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Ambiguidade genital em uma série de casos do ambulatório de distúrbios/diferenças do desenvolvimento do sexo do Hospital Universitário Prof. Alberto Antunes da UFAL – 2008 - 2018

Maceió
2021

REINALDO LUNA DE OMENA FILHO

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Dissertação submetida ao exame de defesa em nível de mestrado do Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal de Alagoas em 10 de setembro de 2021.

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RESUMO

A ambiguidade genital (AG) é um fenótipo inserido no grande grupo dos distúrbios/diferenças do desenvolvimento do sexo (DDS), no qual a atribuição do sexo anatômico do sujeito não é óbvia. Com prevalência de 1:4.500 nascidos vivos e etiologia predominantemente genética, a AG pode ocorrer em pessoas com par cromossômico sexual XX, XY ou com alterações do número ou estrutura destes cromossomos. A partir de 2008, no Hospital Universitário Professor Alberto Antunes da Universidade Federal de Alagoas (HUPAA-UFAL), inicia-se um ambulatório direcionado ao atendimento destes casos, que hoje encontra-se consolidado com uma equipe multidisciplinar de profissionais. O objetivo deste trabalho foi descrever o perfil demográfico e genético-clínico de sujeitos atendidos no período de 2008 a 2018. As informações foram colhidas ao longo dos anos utilizando o mesmo protocolo clínico e compõem um único banco de dados. O cariótipo foi exame universal e a análise dos genes *AR*, *SRD5A2*, *HSD17B3*, *NR5A1* e *CYP21A2* foi realizada conforme a indicação clínica, após o resultado do cariótipo. Neste período foram atendidos 146 indivíduos com DDS, 73 (50%) dos quais com AG, pertencentes a 71 famílias, 21,9% das quais residentes em Maceió. A consanguinidade parental e a recorrência do fenótipo foram observadas em 16,9% e 14,1%, respectivamente. A idade na primeira avaliação especializada foi superior a 90 dias de vida (inadequada) em 56,2% da amostra e 78,1% apresentavam AG moderada a grave. Os defeitos de síntese ou ação de andrógenos (DDS XY) compuseram o maior grupo (50,7%), seguidos pelo grupo de excesso de andrógenos (32,9%) e pelos distúrbios da diferenciação gonadal (DDG) (16,4%). A hiperplasia adrenal congênita foi a nosologia mais prevalente (32,9%). Foram identificadas novas variantes nos genes *HSD17B3, NR5A1* e *CYP21A2*. Entre os casos de DDG destacaram-se um caso de Síndrome de Klinefelter com DDS ovário-testicular e outro com disgenesia gonadal mista e Síndrome de Down, situações raras na literatura. Esta pesquisa descreveu pela primeira vez em Alagoas o perfil demográfico e genético-clínico de sujeitos com AG, cujos resultados corroboram a literatura. A pesquisa também identificou casos raros e novas variantes em genes relacionados à AG que contribuem para o melhor entendimento dos DDS. O conjunto dos resultados fornece subsídios para o planejamento da atenção à saúde a pessoas com AG em Alagoas.

Palavras-chave: distúrbios da diferenciação do sexo; ambiguidade genital; defeitos de síntese ou ação de andrógenos; hiperplasia adrenal congênita; distúrbios da diferenciação gonadal.

ABSTRACT

Genital ambiguity (GA) is a phenotype in which the anatomic sex attribution is not clear and belongs to the wide group of disorders/differences of sex development (DSD). The prevalence is of 1:4,500 live births and its aetiology is predominantly genetic. The GA occurs either in individuals with XX, XY normal sexual chromosomes or among those with numerical or structural sexual chromosomes abnormalities. In 2008, at the University Hospital of the Federal University of Alagoas, the DSD ambulatory was initiated. Nowadays this is a regular service delivered by a multidisciplinary team. The aim of this study was to describe the demographic, genetic, and clinical profile of individuals with GA assisted from 2008 to 2018. Data was gathered using a unique protocol and database. The karyotype was a universal test. The *AR*, *SRD5A2*, *HSD17B3*, *NR5A1*, and *CYP21A2* genes analyses were performed according to clinical indication, after the karyotyping. During this period, 146 individuals with DSD were seen, 73 (50%) of which with GA, belonging to 71 families, 21.9% living in Maceió. Parental consanguinity and phenotype recurrence were observed in 16.9% and 14.1%, respectively. Age at the first specialized assessment was over 90 days of life (inadequate) for 56.2% of the sample, and 78.1% presented moderate to severe AG. Disorders of androgen synthesis or action (DSD XY) were the largest group (50.7%), followed by androgen excess (32.9%) and the disorders of gonadal differentiation (DGD) (16.4%). Congenital adrenal hyperplasia was the most prevalent nosology (32.9%). New variants in *HSD17B3*, *NR5A1*, and *CYP21A2* genes were identified. Among DGD, two rare cases of Klinefelter Syndrome plus ovotesticular DSD, and gonadal mixed dysgenesis plus Down Syndrome were identified. This is the first description of the demographic, genetic, and clinical profile of GA in Alagoas. Overall data corroborate the literature. The study has also identified rare and new aetiologies which may contribute to rise the knowledge on DSD. These results inform the debate on health policies for patients with GA in Alagoas.

Keywords: disorders of sex development; genital ambiguity; androgen synthesis or action disorders; congenital adrenal hyperplasia; disorders of gonadal differentiation.

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LISTA DE ABREVIATURAS E SIGLAS

AG	Ambiguidade Genital
AR	*Androgen Receptor*
CEP	Comitê de Ética em Pesquisa
CYP21A2	*Cytochrome P450, Family 21, Subfamily A, Polypeptide 2*
DDS	Distúrbios/Diferenças do Desenvolvimento do Sexo
DNA	Ácido Desoxirribonucleico
HAC	Hiperplasia Adrenal Congênita
HSD17B3	*17-Beta Hydroxysteroid Dehydrogenase III*
HUPAA	Hospital Universitário Professor Alberto Antunes
MLPA	*Multiplex Ligation-dependent Probe Amplification*
NCBI	*National Center for Biotechnology Information*
NR5A1	*Nuclear Receptor Subfamily 5, Group A, Member 1*
OT	Ovário-Testicular
PCR	Reação em Cadeia da Polimerase
PCR-AS	Reação em Cadeia da Polimerase - Alelo Específico
SRD5A2	*Steroid 5-Alpha-Reductase 2*
TCLE	Termo de Consentimento Livre e Esclarecido
UFAL	Universidade Federal de Alagoas

SUMÁRIO

1. INTRODUÇÃO

A ambiguidade genital (AG) refere-se a um grupo variado de condições clínicas associadas a anormalidades da genitália externa, nas quais a atribuição do sexo não é óbvia (LAINO et al., 2014; THYEN et al., 2006). Apresenta uma prevalência estimada de 1 em cada 4.500 nascidos vivos (HUGHES et al., 2006).

Trata-se de uma situação complexa não só por envolver condições que trazem risco à vida do recém-nascido, como a hiperplasia adrenal congênita (HAC) e quadros malformativos, mas também pelos efeitos psicossociais relacionados à indefinição do sexo. Sua abordagem requer, portanto, ações multidisciplinares iniciadas logo após o nascimento (DE PAULA et al., 2016; HUGHES, 2008).

Existem sistemas para valorar a gravidade da AG como as escalas de Prader (Figura 1), elaborada em 1954 incialmente para os casos de HAC, e de Quigley, apresentada em 1995 para insensibilidade androgênica (Figura 2) (DAMIANI et al., 2001; QUIGLEY et al., 1995). Ambos apresentam graus progressivos de AG, estendendo-se desde a aparência predominantemente feminina (P1/Q5-7) à predominantemente masculina (P5/Q1).

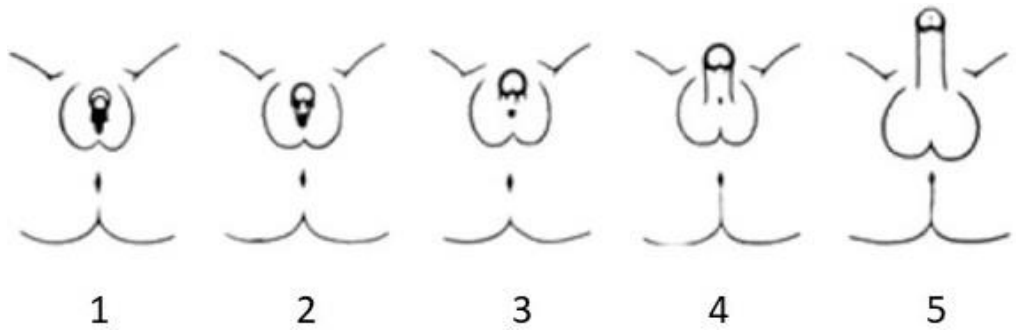

Figura 1. Escala de Prader: 1 – aumento isolado do clitóris; 2 - aumento do clitóris associado a um introito vaginal em forma de funil, podendo visualizar-se aberturas uretral e vaginal distintas; 3 – aumento de clitóris associado a um introito profundo, em forma de funil, com a uretra esvaziando-se na vagina, como um pseudo seio urogenital; 4 - clitóris fálico com abertura urogenital em forma de fenda na base do falo; 5 – fusão lábio-escrotal completa e uretra peniana (figura obtida a partir de https://www.ncbi.nlm.nih.gov/books/NBK278953/).

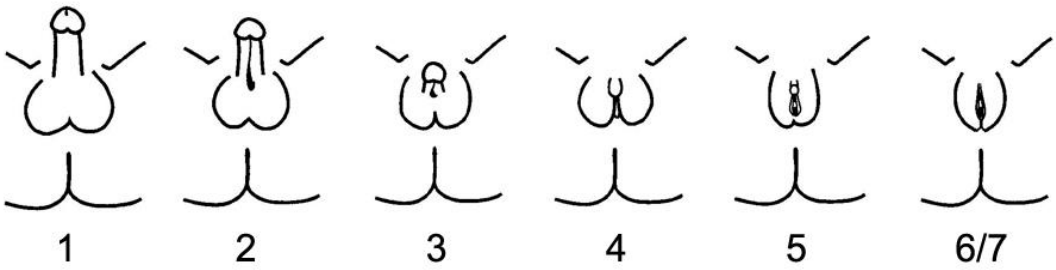

Figura 2. Escala de Quigley: 1 – sujeitos com genitália masculina normal, mas azoospérmicos e com perfil hormonal de resistência androgênica, e também os sujeitos com virilização reduzida na puberdade; 2 – sujeitos com fenótipo masculino, porém com distúrbios leves da masculinização, como hipospadias isoladas; 3 – sujeitos com fenótipo masculino, porém com distúrbios mais graves da masculinização como hipospadia perineal e micropênis com criptorquidia e/ou escroto bífido; 4 – genitália ambígua, com estrutura fálica, geralmente acompanhada por seio urogenital com orifício perineal, saliências labioescrotais e fusão posterior; 5 – fenótipo feminino, inclusive com orifícios uretral e vaginal separados, leve clitoromegalia e/ou pequeno grau de fusão labial posterior; 6 – fenótipo feminino, mas na puberdade há desenvolvimento de pelos pubianos e axilares dependente de andrógeno; 7 – fenótipo feminino, sem desenvolvimento de pelos na puberdade (figura obtida a partir de https://en.wikipedia.org/wiki/Quigley_scale).

A AG tem etiologias variadas cuja definição contribui para a elucidação diagnóstica, definição do sexo de criação, planejamento individualizado do tratamento e do acompanhamento, além de auxiliar à família e/ou sujeito no seu planejamento familiar a partir do aconselhamento genético (AHMED et al., 2016; BAXTER; VILAIN, 2013; LAINO et al., 2014). Ao lado dos aspectos médicos, o conhecimento da etiologia auxilia na compreensão do processo normal da diferenciação do sexo na espécie humana (AHMED et al., 2013; ARBOLEDA; SANDBERG; VILAIN, 2014; BAXTER; VILAIN, 2013; DÉLOT et al., 2017).

Uma primeira abordagem para esclarecer a etiologia é posicionar a AG como um fenótipo do grupo dos Distúrbios/Diferenças do desenvolvimento do Sexo (DDS). Estes são condições congênitas nas quais há discordância entre os achados cromossômicos, gonadais e anatômicos da genitália (HUGHES, 2008; HUGHES et al., 2006; LEE et al., 2016). Os DDS podem ser identificados ao nascimento em casos com AG. Contudo, quando esta não está presente, os DDS podem se expressar na adolescência como desenvolvimento atípico dos caracteres sexuais secundários e mesmo na idade adulta, com infertilidade (AHMED et al., 2016; LEE et al., 2016).

Em 2006, na cidade de Chicago, impulsionados pelo avanço das técnicas de diagnóstico e cirúrgicas e, também, pelo entendimento das questões psicossociais e dos direitos dos pacientes, especialistas em DDS elaboraram o documento que ficou conhecido

como “Consenso de Chicago”. Uma nova classificação de DDS baseada no resultado do cariótipo foi definida naquela reunião, sendo ratificada em 2016 (Quadro 1) (HUGHES, 2008; HUGHES et al., 2006; LEE et al., 2016).

Quadro 1. Classificação dos DDS conforme o cariótipo

DDS		
Anormalidades dos cromossomos sexuais	DDS XY	DDS XX
• Disgenesia gonadal mista – 45,X/46,XY • Síndrome de Turner – 45,X • Síndrome de Klinefelter – 47,XXY • Quimeras – 46,XX/46,XY	• Distúrbios da diferenciação gonadal (testicular) - Disgenesia gonadal completa - Disgenesia gonadal parcial - Regressão gonadal - DDS ovário-testicular • Distúrbios de síntese ou ação de andrógenos - Defeito da biossíntese de andógenos (ex.: deficiência de 17-hidroxiesteroide desidrogenase, deficiência de 5-alfa-redutase tipo 2) - Defeitos na ação do andrógeno (ex.: insensibilidade androgênica) - Defeitos de receptor do LH (ex.: hipoplasia / aplasia das células de Leydig) - Distúrbios do hormônio anti-mülleriano e de seu receptor. • Outros: hipospadias severas, extrofia de cloaca.	• Distúrbios da diferenciação gonadal (ovariano) - DDS ovário-testicular - DDS testicular (ex.: *SRY*+, dup *SOX9*) - Disgenesia gonadal • Excesso de andrógenos - Fetal (ex.: hiperplasia adrenal congênita, deficiência de 11-hidroxilase) - Fetoplacentária (ex.: deficiência de aromatase, POR) - Materna (ex.: luteoma, exógeno, etc.) • Outros: extrofia de cloaca, atresia vaginal, MURCS, outras síndromes

Fonte: (HUGHES, 2008; HUGHES et al., 2006; LEE et al., 2016)

Segundo a classificação apresentada no Quadro 1, no grupo dos DDS com anormalidades dos cromossomos sexuais, os casos de disgenesia gonadal mista (45,X/46,XY) e os casos de quimeras 46,XX/46,XY apresentam AG (HUGHES et al., 2006; HUGHES, 2008).

Os DDS XY, como grupo, apresentam maior número afetados. No entanto, a maioria dos casos permanece sem diagnóstico etiológico e nosológico, mesmo com todo o avanço tecnológico no processo de investigação (DE PAULA et al., 2016; GAZZANEO et al., 2016; LAINO et al., 2014). Como responsáveis por casos de AG, destacam-se os defeitos de síntese ou ação de andrógenos, com a deficiência de 5-alfa-redutase 2 (*SRD5A2*) e a insensibilidade androgênica (*AR*), respectivamente, como principais exemplos, e as disgenesias gonadais parciais que têm mais de 10 genes implicados. Entre estes, está o gene *NR5A1*, cuja primeira variante patogênica foi descrita em 1999. Diversos estudos têm mostrado que este gene também se associa com anorquia bilateral, hipospadia isolada, infertilidade masculina e em alguns casos de tumores adrenais e endometriose, o que o torna um gene de particular interesse (ACHERMANN et al., 1999, 2002; ACIÉN; ACIÉN, 2020; FABBRI et al., 2016; HUGHES, 2008; HUGHES et al., 2006; ONO; HARLEY, 2013).

Já nos casos de DDS XX, destaca-se a HAC como principal responsável por casos de AG. Como entidade nosológica, é a principal causa de DDS, em especial pela deficiência da enzima CYP21A2, codificada pelo gene *CYP21A2* (DE PAULA et al., 2016; GAZZANEO et al., 2016; LAINO et al., 2014). Existem 378 variantes patogênicas descritas para este gene, estando as variantes p.Pro30Leu, c.290-13A/C>G, p.Gly110fs, p.Ile172Asn, Cluster6 (p.Ile236Asn+p.Val237Glu+p.Met239Lys), p.Val281Leu, p.Gln318* e p.Arg356Trp entre as mais comumente encontradas em pacientes com deficiência de CYP21A2 (“HGMD® gene result”, [s.d.]; NEW et al., 2013).

Os casos de DDS ovário-testicular associados à AG encontram-se tanto no grupo DDS XY quanto DDS XX (HUGHES, 2008; HUGHES et al., 2006; LEE et al., 2016). Alguns genes estão relacionados a estes quadros, como por exemplo: *DMRT1*, *RSPO1*, *SOX9*, *SRY*, *WNT4* (KREMEN; CHAN, 2019; ONO; HARLEY, 2013).

Em Alagoas, a atenção à saúde de pessoas com DDS era realizada de forma fragmentada, sem discussão entre os profissionais que prestavam assistência a estes pacientes. Entre os anos de 2007 e 2008 foi desenvolvida a pesquisa “Ambiguidade Genital no Hospital Universitário Professor Alberto Antunes da Universidade Federal de Alagoas (HUPAA – UFAL)”. Esta foi a primeira iniciativa direcionada aos casos de AG / DDS neste hospital (ZANOTTI; DA SILVA XAVIER, 2011).

