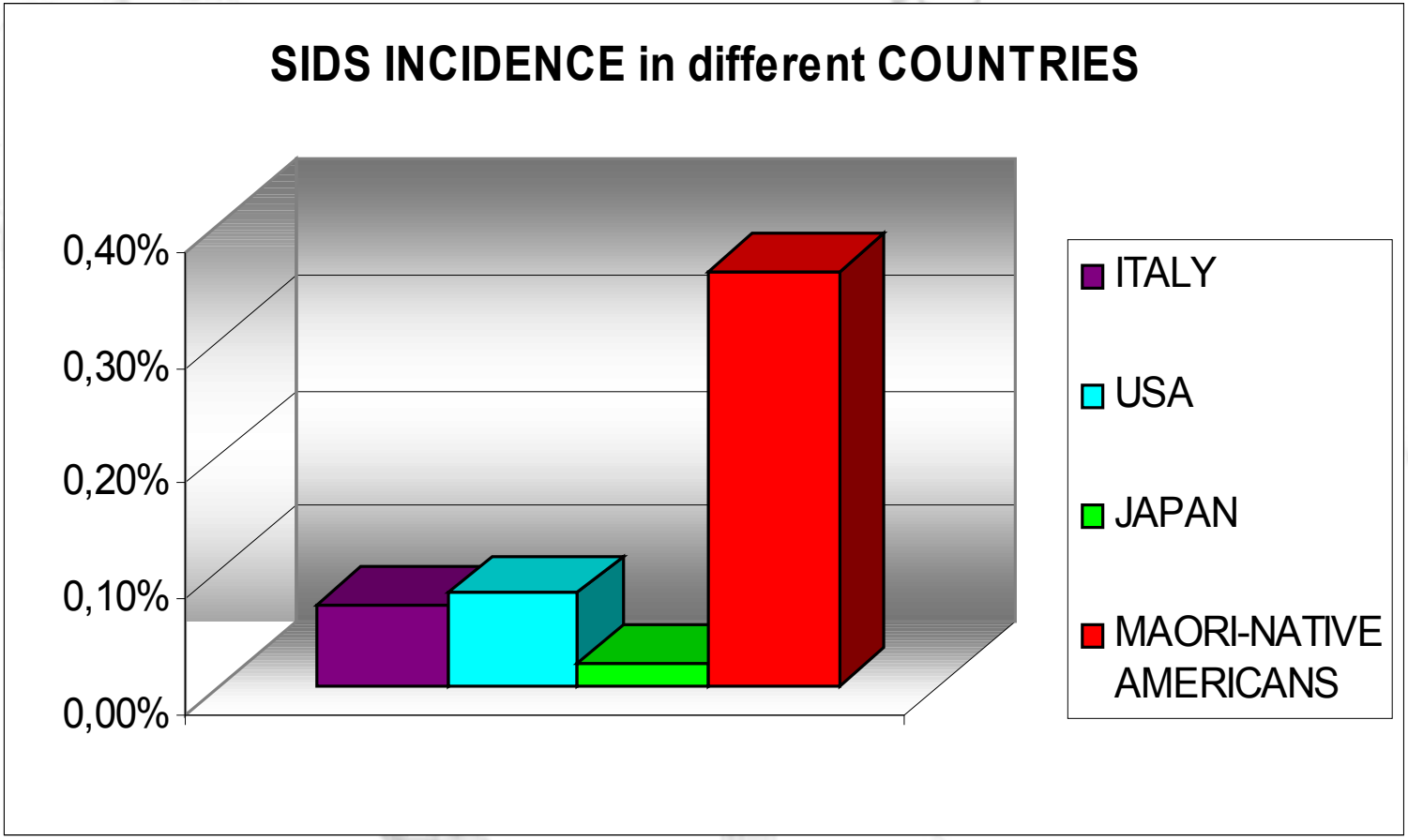
Componente biologica



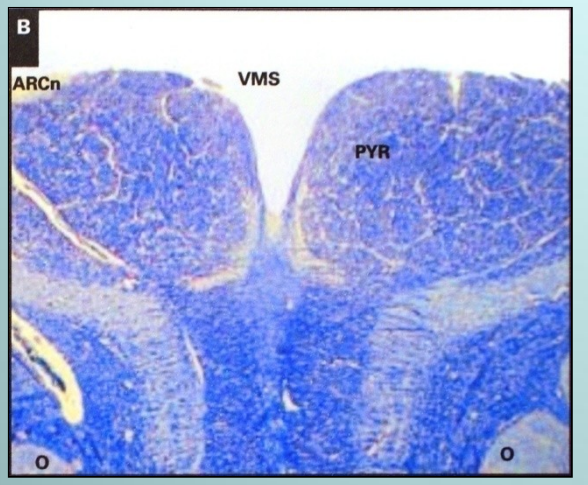
GENETICS LOADS THE GUN
AND
THE ENVIRONMENT PULLS THE TRIGGER

Componente ambientale

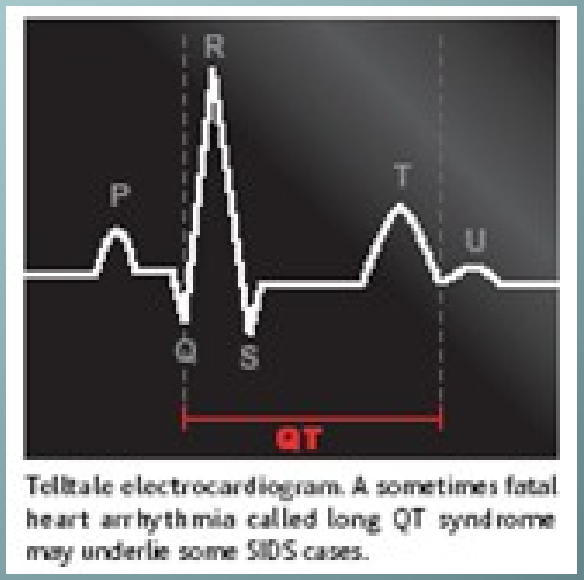
SIDS


SIDS INCIDENCE in different COUNTRIES
0,40%
0,30%
0,20%
0,10%
0,00%
ITALY
USA
JAPAN
MAORI-NATIVE AMERICANS

Affinità patogenetica
BULBO-SPINALE (Matturri, 2005)



Affinità patogenetica
CARDIACA - ARITMOGENA
(Schwartz, 2001)



Telltale electrocardiogram. A sometimes fatal heart arrhythmia called long QT syndrome may underlie some SIDS cases.

Raccolta dei campioni

20 SIDS
5 SIUD

→ **Campioni autoptici**

- **Centro di Ricerca “Lino Rossi” (Università di Milano)**
- **Dip. Patologia e Medicina di Laboratorio (Università di Parma)**

28 ALTE
48 IALTE

→ **Sangue intero**

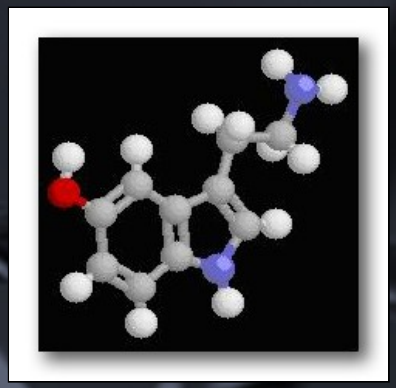
- **U.O. Neonatologia (Ospedale di Parma)**
- **U.O. Pediatria (Ospedale di Varese)**

