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EDITORIAL

Newborn screening of congenital adrenal hyperplasia☆

Cribado neonatal de hiperplasia suprarrenal congénita

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Congenital adrenal hyperplasia (CAH) is a autosomal recessive hereditary disorder due to steroid production enzyme deficiency. Ninety-five percent of all such hyperplasias are attributable to 21-α-hydroxylase (21OHD, OMIM #201910) deficiency. The decrease in cortisol synthesis causes an increase in ACTH, with the accumulation of 17-hydroxyprogesterone (17OHP), a metabolite preceding the point of enzymatic block. If the enzyme deficiency affects the aldosterone production pathway, an alteration to the water-electrolyte balance occurs. The enzymatic block gives rise to an increase in adrenal gland androgen synthesis, which is a metabolic pathway not affected by such a deficiency.[1]

Clinically the disease is classified into two forms: classical and non-classical. The classical forms in turn are divided into two presentations: with electrolyte loss (EL) and the simple virilizing form (SV).

An excessive androgen increment in the early fetal period gives rise to virilization of the external genitals in females. The degree of virilization is variable in such cases (Prader classification). Macrogenitosomia may develop in male fetuses, though not in all cases.

Electrolyte loss occurs in 75% of the patients with classical forms of the disorder. The affected newborn infants suffer progressive anorexia, a lack of weight gain, weakening, polyuria and vomiting. Hypotonic dehydration, cardiogenic shock and death result if the condition is not adequately recognized and treatment provided immediately. The electrolyte crisis manifests between day 5 and 10 of life. Treatment in the form of hydrocortisone should be started immediately in order to replace the physiological secretion of glucocorticoids and mineralocorticoids, and avoid electrolyte loss.

The incidence of the disease (classical forms) varies between 1/10,000 and 1/20,000, depending on the population considered.[2]

The early detection of the classical presentations of CAH is contemplated in the Newborn Screening Programs (NSPs) of many countries, and is based on the classical NSP inclusion criteria. These criteria, established by Wilson and Junger,[3] remain valid today, and can be summarized in terms of four main points:

1 The disease causes severe morbidity (with possible death), and clinically is not easily recognizable in the neonatal period.

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2 Effective, immediate and easily administered treatment is available. Adequate medical intervention reduces morbidity and the possible associated disabilities.
3 The frequency of the disease is relatively high (>1/10,000–1/15,000).
4 A screening parameter and a sensitive and specific analytical procedure that is simple, reliable, rapid and inexpensive is required.

Newborn Screening Programs are regarded as an essential element in Public Health, and seek to allow the presymptomatic identification of certain endocrine-metabolic disorders based on the use of tests that can be applied to all the population subjected to screening. Early detection, adequate medical intervention and immediate treatment avoid neurological damage, reduce morbidity and mortality, and lessen the possible associated disabilities.

Newborn Screening Programs should be clearly identified in the Public Health policies of the different Spanish Autonomous Communities. The Public Health authorities have a decisive role in the promotion of these programs, regarding decisions and planning, and need to take a number of essential aspects into account: 1) the provision of information targeted at parents regarding the objectives, the diseases included under the program, the tests performed and the benefits of screening for the newborn infant; 2) the availability of a Newborn Screening laboratory; 3) the availability of a Differential Diagnosis laboratory; and 4) the integration of Clinical Monitoring Units to complete and validate the benefits of the program. In addition, the NSPs should be globally financed by the corresponding Autonomous Communities.

The objectives of the early detection of CAH are:

1 To identify the severe classical forms of the disease, avoiding the development of severe dehydration, shock and death, particularly in male infants with electrolyte loss (EL).
2 To avoid incorrect gender designation in newborn girls with highly virilized genitals and the resulting sequelae.
3 To detect simple virilizing forms (SV) in order to avoid hyperandrogenization.

The benefits of screening for CAH show that:

1 Patient survival is improved. In countries where newborn screening for CAH is not available, the reported incidence of the disorder is higher in girls than in boys.[4]
2 Hyponatremia, which over the long term causes mental disabilities and learning problems, is avoided.
3 Incorrect gender designation can be remedied more rapidly.