Como resultado, no ano de 2008 inicia-se o ambulatório específico para sujeitos com DDS no HUPAA – UFAL que, de modo integrado, passa a oferecer atendimento nas áreas de genética e psicologia (ZANOTTI e MONLLEÓ, 2012). A partir de 2016 este

ambulatório passou a contar com equipe multidisciplinar para discussão conjunta dos casos em uma abordagem médica, psicossocial e bioética.

Neste estudo, descreve-se o perfil demográfico e genético-clínico dos sujeitos com AG atendidos no ambulatório DDS do HUPAA-UFAL entre os anos de 2008 e 2018.

2. OBJETIVOS

2.1. Objetivo geral: Descrever o perfil demográfico e genético-clínico de pessoas com AG atendidas no ambulatório de DDS do HUPAA – UFAL de maio de 2008 a dezembro de 2018.

2.2. Objetivos específicos:

2.2.1. Descrever as características demográficas, clínicas e familiais dos participantes com AG.

2.2.2. Investigar os genes *AR*, *SRD5A2*, *NR5A1*, *HSD17B3* e *CYP21A2* e estabelecer correlações genótipo-fenótipo para os casos com variante patogênica identificada.

3. METODOLOGIA

3.1. Delineamento do estudo

Estudo transversal, descritivo, com amostra de conveniência, constituída a partir de 146 sujeitos com DDS participantes de pesquisas realizadas entre maio de 2008 e dezembro de 2018, com os seguintes protocolos de aprovação pelo CEP-UFAL: 010367/2009-29; 19144013.5.0000.5013; CAAE: 59929716.8.0000.5013 e 59931616.6.0000.5013 (Anexos B – E).

Ao longo dos 10 anos de existência do ambulatório de DDS do HUPAA – UFAL, a investigação clínica e etiológica da totalidade dos sujeitos utilizou o mesmo protocolo (Anexo A), característica que contribui para homogeneidade e consistência dos dados coletados. Este protocolo encontra-se arquivado junto com o prontuário do paciente e Termo de Consentimento Livre e Esclarecido (TCLE) no Serviço de Genética Clínica do HUPAA-UFAL. As informações obtidas, assim como atualizações de resultados de testes genéticos, são continuamente inseridas no banco de dados geral de DDS.

Para esta pesquisa, foram selecionados participantes com diagnóstico de AG categorizados de acordo com os critérios de Prader (DAMIANI et al., 2001) e Quigley (QUIGLEY et al., 1995). Com exceção de casos Disgenesia Gonadal Mista, todos os demais quadros sindrômicos foram excluídos. Aplicados estes critérios, a amostra final foi de 73 indivíduos, o que corresponde a 50% do total de casos de DDS.

As seguintes variáveis foram selecionadas:

a) Demográficas:
    - Idade na primeira consulta registrada em dias, meses ou anos de vida. Para a análise da adequação da idade na primeira consulta, tomou-se como base a importância de realizar a avaliação no período da “minipuberdade” (30 a 100 dias de vida) (BIZZARRI; CAPPA, 2020). Assim, a amostra foi reagrupada em duas categorias: (i) idade adequada: criança com até 90 dias de vida na primeira consulta; (ii) idade inadequada: criança acima de 90 dias. Considerou-se o ponto de corte para a idade adequada como sendo até 90 dias para haver tempo hábil para realização dos exames hormonais ainda na janela da “minipuberdade”.
    - Naturalidade, procedência, registro civil e nome social.

b) Clínicas:

- Gravidade da AG conforme as classificações de Prader (P) e Quigley (Q): ambas as escalas eram aplicadas no momento da primeira avaliação. Após o resultado do cariótipo, mantinha-se a classificação de Prader para os casos 46,XX e a de Quigley para os casos com a presença do cromossomo Y ou marcadores de Y. Graduou-se a AG como leve (P1/Q5 e P5/Q1); moderada (P2/Q4 e P4/Q2) e grave (P3/Q3).
- Cariótipo: 46,XX; 46,XY; 45,X/46XY e variações.
- Grupos diagnósticos: distúrbios da síntese ou ação de andrógenos, excesso de andrógenos e distúrbios da diferenciação da gônada.
- Diagnóstico nosológico.

c) Familiais: consanguinidade parental e recorrência. Avaliadas a partir da análise do heredograma obtido durante o atendimento de cada paciente como etapa obrigatória do protocolo clínico. Em todos os casos é composto de, pelo menos, três gerações – Anexo A).

3.2. Ensaios laboratoriais

a) Estudo citogenético: foi realizado cariótipo de linfócitos com bandamento G e resolução 400 bandas (SCHRECK; DISTÈCHE, 2001) no Laboratório de Citogenética Humana da Universidade Estadual de Ciências da Saúde de Alagoas, por único examinador. Em todos os casos foram analisadas no mínimo 40 metáfases.

b) Obtenção de amostras e extração de DNA:

Foram colhidos 10 mL de sangue total em EDTA do participante e seus genitores para obtenção de DNA genômico. Quando possível, também foram colhidas amostras de irmãos. A metodologia de extração de DNA utilizada foi a de lise com Proteinase K, seguida de purificação fenólica, conforme SAMBROOK; FRITSCH; MANIATIS, 1989. Posteriormente foi realizada a amplificação por Reação em Cadeia da Polimerase (PCR) e sequenciamento pelo método Sanger.

c) Estudo molecular:

Para casos sem anormalidades cromossômicas, a análise dos diagnósticos nosológico e etiológico assim como as correlações genótipo-fenótipo foram realizadas após a investigação dos genes *AR* (OMIM * 313700), *SRD5A2* (OMIM

* 607306), *HSD17B3* (OMIM * 605573), *NR5A1* (OMIM * 184757) e *CYP21A2* (OMIM * 613815).

A escolha dos três primeiros genes citados e do *CYP21A2* baseou-se na sua relação com as principais causas de ambiguidade genital no grupo DDS XY e DDS XX, respectivamente. Já o gene *NR5A1* foi selecionado devido ao destaque mais recente pela literatura de sua relação com casos de disgenesias gonadais parciais no DDS XY. Esta investigação foi direcionada de acordo com as suspeitas clínicas, após o resultado do cariótipo.

A investigação dos genes relacionados ao DDS XY foi integralmente realizada no Centro de Biologia Molecular e Engenharia Genética (CBMEG) – Unicamp, por meio de Sequenciamento Sanger. Foram estudados todos os éxons e junções éxon / íntron dos genes *AR* (conforme PETROLI, 2010), *SRD5A2* e *HSD17B3* (CALAIS, 2010) e *NR5A1*, (FABBRI-SCALLET, 2013).

Para a análise do *CYP21A2*, nos sujeitos do grupo DDS XX e diagnóstico de HAC, foram empregados os seguintes métodos:

- PCR alelo específico (PCR – AS) para rastreamento das variantes p.Pro30Leu, c.290-13A/C>G, p.Gly110fs, p.Ile172Asn, Cluster6 (p.Ile236Asn+p.Val237Glu+p.Met239Lys), p.Val281Leu, p.Gln318* e p.Arg356Trp, todas alterações provenientes do pseudogene. Estas análises foram realizadas no Laboratório de Genética Molecular Humana do HUPAA-UFAL.
- Ensaios com enzimas de restrição e o sequenciamento do gene *CYP21A2* foram realizadas no CBMEG – Unicamp, conforme MICHELATTO, 2016, com todos os éxons e íntrons estudados. Estas análises foram realizadas no CBMEG – Unicamp.

As sequências obtidas foram analisadas e comparadas com a sequência normal de cada gene, com o auxílio dos programas Chromas e CLC Sequence Viewer 6.

Para as variantes novas encontradas na amostra, foram realizadas análises preditivas utilizando SIFT, PolyPhen-2, Mutation Taster, DUET, Align GVGD ou SNPs&GO, e a modelagem molecular foi obtida através do MODELLER web server program ou PyMol Viewer.

3.3. Análise estatística

As informações referentes às variáveis de interesse foram extraídas do banco de dados geral do ambulatório DDS do HUPAA-UFAL. Estas foram revisadas, codificadas e

tabuladas em planilha própria, utilizado o programa Microsoft Excel. Os resultados das análises moleculares foram inseridos nesta planilha. Para a análise descritiva, foi empregado o pacote estatístico Epi-Info™.

4. RESULTADOS

No período 2008-2018, 146 sujeitos foram atendidos no ambulatório de DDS do HUPAA-UFAL, 73 (50,0%) dos quais compõem a presente amostra. A distribuição dos casos de DDS ao longo dos anos é apresentada na Figura 3, sendo destacado entre esses os casos com AG. Nota-se que a contribuição relativa dos casos de AG diminuiu na medida em que aumentou o volume de casos no ambulatório, porém se manteve acima de um terço em todos os períodos.

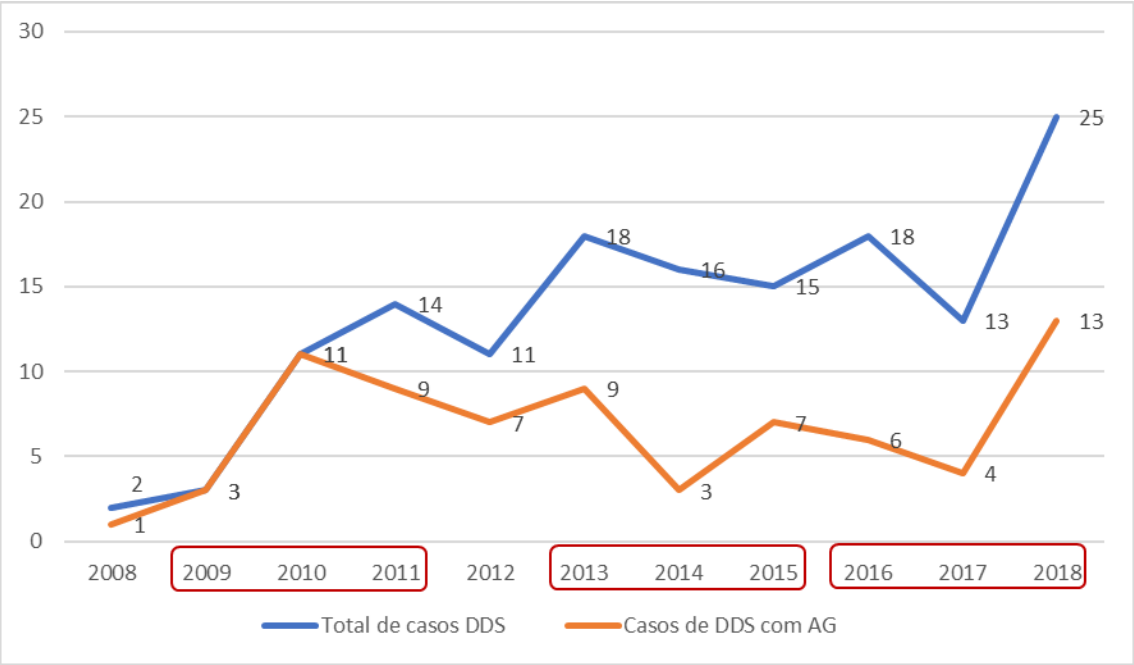

Figura 3. Distribuição do quantitativo de pacientes atendidos no ambulatório DDS do HUPAA entre os anos de 2008 e 2018. Em azul a distribuição do total de casos atendidos nesse período. Em laranja, o quantitativo de DDS com AG. Destacado com os retângulos em vermelho os períodos em que houve financiamento vigente para a pesquisa.

As características demográficas, clínicas e familiais da amostra estão detalhados na Tabela 1. A idade na primeira consulta variou de 6 dias a 34 anos. Um total de 32 (43,8%) sujeitos chegou para a primeira consulta em idade adequada (≤ 90 dias), com média de 30,47, moda de 22 e mediana de 25 dias de vida. Houve predomínio de participantes na faixa etária inadequada (> 90 dias de vida), num total de 41 sujeitos. Apenas dois participantes nasceram em outro estado, mas todos os 73 residiam em Alagoas, sendo 16 (21,9%) na capital, Maceió, e 57 (78,1%) em outros municípios.

Os 73 participantes do estudo pertencem a 71 famílias distintas. A consanguinidade parental foi observada em 12 (16,4%) famílias, nenhuma procedente de Maceió. Houve recorrência de AG em 10 (13,7%) famílias, porém entre estas apenas duas duplas de irmãs (famílias distintas) foram examinadas.

O espectro de manifestações genitais externas foi amplamente variável (Figura 4). Embora AG moderada a grave tenha sido verificada em quase 80% da amostra, os resultados sugerem que não houve associação entre maior gravidade da AG e idade adequada na primeira consulta. Por outro lado, a ausência de registro civil foi maior entre crianças atendidas em idade adequada. Registro civil masculino foi informado 29/73 casos, feminino em 21/73 e em 23/73, ainda não havia registro. A despeito disto, apenas uma criança chegou ao atendimento sem nome social definido. Entre 43/73 casos com nome social masculino, três foram modificados para feminino ao longo do acompanhamento. Entre os 29/73 participantes com nome social feminino, quatro mudaram para masculino.

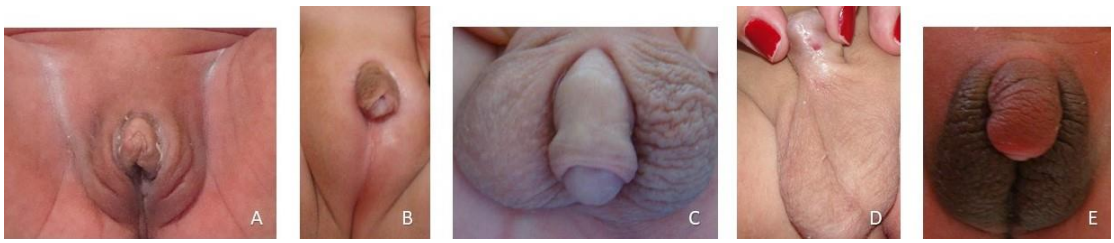

Figura 4. Variabilidade do espectro de manifestações da genitália externa encontradas: (A) Q5/P1, (B) Q4/P2, (C) Q3/P3, (D) Q2/P4 e (E) Q1/P5.

Na amostra como um todo, predominaram casos com aparência genital externa gravemente ambígua (P3/Q3), gônada palpada, ausência de útero, com cariótipo 46,XY, atendidos em idade inadequada.

A Figura 5 apresenta um diagrama com a distribuição dos casos, de acordo com o resultado do cariótipo, grupo diagnóstico, genes estudados e resultados.

Os defeitos de síntese ou ação de andrógenos compuseram o grupo diagnóstico mais frequente (37; 50,7%), seguido pelo excesso de andrógenos (24; 32,9%) que, nesta amostra, correspondeu integralmente à HAC. O terceiro grupo foi representado pelos distúrbios da diferenciação gonadal (12; 16,4%), correspondendo à disgenesia gonadal parcial XY (5/12), três deles com histopatológico da gônada; disgenesia gonadal mista (4/12) – sendo três 45,X/46,XY e um 45,X/47,XY,+21; DDS OT (2/12), um 46,XX e um 46,XX/47,XXY. O caso restante tinha hipogonadismo hipergonadotrófico 46,XY, sem derivados mullerianos ou gônadas detectáveis por exames de imagem. Este pode representar uma

situação rara de regressão testicular que, por limitações técnicas, não pode ser confirmada. O paciente havia sido avaliado aos 14 anos, com criptorquidia bilateral, fusão completa das saliências lábio-escrotais e meato uretral tópico. As gônadas não foram localizadas através da ultrassonografia da região abdominal e inguinal. Foi submetido à videolaparoscopia que não encontrou gônadas bilateralmente. Atualmente com 20 anos, é acompanhado pelo ambulatório de endocrinologia.

Tabela 1. Distribuição dos dados demográficos, clínicos e familiais dos participantes

Variáveis	Idade na 1ª consulta		
	Adequada	Inadequada	Amostra total
	32 (43,8%)	41 (56,2%)	73 (100%)
Naturalidade			
Maceió	18 (56,3%)	21 (51,2%)	39 (53,4%)
Outras cidades	14 (43,7%)	20 (48,8%)	34 (46,6%)
Procedência			
Maceió	3 (9,4%)	13 (31,7%)	16 (21,9%)
Outras cidades	29 (90,6%)	28 (68,3%)	57 (78,1%)
Consanguinidade parental			
Sim	8 (25,0%)	4 (10,3%)	12 (16,9%)
Não	24 (75,0%)	35 (87,4%)	59 (83,1%)
Recorrência de AG			
Sim	4 (12,5%)	6 (15,4%)	10 (14,1%)
Não	28 (87,5%)	33 (84,6%)	61 (85,9%)
Gravidade da AG			
Leve (P1/Q5 e P5/Q1)	8 (25,0%)	8 (19,5%)	16 (21,9%)
Moderada (P2/Q4 e P4/Q2)	9 (28,1%)	17 (41,5%)	26 (35,6%)
Grave (P3/Q3)	15 (46,9%)	16 (39,0%)	31 (42,5%)
Registro civil			
Sim	13 (40,6%)	37 (90,2%)	50 (68,5%)
Não	19 (59,4%)	4 (9,8%)	23 (31,5%)
Gônada			
Palpável	18 (56,2%)	23 (56,1%)	41 (56,2%)
Não palpável	14 (43,8%)	18 (43,9%)	32 (43,8%)

Útero			
Sim	13 (40,6%)	17 (41,5%)	30 (41,1%)
Não	18 (56,3%)	22 (53,6%)	40 (54,8%)
Não avaliado	1 (3,1%)	2 (4,9%)	3 (4,1%)
Cariótipo*			
46,XY	19 (61,3%)	24 (58,5%)	43 (59,7%)
46,XX	12 (38,7%)	12 (29,3%)	24 (33,3%)
Outros**	-	5 (12,2%)	5 (6,9%)
Grupos diagnósticos			
Distúrbio de síntese ou ação de andrógeno	16 (50,0%)	21 (51,2%)	37 (50,7%)
Excesso de andrógenos	12 (37,5%)	12 (29,3%)	24 (32,9%)
Distúrbio da diferenciação gonadal	4 (12,5%)	8 (19,5%)	12 (16,4%)

AG: ambiguidade genital. P: escala de Prader. Q: escala de Quigley. *Um lactente foi a óbito antes da realização da coleta. ** Três casos: 45,X/46,XY, um caso: 45,X/47,XY,+21, um caso: 46,XX/47,XXY.