150 Controlli

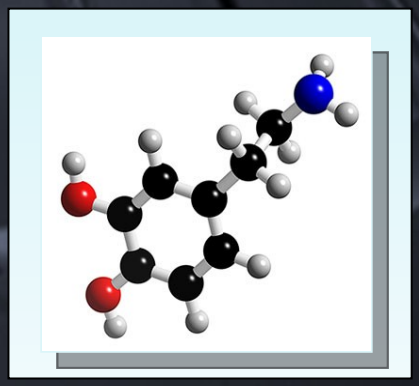
→ **Sangue intero, Saliva**

Polimorfismi genici e metabolismo di neurotrasmettitori

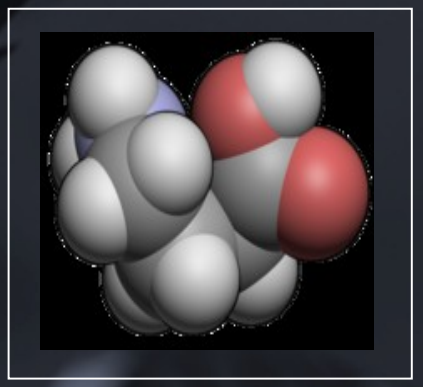
Serotonina



Dopamina

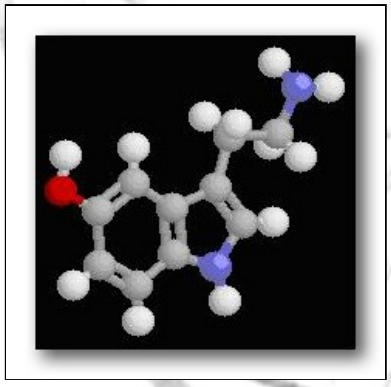


GABA



Polimorfismi genici di neurotrasmettitori

Serotonina



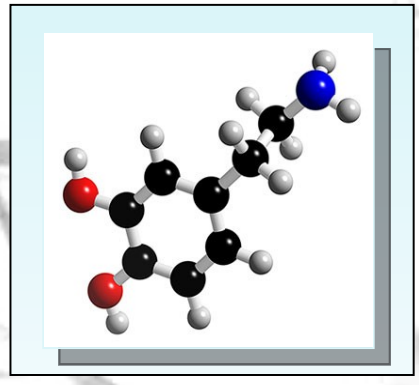
TPH2

5HTT

MAOA

