Absence of Maternal Methylation in Biparental Hydatidiform Moles from Women with NLRP7 Maternal-Effect Mutations Reveals Widespread Placenta-Specific Imprinting.

Sanchez-Delgado M, Martin-Trujillo A, Tayama C, Vidal E, Esteller M, Iglesias-Platas I, Deo N, Barney O, Maclean K, Hata K, Nakabayashi K, Fisher R, Monk D. PLoS Genet. 2015 Nov 6;11(11):e1005644. doi: 10.1371/journal.pgen.1005644. eCollection 2015 Nov.

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8th July 2016

Background: Hydatidiform mole, genomic imprinting, NLRP7

Methods: Methyl-Seq, RT-PCR, methylation sensitive genotyping assay, mouse crossing analysis (C57BL/6 x JF1)

Results: Comparison of hydatidiform (CHM and RHM) with healthy placenta samples

Bioinformatic screening and confirmation of novel identified maternal DMRs

Discussion: Molar phenotypes

Conclusion: The main findings of the study

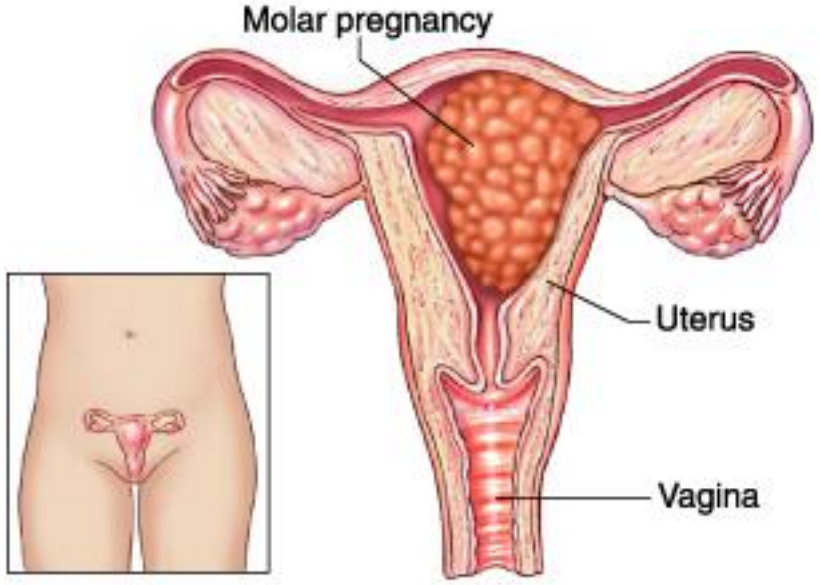
HYDATIDIFORM MOLE

Gestational trophoblastic disease

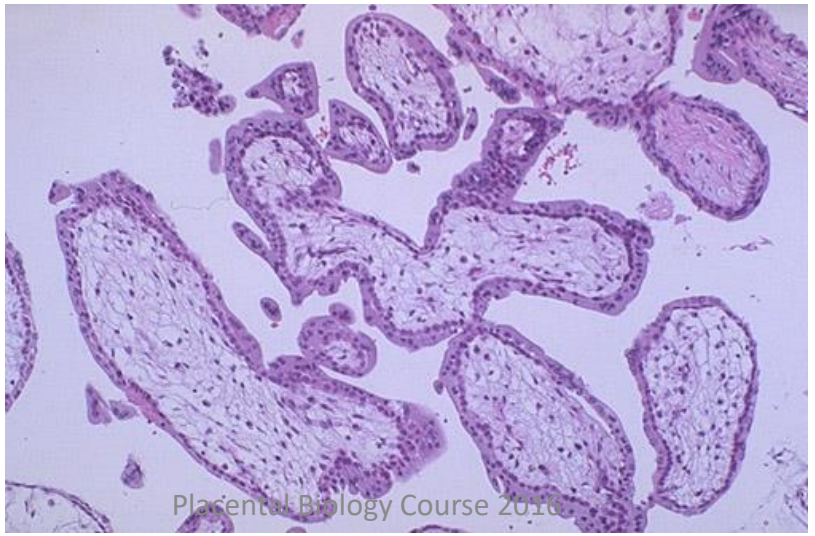
Epidemiology: sporadic, and rarely recurrent and familial(RHM)

Etiology: abnormal genomic imprinting (due to lack of maternal chromosome set)