Entre os casos sem anormalidades do par cromossômico sexual (n = 67), 66 (98,5%) foram submetidos a análises moleculares conforme a suspeita clínica. O caso restante, com diagnóstico de DDS OT XX, não está associado a alterações nos genes incluídos neste estudo.

Genes *AR*, *SRD5A2* e *HSD17B3:* defeitos de síntese ou ação dos andrógenos (n = 37)

Por questões epidemiológicas e de custos, optou-se por iniciar a investigação deste grupo pelo sequenciamento dos genes *AR* e *SRD5A2*. A Figura 5 apresenta um sumário dos experimentos realizados.

Foram identificados 2/21 (9,5%) casos com variantes patogênicas envolvendo os genes *AR* e *SRD5A2*. O gene *HSD17B3* foi sequenciado em apenas dois casos, tendo sido encontrada variante patogênica em ambos (Figura 6). A variante p.Gli262Val é nova (em processo de validação no *National Center for Biotechnology Information* – NCBI). As análises preditivas foram compatíveis com uma variante deletéria do tipo "sentido trocado" e indicaram que a valina 262 desestabiliza a estrutura da proteína (maiores detalhes no artigo apresentado no Capítulo 6).

Gene *NR5A1*: disgenesia gonadal (n = 6)

Foi encontrada uma variante patogênica em heterozigose em um participante e uma heterozigose composta (variante de significado incerto/polimorfismo) em outro (Figura 5). Esta última requer estudos funcionais.

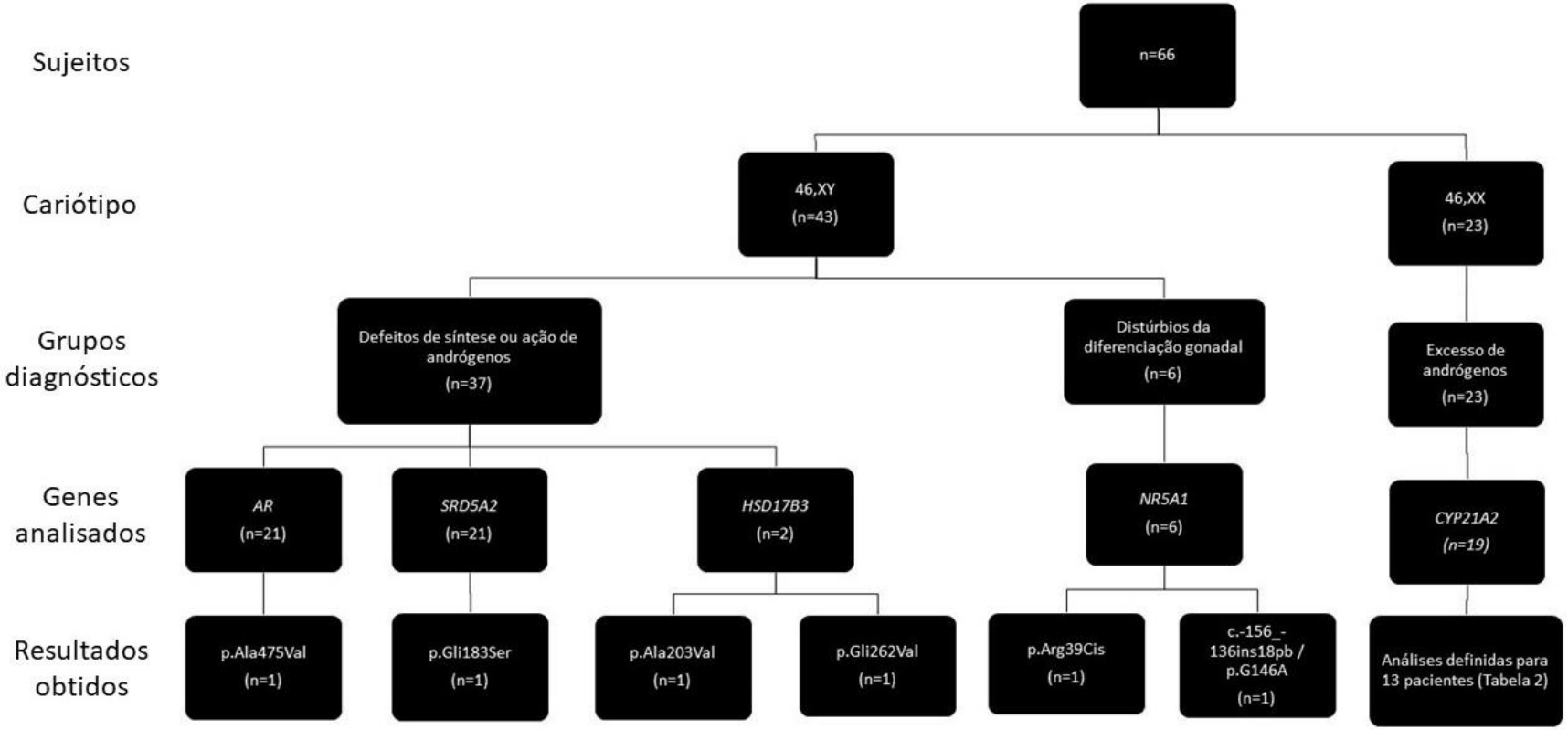

Figura 5. Distribuição dos participantes conforme o cariótipo e os grupos diagnósticos, com as variantes encontradas nos genes *AR*, *SRD5A2*, *HSD17B3*, *NR5A1* e *CYP21A2*.

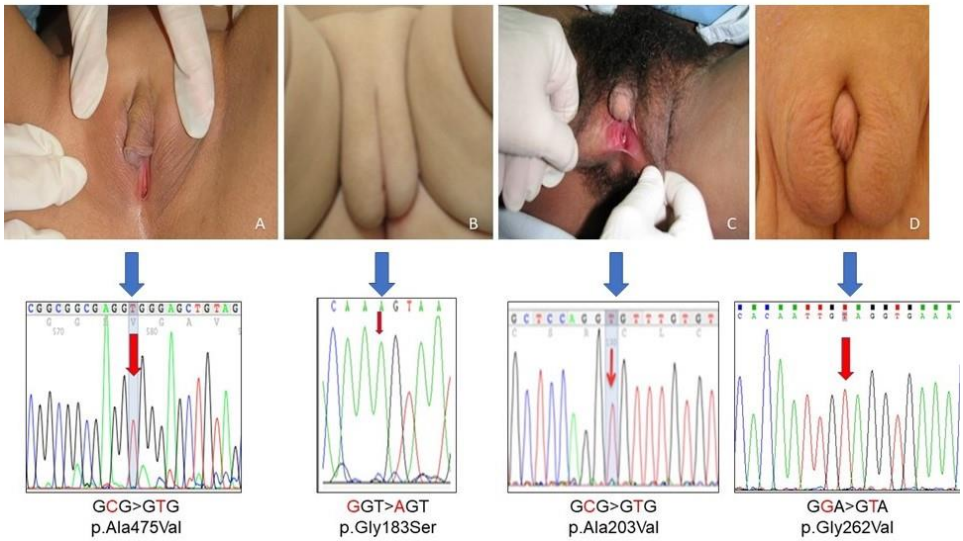

Figura 6. Genitália dos pacientes com defeitos de síntese ou ação de andrógenos com variantes encontradas nos genes: (A) *AR*, (B) *SRD5A2*, (C) e (D) *HSD17B3* e seus respectivos eletroferogramas.

Gene *CYP21A2:* Hiperplasia Adrenal Congênita (n = 24)

Neste grupo, predominaram casos com ambiguidade genital grave, com fenótipo perdedor de sal. Entre 19 participantes (79,2%) submetidos à análise molecular do gene *CYP21A2,* 13 (68,4%) apresentaram variantes patogênicas, sendo 12 já descritas e uma nova. Entre as variantes já descritas, quatro estão entre as mais comumente relatadas e uma, a variante p.Ser170Lysfs*124, é rara. A variante nova, p.Ser301Pro, está embargada no NCBI – (SNP ID NG_007941:g.1748T>C), submitted SNP(ss) Details: ss2137544212 (RefSNP: clustering in process). As análises preditivas indicaram tratar-se de uma variante danosa do tipo "sentido trocado" e que a prolina 301 modifica as interações entre os aminoácidos da proteína (discussão detalhada no artigo apresentado no Capítulo 7). A correlação genótipo-fenótipo foi incoerente em 1/12 (caso 224), e a frequência de heterozigotos compostos foi de 3/13 (23,1%) (casos 25, 75 e 81) (Tabela 2).

Os seis casos (6/19) restantes (casos 29, 79, 131, 155, 169 e 230) necessitam de complementação das análises.

a) Casos 131, 155, 169 e 230 têm análise através da PCR-AS sem variantes patogênicas detectadas.
b) Caso 29 com probando falecido antes da realização das análises moleculares. Optou-se pelo sequenciamento do *CYP21A2* dos genitores e de um irmão sem diagnóstico de HAC. O pai e o irmão são heterozigotos, porém não foram encontradas variantes patogênicas na genitora.

c) Caso 79 com apenas variante patogênica identificada por sequenciamento no probando. A genitora é heterozigota e nenhuma variante patogênica foi encontrada no genitor.

Tabela 2. Variantes encontradas no gene *CYP21A2*.

Código do paciente	Variantes encontradas	Correlação genótipo-fenótipo		Consanguinidade	Recorrência	Frequência populacional		
		PS	VS			Comum	Rara	Nova
20	c.290-13A/C>G	X			X	X		
25	p.Gln318* p.Arg356Trp c.290-13A/C>G	X		X	X	X		
30	p.Ser170Lysfs*124	X		X	X		X	
37	p.Ile172Asn		X		X	X		
41	c.290-13A/C>G	X		X	X	X		
75	p.Gln318 p.Arg356Trp c.290-13A/C>G	X		X	X	X		
81	p.Gln318+p.Arg356Trp/p.Arg356Trp	X				X		
147	p.Ser301Pro	X		X				X
158	p.Ser170Lysfs*124	X		X			X	
161	p.Gln318*	X				X		
170	c.290-13A/C>G	X		X		X		
214	p.Ile172Asn		X			X		
224	c.290-13A/C>G		X			X		

Pacientes 25 e 75 são irmãs. PS: perdedor de sal; VS: virilizante simples.

5. DISCUSSÃO

O atendimento sistematizado a pessoas com DDS em Alagoas, iniciado em 2008, se consolidou ao longo dos anos como atividade de assistência, ensino e pesquisa em um ambulatório específico, implantado no HUPAA, unidade referência na rede SUS do estado. Como se observa na Figura 1, os períodos de maior volume de atendimentos coincidem com a vigência de pesquisas financiadas pelo Programa de Pesquisas para o SUS, o que ressalta o papel indutor do Ministério da Saúde na produção de conhecimento aplicado ao SUS (BRASIL. MINISTÉRIO DA SAÚDE. DECIT., 2006a, 2006b; SOUZA; CALABRÓ, 2017).

Além do aspecto do financiamento, destaca-se 2016-2018 como o período de maior volume de pacientes, provavelmente devido à maior difusão da existência do ambulatório DDS e à sua configuração multidisciplinar que passou a contar com profissionais não só da psicologia e genética médica, mas da endocrinologia pediátrica, cirurgia pediátrica, patologia, enfermagem, biologia, biomedicina e direito, e de acadêmicos da psicologia, biologia e medicina. Esta configuração pode ter sido responsável pela diminuição relativa da frequência de AG, resultado semelhante ao encontrado por um grupo especializado, referência em DDS no Brasil (BECK et al., 2020). O *modus operandi* deste grupo multidisciplinar é descrito no Apêndice A sob forma de artigo.

Com relação ao início da avaliação especializada, nesta pesquisa 56,2% dos sujeitos chegaram para primeira consulta em idade inadequada. Alguns aspectos subjetivos individuais, características sociodemográficas e estruturais do sistema de saúde devem ser considerados na análise deste resultado.

O impacto psicossocial da indefinição de sexo nas AG geralmente leva as famílias a buscar atendimento o mais precocemente possível (DE PAULA et al., 2016; HUGHES, 2008). Contudo, medo e ansiedade são situações de destaque entre os pais de um RN com AG. A negação e fuga são formas comuns de enfrentar essas situações (SILVA et al., 2006). Aventa-se a possibilidade de que, em alguns casos, a dificuldade em lidar com o diagnóstico tenha contribuído para o atraso da primeira avaliação em idade adequada.

Em Guerra-Júnior e Guerra, 2007 e Beck et al., 2020 é enfatizada, também, a importância do reconhecimento e valorização dos sinais de AG pelos profissionais que avaliam rotineiramente a criança na maternidade ou na atenção primária à saúde. Neste sentido, a necessidade de educação permanente paras pediatras / neonatologistas em relação à AG é outro aspecto crítico. Subdiagnóstico de sinais sugestivos de AG foi

verificado em estudo deste grupo com neonatos de duas maternidades de referência de Alagoas (MONLLEÓ et al., 2012) e pode estar implicado no atraso para realizar a avaliação em idade adequada.

Outro fator a ser considerado é a possível dificuldade de acesso ao ambulatório DDS do HUPAA que, embora seja de fato a referência em Alagoas para estas situações e venha se tornando cada vez mais conhecido, ainda não está incorporado na estrutura do SUS e na rotina dos profissionais das maternidades e da atenção primária à saúde no Estado.

Como o ambulatório DDS está situado na capital, era de se esperar que este fator favorecesse o acesso em idade adequada aos domiciliados em Maceió, o que não foi observado. Este resultado reforça a existência de falhas na articulação entre as redes de atenção primária e secundária com a rede terciária, o que é reconhecidamente um problema do SUS em diferentes áreas da atenção especializada à saúde (CASTRO et al., 2019).

A consanguinidade e a recorrência de distúrbio congênito são sinais de alerta para condições de etiologia genética (BITTLES; BLACK, 2010; CARDOSO et al., 2019; WELLER et al., 2012). A presença destes fatores deveria favorecer o encaminhamento precoce dos pacientes com AG ao serviço especializado. Os resultados obtidos neste estudo indicam que a associação entre AG e consanguinidade parental favoreceram a chegada dos sujeitos em idade adequada para primeira avaliação. No entanto, isto não foi o observado em relação à recorrência. É necessário reforçar a importância da associação entre fatores que elevam o risco de distúrbio genético e a condição apresentada, no caso AG, junto aos profissionais de saúde que atuam nas maternidades e na atenção primária.

A maior gravidade da AG também não parece estar associada com a abreviação do tempo entre o nascimento e avaliação genética da criança, uma vez que entre os 31/73 (42,5%) com AG grave, 16 (51,6%) chegaram em idade inadequada. Por outro lado, a ausência de registro civil prevaleceu no grupo de crianças com atendimento em idade adequada. Provavelmente este resultado se deve à importância do registro civil como documento básico do cidadão para ter acesso a direitos no Brasil, já que para realizar o registro civil é necessária a informação do sexo do recém-nascido que consta na Declaração de Nascido Vivo. Nos casos em que o sexo é registrado como ignorado, na maioria dos estados brasileiros, não é possível realizar o registro até que se tenha a definição do sexo.

Exceto no grupo diagnóstico de distúrbios da diferenciação gonadal, em que predominaram casos com ambiguidade genital moderada, tanto nos casos de distúrbios de síntese ou ação de andrógenos, como no grupo de excesso de andrógenos, predominaram as

situações com ambiguidade genital grave. Em face desta sobreposição clínica, apenas a avaliação através do exame físico não é suficiente para definição diagnóstica, nem mesmo para identificação do grupo diagnóstico. A AG é um fenótipo em que é fundamental a associação da avaliação clínica com os resultados do cariótipo, exames hormonais e a análise molecular para alcançar o diagnóstico nosológico (LAINO et al., 2014). Nesta amostra a caracterização hormonal ficou prejudicada porque a maioria dos sujeitos chegou em idade fora da "minipuberdade" e não havia recursos para realização de testes de estímulo.

Em relação à distribuição dos participantes conforme o cariótipo, a amostra seguiu o esperado pela literatura, onde o maior número de indivíduos com AG apresenta cariótipo 46,XY e está relacionado com defeitos de síntese ou ação de andrógenos. Assim como em outros estudos, chama a atenção o grande número de indivíduos sem diagnóstico nosológico neste grupo, mesmo após a análise molecular dos principais genes envolvidos (DE PAULA et al., 2016; GAZZANEO et al., 2016; LAINO et al., 2014; MURPHY; ALLEN; JAMIESON, 2011).

Nesta amostra, os diagnósticos concluídos compreenderam um caso de síndrome da insensibilidade parcial aos andrógenos (*AR*), um de deficiência de SRD5A2 (*SRD5A2*), dois de deficiência de HSD17B3 (*HSD17B3*) e dois de disgenesia gonadal parcial (*NR5A1*) (Figura 5). Destaca-se que entre os seis casos concluídos, dois apresentam variantes novas, c.115C>T e c.785G>T que correspondem, respectivamente, à disgenesia gonadal parcial relacionada ao gene *NR5A1* e à deficiência de HSD17B3, relacionada ao gene *HSD17B3*.