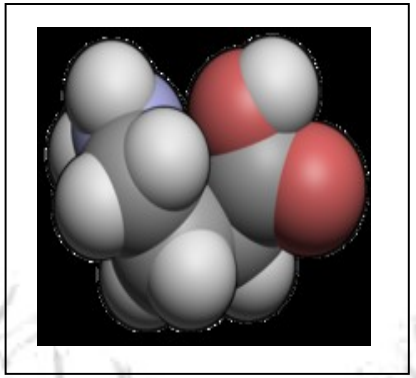
Htr 1a

Dopamina



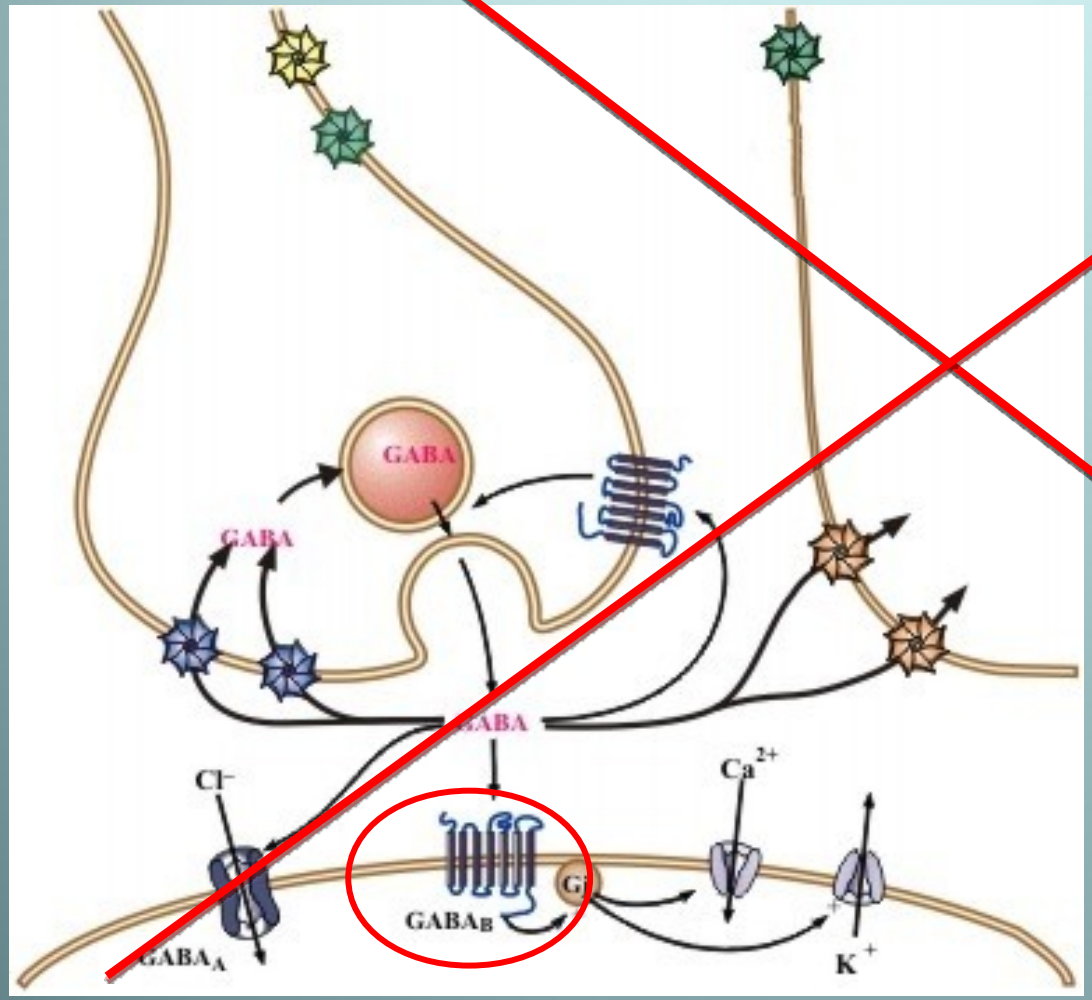
DAT

GABA



GABA(B)R1

GABA(B)R1
Recettore del GABA



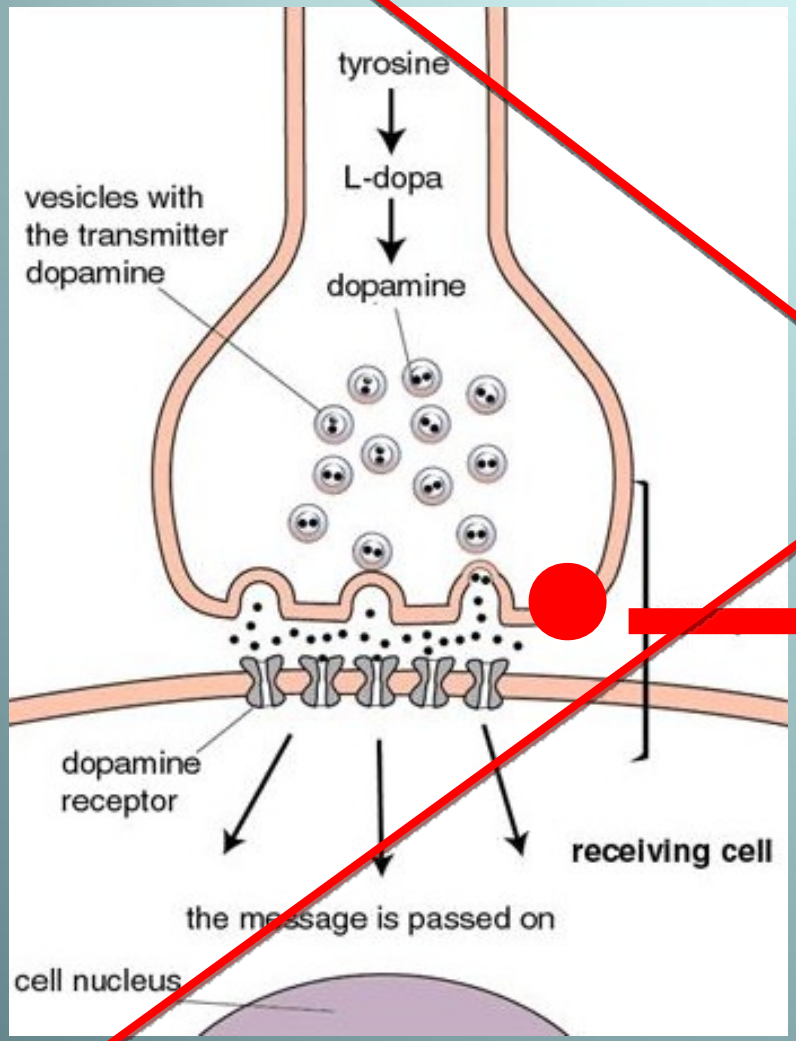
Mutazione puntiforme

G1465A

Esone 7

↓ espressione del recettore

PATHWAY METABOLICO della DOPAMINA



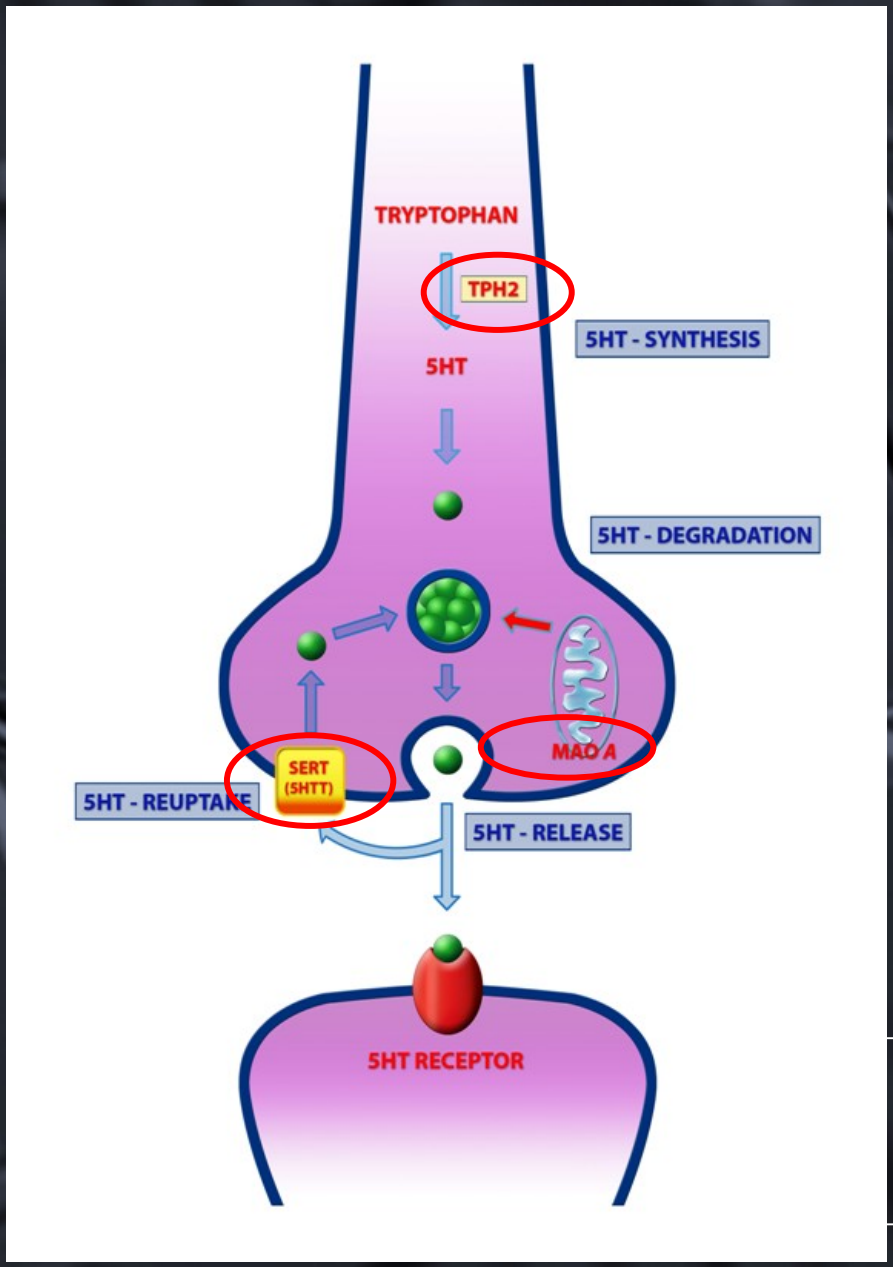
DAT **- trasportatore della Dopamina**

VNTR - 40 bp (alleli 3-11)