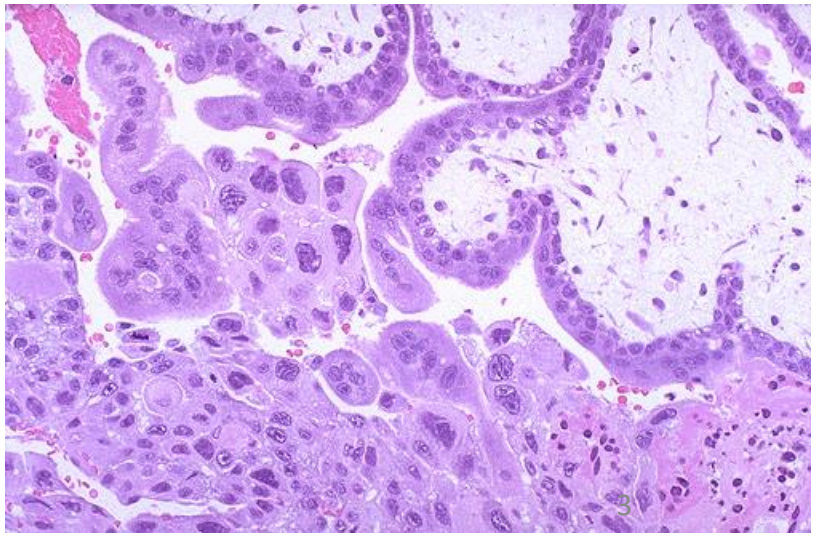
Karyotype: 1. 46,XX (uniparental paternal iso-/heterodisomy)
2. 46,XX or 46,XY (biparental, with mutations in *NLRP7 (AR)* and *KHDC3L*)



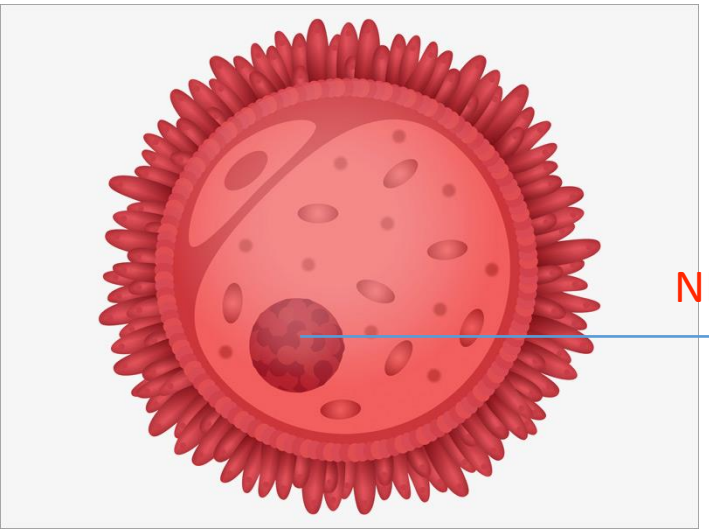
Normal placental villi, 1st trimester



Hydatidiform mole, 1st trimester

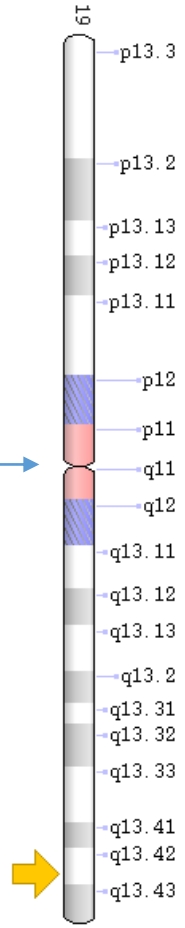


NLRP7 ROLE IN FAMILIAL RECURRENT HYDATIDIFORM MOLE

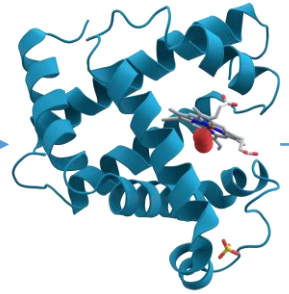


Oocyte

NLRP7



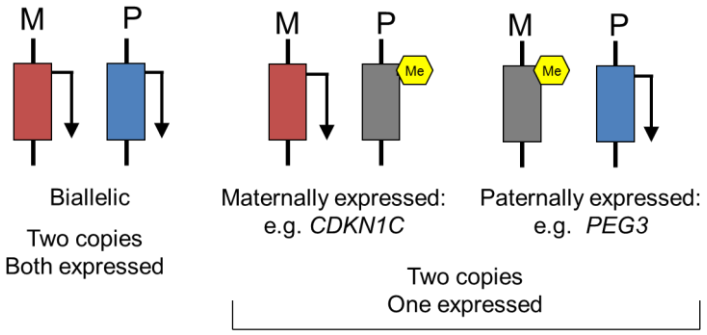
NLRP7 (NACHT, leucine rich repeat, and PYD domain containing 7)



NLRP7 protein

Involve in imprinting multiple maternal genes in oocytes

In mammals, some genes are switched off by DNA methylation on one parental allele