Detection is based on the measurement of 17OHP in capillary blood samples obtained from the heel of the patient and impregnated in blotting paper at 48 h of life. The test is based on time-resolved fluorescence immunoassay (AutoDelfia, PerkinElmer Life Sciences). Each laboratory needs to establish its own cut-off points according to the target population involved, stratified according to gestational weeks and gender. Statistically significant gender differences in 17OHP levels are observed in infants born to term.

The urgent management of patients with electrolyte loss (EL) requires a Clinical Monitoring Unit including pediatricians with expertise in this disease, as well as an Intensive Care Unit where the infants can be admitted, if necessary.

In Spain, the efficacy/effectiveness[2] and cost/effectiveness studies made[5] have generated evidence in favor of the introduction of screening for CAH, though there are also some doubts.

The evidence in favor of screening is as follows:

1 Adrenal crises are avoided in EL forms of the disease.
2 Incorrect gender designation is avoided.
3 Hyponatremia is less pronounced in cases detected through screening.
4 The hospital admission time is shortened.
5 Screening for CAH is cost-effective given a willingness to pay 30,000 €/QALY (quality-adjusted life years gained).
6 Assuming a clinical sensitivity of 85% for the EL forms of the disease in the absence of screening, it is possible to recommend the inclusion of CAH in the Spanish NSPs.

Doubts regarding screening:

1 The latency period of the disease requires a short response time. Those NSPs that include this disease must meet the quality standards and optimize the response time, ensuring that case detection occurs in the first week of life (7–8 days) in order for the program to be effective.
2 Cut-off points and percentage false-positive results. Compliance with the quality criteria includes the availability of proprietary cut-off points established according to gestational weeks and gender. This makes it possible to reduce the number of false-positive cases and increase the positive predictive value of the test.

The mortality rate due to adrenal crises in non-screened patients varies between 4 and 11.9%.[6] Most cases detected through screening are already at home by the time the diagnosis is established, affected males being the patients at highest risk.

The early detection of CAH is internationally recommended with a level of evidence of 1/++.[7]

Congenital adrenal hyperplasia is one of the candidate diseases for inclusion in[7,8] NSPs. In Spain, the decentralization of Public Health, with the corresponding competences being the responsibility of the Autonomous Communities, has allowed the incorporation over the years of new early detection programs that differ in terms of the range of diseases subjected to screening. The Community of Madrid incorporated the early detection of CAH to its NSP in 1990. From its start until December 2016, a total of 1,661,554 newborn infants had been analyzed, with the detection of 79 cases of classical CAH (EL + SV), the incidence of the disease being 1/21,032.

At present (2017), screening for CAH has been introduced in 6 Autonomous Communities, covering 29.8% of all newborn infants. The website of the Spanish Newborn Screening Association (*Asociación Española de Cribado Neonatal* [AECNE])[9] reports the activity of the Newborn Screening Centers. Between the introduction of CAH screening and December 2016 (data pending incorporation to the website), a total of 3,086,015 newborn infants had been analyzed,

with an estimated incidence of the classical forms of the disease (EL + SV) of 1/21,732. There may be a degree of underestimation in these data, since positive cases that do not exhibit neonatal clinical manifestations (SV forms in males; see below) are not included.

Contributions of CYP21A2 genotyping to newborn screening of congenital adrenal hyperplasia

Genetic analysis is a diagnostic confirmation tool for monogenic diseases characterized by a strong genotype/phenotype correlation. Such analysis can moreover rule out the disease if a high diagnostic yield can be guaranteed, free of data of uncertain interpretation (see Annex 1 of the supplementary material). When CAH was incorporated into screening, its molecular basis was not fully understood; alterations of the *CYP21A2* gene (NM_000500) began to be described in 1990,[10] revealing that a series of alterations allowed for the characterization of the deficient alleles with good genotype/clinical correlation in our population, also.[11] Compared with the determination of 17OHP, which in the perinatal period is interfered with by adrenal gland immaturity, situations of stress, etc., genotyping is acknowledged as a help in the management of patients with CAH, also in the context of newborn screening.[12,13]

Although 17OHP is the marker used, other defects can also be detected, including those in which the implicated enzymes are located beyond the level of the metabolite. We have found at least 8 cases in the literature, the latest being published in 2016.[14] This capacity is an advantage, since it facilitates the detection of other rarer forms of CAH, though it is also evidence of the existence of analytical interference. False-positive results in direct immunoassays are a recognized fact in perinatal samples.[15,16]