Polimorfismo funzionale

esone 15

PATHWAY METABOLICO della SEROTONINA



TPH2

triptofano idrossilasi 2

SINTESI

5-HTT

trasportatore della serotonina

RE-UPTAKE

MAOA

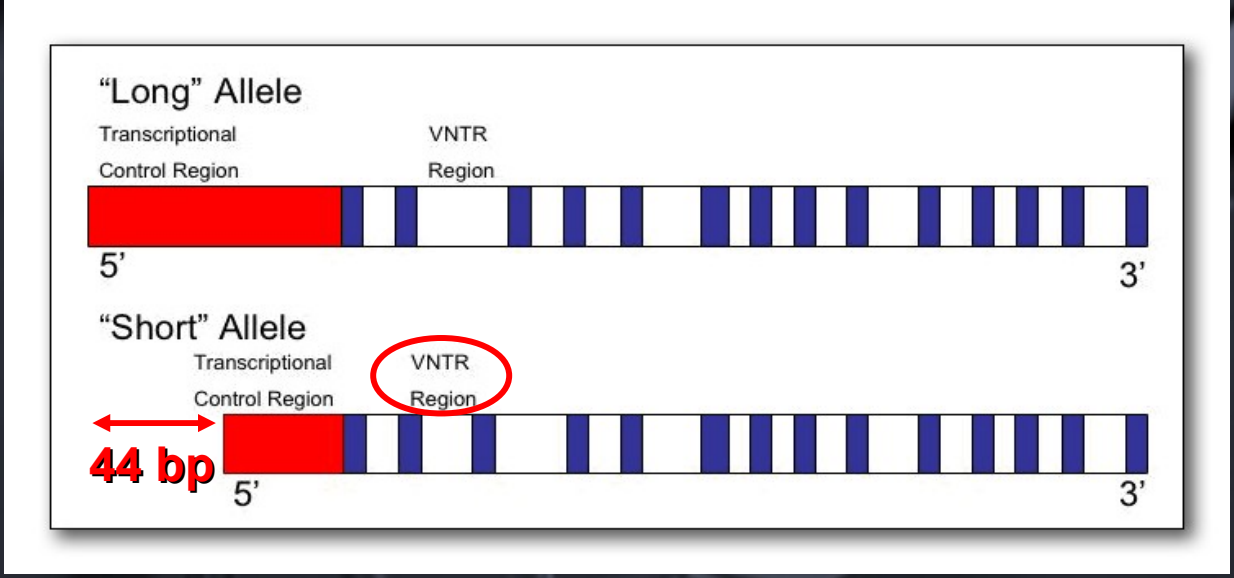
monoamino ossidasi A

DEGRADAZIONE

Serotonin Receptor 1A

Recettore della serotonina

5HTT- trasportatore della Serotonina

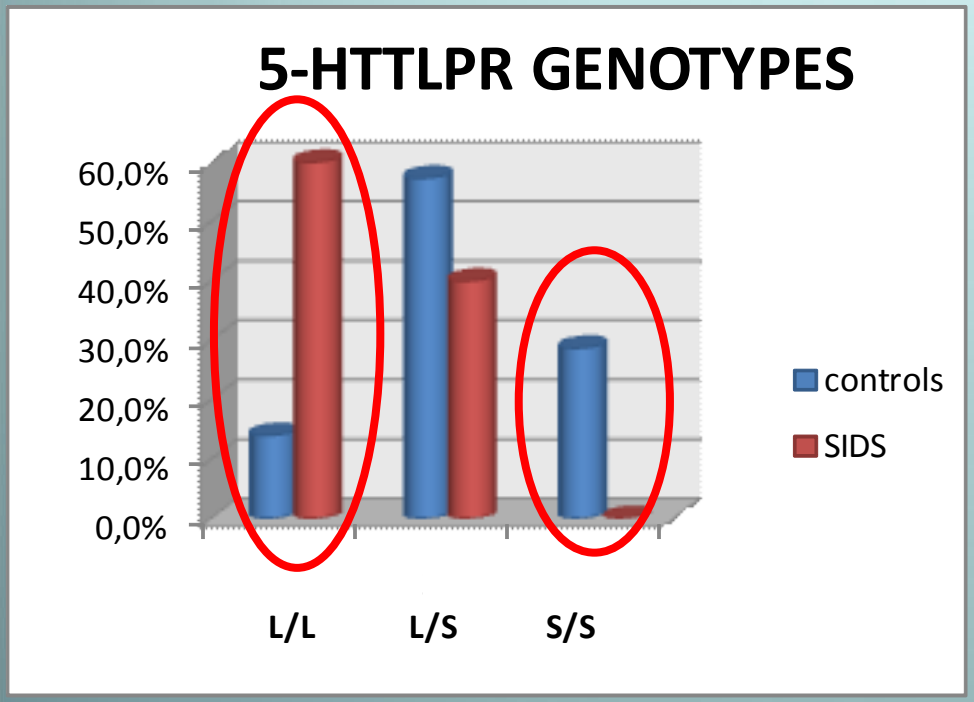


GENOTIPO L/L ***Fattore di rischio***

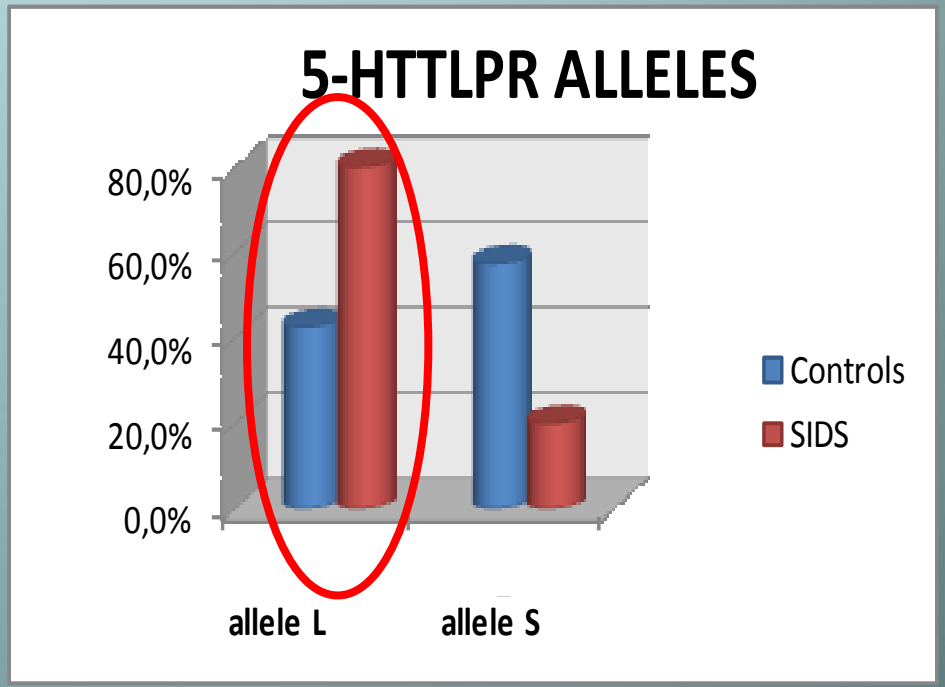
GENOTIPO S/L
GENOTIPO S/S

5HTTLPR
Frequenze alleliche e genotipiche

SIDS



p=0.001



GENOTIPO L/L

60% vs. 14%

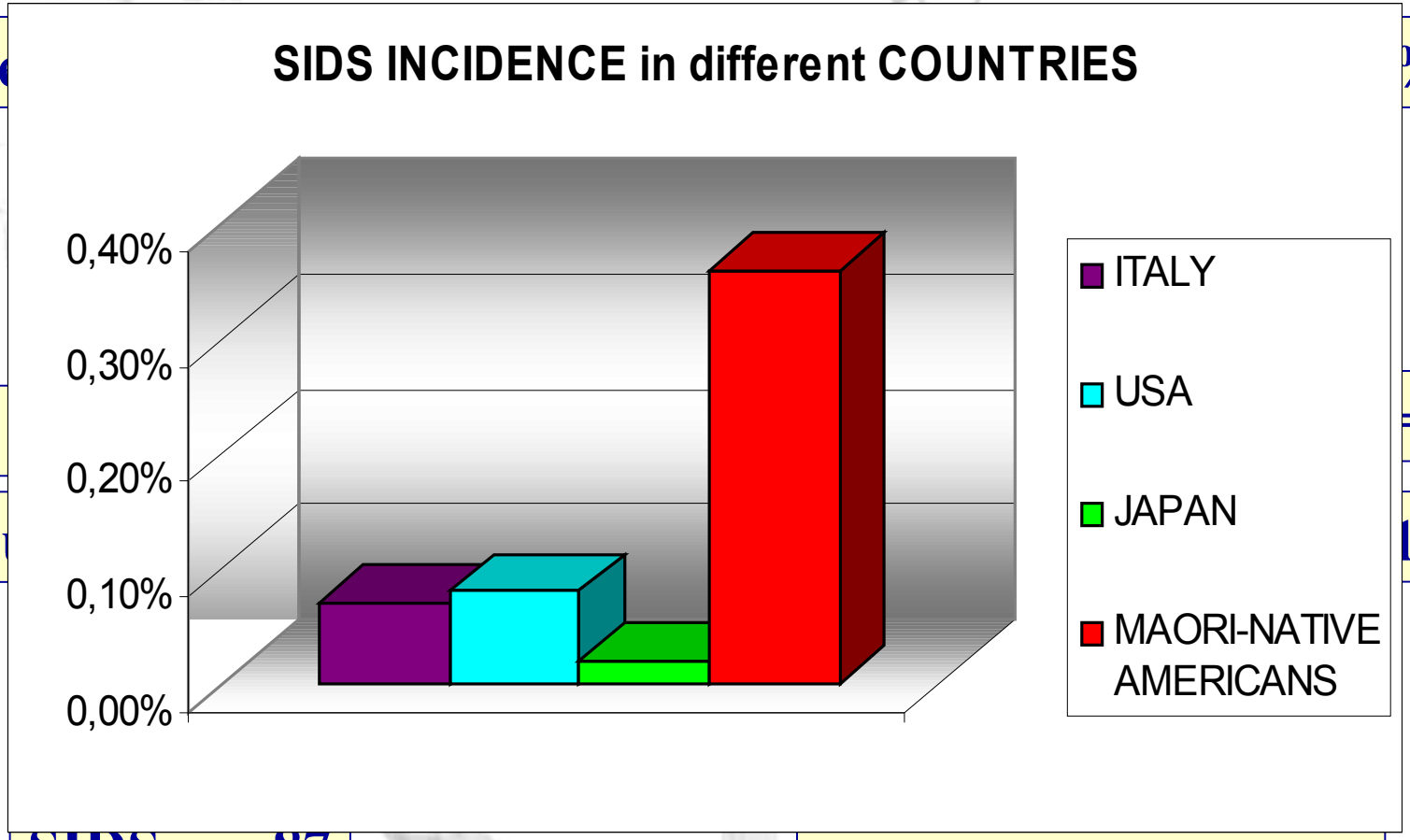
SIDS controlli

p<0.001

5-HTT FREQUENZE ALLELICHE

SIDS n=20

CONTROLLI n=150



SIDS n=87

Frequenza allele L 73%

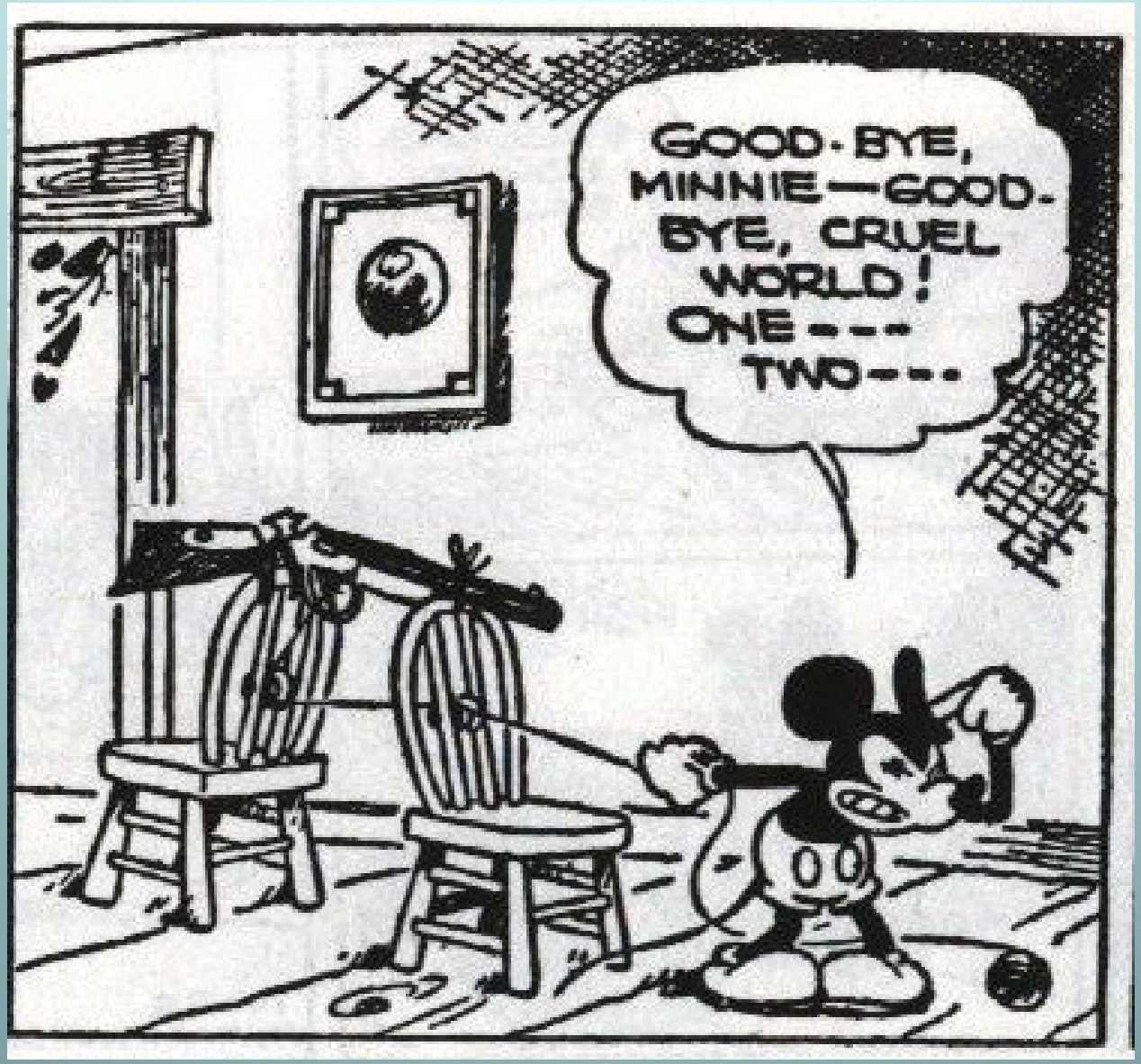
CONTROLLI SANI

Frequenza allele L 35%

SEROTONINA (5 Idrossitriptamina)

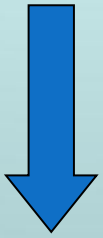