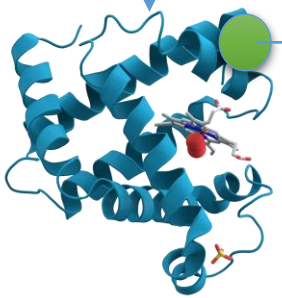


Imprinted genes

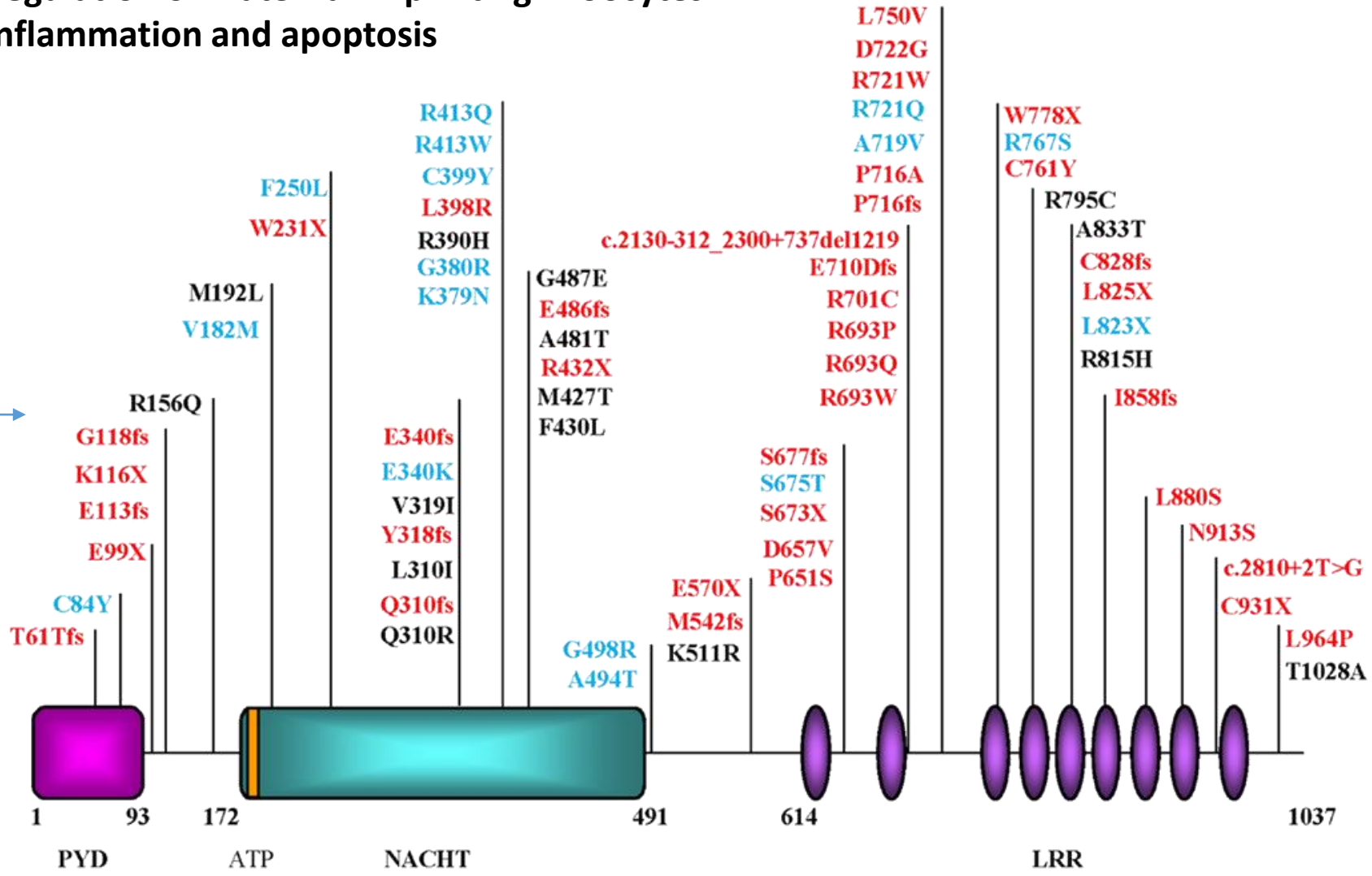
*NLRP7 (*NACHT, leucine rich repeat, and PYD domain containing 7)

Role:

Regulation of maternal imprinting in oocytes
Inflammation and apoptosis



Mutations



Protein with impaired function

Carey et al. (2015); Slim R. and Wallace E. (2013)

AIM OF THE ARTICLE

To analyse imprinting defects in molar biopsies through genome-wide methylation profiling

METHODS

Methylation profiles:

a.) 4 androgenetic moles (CHM)

b.) 4 NLRP7 mutated samples (FHM)