Alterações do gene *NR5A1* são observadas em pacientes com disgenesia gonadal parcial 46,XY (GOMES et al., 2018). A variante de sentido trocado c.115C>T, leva à substituição da arginina por uma cisteína no resíduo 39 da SF-1 (p.Arg39Cis). Estudos funcionais demostraram que a variante p.Arg39Cis tem efeito patogênico uma vez que promove uma redução importante na capacidade de transativação de promotores, além de reduzir a capacidade de reconhecimento e ligação ao DNA, uma vez que essa alteração se localiza no domínio de ligação ao DNA da proteína SF-1 (FABBRI-SCALLET et al., 2018). A criança, com idade de um mês na primeira consulta, tinha AG moderada (Quigley 4) estava sendo criada como menina, teve sexo social modificado para masculino após o diagnóstico.

A descrição detalhada do caso e da variante nova identificada no gene *HSD17B3* será apresentada no Capítulo 6.

No grupo DDS 46,XX, conforme esperado, a HAC foi a nosologia mais frequente (DE PAULA et al., 2016; GAZZANEO et al., 2016; HUGHES, 2008) e, nesta amostra, representou quase a totalidade dos participantes deste grupo diagnóstico.

A HAC devida a alterações do gene *CYP21A2* é uma condição incluída nos testes de triagem neonatal devido à elevada letalidade, tratamento e prevalência de 1:10.000 a 1:20.000 (SPEISER et al., 2010). Nesta condição clínica, são reportadas frequências de heterozigotos tão altas quanto 1:55 e 1:70 (GUARNOTTA et al., 2020; NORDENSTRÖM et al., 2019). Assim, espera-se que a maioria dos casos resulte de heterozigose composta (GUARNOTTA et al., 2020; MERKE; AUCHUS, 2020; NORDENSTRÖM et al., 2019), sendo a homozigose mais comum em casos de uniões consanguíneas (NEW et al., 2013).

No presente estudo, apenas três sujeitos eram heterozigotos compostos (casos 25, 75 e 81), o que difere da literatura. Curiosamente, nos casos 25 e 75, o genótipo foi heterozigoto a despeito da existência de consanguinidade parental. Estes resultados podem refletir o pequeno tamanho amostral com estudo molecular completo do gene *CYP21A2*.

Entre os 10 casos homozigotos, cinco (casos 30, 41, 147, 158 e 170) apresentavam consanguinidade.

Os casos 30 e 158 têm a variante c.508_509insA classificada como rara. A inserção de uma adenina modifica o quadro de leitura e introduz um códon de parada prematuro, promovendo a troca de uma serina por uma lisina no resíduo 170 (p.Ser170Lysfs*124) da CYP21A2, deixando a proteína truncada. Está associada principalmente ao fenótipo perdedor de sal (ZENG et al., 2004). As duas crianças deste estudo não relacionadas e têm genitores oriundos de municípios distantes entre si.

No caso 147 foi identificada a variante nova p.Ser301Pro em homozigose. A descrição completa do estudo é apresentada no Capítulo 7.

Apenas no caso 224 a correlação genótipo-fenótipo divergiu do esperado para a maioria dos casos. A variante c.290-13A/C>G está relacionada principalmente ao fenótipo perdedor de sal da HAC. Por ser uma variante que leva à alteração no sítio de *splicing*, há a possibilidade da formação de *splicing* alternativos permitindo a produção da enzima CYP21A2 com alguma atividade residual (WITCHEL et al., 1996). A hipótese é de que esta atividade enzimática tenha sido suficiente para evitar o fenótipo perdedor de sal neste caso.

À parte os casos de HAC, no grupo 46,XX existe um caso de DDS OT sem marcadores de Y (DYZ1, DYZ3, SRY e ZFY, pesquisados através da PCR). Trata-se de condição rara, com incidência estimada em menos de 1:20.000 (“Ovotesticular Disorder of

Sex Development - NORD (National Organization for Rare Disorders)", [s.d.]). A criança atendida aos 12 dias de vida, tinha sexo social feminino, AG grave (Prader 3), gônada palpável em região inguinal esquerda e heterocromia de íris. A biópsia gonadal foi compatível com tecido ovariano à direita e ovotestes à esquerda (Figura 7) (GAZZANEO et al., 2013 – Apêndice B).

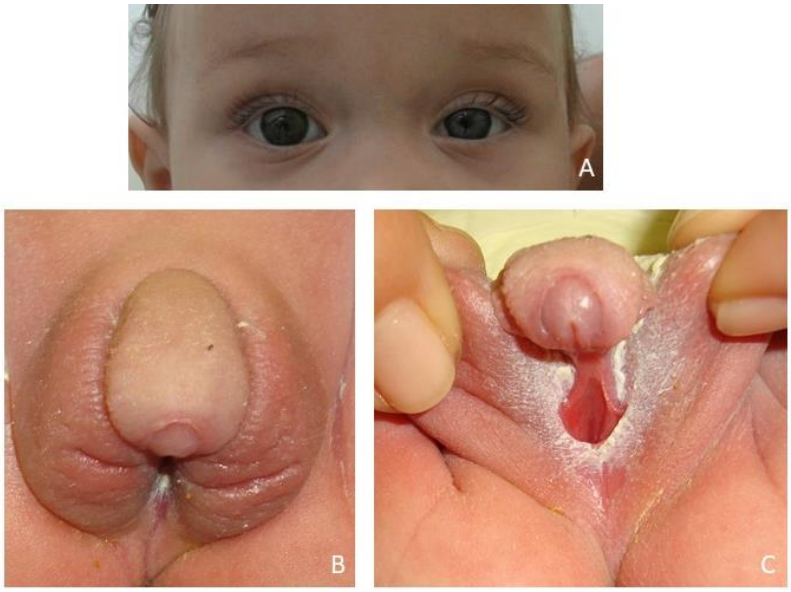

Figura 7. Em A observa-se a heterocromia de íris. Em B e C a genitália ambígua da paciente com DDS OT e cariótipo 46,XX.

No grupo diagnóstico dos DDS com alterações dos cromossomos sexuais houve um participante com cariótipo 46,XX/47,XXY (Síndrome de Klinefelter e DDS OT). Os casos de DDS OT apresentam mais frequentemente o cariótipo 46,XX. A criança foi atendida aos 10 anos de idade e já havia sido submetida a quatro genitoplastias masculinizantes. Naquele momento, apresentava hipospadia glandar, gônada palpável à esquerda (Quigley 2) e não havia derivados mullerianos. A biópsia gonadal evidenciou tecido ovariano com cistos foliculares e tuba uterina congesta à direita e tecido testicular atrófico à esquerda (Figura 8) (OMENA FILHO et al., 2021 – Apêndice C). Este caso, configura uma situação extremamente rara, com 19 relatos na literatura, até o momento (TANGSHEWINSIRIKUL et al., 2020).

Figura 8. Paciente com Síndrome de Klinefelter e DDS OT (46,XX/47,XXY) em A, onde observam-se os cúbitos valgos e a cintura ginecoide. Em B, a genitália do paciente (após quatro genitoplastias), com a presença de gônada à esquerda

Entre os quatro casos que apresentam disgenesia gonadal mista, um também tem Síndrome de Down. Trata-se de uma criança atendida com 1 ano e 4 meses de idade, com ambiguidade genital moderada (Quigley 2), com gônada palpada no escroto à direita. Seu cariótipo foi 45,X/47,XY,+21 (Figura 9).

A associação de Síndrome de Down e DDS também é rara e está mais frequentemente relacionada ao grupo de DDS com anormalidades dos cromossomos sexuais. Há menos de 200 relatos desta associação, 12 (6%) dos quais são situações em que o paciente também apresenta disgenesia gonadal mista (SANTOS-NETO et al., 2021) como se observa no caso aqui descrito.

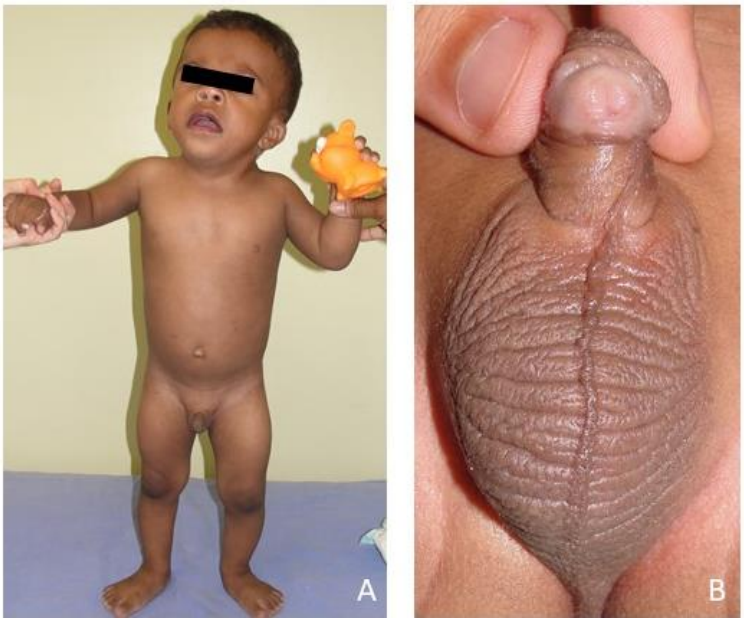

Figura 9. Paciente com Síndrome de Down e Disgenesia Gonadal Mista (45,X/47,XY,+21) em A. Em B a genitália do paciente, com hipospadia glandar e gônada palpada à direita.

6. ARTIGO 1

Title: So, and if it is not congenital adrenal hyperplasia? Addressing an undiagnosed case of genital ambiguity: case report

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Abstract

The Congenital Adrenal Hyperplasia due to 21 hydroxylase deficiency is the most common cause of genital ambiguity in persons with XX sexual chromosomes. Genital ambiguity among persons with XY sexual chromosomes comprises diverse and rare etiologies. The deficiency of 17-beta-hydroxysteroid dehydrogenase type 3 enzyme (HSD17B3) is a rare autosomal recessive disorder due to functionally altered variants of the *HSD17B3* gene. In this disorder/difference of sex development, the conversion of androstenedione into testosterone is impaired. The appearance of external genitalia of 46,XY individuals varies from typically male to almost female. We report on a child presenting severe ambiguous genitalia. Due to access constraints, specialized care did not start until the child was 10 months old. Parents are consanguineous and were born in an area of high isonymy that is a cluster for rare recessive diseases. A new homozygous missense variant c.785G>T was found in exon 10 of the *HSD17B3* gene. Researchers-clinicians and researchers-researchers collaborative efforts to elucidate the genetic basis of this disease were critical since this etiologic investigation is not available through the public health system. This case exemplifies the families' pilgrimage in cases of genital ambiguity due to a rare genetic condition. Recognizing the etiology was the baseline to provide information on prognosis and treatment options, and to shelter family and child doubts and hopes in order to better support their decisions.

Keywords: rare disease; ambiguous genitalia; HSD17B3 deficiency; novel variant; case report.

1. Background

Rare diseases are always challenging for patients, their families and health professionals. When it comes to those that affect sexual development/differentiation, patients face other still complex vulnerabilities such as seeing himself/herself as different

from the others, being targets of bullying in school age, and finding difficulties in understanding the situation [1].

The Congenital Adrenal Hyperplasia due to 21 hydroxylase deficiency is the most common cause of genital ambiguity in persons with XX sexual chromosomes. Genital ambiguity among persons with XY sexual chromosomes comprises diverse and rare etiologies [2].

The deficiency of 17β-hydroxysteroid dehydrogenase type 3 (HSD17B3; OMIM # 264300) is a good example. This 46,XY disorder/difference of sex development (DSD) is due to disruption of *HSD17B3* gene (*OMIM* * 605573) that impairs the conversion of androstenedione into testosterone mediated by the HSD17B3 enzyme. Inherited as an autosomal recessive condition, it shows a wide prevalence variation, ranging from 1:100-300 in Gaza Strip, where consanguineous marriages are frequent, to 1:147,000 in the Netherlands [3–7].

In cases of HSD17B3 deficiency, the external genitalia may range from male to almost female appearance including several degrees of ambiguity. Testes usually are in the inguinal canal or in the labioscrotal folds. Wolffian derivates are present, suggesting that low testosterone concentration is sufficient for internal male genitalia differentiation [5–8].

The recognition of genital ambiguity facilitates early diagnosis in childhood. On the other hand, individuals presenting minor genital changes and reared as girls may not be diagnosed until adolescence or adulthood. At this stage, primary amenorrhea and virilization of external genitalia may arise due to peripheral testosterone conversion [6,9–11]. At any stage of life, this is a stressful situation that should be addressed carefully by an experienced multidisciplinary team in a patient-centered manner.

2. Case report

A 10-month-old child, assigned as female at birth, resident in the countryside of the Northeast region of Brazil, was referred to our team for genetic assessment due to genital ambiguity. Pregnancy and delivery were uneventful. Parents reported a clitoris enlargement and gonadal descent when the child was 30 days old. Afterward, they decided to rear him as a boy despite the maintenance of female legal sex. The patient is the second child in a consanguineous marriage (double first cousins once removed), with no familial recurrence of the disorder in the family (Figure 1).

Upon physical examination the genital ambiguity was Quigley's type 4 [12]. There was a phallus measuring 25 mm x 10 mm, palpable gonads at labioscrotal folds (1 cm^3 left-sided and 0.5 cm^3 right-sided), and an urogenital sinus with a perineal opening (Figure 2). The karyotype of peripheral lymphocytes was 46,XY. Basal FSH and LH levels were normal while those for testosterone were low. Abdominal ultrasound showed no Mullerian derivates.

The etiologic investigation included androgen receptor (*RA* – OMIM * 313700), steroid 5α-reductase 2 (*SRD5A2* – OMIM * 607306), nuclear receptor subfamily 5 group A member 1 (*NR5A1*– OMIM * 184757), and *HSD17B3* genes sequencing, and predictive analyzes (Additional file 1).

Sequencing studies showed a novel homozygous c.785G>T nucleotide change in exon 10 of the *HSD17B3* gene (Additional file 2), which was inherited from both heterozygous parents. The c.785G>T substitution leads to the replacement of glycine by valine in residue 262 (p.Gly262Val).

Predictive analyzes were compatible with a damaging missense variant and indicated that valine 262 destabilizes the protein structure. Glycine 262 is a highly conserved residue

(Additional file 3) and the comparison between them did not reveal changes on the protein internal contacts (Additional file 4).

3. Discussion and conclusion

3.1. Clinical issues that impact on the family

The main cause of genital ambiguity is congenital adrenal hyperplasia, which can be lethal in the neonatal period if not diagnosed and timely treated. In this sense, neonatal screening represents a significant improvement in health care [2,13].

On the other hand, genital ambiguity due to non-life-threatening etiologies is also an urgent situation because of the devastating impact it can have on the family and throughout the life of the person with this clinical condition. Although these children may be recognized at birth, they are not usually diagnosed promptly, either because of the lack of knowledge of health professionals or the unavailability of genetic tests. The last may comprise diverse and complex molecular methods from peripheral karyotype to exome sequencing [14–19]. For many persons living in middle and low income countries, a simple karyotype may be unreachable.

The 46, XY DSD group is particularly challenging since different diseases share the same clinical features. This is the case of HSD17B3 deficiency that overlaps with other conditions that affect both the androgens synthesis or action. In the clinical setting, it is not always easy to establish a specific diagnosis since ambiguous genitalia with no Mullerian structures is a common feature in DSD, and basal hormonal probes are age-dependent. In cases of HSD17B3 deficiency, a testosterone/androstenedione ratio lower than 0.8 has shown a 100% diagnosis sensitivity in children up to six months old, illustrating the importance of early diagnosis. In any case, the molecular analysis of sex-related developmental genes is a critical tool not only for diagnostic purposes but also for genetic counseling [6,9–11].

It is noteworthy that although parents had noticed the genital ambiguity when the child was 30 days old, our patient was ten months old at first genetic assessment. The time elapsed illustrates the difficulty of accessing specialized care through the Brazilian Unified Health System (SUS). Some of the reasons that may explain this situation are practitioners' lack of knowledge on rare diseases, incoordination between the different levels of the SUS, shortfall of geneticists, and economical constraints [14].

The SUS assists around 80% of the Brazilian population which is an important achievement considering Brazil's extension and complexity. Significant improvement in the care of persons living with genetic disorders was gained since the implementation of the national policy on rare diseases in 2014. Currently, the country has 17 rare diseases enabled services randomly distributed in the national territory. Furthermore, the unavailability of genetic tests, including those for DSD, remains a bottleneck [15–18].

Our team is a multidisciplinary and voluntary group that provides open door care to DSD patients at the University Hospital from the Federal University of Alagoas. The genetic investigation is performed as a research protocol mainly in partnership with colleagues from the State University of Campinas. This collaboration eventually allowed us to investigate DSD etiology in this family.

Three years have elapsed from the first consultation with us to the conclusion of genetic tests due to personnel and financial constraints. Meanwhile, the family gave up the follow up restarting it when the child was six years old. Although they continue rearing the child as a boy, they have not decided on treatment and legal sex yet, which remains as female. Currently, they are engaged with our team psychologist.

3.2. Molecular and genetic studies provision

The variant c.785G>T herein described causes the replacement of glycine by valine in the residue 262 of HSD17B3 as checked against The Human Gene Mutation Database [20],

ClinVar [21], Genome Aggregation Database [22], and Brazilian Genomic Variants [23]. As far as we know, this variant has never been reported before.