In a recessive disease, genotyping is considered to rule out the disorder in >95% of the cases provided it encompasses no less than 80% of the causal alterations in the analyzed population (two alleles to be characterized, $0.2 \times 0.2 = 0.04$; <5% false-negative results of genotyping). Carriers will be detected which, while not affected by the disease, will prove positive, and patients that are homozygous for a rare alteration will escape this calculation, thereby increasing the number of false-negative results. In comparison with *CFTR* genotyping included in the screening for cystic fibrosis, *CYP21A2* offers the advantage that a limited battery of frequent alterations (point alterations and deletions) guarantees greater coverage (>90%), with a lesser frequency of carriers. The inconveniences of *CYP21A2* are the complexity of the locus to be analyzed, poor adaptability to high-performance techniques, and the need for experience with this concrete locus.[15,17–22] The *CYP21A2* locus includes a pseudogene characterized by the pre-existence of most of the causal mutations, and there are complex rearrangements in the normal and mutated alleles.

An analysis targeted at the clinically validated frequent *CYP21A2* alterations offers the advantage of avoiding uncertainty (polymorphisms and rare variants of uncertain interpretation), thereby facilitating the exclusion of the disease. We must ensure the required coverage, especially in relation to severe mutations,[18,19,21] with correct characterization of the alleles[11,17–20] (see Annex 2 of the supplementary material).

The usefulness of analyzing *CYP21A2* was demonstrated[23] by the monitoring of 76 positive cases in the newborn screening program for CAH of the Community of Madrid; 43 patients in which the disease was finally discarded and who were classified as transient 17OHP elevations, had yielded negative results in *CYP21A2* genotyping. Normalization of the 17OHP determinations was not achieved until after 6 months in 50% of the cases, and after one year in the remainder. Of the 33 cases in which *CYP21A2* proved positive, 24 were patients with classical forms of the disease, while 9 were also CAH cases, though of a ''cryptic'' nature in the neonatal period (virilizing cases in males and non-classical forms in both genders); genotyping had correctly classified these patients. In the global series of positive cases at screening which we have been able to analyze to date (176 cases in the Community of Madrid and 41 [up until 2012] from other Autonomous Communities), 47 corresponded to classical disease genotypes (16 SV) while 170 proved negative for the classical forms: 25 non-classical presentations and 145 negative cases (6 carriers of mild alterations, 3 carriers of severe mutations,[22] and 6 cases presenting a false severe allele with gene duplication including p.Gln319X[20]).

The *CYP21A2* genotype offers a great deal of information regarding CAH and can be used in the positive cases of newborn screening (with or without clinical manifestations) to discard, confirm and classify the disease. However, expert analysis and interpretation is required, in view of the special characteristics of the locus.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.endien.2017.11.015.

References

1. Spencer PW, Azziz R, Baskin LS, Ghizzoni L, Hensle TW, Merke DP, et al. Congenital adrenal hyperplasia due to steroid 21-hidroxylase deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2010;95:4133–60.
2. Paz-Valiñas L, Varela-Lema L, Atienza G. Cribado neonatal de la hiperplasia suprarrenal congénita. Revisión sistemática, Red Española de Agencias de Evaluación de Tecnologías y Prestaciones del Sistema Nacional de Salud. Axencia de Avaliación de Tecnoloxias Sanitarias de Galicia; 2014. Informes de Evaluación de Tecnologías Sanitarias.
3. Wilson JM, Junger G. Principles and practice of screening for disease. Public Health Papers. Núm. 34. Geneva: World Health Organization; 1968.
4. Nordenström A, Ahmed S, Jones J, Coleman M, Price DA, Clayton PE, et al. Female preponderance in congenital adrenal hyperplasia due to CYP21 deficiency in England: implications for neonatal screening. Horm Res. 2005;63:22–8.
5. Castilla I, Vallejo-Torres L, Rica-Echevarría I, Rodríguez-Sánchez A, Dulín-Íñiguez E, Espada M, et al. Coste-efectividad del cribado neonatal de la hiperplasia suprarrenal congénita. Ministerio de Sanidad, Servicios Sociales e Igualdad. Servicio de Evaluación del Servicio Canario de la Salud; 2013. Informes de Evaluación de Tecnologías Sanitarias.

