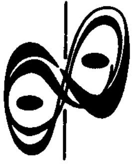

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Phenotype-Karyotype-Genotype Correlations in Prader-Willi and Angelman Syndromes: Preliminary Results

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Prader-Willi Syndrome (PWS) and Angelman Syndrome (AS) are well established as models of Genomic Imprinting in humans, since completely different phenotypes are generated by the absence of paternal (PWS) or maternal (AS) contribution to the q11-13 region of chromosome 15 as a result of deletion or uniparental disomy. We report a preliminary study based on our experience of more than 20 years of research into the genetics of PWS and AS syndromes.

Thirty nine subjects, referred from a number of Centers and Medical Doctors have been examined to either confirm or rule out a diagnosis of PWS or AS.

Patients were evaluated through the Clinical Genetics and Dysmorphology Program at the Human Genetics Center, Dept. of Paediatrics, University of Florence.

Clinical evaluation showed that 10 of these patients fulfilled diagnostic criteria for PWS and 8 for AS.

All patients were isolated cases and the 18 nuclear families were unrelated.

We adopted the staged diagnostic approach for all our families diagnosed PWS or AS families, moleucular using cytogenetic, genetic and molecular cytogenetic techniques.

CYTOGENETICS

Patients were screened cytogenetically for chromosomal rearrangements such as inversions, deletions, and balanced translocations. Peripheral blood lymphocytes from patients and parents were cultured. Complete karyotype analysis of G-banded and Q-banded chromosomes was performed. Not in all cases, high resolution banding was possible.

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In few cases, where fresh preparations were available, the microscopic appearance of the short arms of chromosome 15 was recorded and silver staining was carried out to study the parental origin.

In conclusion, we confirm that chromosome analysis is not always optimal in quality and the interpretation is frequently ambiguous and sometimes incorrect. The results of cytogenetic investigations, also in our experience as reported in literature, were often inconclusive.

MOLECULAR GENETICS

Genomic DNA was obtained from peripheral blood leukocytes according to standard procedures, from patients and parents of all available nuclear families. RFLPs analysis by Southern blotting and hybridisation, using standard methods, involved the following DNA Markers: IR39, ML34, IR4-3R, PW71, TD189-I, TD3-21, IRIO-I, CMWI.

Distal dinucleotide repeat (CA) markers GABRB3 and GABRA5 and LS6-1 were analysed using radiolabelled PCR conditions, essentially as previously described.

Non-radioactive PCR method was used to study a four nucleotides (AAAT) sequence polymorphic STR marker, FES, localized at 15q25qter).

FISH

Fluorescent In Situ Hybridization was performed using biotin labelled DNA kits following the protocols provided by the Oncor manufacturer. For samples referred to confirm or rule out a clinical diagnosis of PWS, FISH was initially carried out using Oncor Region A probe (D15S11) which contains approximately 60 kb of DNA sequence identified by hybridization to a D15S11 probe. For patients with AS clinical diagnosis, initial analysis was performed with the Oncor Region B probe (GABRB3) containing approximately 80 kb of DNA sequence identified by hybridization to a GABRB3 probe. For all patients who appeared to have a deletion based on the study with Region A or Region B, the analysis was repeated using the probe not already utilized in the investigation.

RESULTS

A) PWS Syndrome

Patient PWS-1, L.S., Female, classical phenotype.
Karyotype: 45,XX,t(15;20)(15q12,20q13.3). De novo, unbalanced reciprocal translocation.
The pattern of the marker PW71, after digestion with HindIII and the methylation sensitive enzyme HpaII, is in agreement with the presence of only a maternal allele in the 15 critical region.
DELETION OF PATERNAL ALLELE IN THE 15q11-q113 REGION BREAKPOINT

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Patient PWS-2, M.B., Female, classical phenotype.
Both PW71 and IR4-3R (Sty polymorphism) showed maternal allele only.
DELETION OF PATERNAL ALLELE IN THE 15q11-q13 REGION

Patient PWS-3, E.D., Male, classical phenotype.
IR4-3R (StyI polymorphism), PW71, LS6-I and GABRB3 showed maternal allele only.
DELETION OF PATERNAL ALLELE IN THE 15q11-q13 REGION