Glycine 262 is a conserved residue. Its side chains contain hydrogen, which provides conformational flexibility, while valine is a Cβ branched and hydrophobic amino acid with less conformational flexibility [24]. Therefore, the variant valine 262 can affect the protein due to the amino acid structure. The alignment between the wild-type and the variant protein (Additional file 4) shows that the amino acid change could affect the protein structure, although the internal contacts have not been affected.

Upon these predictive analyzes, we hypothesize that the p.Gly262Val variant has led to a decrease in the HSD17B3 activity. As a consequence, testosterone synthesis was lowered to an insufficient rate to ensure our patients' complete genital masculinization. Thus, we suggest the p.Gly262Val variant is pathogenic, although *in vitro* protein function studies should be carried out in order to validate these data.

The heterozygosis of this variant was found in both parents, who are double first cousins once removed as shown in Figure 1. Consanguineous unions (those between persons with a common ancestor) and endogamy (union between persons belonging to the same community or social/ethnic group) are well-established risk factors for rare genetic conditions. These relationships have been studied for years in Brazil, with evidence of a significant impact in the Northeast region. By the end of 1990, the analysis of shared surnames (isonymy) arose as a powerful method to investigate migration, miscegenation, and isolation. Such population behaviors may be additional factors favoring the occurrence of rare diseases [25–29].

Our patient's parents, as well as those of another case of HSD17B3 deficiency reported by the authors a few years ago [30], are consanguineous. Both families come from an area with the highest rate of isonymy in Brazil [29].

The case presented here exemplifies how challenging the care of persons with rare DSD can be. From the patients' perspective the first vulnerability lies in the search for the correct and early diagnosis, which may not be reached before a pilgrimage through specialists and health services, the amount of analysis and costs of all kinds - time, emotional, and why not, financial [31,32].

Additionally, patients and families need accurate information on diagnosis and prognosis to make complex decisions on rearing sex, and clinical and surgical treatment [33–35]. These challenges are even higher in countries with abyssal inequities, such as Brazil, where patients' pilgrimage is usually arduous.

In this case, the diagnosis of HSD17B3 deficiency was reached when the child was four-years-old as a result of a collaborative effort of researchers involved with DSD investigations. Nonetheless, the time elapsed has left its mark. The child is already six and is being reared as a boy, however, he is beginning to perceive differences between himself and other children. This situation is making the family's suffering a continuous cycle and should be put on the table when discussing the wide impacts of undiagnosed rare diseases.

From a genetic viewpoint, the novel homozygous variant c.785G>T of the *HSD17B3* gene widen the molecular knowledge on this rare 46,XY DSD. Family pedigree alongside data on consanguinity and isonymy in Brazil corroborates the importance of our region as a cluster for autosomal recessive diseases in the country.

List of abbreviations

DSD: disorder/difference of sex development

HSD17B3: 17-beta-hydroxysteroid dehydrogenase type 3

NR5A1: nuclear receptor subfamily 5 group A member 1

RA: androgen receptor

SRD5A2: steroid 5α-reductase 2

SUS: Brazilian Unified Health System

Declarations

Ethics approval and consent to participate

Participant's mother provided written informed consent and the father's consent was verbal. The informed consent approved by the Research Ethics Committee of the Federal University of Alagoas (# 19144013.5.0000.5013 - Comitê de Ética em Pesquisa of the Universidade Federal de Alagoas) states that one of the aims of the study is to reach the diagnosis to provide treatment and genetic counseling for the participant family. Since this case refers to an autosomal recessive disease, the study of genetic variants in both parents is an inherent part of the diagnostic investigation. Without this, genetic counseling is not possible.

According to Brazilian law, in the case of minors (individuals younger than the age of 18), consent for participation in research protocols must be given by one of the parents or legal guardians. In line with this regulation, it was obtained the mother's written consent. By consenting to the participation of the child, the parents agree to the investigation of the causative variant in their genetic material.

Consent for publication

Participant's mother provided written informed consent and the father's consent was verbal following Brazilian regulations above mentioned. The consent clearly states that information will be used for scientific purposes, and no personal information will be disclosed.

Availability of data and materials

The datasets generated and analyzed during the current study are not publicly available due individual privacy issues, however they may be available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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None.

Authors' contributions

RLOF, RJP and ILM: study conception and design; collection, analysis and interpretation of data; manuscript drafting and review, patients' clinical review and sampling. FCS, DPM, TNM, HFS and MPM: patients' genetic testing. SVZ and ICG: manuscript drafting and review. All authors read and approved the final manuscript.

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Figure 1. Family pedigree showing complex parental consanguinity.

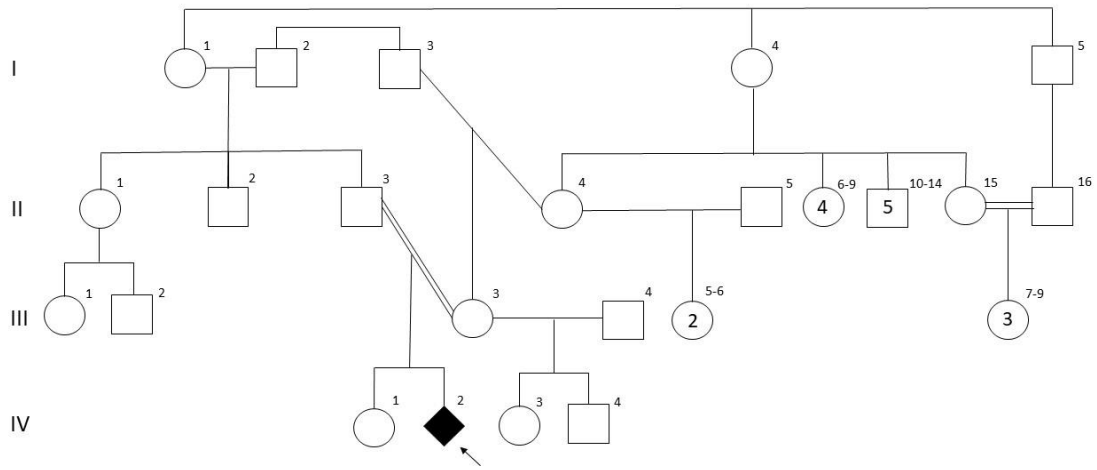
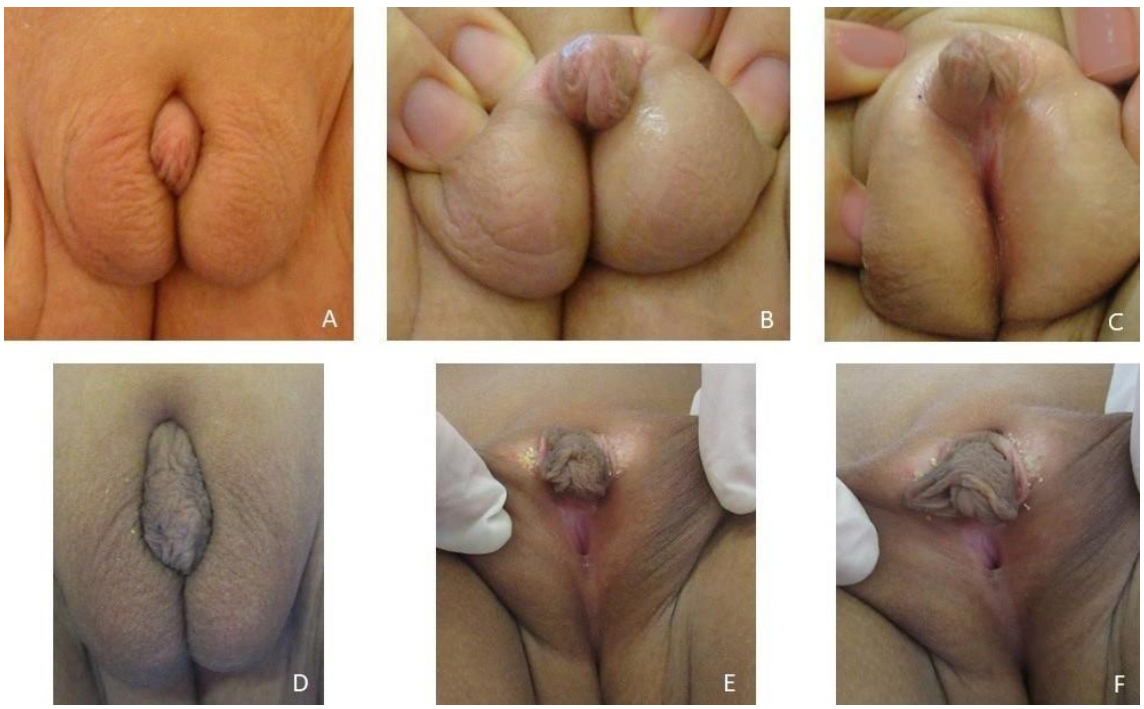


Figure 2. The external genitalia of our patient at 10 months (A, B, C) and at 6 years old (D, E, F).



Additional file 1

Methods

The HSD17B3 gene (OMIM * 605573), located on 9q22.32, spans by 1,159 kb, comprises 11 exons and encodes a 310 amino acid protein. Sixty-seven pathogenic variants have already been described. Roughly, 66% of them are single-base changes, mainly missense/nonsense [1,2]

Genomic DNA was isolated from peripheral blood leukocytes using the proteinase K lysis and phenol/chloroform method. The eleven exons and exon-intron junctions of the HSD17B3 gene were amplified by Polymerase Chain Reaction (PCR). Amplicons were purified and sequenced using both sense and antisense primers. The sequences obtained were compared to the HSD17B3 reference sequence at the Ensembl database (ENSG00000130948) using Chromas and CLC Sequence Viewer v.6.6.2, both free software.

SIFT, PolyPhen-2, Mutation Taster, and DUET were used to perform predictive analyses of the novel variant identified. Molecular modeling was performed using the MODELLER web server program. The 17-beta-hydroxysteroid dehydrogenase X-ray crystal structure available at PDB (ID: 5FYD) was used as a template, and Kalign Multiple Sequence Alignment (free access) was used to compare human HSD17B3 and mammalian protein sequences.

Reference

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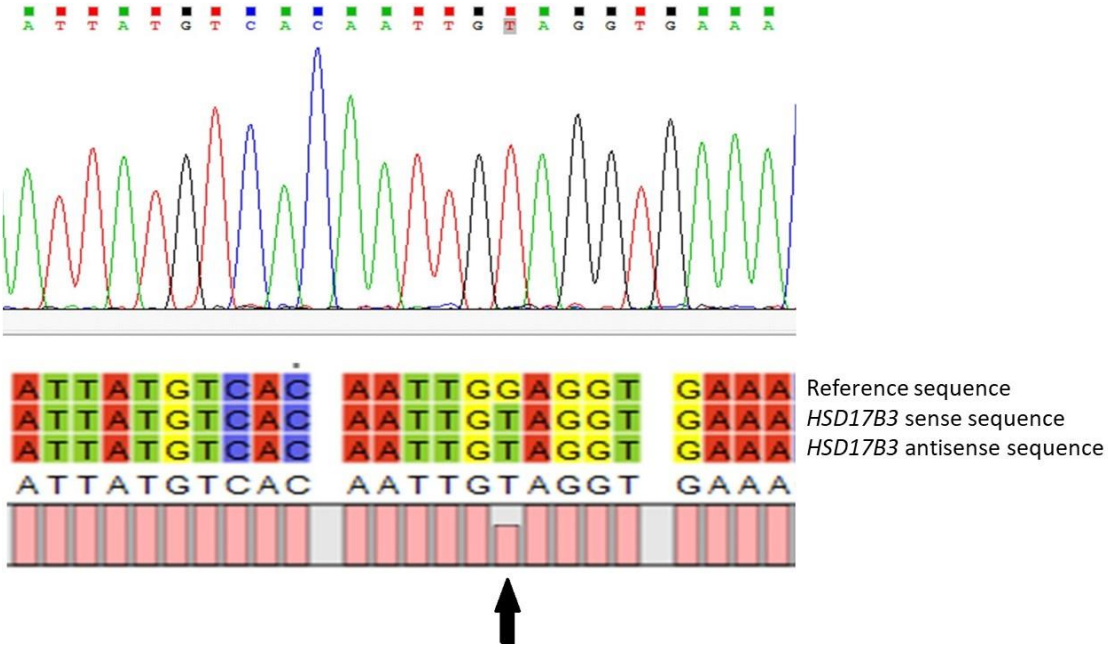
Additional file 2

Part of the electropherogram showing the homozygous change c.785G>T in exon 10 of the *HSD17B3* gene.



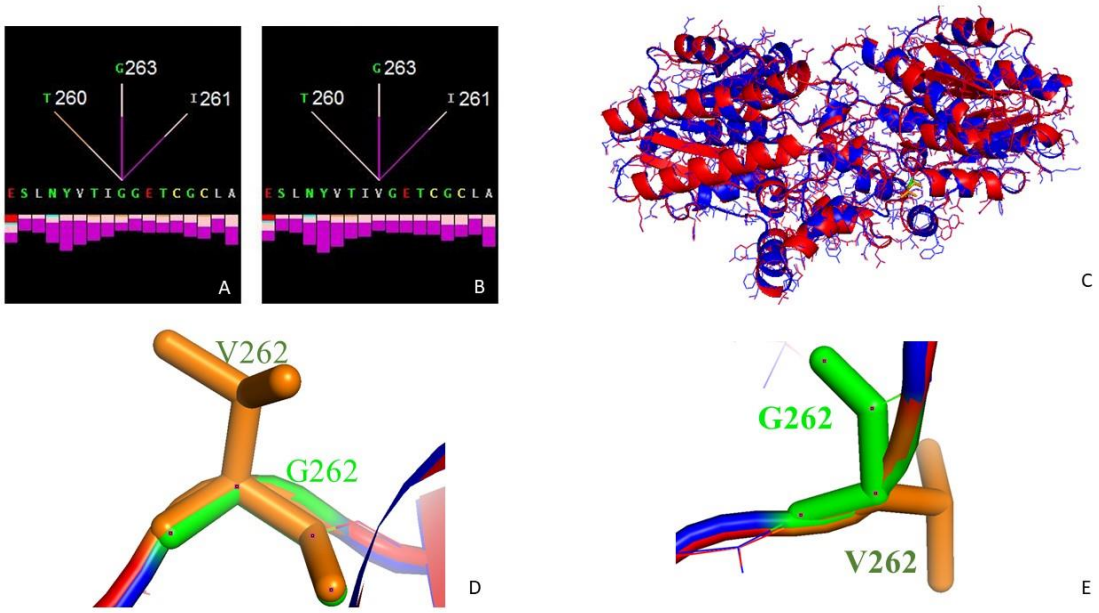
Additional file 3

Comparison between human and different mammalians HSD17B3 showing the conserved glycine 262 residue.

	262
Homo sapiens	A D E F V K E S L N Y V T I G G E T C G C L A H E I L A G
Pan troglodyte	A D E F V K E S L N Y V T I G G E T C G C L A H E I L A G
Papio anubis	A D E F V Q E S L N Y V T I G G E T C G C L A H E I L A G
Chlorocebus sabaeus	A D E F V Q E S L N Y V T I G G E T C G C L A H E I L A G
Macaca muallata	A D E F V Q E S L N Y V T I G G E T C G C L A H E I L A G
Pongo abelii	A D E F V K E S L N Y V T I G G E T C G C L A H E I L A G
Rattus novergicus	A D E F V K E S L K Y V T I G A E T C G C L A H E I L A I
Sus scrofa	A D E F V K E S L K Y V T I G D E T C G C L A H E I L A I
Ovis aries	A D E F V K E S L N Y V T I G D E T C G C L T H E I L A A
Ailuropoda melanoleuca	A D E F V K E S L N Y V T I G D E T C G C L T H E L L R T
Oryctolagus cuniculus	A D E F V K E S L N Y I T I G D E T C G C L A H E I L A G
Sarcophilus harrisii	A D E F V K E S L D F V A V G D E T C G C L A H E I L A H
Felis cattus	A D E F V K E S L N Y V M I G D E T C G C L I H E I L R S
Canis lupus familiaris	A D E F V K E S L N Y V T I G D E T C G C F T H E I L R I
Equus caballus	A D E F A K E S L N Y V T I G D E T C G C L V H E I L V I
Bos taurus	A D E F V K E S L N Y V T I G D E I C G C L T H E I L A V
Loxodonta africana	A D E F V Q E S L N Y V T N G D E N C G C L A H E I L A G

Additional file 4

Internal contacts established by HSD17B3 residue 262: A: wild type Gly262, B: variant Val262. C: 3-D structure modelled for wild-type HSD17B3 in blue and variant in red. D and E: zoom in the protein structure showing structural changes in the micro environment in residue 262, wild-type Gly represented in green and variant Val in orange.



7. ARTIGO 2

Title: A novel *CYP21A2* variant causing severe congenital adrenal hyperplasia due to 21-hydroxylase deficiency in a Brazilian high consanguinity region

Short title: A novel *CYP21A2* variant in Brazil

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Funding source: none.

Ethical approval: Research protocol was approved by the Research Ethics Committee of the Federal University of Alagoas (# 19144013.5.0000.5013). Participant's mother provided written informed consent, stating that information would be used only for scientific purposes, and no personal information would be disclosed.

Conflict of interest: No financial benefits have been received from any party related directly or indirectly to the subject of this article.

Contributors' statement: RLOF, DPM, RJP and ILM: study conception and design; collection, analysis and interpretation of data; manuscript drafting and review, patients' clinical review and sampling. LGS and MPM: patients' genetic testing. SVZ: manuscript drafting and review. All authors read and approved the final manuscript.