Neuroni serotoninergici esercitano effetto modulatorio su:

FUNZIONALITA' CARDIOVASCOLARE
TONO MUSCOLARE
RITMO RESPIRATORIO
FUNZIONALITA' GASTRO-ESOFAGEA
TERMOREGOLAZIONE
RITMI CIRCADIANI
STATI COMPORTAMENTALI



Genotipo S/S - INSONNIA

SIDS **Genotipo L/L**



IPERSONNIA

DEFICIT DI AROUSAL

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ScienceDirect
Genomics 91 (2008) 485–491
GENOMICS
www.elsevier.com/locate/ygeno

Genes regulating the serotonin metabolic pathway in the brain stem and their role in the etiopathogenesis of the sudden infant death syndrome

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[b] *"Lino Rossi" Research Center, Institute of Pathology, University of Milan, 20122 Milan, Italy*
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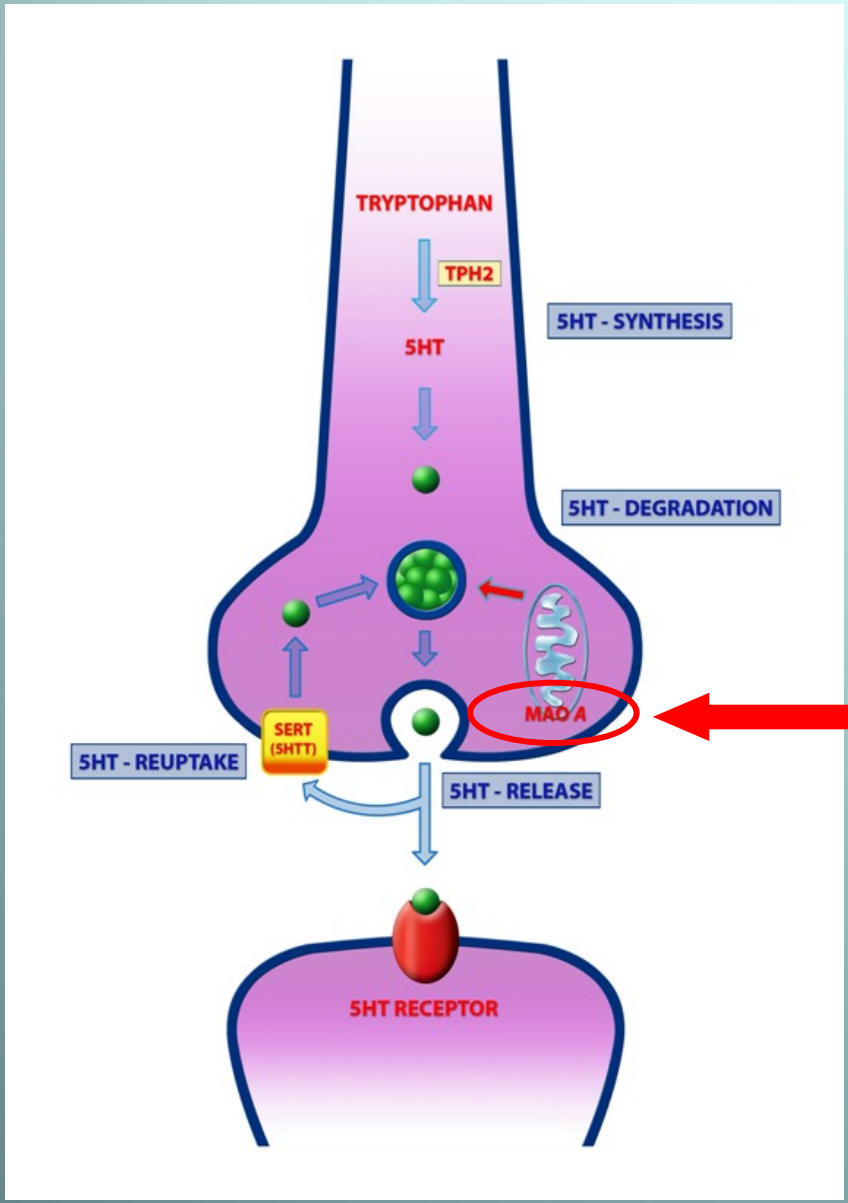
Available online 2 April 2008