Patient PWS-4, P.P., Female, classical phenotype.
D15S11, D15S97 and GABRB3 showed maternal allele only
DELETION OF PATERNAL ALLELE IN THE 15q11-q13 REGION

Patient PWS-5, M.M., Female, classsical phenotype.
IR39, TD189.1, ML34, IR4-3R (RsaI and StyI), Td3-21 (TaqI), TD3-21(CA), GABRB3 and IR10.1 clearly indicated the presence of only one allele. The marker TD3-21 (CA repeats) showed that deletion is of paternal origin.
DELETION OF PATERNAL ALLELE IN THE 15q11-q13 REGION

Patient PWS-6, S.M., Male, mild phenotype.
IR4-3 (Sty I Polymorphism), PW71, Ls6-1 and GABRB3 indicated the presence of only one allele, which is of maternal origin.
DELETION OF PATERNAL ALLELE IN THE 15q11-q13 REGION

Patient PWS-7, E.P., Female, classical phenotype.
IR4-3R (StyI), PW71 (HindIII and methylation sensitive enzyme HpaII), LS6-1 and GABRB3 indicated the presence of only one allele, which is of maternal origin.
DELETION OF PATERNAL ALLELE IN THE 15q11-q13 REGION

Patient PWS-8, L.P., Male, classical phenotype.
IR39 (SSCI), TD189.1, ML34, IR4-3R (RsaI and StyI), TD3-21(EcoRV), IR10.1, CMW-1 revealed the presence of both maternal alleles.
MATERNAL ETERODISOMY

Patient PWS-9, P.P., Male, classical phenotype, and Patient PWS-10, M.M., Female, classical phenotype.
IR4-3R (StyI), PW71 (HindIII and HpaII), LS6-1, GABRB3 and GABRA5 excluded the abnormalities characteristic for most PWS. A small deletion or disomy between the markers already examined or a different mutation cannot be excluded.

B) AS Syndrome

Patient AS-1, M.G., Female, classical phenotype.
PW71 (HindIII and HpaII) and LS6-1 showed paternal alleles only.
The pattern of the marker IR4-3R is in agreement with the presence of only one allele.
DELETION OF MATERNAL ALLELE IN THE 15q11-q13 REGION

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Patient AS-2, L.R., Female, classical phenotype. PW71 and GABRB3 showed paternal allele only MATERNAL DELETION or PATERNAL ISODISOMY?

Patient AS-3, M.G., Male, non-classical phenotype, dark hair. GABRB3 and GABRA5 showed paternal alleles only. MATERNAL DELETION or PATERNAL ISODISOMY?

Patient AS-4, A.S., Female, non-classical phenotype, dark hair.
The patterns of IR10.1, TD189.1 and TD3-21 are consistent with a deletion of maternal allele. At the telomeric side the boundary of the deletion is between the markers IR10.1 and CMW-1
DELETION OF MATERNAL ALLELE IN THE 15q11-q13 REGION

Patient AS-5, A.B., Male, classical phenotype.
PW71 (Hind III and HpaII), LS6-1 and IR4-3R showed paternal allele only.
DELETION OF MATERNAL ALLELE IN THE 15q11-q13 REGION

Patient AS-6, M.B., Male, classical phenotype.
PW71 and GABRB3 showed paternal alleles only.
DELETION OF MATERNAL ALLELE IN THE 15q11-q13 REGION

Patient AS-7, P.C., Female, classical phenotype, and Patient AS-8, E.D., Male, classical phenotype.
PW71, LS6-1 and GABRB3 showed the presence of both maternal and paternal, normal alleles. This result does not exclude a small deletion or disomy between PW71 and LS6-1 or an abnormality of a different nature.

FINAL COMMENTS

The preliminary data we report on two groups of patients, 10 PWS and 8 AS with consistent clinical diagnosis, are presently being completed, using the most recent DNA markers (e.g. PW71 with the double digestion Hind III and HpaII, for patients studied several years ago; additional STR markers for all cases).

The completion of such tests is particularly interesting for those cases where no laboratory result confirming the typical clinical phenotype has up to now been obtained.

However, continuation in greater depth of molecular investigations is important also in patients whose diagnosis has already been confirmed, in order to define more precisely the size of molecular deletion, and to obtain a better phenotype/genotype correlation.

Our results on the frequency of molecular deletion and UPD are consistent with the previous data of the literature.

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