Title: A novel *CYP21A2* variant causing severe congenital adrenal hyperplasia due to 21-hydroxylase deficiency in a Brazilian high consanguinity region

Short title: A novel *CYP21A2* variant in Brazil

Abstract

This article reports on a 7-days-old newborn, raised as a girl, presenting Congenital Adrenal Hyperplasia due to 21-hydroxylase deficiency (21OHD). She was the second child of a consanguineous couple from a semi-arid and poor zone of Brazil. The *CYP21A2* gene sequencing revealed homozygous missense variant NM_000500.9:c.901T>C in exon 7. As far as we know, this variant was not described before. According to predictive analysis the NP_000491.4:p.Ser302Pro variant leads to a significant decrease of CYP21A2 activity that corroborates the child's salt-wasting phenotype. Before genetic assessment and counseling, parents were unaware of diagnosis, implications, treatments, consanguinity influence, and recurrence risk of 21OHD. Besides information on a novel causative variant of the *CYP21A2* gene, this report exemplifies the diagnostic and therapeutic trajectory of persons

with rare genetic diseases living in poor areas. The familial genetic background knowledge may improve genetic literacy and empowerment in low and middle-income countries.

Keywords: congenital adrenal hyperplasia; genital ambiguity; consanguinity; therapeutic trajectory.

1. Introduction

Congenital adrenal hyperplasia (CAH), an inborn metabolism error is caused by enzymatic defects involved in the adrenal steroid biosynthesis and inherited in an autosomal recessive manner [1]. Approximately 95% of CAH cases present 21-hydroxylase deficiency (21OHD) (*OMIM* # 201910) which are related to classical and non-classical forms. 21OHD results from cortisol and aldosterone deficiency that increases the adrenocorticotrophic hormone through negative feedback and, ultimately, leads to an excessive adrenal androgen synthesis [2].

The clinical picture reflects the enzyme residual activity and is classified as classical (CL: enzymatic residual activity 0-2%) and non-classical form (NC: enzymatic residual activity ≥10%). In the more severe phenotype, the classical salt-wasting form (CL-SW), the enzymatic activity is null. The aldosterone deficiency results in adrenal crisis, shock and can lead to death when untreated. 46,XX children present with genital ambiguity due to high androgen exposure during prenatal life while 46,XY children present early virilization only in the postnatal period. As genital ambiguity does not occur, non-specific signals of adrenal crisis such as vomiting, lethargy and inability to gain weight may be misinterpreted as infectious symptoms [2–4].

The CL-HAC is an example of rare and treatable genetic disease, with global prevalence of 1:15,000 [5]. Although classified as a rare disease, the prevalence of heterozygous individuals carrying pathogenic *CYP21A2* variant is as high as 1:55 to 1:70 [6, 7]. With such a prevalence, it is expected the majority of cases to be compound

heterozygotes for whom parental consanguinity is not relevant. However, populations with high levels of consanguinity it is expected an increased homozygosity of pathogenic variants.

In this study, we report on a child of consanguineous parents from the Northeast region of Brazil presenting CL-SW 21OHD phenotype due to a novel homozygous *CYP21A2* variant (Ethics approval # 19144013.5.0000.5013).

2. Patient and methods

Patient

A child raised as a girl was referred to our team due to genital ambiguity when she was 7 days old. Upon physical examination, she weighted 2,685 g (5^{th} percentile), and was lethargic and dehydrated. There was a 2.2 mm x 1.2 mm phallus with an urogenital sinus opening in its base, partial labia majora fusion, and wrinkled and hyperpigmented genital skin with no palpable gonads (Prader 4) [8] (Figure 1). Plasmatic sodium was 133 mEq/L (normal: 135–150 mEq/L), potassium 8.1 mEq/L (normal: 3.5–5.1 mEq/L) and 17OHP 70.9 ng/mL (normal: < 2.5 ng/mL). The karyotype was 46,XX. She is the second child of a consanguineous couple (Figure 2).

Analysis of the *CYP21A2* gene and protein structure

Proteinase K lysis and phenol/chloroform method was used to obtain genomic DNA from peripheral blood leukocytes [9]. The *CYP21A2* gene was specifically amplified in two or four fragments, depending on the absence or presence of intron 2 variant c.290-13C respectively. Amplicons were purified with Wizard SV Gel and PCR Clean-UP System (Promega®) and directly sequenced with Big Dye Terminator Cycle Sequencing Kit V3.1 Ready Reaction (ABI PRISM/PE Biosystems, Foster City, CA, USA) using internal primers in addition to sense and antisense primers used in the PCRs [10].

Sequencing electropherograms were analyzed against the reference sequence NM_000500.6 (Ensembl database), but removing 3 base pairs of the CTG repeat at the beginning of the gene thus keeping the numbering of the variants as previously stated in the literature (e.g. p.Pro30Leu and not p.Pro31Leu), using Chromas and CLC Sequence Viewer, both free software. Segregation analyses were performed by sequencing parental samples.

PolyPhen-2, Align GVGD, and SNPs&GO were used as prediction algorithms to analyze the novel variant identified. The molecular modelling was obtained by PyMol Viewer.

3. Results

The *CYP21A2* gene sequencing revealed a novel homozygous NM_000500.9:c.901T>C nucleotide substitution in exon 7 inherited from her heterozygous parents (SNP ID NG_007941:g.1748T>C), submitted SNP(ss) Details: ss2137544212 (RefSNP: clustering in process). This substitution led to the replacement of serine by proline in the residue 301 (NP_000491.4:p.Ser301Pro) of the protein CYP21A2 (Figure 3). It also showed homozygous of unusual *CYP21A1P*-derived single nucleotide variants (SNV) in 5' region and in intron 2. These SNVs were transmitted through the parental alleles carrying the NM_000500.9:c.901T>C variant (Table I).

PolyPhen-2, Align GVGD, and SNPs&GO scores indicated a damaging missense variant. In silico studies suggested that NP_000491.4:p.Ser301Pro introduces kinks into the α-helices. Structural modelling analysis (PyMol Viewer) did not show α-helix disruption. However, the exchange of a serine for a proline at position p.301 creates one hydrophobic interaction with the T299 and four hydrophobic interactions with the N298; exchange one hydrogen bond (main chain – main chain) and one hydrogen bond (side chain – main chain)

interactions with the A297 to two hydrophobic interactions; and maintains interactions with the V305, V304, M473, W302 and L300.

4. Discussion

The *CYP21A2* gene is located within a region of approximately 60 kb on 6p21.3 in the major histocompatibility complex. It spans more than 2,000 kb, comprises 10 exons and encodes a protein of 495 amino acids. This 60 kb region also includes the pseudogene *CYP21A1P* that maps in tandem with *CYP21A2* and presents 98% of homology with the active gene [2].

The variant NP_000491.4:p.Ser301Pro herein described was not found in The Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), Genome Aggregation Database (https://gnomad.broadinstitute.org/), Brazilian Genomic Variants (https://abraom.ib.usp.br/), and Pharmacogene Variation Consortium (https://www.pharmvar.org/) accessed on August 18, 2021.

Stikkelbroeck et al.(2003) reported on three patients from two unrelated families with the variant NM_000500.9:c.902C>A which causes the replacement of serine by tyrosine in the same residue 301 (NP_000491.4:p.Ser301Tyr) on CYP21A2. Their patients were compound heterozygotes with NM_000500.9:c.290-13A/C>G variant, and presented NC 21-OHD phenotypes [11].

Serine is a small, polar and neutral charged amino acid that is seen within the interior and on protein surface. It may build hydrogen bonds with the protein backbone within tight turns on the protein surface. Occasionally, it may be substituted by other small or polar amino acid without serious damage to protein structure [12]. Tyrosine is an aromatic amino acid, partially hydrophobic and neutral charged. Similarly to serine, it has a reactive hydroxyl group and is responsible for intracellular protein phosphorylation [12] however,

the much larger tyrosine side chain cannot be accommodated at this site without clashes. So it is conceivable that the β-strand and the loop region preceding it could move somewhat to accommodate the bulkier tyrosine, which appears to be of little consequence for enzyme activity being associated with the NC 21OHD phenotype [13]. On the other hand, the variant c.290-13A/C>G is associated with the CL 21OHD phenotype [14]. Therefore, the NC 21OHD phenotype of their compound heterozygous patients is likely associated with the p.Tyr301 allele.

Serine and proline are chemically diverse. Proline is an apolar amino acid unable to adopt a normal helical conformation. It is the only amino acid presenting a side chain that connects twice with the protein backbone. It is often seen producing kinks into the α-helix protein structure and rarely involved in protein activation or binding sites [12]. Based on the predictive analyses, p.Pro301 did not lead to a α-helix disruption however it caused significant internal contact changes (Figure 4), as described before.

Accordingly, we infer that p.Ser301Pro variant has led to significant decrease of CYP21A2 enzymatic activity prompting to SW 21OHD phenotype in our homozygous patient. Interestingly, unusual SNVs were also identified in the *CYP21A2* sequencing co-segregate with the causative variant inherited from both parents, reinforcing the role of consanguinity in this case (Table I). Further studies are necessary to determines if the severe phenotype is entirely due to the novel variant or if the SNVs have a synergistic/additive action.

Our patient inherited the novel variant from her consanguineous parents who are from a region of high consanguinity [15, 16]. They live in one out 26 municipality in the Sertão of Alagoas, a semi-arid and poor zone of the Brazil, with 433,067 inhabitants, population density of 49.37 inhabitants/km^2 according to the latest national demographic census (2010) [17].

Accessing genetic assessment, tests, counseling and high-cost therapies, maintaining follow-up, and understanding the genetic terms are among difficulties faced by families living with rare genetic diseases. The burdensome of the diagnosis and its implications on patients and caregivers may involve frustration, fear, incomprehension, and financial restraints [18, 19]. These issues are present in the history of this family and should be highlighted.

Parents have low educational level and face financial difficulties. They reported their first child deceased at five months due to malnutrition and vomits. These are unspecified symptoms that may be related to many etiologies including infectious and respiratory diseases (ICD-10 chapters 1 and 10), which were the third cause of infant mortality in Sertão of Alagoas in 2018, when the infant mortality rate was 14.42 [20]. However, they also suggest adrenal crisis related to 21OHD that may have been the real cause of his precocious death. Before genetic assessment and counseling, parents were unaware of diagnosis, implications, treatments, consanguinity influence, and recurrence risk of 21OHD. For their second child this meant surviving.

This report brings information on a novel causative variant of 21OHD which is essential for a personalized medicine approach. It also exemplifies how the familial genetic background knowledge may improve genetic literacy and empowerment of those living in low and middle-income countries.

Acknowledgments

Authors thank the patient's family for enrolling the study, the laboratory personnel from Human Cytogenetic Lab of the State University of Health Sciences of Alagoas, specifically to Diogo Nascimento and Michel Alves, for technical support.

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2021

Table

Table I.

SNV*	Genomic position	Allele 1	Allele 2
**(rs3130677)	5'regulatory -296T>C	C	C
**(rs3130495)	5'regulatory -295A>C	C	C
**(rs78873743)	5'regulatory -284A>G	G	G
**(rs79899926)	5'regulatory -282T>G	G	G
(rs11449852)	5'regulatory -196T>C	C	C
**(rs6462)	Intron 2 c.289+9T>C	C	C
(rs6451)	Intron 2 c.290-67C>G/A	A	A
**(rs59064806)	Intron 2 c.290-48A>G	G	G
**(rs6453)	Intron 2 c.290-44G>T	T	T
**(rs35147842)	Intron 2 c.290-39_38delCAinsGG	GG	GG
(rs6466)	Intron 3: c.444+38C>T	T	T
***	**Exon 7 c.901T>C**	**C**	**C**
(rs1058152)	3'UTR: c.*52C>T	C	C
**(rs6457475)	3'UTR c.*440C>T	T	T
**(rs6457476)	3'UTR c.*443T>C	C	C
**(rs7756934)	3'UTR c.*464T>C	C	C
**(rs7383707)	3'UTR c.*474C>T	T	T

(*) Single Nucleotide Variants bank access number (http://www.ncbi.nlm.nih.gov/sites/entrez). (**) Pseudogene variants. (***) New variant. Allele 1: inherited from her father. Allele 2: inherited from her mother. RefSeq NM_000500.9.

Figure legends

Figure 1. The external genitalia of our patient when she was seven days old.

Figure 2. Family pedigree. The patient also has a younger brother with no signs of 21-OH deficiency.

Figure 3. Part of the *CYP21A2* electropherogram showing (A) a normal sequencing and (B) the homozygous change NM_000500.9:c.901T>C in exon 7.

Figure 4. Visualization of the structural changes caused by the mutation p.Ser301Pro: (A) wildtype p.Ser302; (B) pathogenic variation p.Pro302; (Serine and Proline both in red); Graph internal contacts lost or established by the exchanges of amino acids (provided by the program STING Report); (C) wildtype; (D) pathogenic variation p.Pro302. Rose:

hydrogen bonds (main chain - main chain); Orange: hydrogen bond (side chain – main chain); Purple: hydrophobic interaction.

Figures

Figure 1.

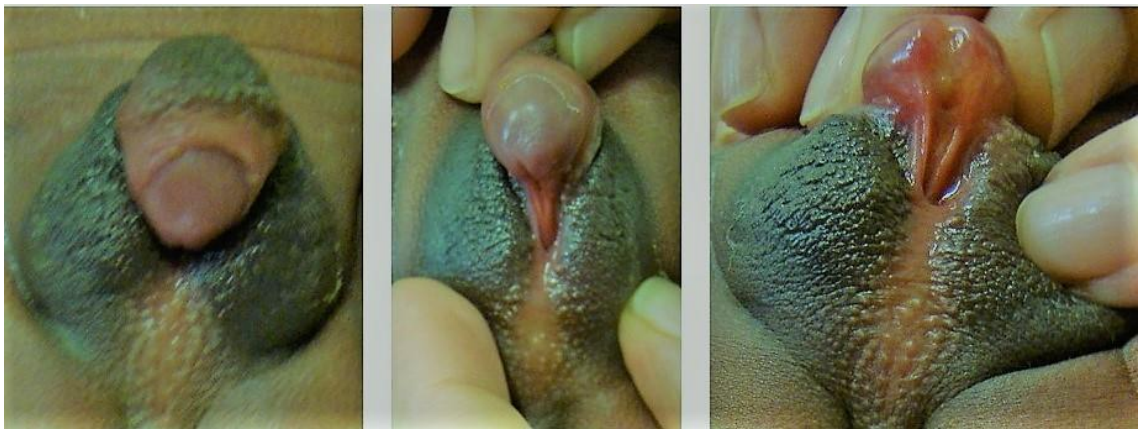


Figure 2.

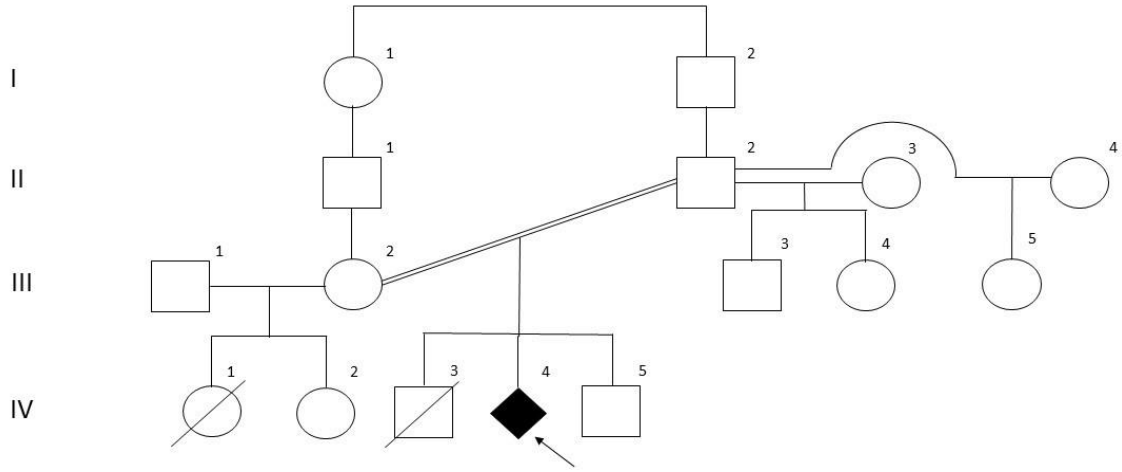


Figure 3.

