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Molecular Confirmatory Test Improves the Accuracy of Congenital Adrenal Hyperplasia Diagnosis in Newborn Screening Program

Mirela Costa de Miranda[1], Eliane Pereira dos Santos[2], Daniel Fiordelisio de Carvalho[1], Andresa De Santi Rodrigues[1], Ivana Van Der Linden Nader[2], João Amélio da Silva Junior[2], Berenice B Mendonca[1] and Tania A Bachega[1]

[1]Laboratório de Hormônios e Genética Molecular- LIM/42, Unidade de Adrenal, Disc. de Endocrinologia e Metabologia, Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil, [2]ASSOCIAÇÃO DE PAIS E AMIGOS DOS EXCEPCIONAIS (APAE) do Estado de Goiás

All authors declare no conflicts of interest

Introduction and Objective

Congenital adrenal hyperplasia (CAH) is a life threatening disorder presenting the criteria for inclusion in newborn screening (NBS) programs. NBS is highly effective in identifying the severe cases; however, the high rate of false-positive (FPR) results remains an important issue. Therefore, positive neonatal results must be confirmed by serum 17OHP levels, which present, though, a great overlap among cases with SW, SV and NC forms, leading to therapeutic implications. Additionally, some stressed NB remains with increased hormonal confirmatory tests, needing prolonged follow up.

Objective: To evaluate the utility of molecular analysis to improve CAH diagnosis in our NBS program.

Methods

Between 1999-2014, 86 newborns (NB) were submitted to DNA analysis due to positive tests in NBS program of Goias State – Brazil. Molecular study was performed using peripheral DNA samples. Neonatal 17OHP levels were measured by IFMA assay (*Autodelfia-Perkin Elmer*) and adjusted for birth-weight. Confirmatory tests included serum 17OHP, androstenedione, testosterone measurements. *CYP21A2* genotypes were determined by entire *CYP21A2* sequencing and MLPA technique.

Results

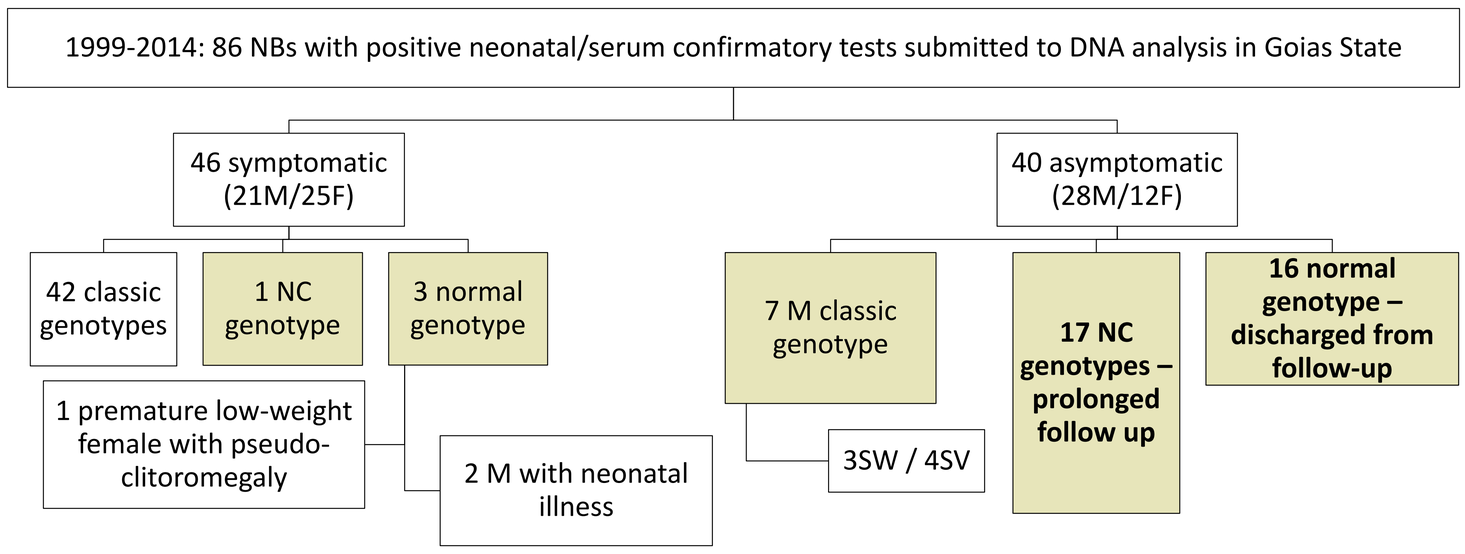
- ✓ 46 NB were described as presenting some sign of dehydration/mild hyponatremia and/or atypical genitalia:
  - ➢ 42 of them presented genotypes predicting severe classical forms (19 males)
    - Among patients with the classical form, 4 females with severe external genitalia virilization were assigned in male social sex, being subsequently corrected.
  - ➢ 1 presented with NC genotype (girl with isolated clitoromegaly) and 3 presented normal genotype: 1 premature girl with pseudo-clitoromegaly and 2 males with loss of weight due to neonatal stress conditions
- ✓ 40 patients were asymptomatic:
  - ➢ 7 males with the classical genotypes, 3 SW and 4 SV
  - ➢ 33 non-affected patients were prevented to receive unnecessary treatment, 16/33 with normal genotype were discharged from follow-up