6. Grosse SD, van Vliet G. How many deaths can be prevented by newborn screening for congenital adrenal hyperplasia? Horm Res. 2007;67:284–9.
7. Rodríguez Arnao MD, Rodríguez Sánchez A, Dulín Iñiguez E. Detección precoz de alteraciones endocrinas. Rev Esp Endocrinol Pediatr. 2013;4 Suppl.:59–71.
8. Dulín Iñiguez E, Espada M, Eguileor Gurtubai I. Programas de Cribado Neonatal. An Pediatr Contin. 2006;4:61–5, http://dx.doi.org/10.1016/S1696-2818(06)73590-9
9. Asociación Española de Cribado Neonatal (AECNE). Datos acumulados de Programas de Cribado Neonatal en España, actualizado a diciembre de 2016. Available from: http://aecne.es/datos.html [accessed 30.09.16].
10. White PC, Speiser PW. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Endocr Rev. 2000;21:245–91.
11. Ezquieta B, Oliver A, Gracia R, Gancedo PG. Analysis of steroid 21-hydroxylase gene mutations in the Spanish population. Hum Genet. 1995;96:198–204.
12. Nordenström A, Thilén A, Hagenfeldt L, Larsson A, Wedell A. Genotyping is a valuable diagnostic complement to neonatal screening for congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency. J Clin Endocrinol Metab. 1999;84:1505–9.
13. Therrell BL. Newborn screening for congenital adrenal hyperplasia. Endocrinol Metab Clin North. 2001;30:15–30.
14. Levy-Shraga Y, Pinhas-Hamiel O. High 17-hydroxyprogesterone level in newborn screening test for congenital adrenal hyperplasia. BMJ Case Rep. 2016, published online 24.02.16.
15. Choi JH, Kim GH, Yoo HW. Recent advances in biochemical and molecular analysis of congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Ann Pediatr Endocrinol Metab. 2016;21:1–6.
16. Honour JW. 17-Hydroxyprogesterone in children, adolescents and adults. Ann Clin Biochem. 2014;51:424–40.
17. Kolahdouz M, Mohammadi Z, Kolahdouz P, Tajamolian M, Khanahmad H. Pitfalls in molecular diagnosis of 21-hydroxylase deficiency in congenital adrenal hyperplasia. Adv Biomed Res. 2015;4:189–98.
18. Santomé Collazo JL, Cirujano Segura A, Ferreiro Fernández B, Casado Fúnez C, Muñoz-Pacheco R, Ezquieta Zubicaray B. [Simple virilizing forms of congenital adrenal hyperplasia: adaptation and prospective validation of the molecular screening]. Med Clin (Barc). 2010;135:195–201.
19. Ezquieta B, Santomé L, Barrio R, Barrionuevo JL, López-Siguero JP, Oliver A, et al. Carrier detection and prenatal diagnosis of congenital adrenal hyperplasia must identify'apparently mild' CYP21A2 alleles which associate neonatal salt-wasting disease. Prenat Diagn. 2010;30:758–63.
20. Ezquieta B, Beneyto M, Muñoz-Pacheco R, Barrio R, Oyarzabal M, Lechuga JL, et al. Gene duplications in 21-hydroxylase deficiency: the importance of accurate molecular diagnosis in carrier detection and prenatal diagnosis. Prenat Diagn. 2006;26:1172–8.
21. Ezquieta B, Oyarzábal M, Jariego CM, Varela JM, Chueca M. A novel frameshift mutation in the first exon of the 21-OH gene found in homozygosity in an apparently nonconsanguineous family. Horm Res. 1999;51:135–41.
22. Soriano Guillén L, Velázquez de Cuellar Paracchi M, Ezquieta B. [Usefulness of molecular analysis in the differential diagnosis of congenital 21-hidroxylase deficiency detected in neonatal screening]. Med Clin (Barc). 2011;136:313–4.
23. Huidobro Fernández B, Echeverria Fernández M, Dulin Iñiguez E, Ezquieta Zubicaray B, Roldán Martín MB, Rodríguez Arnao MD, et al. Neonatal screening for congenital adrenal hyperplasia: transitory elevation of 17-hydroxyprogesterone. J Pediatr Endocrinol Metab. 2011;24:155–62.