Abstract

Genotypes and allelic frequencies of *TPH2, 5-HTTLPR,* the *5-HTT* (*SLC6A4*) intron 2 variable-number tandem repeat (VNTR) region, and the *MAOA* VNTR region were determined in brain-stem samples of 20 "genuine" SIDS cases and compared with results obtained from 150 healthy controls. The SNP G1463A responsible for 80% functionality loss of *TPH2* (tryptophan hydroxylase 2) was not detected, neither in SIDS infants nor in the controls. In contrast, a strict relation was found between the *5-HTTLPR* genotype and its allelic frequencies with SIDS cases. The L/L genotype and the long allele (L) of the promoter region of the serotonin transporter were significantly associated (likelihood ratio (LR) test, $p<0.001$) with the syndrome (L/L, 60% SIDS vs 14% controls; L, 80% SIDS vs 42.6% controls). Polymorphisms of the intron 2 VNTR of the same gene showed a trend for significant differences between genotypes 10/10 and 12/12 (LR test, $p=0.068$), with the L-12 haplotype being almost twofold in SIDS (44.5%) with respect to controls (23.4%). Differences were even higher considering the genotype combination L/L-12/12 (20% SIDS vs 2.6%), and variations among categories were statistically highly significant ($p<0.001$). Although additional differences were observed in the frequency of the *MAOA* (monoamine oxidase A) VNTR genotype 3R/3R between SIDS and controls (respectively 15% vs 26%), the results were not supported by statistical significance. Molecular polymorphisms are discussed considering their functional role in regulating serotonin synthesis (*TPH2*), neuronal reuptake (*5-HTTLPR* and *5-HTT* intron 2), and catabolism (*MAOA*) in the nervous system of Italian SIDS infants. Comparisons are made with previous data obtained in different ethnic groups.

Keywords: *5-HTT*; *MAOA*; *TPH2*; Molecular polymorphisms; SIDS; Neurotransmitter

MAOA - monoamine oxidase A



***MAOA* VNTR**

2, 3, 3.5, 4, 5 repeats

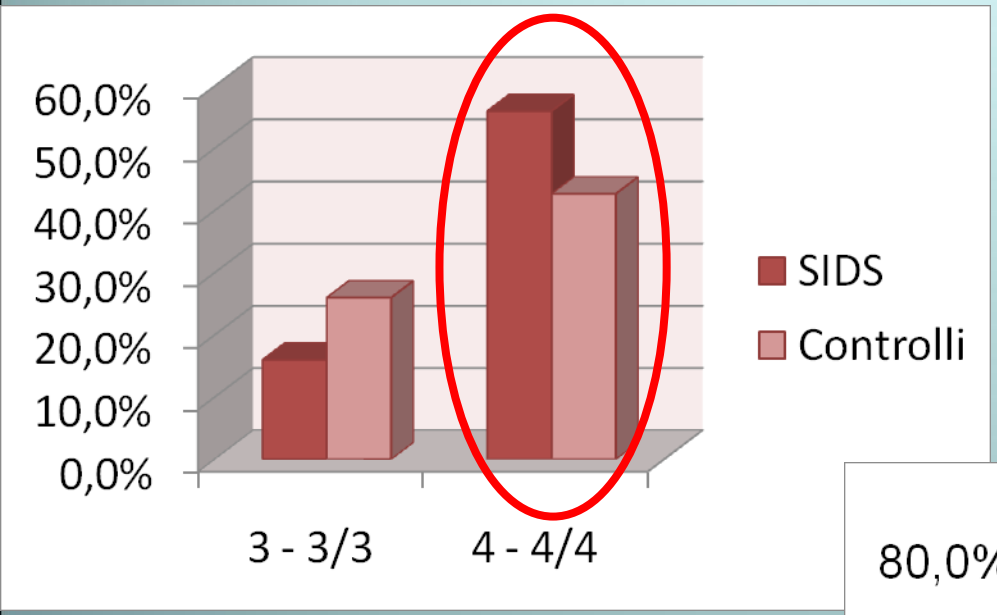
polimorfismo funzionale

PROMOTORE

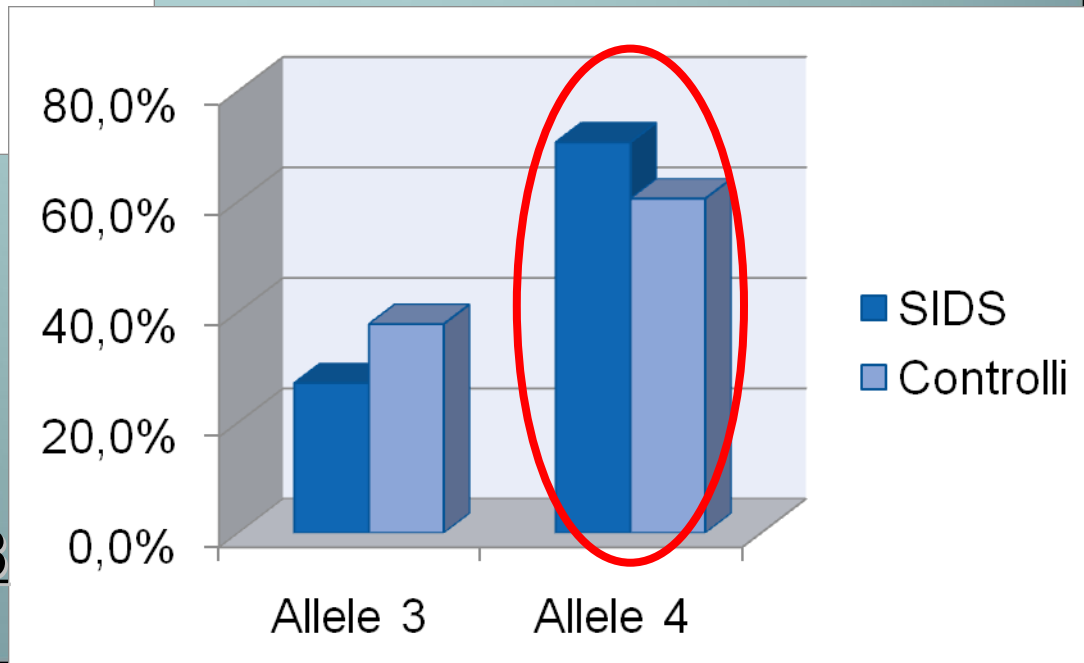
MAOA

Frequenze genotipiche e alleliche

SIDS = IALTE



p=0.047



p=0.018

Neurogenetics
DOI 10.1007/s10048-008-0149-x

ORIGINAL ARTICLE

Association of dopamine transporter and monoamine oxidase molecular polymorphisms with sudden infant death syndrome and stillbirth: new insights into the serotonin hypothesis

Laura Filonzi · Cinzia Magnani · Anna Maria Lavezzi · Guido Rindi · Stefano Parmigiani · Giulio Bevilacqua · Luigi Matturri · Francesco Nonnis Marzano