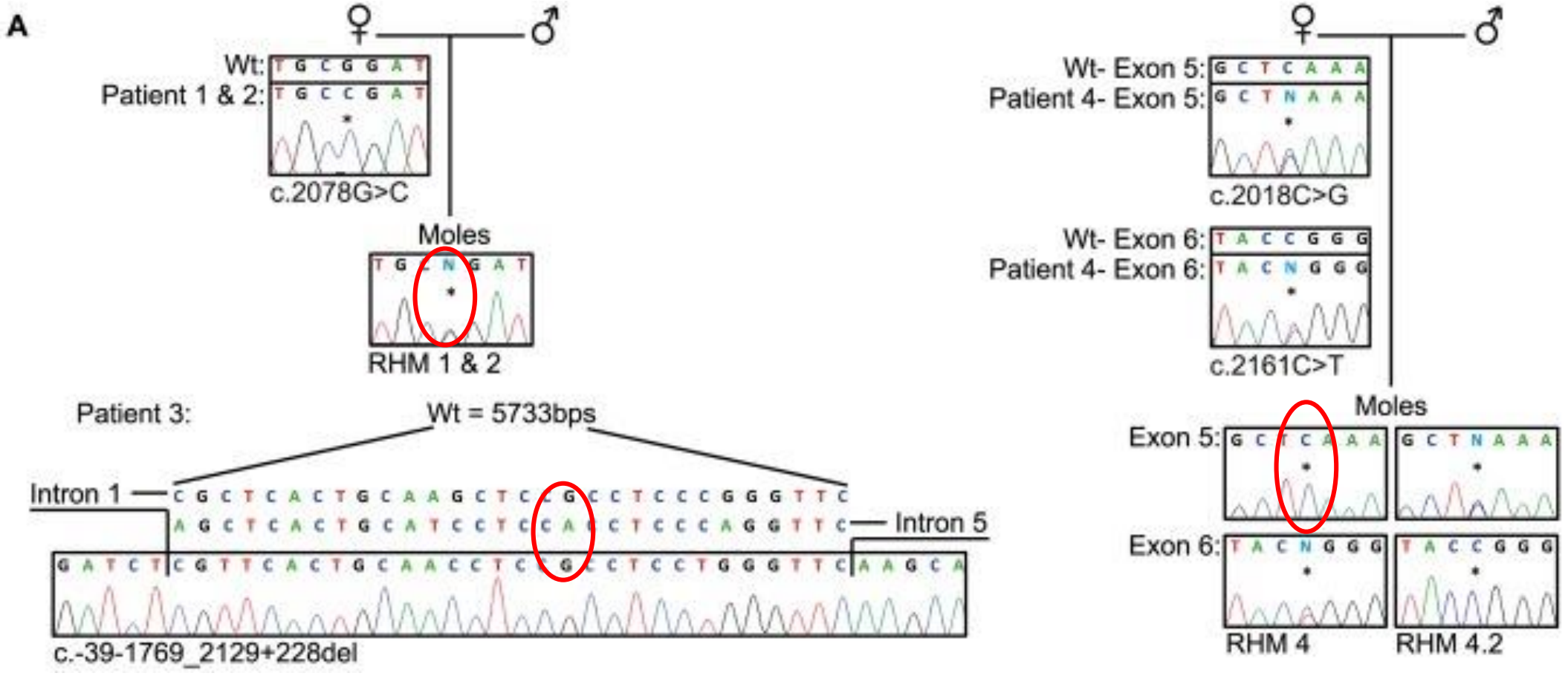
c.) 7 normal placentas

Methyl-Seq → Genome-wide methylation sequencing

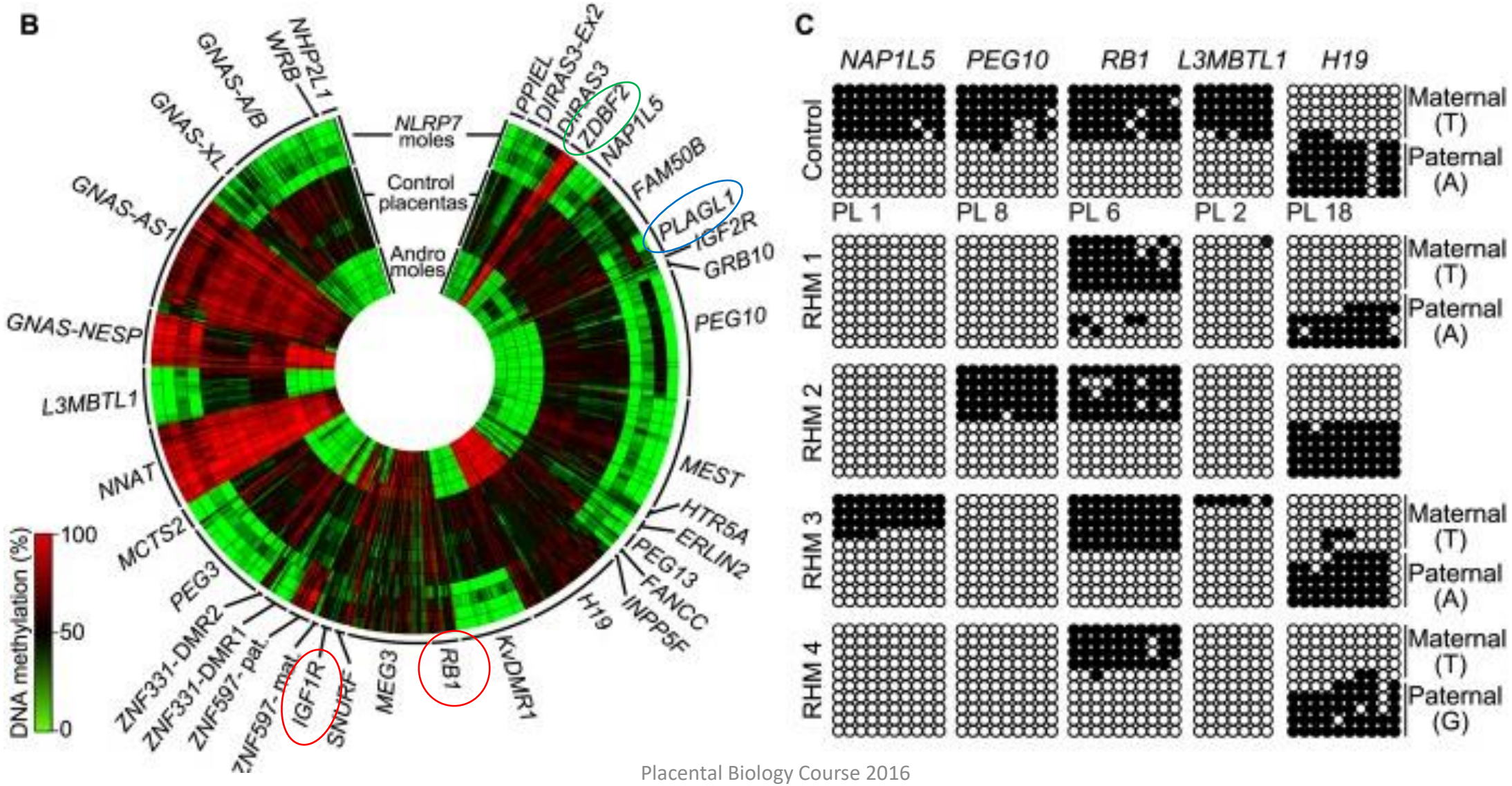
Pyrosequencing and standard allele-specific bisulphite PCR → Confirm ubiquitous imprinted DMRs