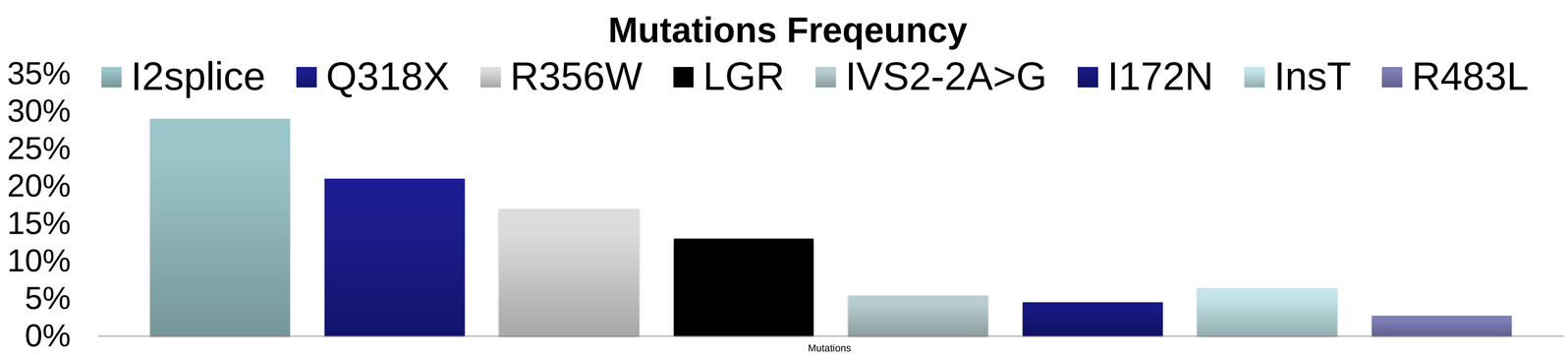
Among patients with classical genotype, 18 had the SW genotype, 23 genotypes predicting SW or SV forms and 5 had the SV genotype.