Neurogenetics
DOI 10.1007/s10048-010-0257-2

LETTER TO THE EDITORS

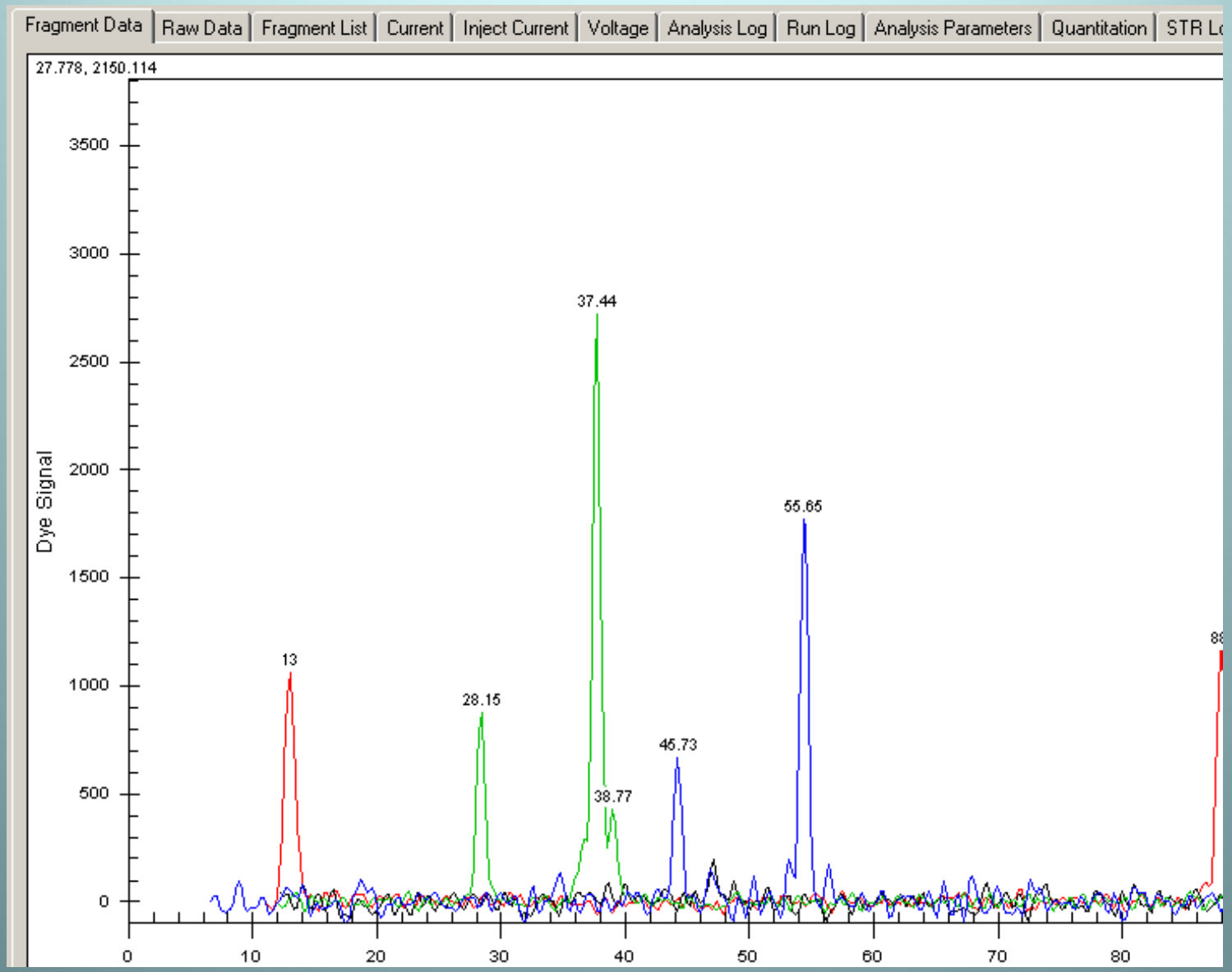
Confirmed association between monoamine oxidase A molecular polymorphisms and Sudden Infant Death Syndrome

Laura Filonzi · Cinzia Magnani · Francesco Nonnis Marzano

Received: 30 July 2010 / Accepted: 19 August 2010

SINDROME DEL QT-LUNGO

Geni KVLQT1, HERG, SNC5A, KCNE1 e KCNE2



ASSOCIAZIONE SEMI PER LA SIDS

MADRE con allele mutato per LQTS - ASINTOMATICA

PADRE con allele mutato per BRUGADA - ASINTOMATICO

FIGLIA con allele mutato per LQTS e BRUGADA - ASINTOMATICA

FRATELLO deceduto per SIDS 15 anni prima


MasterAmp™
Buccal Swab DNA
Extraction Kit
Cat. No. MB71030
30 Reactions
Store at -20°C
LOT NO. BS-90219
EPICENTRE® Biotechnologies
FOR RESEARCH USE ONLY

Apparent Life-Threatening Event

Eziologia e patogenesi

Fattore rischio SIDS

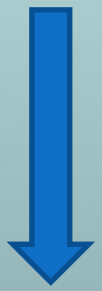
ALTE E' FATTORE DI RISCHIO

SIDS ????

Brooks (1992)	SI
Kahn (2003)	SI
Kiechl-Kohlendorfer (2004)	NO
Edner (2007)	NO
Dageville (2008)	NO
Esani (2008)	FORSE

ANALISI DI I LIVELLO

ANALISI DI II LIVELLO



ANALISI GENETICHE

Linee guida ALTE

Raccolta dei campioni

76 ALTE

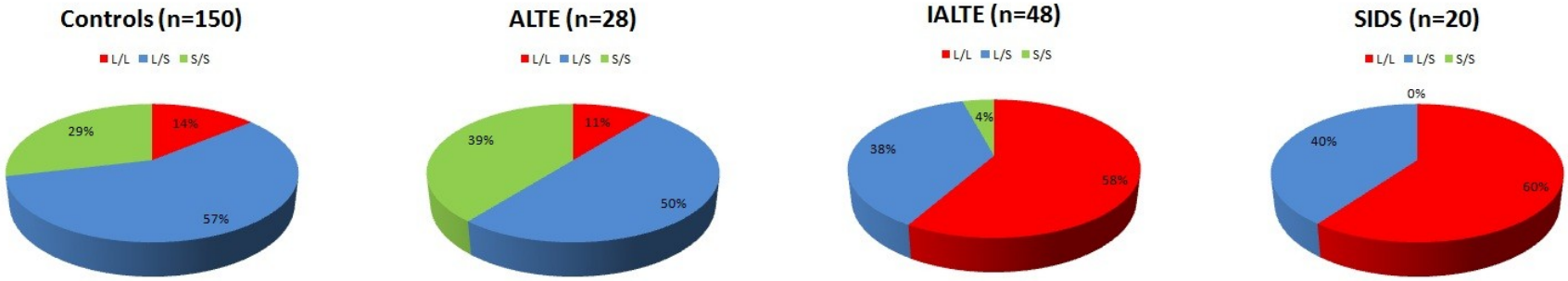
28 ALTE
48 IALTE

→ **Sangue intero**

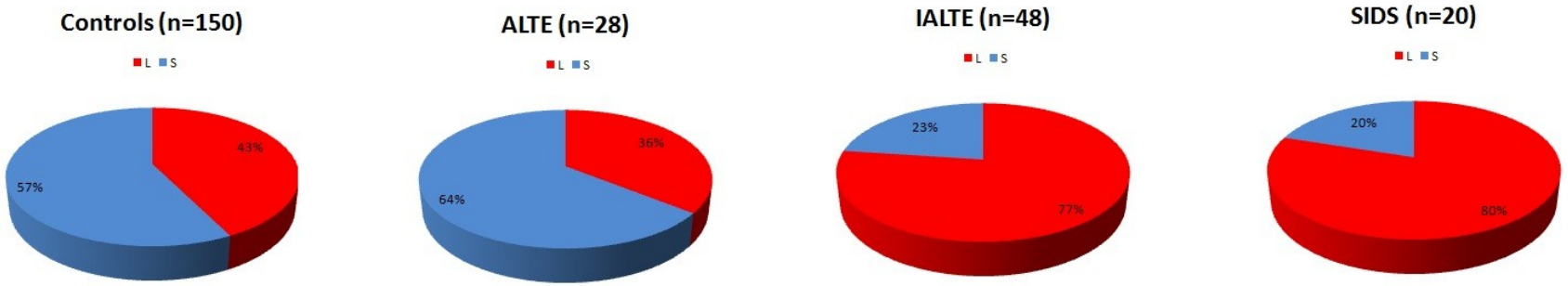
- **U.O. Neonatologia (Azienda Università Ospedale Parma)**
- **U.O. Pediatria (Ospedale di Circolo Varese)**