RT-PCR → Confirmation LOM at imprinted DMR

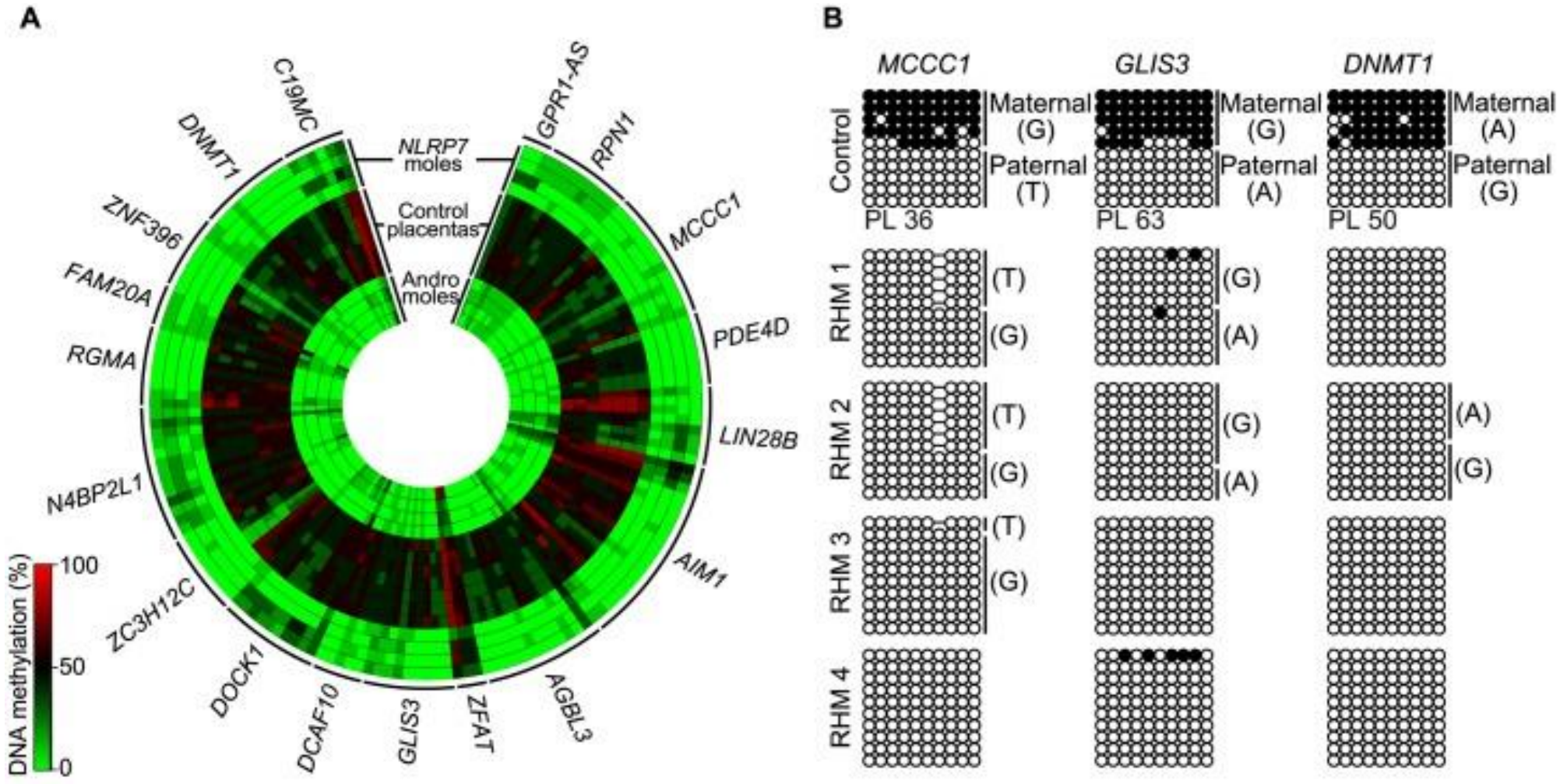
CONFIRMATION OF RECESSIVE NLRP7 MUTATIONS IN PATIENTS



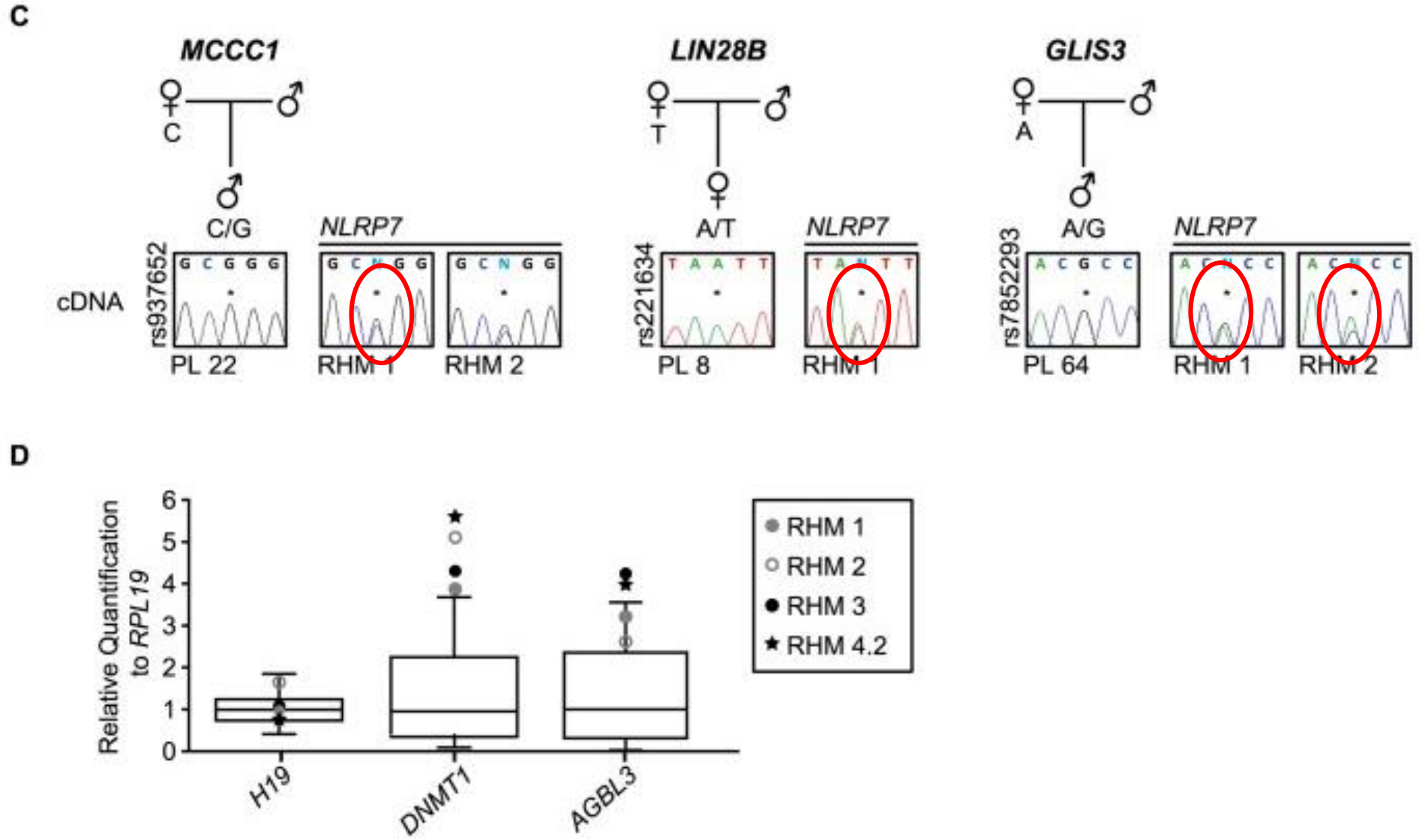
DIFFERENTIALLY METHYLATED REGIONS AND METHYLATION PROFILES



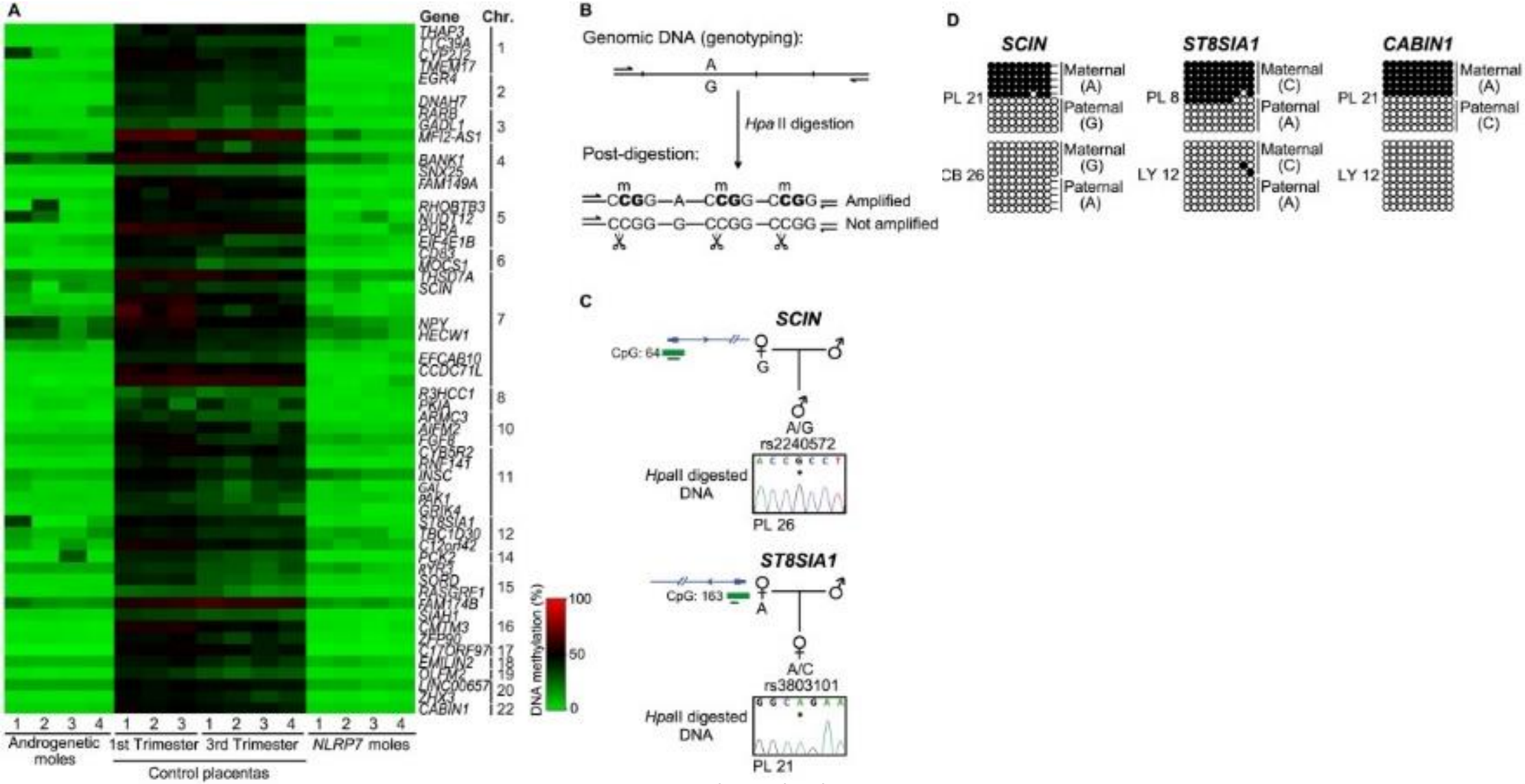
PLACENTA SPECIFIC IMPRINTED LOCI PROFILING



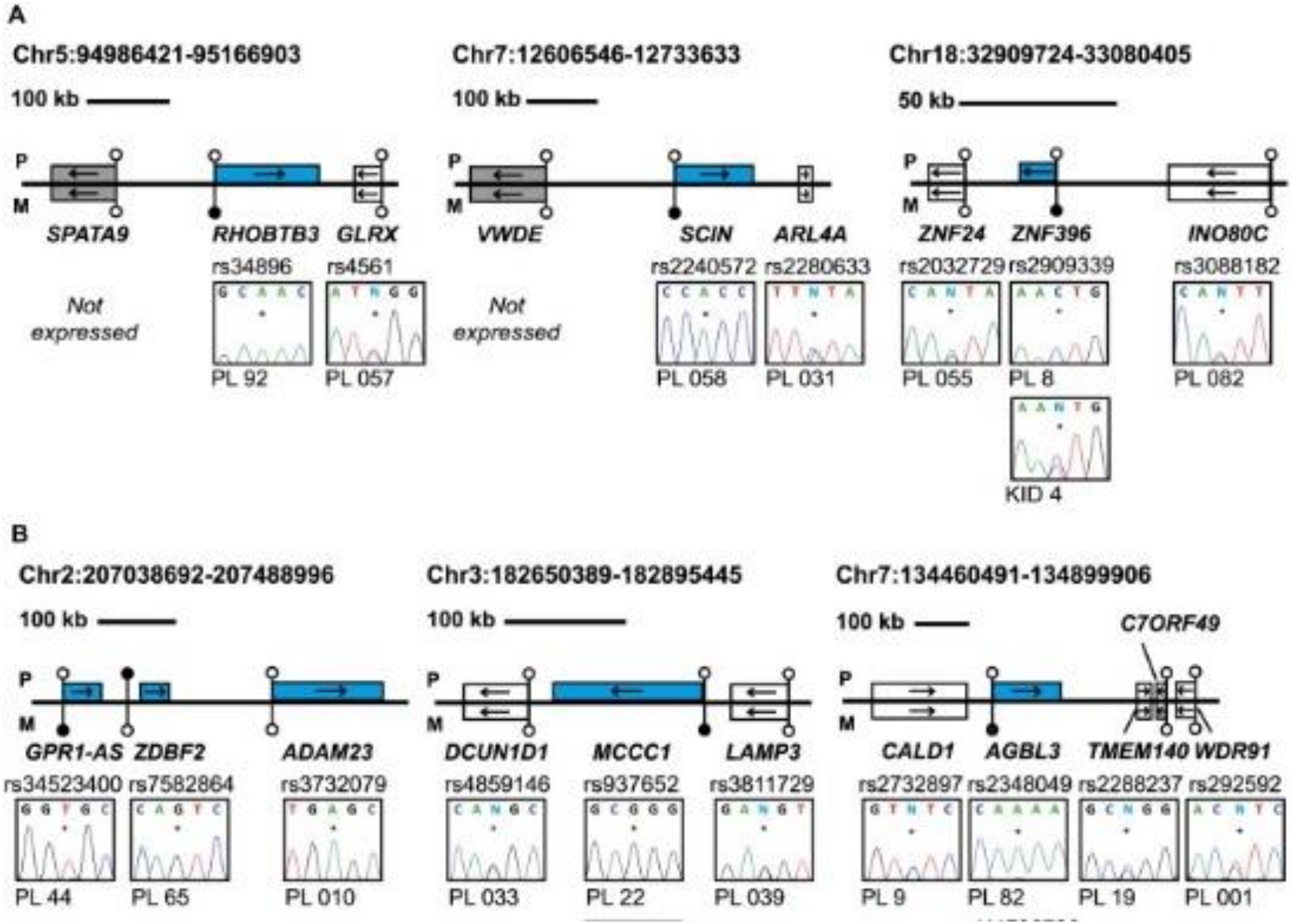
ALLELIC EXPRESSION ANALYSIS OF IMPRINTED GENES AND RT-PCR