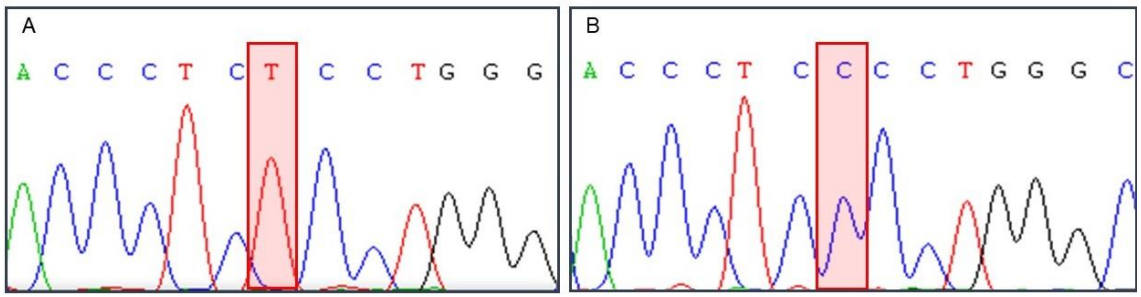
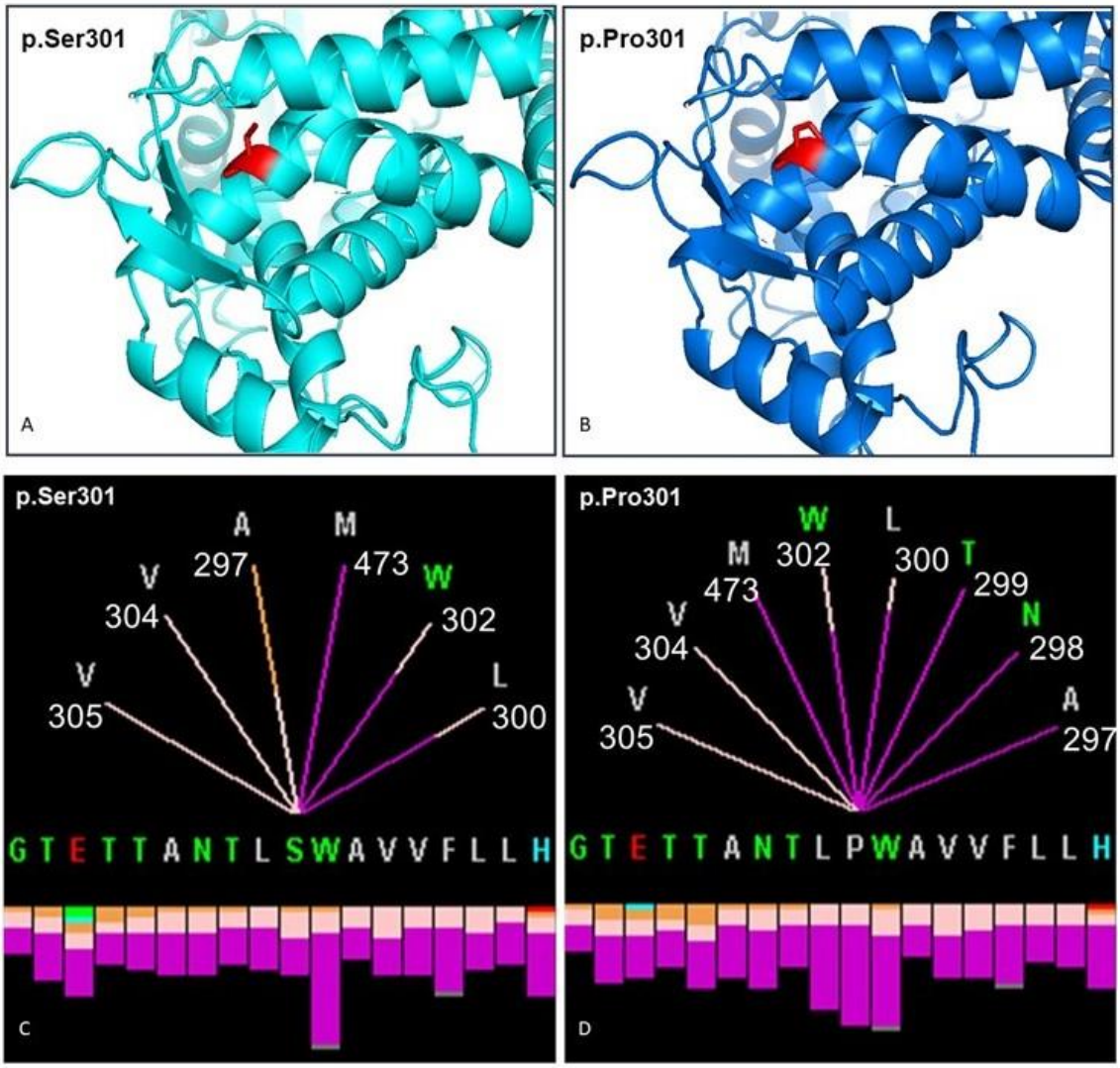


Figure 4.



8. CONCLUSÕES

Esta pesquisa consiste na primeira descrição demográfica, clínica e genética de AG em Alagoas a partir de uma série de casos oriundos do ambulatório de Distúrbios/Diferenças do Desenvolvimento do Sexo do HUPAA-UFAL, no período 2008 – 2018. Os resultados permitem concluir:

- Os primeiros 10 anos de existência deste ambulatório foram marcados pelo atendimento de pessoas com AG em idade inadequada. Este resultado revela que o serviço oferecido não foi incorporado como rotina no cotidiano dos profissionais de saúde, o que expõe falhas na articulação da referência e contrarreferência nesta área dentro do SUS.
- A ausência de registro civil foi o único fator que esteve relacionado à chegada numa idade adequada para a primeira consulta no ambulatório especializado. No nosso meio, este resultado sugere o não reconhecimento dos fatores de risco para distúrbios congênitos, no caso a AG, bem como a não associação destes e a não valorização dos achados genitais como indicativos de necessidade de avaliação por equipe especializada.
- DDS XY se apresentou como grupo com maior número de sujeitos e maior quantitativo sem diagnóstico nosológico e a HAC se apresentou como principal diagnóstico nosológico responsável por AG. Estes resultados são semelhantes aos descritos na literatura.
- Foram identificados dois casos raros de DDG, correspondendo às associações de disgenesia gonadal com Síndrome de Down e de DDS OT com Síndrome de Klinefelter.
- Foram identificados dois casos com variante rara do gene *CYP21A2* em duas famílias não relacionadas e oriundas de municípios 190 Km distantes entre si. Este resultado suscita a investigação de efeito fundador em pesquisas futuras.
- Três novas variantes foram encontradas na amostra para diferentes nosologias: disgenesia gonadal parcial XY (gene *NR5A1*), deficiência de HSD17B3 (gene *HSD17B3*) e HAC (gene *CYP21A2*).
- A variante do gene *NR5A1* foi incluída em um estudo funcional que lançou luzes sobre o conhecimento dos efeitos de alterações deste gene na etiopatogênese das disgenesias gonadais parciais XY.

- As variantes novas dos genes *HSD17B3* e *CYP21A2*, igualmente poderão contribuir para ampliar o conhecimento sobre o desenvolvimento do sexo mediante a realização de estudos funcionais em outras pesquisas.

O conhecimento aportado por esta pesquisa poderá contribuir para a melhoria das estratégias de acolhimento às famílias e para a elaboração de políticas e programas de educação permanente para os profissionais de saúde. O conjunto dessas ações poderão incrementar o reconhecimento, adoção de condutas e encaminhamento adequados e oportunos de pessoas com AG para seguimento especializado.

O ambulatório de DDS do HUPAA-UFAL iniciou esse processo, alcançando nesses primeiros 10 anos, de forma pioneira em Alagoas, um modelo multidisciplinar de atendimentos, com condutas individualizadas para cada família, mediante discussão e pactuação entre os especialistas.

Na perspectiva da pesquisa translacional é ainda necessário aproximar os gestores de saúde e a equipe multidisciplinar, visando ao compartilhamento das experiências e a aplicação dos resultados desta pesquisa na melhoria da atenção à saúde.

9. LIMITAÇÕES E PERSPECTIVAS

Uma limitação importante desta pesquisa foi não ter concluído todas as análises moleculares pretendidas dentro do cronograma planejado. A finalização destas poderá trazer alguma modificação ao perfil genético aqui descrito, em especial para os casos de HAC por alteração do *CYP21A2*, cujos resultados destoaram do esperado conforme a literatura, e dos casos de DDS XY que não puderam ter o gene *HSD17B3* analisado. Além disto, o projeto inicial não previu a realização de estudos funcionais das variantes novas identificadas, o que reduz o impacto científico das respectivas publicações.

Ainda para os seis (6/19) casos de HAC em que há a necessidade de complementação das análises moleculares, há a perspectiva de realizar o sequenciamento completo do gene *CYP21A2* nos casos 131, 155, 169 e 230. Para o caso 29, realizar a técnica de *Multiplex Ligation-dependent Probe Amplification* (MLPA) para investigar a genitora. No caso 79, também utilizar a MLPA para investigação do genitor.

Apesar destas limitações, há perspectivas concretas de manutenção das pesquisas sobre DDS em Alagoas com a recente aprovação do projeto Distúrbios da diferenciação do sexo em Alagoas: da atenção primária ao diagnóstico etiológico e tratamento multidisciplinar pelo Programa de Pesquisas para o SUS (2021-2023).

Neste sentido, já estão em desenvolvimento dois estudos em nível de mestrado, um abordando a HAC relacionada ao gene *CYP21A2* e outro, a análise do gene *HSD17B3* em 17 casos de DDS XY até o momento sem diagnóstico elucidado. Além disto, com o objetivo de ordenar e descentralizar a atenção, estão previstos cursos de capacitação para os profissionais de saúde da linha de frente no atendimento a pessoas com AG em Alagoas.

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APÊNDICES

Apêndice A – A multidisciplinary reflection on congenital adrenal hyperplasia: medical, psychological, and bioethical issues

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Abstract

This report brings a multidisciplinary reflection on genital ambiguity based on the follow-up of three children later diagnosed as having congenital adrenal hyperplasia. The first is a neonate that had been raised by his parents as a girl. The second, a six-year-old boy whose shocked parents decided to keep him as a boy, although he may reassign his gender later. The third, an adolescent reared as a girl who, supported by his social environment, had decided to change his gender. The authors' starting point was to discuss the impact of the first doctor's information and conduct. For psychological issues, it was considered parents'/patients' subjective perspective on the differences between genital ambiguity and binary social gender. From the bioethical perspective, confidentiality, beneficence, and autonomy were analysed considering families' educational, cultural, and economic contexts. The team's experience has shown that caring for these families includes sheltering their subjective solutions. Accordingly, the team has met monthly to analyse and agree upon the best approach on each patient using a case-by-case strategy. As such, authors consider this is the way better care can be offered to patients.

Keywords: congenital adrenal hyperplasia; genital ambiguity; sexual ambiguity; vulnerabilities; autonomy; bioethics.

Introduction

Disorders/differences of sex development (DSD) is the term now used for clinical conditions in which there is a divergence between genetic and phenotypic sex. Congenital adrenal hyperplasia (CAH) is the most common example and includes a group of seven enzymatic deficiencies related to the synthesis of cortisol, inherited in autosomal recessive manner [1].

Alterations in the cytochrome P450 family 21 subfamily A member 2 (*CYP21A2*) gene, result in 21-hydroxilase deficiency (21-OHD) and are responsible for about 95% of CAH

cases. As a consequence, there is glucocorticoid and mineralocorticoid deficiency and excess of androgen [2–4]. The incidence of CAH by 21-OHD ranges from 1:1,000 to 1:15,000 [2,3], according to the severity of enzymatic defect and ethnic-geographic characteristics [5].

A person with XX sexual chromosomes and 21-OHD classical form exhibits genital characteristics that may range from an isolated enlargement of the clitoris up to a male appearance with no palpable gonads. The severity of genital virilisation has been classified in five degrees, according to Prader system [6,7]. In these cases, the gonads are fully differentiated into ovaries and the internal female genitals comprise uterus and Fallopian tubes, with no Wolffian duct development [8,9].

Approximately 75% of individuals with classical 21-OHD have the salt-wasting form, which is a paediatric emergency [2,3,5,9]. Pharmacotherapy is indicated to prevent progressive virilization, precocious puberty, and adrenal crisis that is life-threatening. Furthermore, children timely treated with glucocorticoids and mineralocorticoids may develop spontaneous puberty and normal fecundity [4,10].

On the other hand, there is no consensus on genitoplasty. Some authors and groups of patients have suggested postponing the decision until the child is able to be part of the decision-making process [8,11–13].

Besides medical aspects, it is central to take into account psychological, social, economic, cultural, and religious issues surrounding and modulating patient and family's attitudes and decisions. Communicating the diagnosis and its management is a stressful situation that requires an early and coordinated multidisciplinary approach [14–17].

Delivering a coordinate multidisciplinary care is a good recognized way to deal with vulnerabilities of this population. However, it does not prevent the emergence of many ethic, legal and social issues (ELSI), encompassing not only who will make a decision, but

what to do and when it should be done, as well as how financial costs will be approached, and how satisfied the person will feel with the outcomes [18].

Materials and Methods

In 2008, a pioneering initiative resulted in the establishment of an integrated ambulatory for genetic and psychological care for individuals with genital ambiguity at the University Hospital of the Federal University of Alagoas, Brazil. Over 12 years, more than 250 families with DSD have been followed up and the ambulatory became a reference on DSD. Currently, it is composed by a multidisciplinary group of health professionals, voluntary, and altruistic, comprising psychologists, human and medical geneticists, paediatric endocrinologist, paediatric surgeon, pathologist, nurse, biomedical technician, lawyer, and undergraduate students. Since 2016, team members meet monthly to analyse and agree upon the best approach on each individual patient [19].

This article aims to report medical, psychological, and bioethical aspects underlying the families' decisions based upon the follow up of three cases of CAH due to 21-OHD.

This research was approved by the Research Ethics Committee of the Federal University of Alagoas (CAAE # 59929716.8.0000.5013), and informed consent/assent was obtained from the patients or their parents.

Results

All names presented here are fictitious in order to preserve the persons' identities.

Case 1

Ana is the only child of a healthy non-consanguineous and adolescent couple, with no familial recurrence of CAH. She was a borderline preterm neonate weighing 2,720g, considered a healthy boy with undescended testes by the maternity team and, accordingly, she received male birth certificate. She evolved with dehydration and was admitted to the University hospital of Alagoas when she was 24-days old.

Assessment performed by team's clinical geneticist showed ambiguous and hyperpigmented genitals (Prader 3), with a clitoris measuring 24 x 12 mm (upper to 97th percentile), and no palpable gonads. The diagnosis suspicion and its implications were communicated to the mother at the bedside and she agreed to continue with the team's follow-up after discharge. Laboratory findings confirmed the diagnosis of CAH due to 21-OHD (Table 1).

Case 2

Roberto is the only child of a healthy and non-consanguineous couple, with no familial recurrence of CAH. He looked as a boy with cryptorchidism at birth, received male birth certificate and has been reared as such. Parents were advised to wait testicular descend, however, he developed precocious puberty and gynecomastia during childhood and underwent corticotherapy at the age of six, when he was referred to the DSD multidisciplinary team.

At first genetic assessment, there was ambiguous and hyperpigmented genitals (Prader 4), a clitoris measuring 46 x 24 mm (upper to 97th percentile), and left unilateral gynecomastia; the child weighed 37.7 Kg (upper to 97th percentile) and was 137 cm height (upper to 97th percentile). The existence of DSD was communicated to the mother who agreed with the team's follow up. As pictured in Table 1, laboratory tests corroborate the diagnosis of CAH due to 21-OHD.

Case 3

Antonio is the first of three siblings of a healthy and non-consanguineous couple with no familial recurrence of CAH. The maternity team, at first, concluded he was a boy and then a girl and the parents were communicated of the baby's condition. The child received female birth certificate and was reared as a girl. There is a medical history of three months hospitalization after birth due to low weight. His mother says he has undertaken

hydrocortisone since birth until six years old when therapy was discontinued due to socio-economic problems.

At 15 years old he was an emotionally withdraw and shy adolescent. Upon school request he started psychological follow up in his hometown and made the decision to take on the male gender. At that time, he was referred to the DSD team and clinical assessment revealed ambiguous and hyperpigmented genitals (Prader 4), clitoris measuring 70 x 45 mm (upper to 97th percentile), no palpable gonads or breast tissue. The adolescent weighted 42.9 Kg (5th to 10th percentile) and was 145 cm height (below 3rd percentile).

Laboratory findings confirmed the diagnosis of CAH due to 21-OHD (Table 1). He has been engaged in the team for follow up since then.

Discussion

Medical issues surrounding the CAH diagnosis

Several authors highlight the importance of the "first doctor" in the management of patients with DSD. In CAH, properly facing the condition may mean living or dying [19,20].

This is especially important in regions where prenatal or newborn and screening for CAH is not implemented.

Although Brazil is among upper-middle income countries, there are rough socioeconomic differences inter- and intra- regions. Alagoas, where this study was performed, is in the Northeast part of the country, and formed by 102 municipalities with HDI ranging from 0.7172 to 0.3828 [21]. Neonatal screening for CAH in Alagoas was initiated in 2016, a year after Ana’s birth.

Ana and Roberto were neonates presenting severe genital ambiguity (Prader 3 and 4, respectively) characterized by male genitals appearance plus cryptorchidy. Both received male birth certificate.

Lack of knowledge, inability to recognize genital ambiguity, negligence in examining the genitalia, and failure to deal with the uncertainty about the anatomic sex of the neonate are some possible (and unacceptable) reasons to explain this medical conduct.

It was expected that the “first doctor” would explain the situation to the family, refer them to multidisciplinary expert monitoring, and maintain the child under surveillance due to the chance of an adrenal crisis and death [5,20,22].

Ana was hospitalized when she was 24 days old due to dehydration. Ana's parents were unaware of the diagnosis at that time. The medical decision to intervene during hospitalisation without consent is ethically supported by the imminent risk of death. Thus, it is assumed that no other conduct than the pharmacological intervention to control the metabolic picture would be possible.

The first meeting with the DSD team took place at that time. The genetic tests confirmed the p.Gln318* homozygosis related to the salt-wasting CAH, corroborating the paediatric emergency picture [23]. Although the mother had agreed with the team’s follow up the contact was lost after discharge.

When the child was two years and 10 months old, the father resumed contact with the team. The parental couple had split up and the paternal grandmother is looking after the child since then. At that age, the father said the child was being reared as a girl based on the diagnosis of CAH established during hospitalization, but he had not changed her civil registration yet. The corticosteroid therapy was continued and no genital intervention was done. Currently, grandmother and child are under regular follow-up with the multidisciplinary team.

Roberto has evolved without adrenal crisis, the c.290-13A/C>G variant is related to either the salt-wasting (majority of the cases) or simple virilising phenotypes [24,25]. The baby

grew up healthily, and parents were counselled to wait for the spontaneous testicular descend. This recommendation significantly delayed the CAH diagnosis.