150 Controlli → **Sangue intero, Saliva**

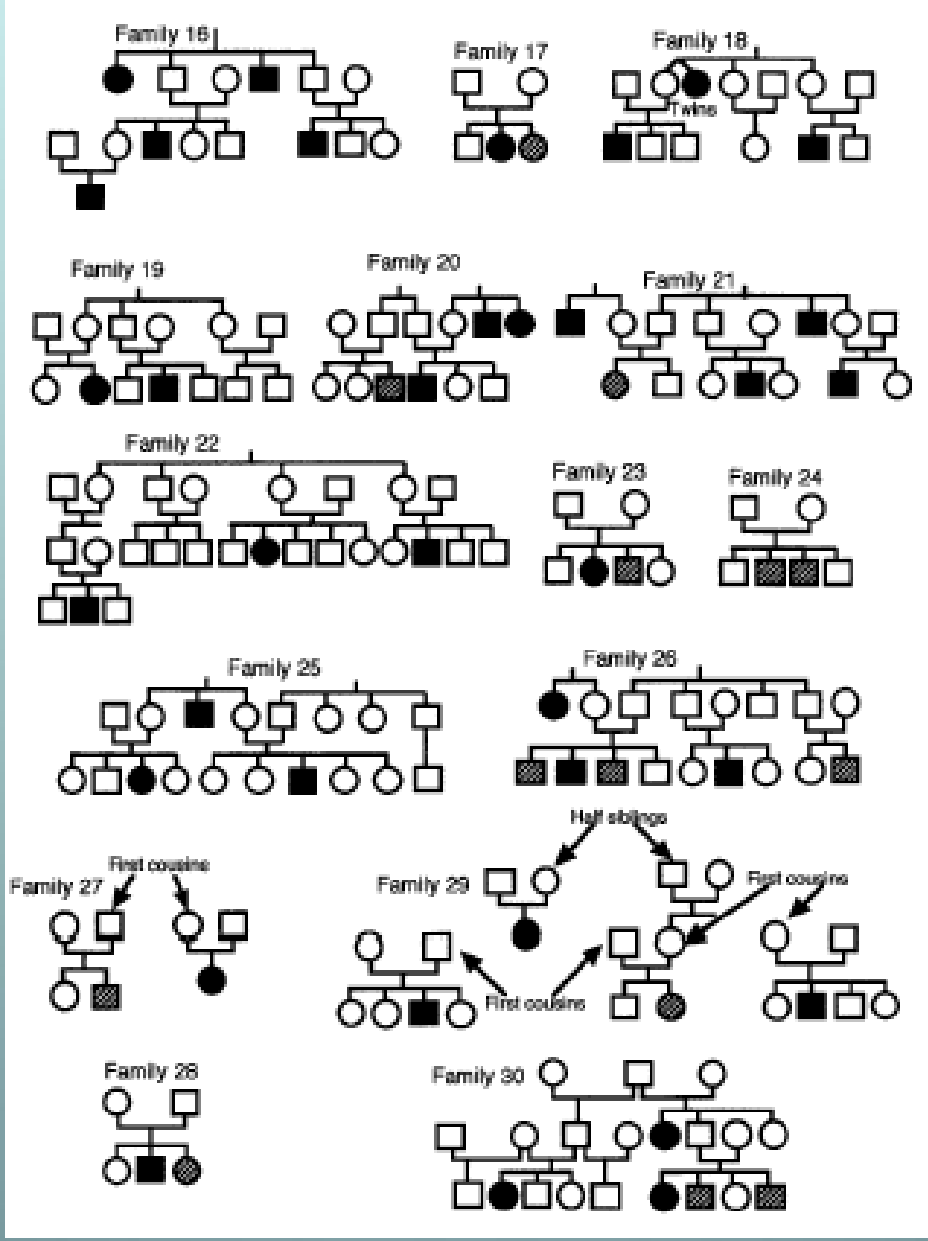
5HTTLPR Genotypes



5HTTLPR Alleles

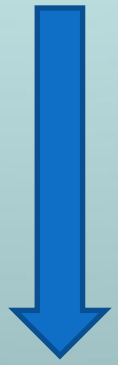


Lenoir Calenda et al. (2000). *Medical Hypotheses* 54: 408-411



FAMILIARITA' SIDS

6 campioni

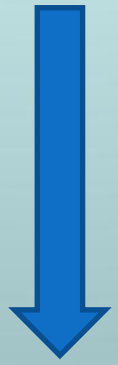


1 Caso IALTE
Sorella della Madre SIDS
Genotipo a rischio L/L

FAMILIARITA' SIDS

ANALISI PRELIMINARI

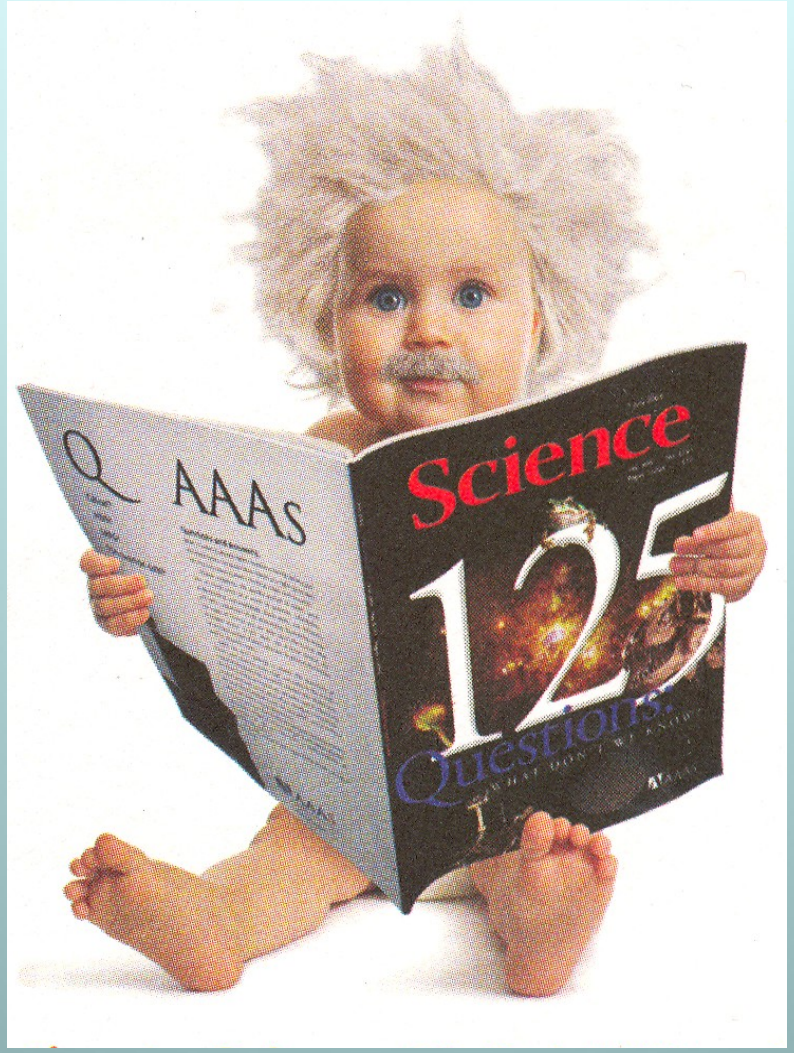
13 campioni



1 Caso IALTE

SIDS intrafamiliare

Genotipo a rischio L/L



In collaborazione con

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