IDENTIFICATION OF NOVEL MATERNAL METHYLATED REGIONS

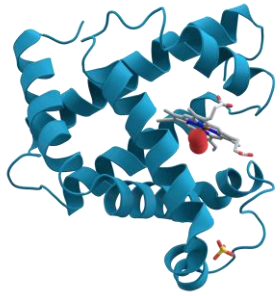


CANDIDATE PLACENTA-SPECIFIC IMPRINTED GENES



DISCUSSION (1)

The results suggest that molar phenotypes are due to defective placenta-specific imprinting and over-expression of paternally expressed transcripts.

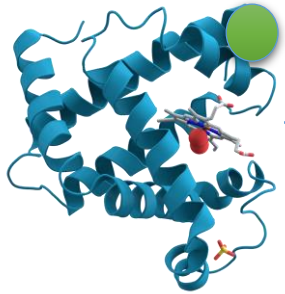


NLRP7 protein

CONTRIBUTES

Placental specific DMR
eg. *NAP1L5, PEG10, L3MBTL1*

Physiologically



Dysfunctional NLRP7 protein

NO CONTRIBUTION

Defective Placental specific DMR

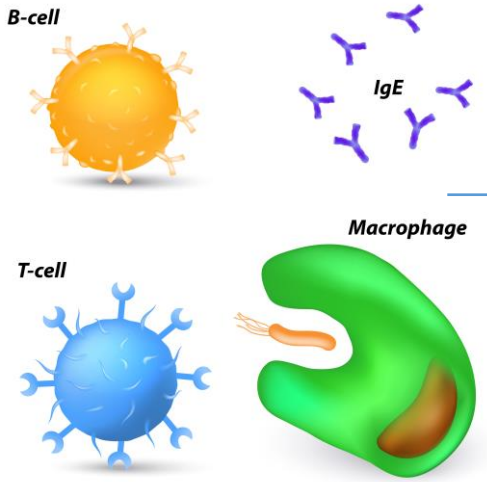
LOM of maternally imprinted genes

Phenotype of HM

RHM

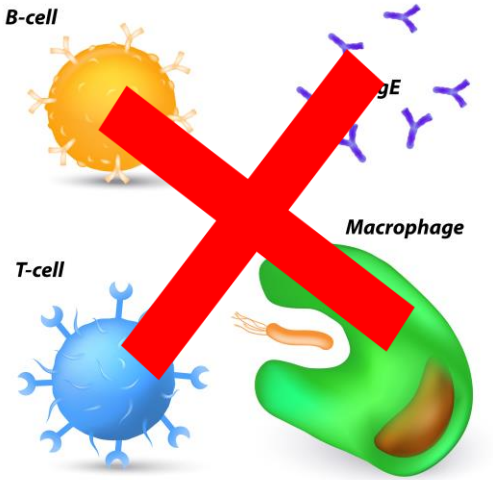
DISCUSSION (2)

NLRP7 plays a role in immune response by regulating release of IL-1β



Normally

Impaired NLRP7 protein slows IL1-Beta release



MAIN FINDINGS

1. Maternal-effect mutations of NLRP7 are associated with the most severe cases of multi-locus imprinting defects in humans.

2. Identified many aberrantly methylated regions.

3. **Bioinformatic screening yielded over 60 loci with methylation profiles consistent with imprinting in the placenta, of which 22 were confirmed as novel maternally methylated loci.**

Thank you for your attention!