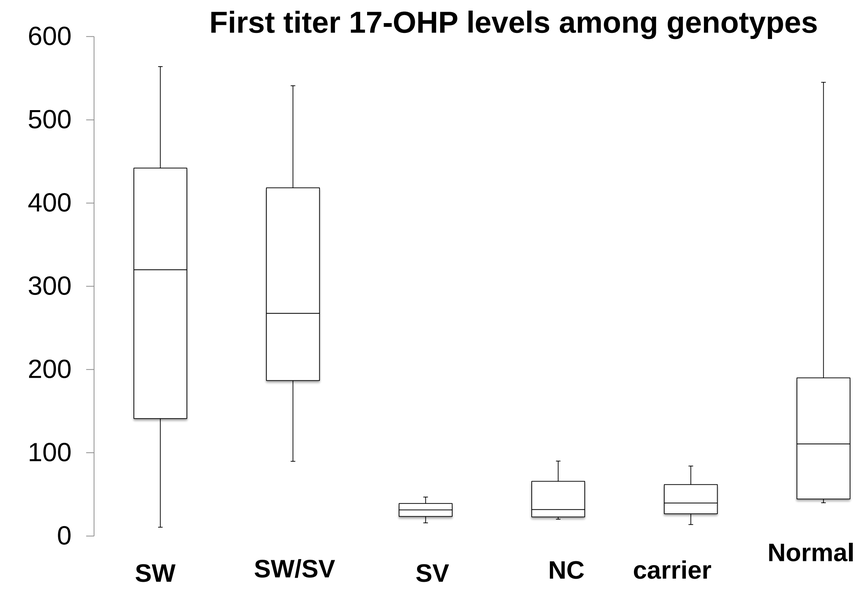
Mean N17OHP levels in classical patients was 271 ng/mL, whereas in the others, including false-positive and non-classical newborns, the mean was 91 ng/mL. Even thought, a great overlap of 17-OHP levels among all genotypes was observed.

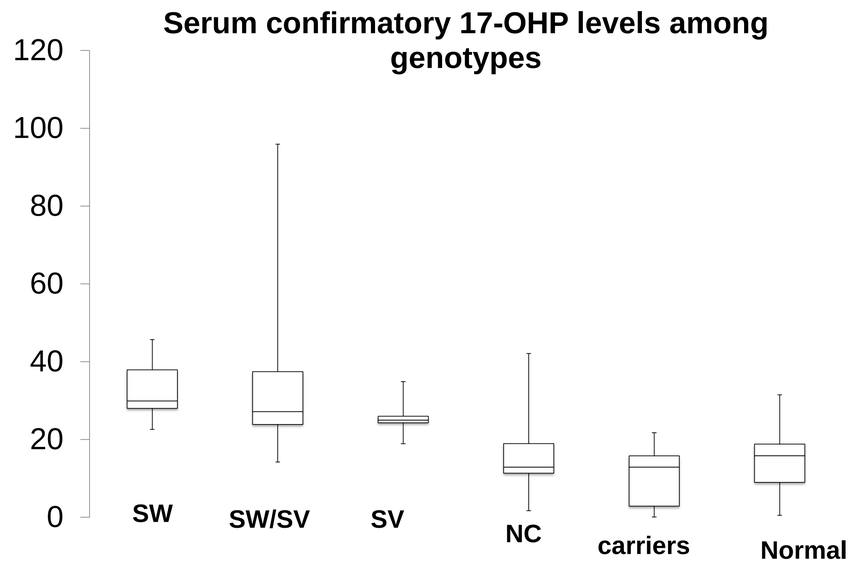
Mutations derived from pseudogene events were found in 88% of the alleles: 13% carried large gene rearrangements and 87% point mutations. Twenty eight percent of patients carried two point mutations in the same allele.

The most frequent point mutations were I2 splice (35%), p.Q318X (23%) and p.R356W (19% of alleles). Novel mutations were found in 12% of the alleles: p.G424S, p.R408C and IVS2-2A>G, all presented with gene founder effect.









Conclusion

We demonstrated that molecular testing was a useful supplemental tool in identifying false-positive results in CAH-NBS, preventing unnecessary follow-up of newborns with inconclusive hormonal tests. Additionally, the high frequency of novel mutations indicated the importance of adding gene sequencing to improve the accuracy of molecular confirmatory tests.

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