At six years old, peripheral precocious puberty and gynaecomastia became evident. Roberto was firstly seen by an urologist and endocrinologist at his hometown where he underwent corticosteroid therapy and, afterward, referred to us. The pharmacological treatment at this stage aims to control adrenal androgenic excess. The consequences of no intervention would be an adrenal crisis, menarche and short final stature. Subfertility and increased adrenal neoplasia risk may also develop [26].

During the first visit with the DSD team, parents were not fully aware of the diagnosis and its implications. After evaluating the impact of the diagnosis on the parents, the team agreed that the best approach would be to bring the family for joint consultation with the geneticist, the psychologist and the paediatric endocrinologist. While the geneticist and the endocrinologist explained the diagnosis, its implications, prognosis, and treatment choices to the parents, the psychologist was interacting separately with the child. Afterward, the parents and child interchanged the rooms. Under such a stressful situation the family has decided continuing clinical follow-up without any surgical intervention or sex reassignment.

Antonio, like the previous children, was born with serious genital ambiguity. Although the "first doctor" identified him as a boy, the baby was discharged as a girl and under corticosteroids due to CAH. According to his mother, the pharmacological treatment was ceased at the age of six due to socio-economic problems. Since then, androgenic effects have increased progressively. He decided to change his social name, clothing, and behaviour from female to male. In order to do so, he relied on the consent and support of his family, school, and religious leader.

Antonio engaged with DSD team when he was 15, showing significant virilisation and short stature. Genetic tests revealed homozygosis p.Ile172Asn alteration. This variant has been associated with the simple virilising form of CAH, meaning that technically there is no chance for adrenal crisis [23,27].

The team agreement was to explain him the diagnosis and its implications such as the presence of a uterus, the possibility of irregular periods and the irreversible short stature. The geneticist made the first approach and the adolescent was asked to carefully think about his life and his clinical condition before making any decision. Therefore, no pharmacological or surgical intervention was suggested at that stage. The risk of adrenal neoplasia [26] has been monitored since then.

Antonio has been followed-up by the team's endocrinologist and psychologist. Upon his request, the team gave him legal support to adopt a male social name. The civil registry, however, remains female.

Besides the impact of the first doctor, these three cases testify how harmful the absence of a timely and multidisciplinary-oriented approach to such situations may be. More than the clinical management, it is critical to support, promote, and facilitate parents' and patients' implication into the therapeutic process.

To deal with this challenge, the DSD team has considered parents' and patients' subjective representations not only on the diagnosis but also on its relationship with sex choice in each social-cultural environment.

Subjective perspective of differences in sexual development

The reference adopted by the DSD team on gender choice is based on the psychoanalytic theory [28,29] which differs from the neurodevelopmental and cognitive approaches [30]. The gender definition process includes biological characteristics and the way the child's psyche is influenced by parents [31]. Therefore, authors will highlight individual subjective

perspectives of parents and/or patients, related to diagnosis, treatment, and choices in cases of genital ambiguity. Original Portuguese transcriptions are given as a supplementary file. Regionalisms and syntax errors were suppressed.

Two subjective aspects are highlighted: 1- The importance of the meaning attributed by parents to the child's body and 2- The absence of linearity between biological sex and gender meaning that each person will recognize himself/herself in his/her sex, whether defined at birth or not. In the following cases, it should be punctuated that the way the family gazes at the child is incessantly marked by the questioning of the child's sex. In this regard, theoretical references based on Lacan (1958/1988, 1969/2003) and Ansermet (2018) [32] and adopted by the DSD team, should be highlighted.

The relationship between family and baby is initially based on the big Other's gaze - subjective instance demarcated by the inscription of language in the constitution of the subject [33]. First, the mother is situated as this Other (maternal function) as she performs the function of caring and supplying the baby´s needs. It is an otherness that introduces the baby into language, in the regime of symbolic interchanges. In this regime of symbolic interchanges, there comes the difference between the sexes, which is socially addressed from the first question asked about the infant body: is it a boy or a girl?

This dynamic points out to the parents' subjective work - the adoption of their children, even permeated by family secrets about their genitalia. Considering the family as a symbolic structure consisting of functions, every subjective constitution depends on a non-anonymous desire which makes such functions operate [28].

According to Ansermet (2018) [32], it is worth distinguishing genital ambiguity from sexual ambiguity. Genital ambiguity is a biological feature, while sexual ambiguity refers to the subjective choice of sex [29,33]. Ansermet (2018) [32] points out that regardless the genetic condition, human being is born in sexual ambiguity, which requires subjective

work. Thus, while genital ambiguity reflects the anatomical differences of sex, sexual ambiguity refers to the assumption of sex, a human subjective and singular phenomenon. Ana's birth, and the many difficulties experienced, radically impacted her teenage parents, who separated a few years after her birth. The paternal grandmother, who currently looks after the child, reports that "Until then he was a little boy and received a male civil registry". At that time, the gender reassignment was assimilated by the grandmother: "it did not matter whether it was a boy or a girl, for me it was a child and that's it".

However, this double inscription, at birth, a boy; almost a month later, a girl, could not be assimilated by the parents. In the grandmother's words "but I think parents saw the baby in a different way. I do not understand it, because the father does not say anything about it". I do not understand, because the father does not say anything". The silence indicated here by the child's grandmother opens an important path for understanding the implications of DSD diagnosis on the parents. The answers range from silence, as something impossible to express, to the status of a family secret [34]. In this family context, the authors highlight the way this genetic condition impacts on each member of the family, specifically the child's grandmother and the father. This silence reflects the family's anguish, meaning it is important they are sheltered and listened to by the team.

The disclosure of a serious illness in a first child of a teenage couple creates for the health professional a dilemma on how to communicate the diagnosis.

The first marks in Ana's life, coming from the parental big Other, plus the insignia of the body image and the hormonal function, resulted in the child's abandonment.

The grandmother's understanding of the information provided by health professionals outlines her gaze on Ana's body, viewing her as a girl or as a boy. "When her hormones levels are high some changes can be seen in her body". This is the grandmother's

knowledge based on the information offered by the endocrine paediatrician, added to the observation, at school, of “a change in this anatomical site”, referring to the genitals.

The uncertainty about Ana’s gender continues to reverberate. If at birth to be simply considered as a child "this is a child and that is it" ensured she was taken care of; today, a simple choice of a toy, considered by the grandmother as a boy’s toy, over a doll, presents itself as an interrogation. “I keep on thinking it’s because of the hormone, which is masculine”. In her search for an answer, the grandmother relies once again on the hormones despite medical information. "... I was already told that her preference for boy’s toys has nothing to do with the hormone levels. But I keep on thinking whether it is due to the masculine hormone or not. But they said it has nothing to do with it. I know, but I am not sure”.

Ana's grandmother's doubt indicates a question about the child’s sex. This doubt is present by the way she sees the child’s playing choices, which brings along the social dimension on the differences between what it is to be a man or a woman. In this case, it is important to consider that the context in which Ana and her grandmother live carries a social mark that reassures the linearity between the genitals and the definitions of man and woman. And Ana, what is she? The grandmother's anguish is in that gap which is marked by her questioning.

Roberto’s birth was accompanied by a particular nomination ('tiny small penis') given by his parents regarding his genital organ. Three moments identified in the speech of his mother, Lucia, marked the definition of sex. The first, at birth “we, once saw the tiny small penis, thought it was a boy, but for us it was normal ...".

Roberto’s parents hold themselves up in the "tiny small penis " he has which represents, in the look of Lucia, the male characteristics. The maternal realization is not reduced to seeing the child, but embodies the big Other as a place of otherness in the baby’s psychic

constitution [35]. This perception has fundamental importance in the approach of the "tiny small penis" that makes Roberto a boy for his mother, showing that "*a posteriori"* the bond between mother and baby took place from this factor.

The second moment, "until the age of 7 for us he was a boy, even with that difference, he was a boy ". The third, "And until today we see him as a boy. And we were shocked to realize that inside he is a girl". It is difficult to specify, between the second and third moments, how these parents came to realize the diagnosis. A fundamental issue for the way in which they deal with something impossible (the diagnosis) to express that affects the relationship between the parents and Roberto.

Despite the sex the child is being raised, the focus is on the fact that the diagnosis includes a totally unexpected event – Roberto, a boy, who now 'inside' is a girl – a situation that the parents did not consider. This case illustrates how a diagnosis binds the child in a family dynamic and the way in which parents adopt their biological children in the light of their own history [19].

The diagnosis is not innocuous for it interferes with the care of the child. However, it is mandatory that the parents be informed of this diagnosis. The appropriation of medical facts and their implications is the only possible means by which free and informed decisions can be made, concerning treating or not-treating the child. The question that remains is how this mother will keep on taking care of this child.

Advising the parents to change their son's name, plus informing that Roberto is not Roberto would be devastating for this family. On the other hand, it is not possible to deny that this child no longer has the "tiny little penis" as a triggering of the relationship with the parents. The liaison now is the "inside he is a girl". Lucia says, "We even talk at home, but we cannot accept what he biologically is, what he was born like".

Roberto's diagnosis shows up in the mother's symbolic universe as a non-inscription of the child's genetic condition, which can be appreciated from the statement that she does not think about her child's condition, otherwise she would not be able to raise him/her. From the psychic mechanism of denial [36] it is evident that this is a way of dealing with unconscious contents, which once denied, may show up or even be said.

Spontaneously saying that she does not think about her son's condition puts in evidence her difficulty in assimilating his condition. Her denial of the condition is reinforced when she manifests that her son's problem is "something from another world, no one believes it. It is so much trouble that we live day by day. I want to see him as a boy, as he sees himself, but if by any chance he chooses to be what he was born, we will see it in the future".

What is possible for the mother before her child's condition? "Keep going." This is an issue that brings into evidence the subjective response to the real of the genital ambiguity, which will have consequences for the child. From a subjective perspective, the dilemma "thinking about the situation of the child" nullifies her belief of being able to take care of Roberto.

No matter what the decision is, either maintaining the male phenotype or reassigning to female, additional interventions will be necessary, which comprise pharmacological and surgical approaches. Who should decide: the parents? The child? The adolescent? The adult?

Believing in another liaison between parents and child, welcoming DSD families as well as a continuous investment in parents' commitment with the children's care has underpinned the DSD team approach in these cases.

In the cases of Ana and Roberto, the authors emphasize on the unexpected situation of the birth of a child with ambiguous genitalia which modulated the relationship between the parents and their children. Antonio's case is different because he is an adolescent. With puberty and the awakening to the uneasiness that arises from it, the issue lies in the way the

subject inscribes the difference between the genital and the gender duality. Thus, the subjective choice of sex falls on his 'becoming sexual'. In this subjective process at least two contingent moments should be considered for a singular response to the sexual impasse [32].

The words of Eduarda, Antonio's mother, outline the marks of the first moment. After giving birth she was told the baby was a boy and would need only a minor 'corrective surgery' due to a 'malformation'. The birth of a boy fulfils the mother's desire: "Even when I was pregnant, I thought it was a boy. I was sure it was a boy". However, during the child's hospitalization "they said he was a girl. I just never believed it because I saw a boyish way".

The mother's desire, in looking at the child, is in itself the big Other's desire whose remnants will constitute the child's psyche [35]. Eduarda looks at her son as a boy; Antonio's "boyish way" allows Eduarda to take care of him and this will certainly have psychic repercussions on the child. To the big Other gaze and the symbolic mark of sex attributed by name, birth certificate and raising is added the sexual impasse of the puberty. Not by chance, Antonio's sex choice happens in adolescence, fixed in a dream: "Then God gave me a dream showing that I was not a woman, that I was truly a man". It is important to consider the sexual difference in the cultural context in which it is inserted. After talking to the clergyman about his dream, and receiving the support of his religious leader, family and school, there is a consolidation of his choice. "The clergyman knew the truth this was not gender reassignment, but a genetic disease. Then I talked, the brothers accepted when I changed, cut my hair, changed clothes. They truly accepted it as something from God and I am going on since then".

Despite the maternal look to his "boyish way" Antonio's choice towards male gender is consolidated from a dream and the acceptance of his social environment. There is not a

direct relationship between the gaze of the maternal big Other and the choice of sex, but of the remnants of the first moment, added to puberty as an awakening [29], a process that will require from Antonio a psychic work. That is the second moment. From there, the adolescent changes his name and appearance. The girl who had a “boyish way” became a boy in her adolescence.

These changes in Antonio’s life indicate that there is no true sex [19]. Linked to biological aspects, the choice of another gender by the adolescent does not necessarily constitute a reassignment of sex, but a subjective assumption of sex. Sex reassignment needs the subject to recognize that his/her body image is of an anatomical sex that does not fit his/her choice. Antonio’s case differs by the fact that his genital is ambiguous.

In the experience of this DSD team, the psychologist’s responsibility is to understand and to bring to the team’s debate the subjective aspects of the double-way relationship between the parents/grandmother and their children/grandchild and how it impacts the care and treatment.

In Ana's case, the grandmother and grandchild relationship is mediated by the question if she is a girl or a boy. The information given on the hormonal physio-pathological effects of CAH seems have not been helpful to positioning the grandchild according to her binary social conception of sex making the doubt perpetual. In the case of Roberto, the parental denial of his genetic condition is critical while in the case of Antonio, the choice to be a man is not a sex reassignment for him because the genital ambiguity is his auto-body image.

Despite the three patients have the same diagnosis, the psychological approach allows the DSD team to recognize singularities. This helps team members’ dialogue and agreement on each patient/family. Moreover, it improves communication to engage patients and families in the treatment and multidisciplinary follow-up.

The bioethical perspective

The three reported cases clearly exemplify the medical and psychological impact underlying the CAH diagnosis. ELSI are also complex and require careful reflection on the options that may be presented for the approach and treatment of these patients, whether neonate, child, or adolescent [18].

ELSI is a widely used acronym in the bioethics literature that encompasses a broad range of research areas involved in examining the various impacts of science and technology on society [37].

As a multidisciplinary field of study, ELSI had a considerable expansion over the last three decades, particularly in the USA, due in large part to the launch of the National Human Genome Research Institute's (NHGRI) ELSI Research Program. Similar efforts have followed in other regions. However when it comes to genetic diseases only recently have they received increased attention as awareness of the unique and sensitive ELSI issues these conditions present [38].

Although bioethics principles as autonomy, beneficence, confidentiality are usually considered in research, in DSD researchers and health professionals still deal with some conflicts, such as parents' consent in the "best interest of the child"; management of genital ambiguity and the consequences of reassigning sex at an early age and postponing interventions until the child/adolescent can take part on the decision.

Some ethical guidelines for addressing children with DSD focusing on principles and processes for the well-being of the child and future adult are presented by Gillam, Hewitt, and Warne (2010) [39] and include minimizing physical and psychosocial risk to child; preserving potential for fertility; preserving or promoting capacity to have satisfying sexual relations; leaving options open for the future, and respecting the parents' wishes and beliefs.

Undoubtedly, vulnerability is one important issue for patients with DSD and since this can be an asymmetrical condition, in which persons are not equally affected, authors adopt the concept of layers of vulnerability [40] to inform the debate on the interlinked ELSI in the reported cases.

Educational, cultural, and economic conditions

The patients here described belong to low-income families, with low educational background, living in rural and poor areas in the Northeast of Brazil. These characteristics substantially compromise their understanding of the medical facts, an issue that may lead to some observed attitudes such as conformism and acceptance based on religious beliefs as in Antonio's case, and fear, incomprehension, and anguish, as in the cases of Roberto and Ana.

Stigma and prejudice, when there is ignorance and lack of understanding of the underlying causes of the condition, result in feelings of guilty on the parents' side, shame for parents and child/adolescent and often bullying in school age.

Another important issue that may impact the process of care is the *modus operandi* of the health system itself and the complexity of the clinical condition. Thus, Thus, lack of coordination between health care levels, centralization of diagnostic tests and specialists in hospitals located in the state capital, and failure to ensure access and follow-up by the public health system can be major barriers. For the families reported here, this meant traveling efforts, repeated consultations, many different kinds of laboratory exams and body exposure to different professionals, which also contributes to discomfort and breach of privacy.

Confidentiality and beneficence

Confidentiality, one of the cornerstones of bioethics, is a conflicting issue in these cases. This is a delicate aspect of health care because at the same time patients affected by DSD

do have the right to know and understand his/her condition the presence of parents, or even other professionals, at the consultation, may turn patients uncomfortable [41,42]. Respect to confidentiality and privacy in the relationship between patient-health professional may contribute to assure confidence of the child towards the professional involved in his/her treatment [41,42]. On the other hand, multidisciplinary is the approach currently recommended for these patients. The question is: how to assure confidentiality in this model of care?

Based upon the reported experience, authors should agree with Kipnis (2004) [41] that sometimes, in order to prevent a major damage, breach of confidentiality may be accepted. However, it is important to consider the meaning and the degree of breach of confidentiality. Farsighted that this is a multidisciplinary service, however, patients with DSD feel very uncomfortable with the exposure of their bodies to students and residents, especially when photographs and images of their inner body parts are to be obtained [43]. In the experience here reported, each health professional does care for privacy; for teenagers one of the parents remains in the consultation room but does not follow visually, unless the adolescent consents. In the case of children parents are asked if they would like to view the genitals and if pictures or images are to be taken, with parents´ consent, there is no one else from the multidisciplinary group in the room, but the attending physician.

The Informed Consent became the gold standard in recruiting patients/participants into a research, by respecting their autonomy and beneficence. However, when it comes to the care of children/adolescents with DSD, there still is an ethical dilemma for health professionals and the children's families [44].

Parental consent can be especially challenging, since it must be a process, an opportunity to clarify the condition in order for the decision to be taken in an informed, free and understood way and in the "best interest of the child". However, when dealing with

poverty, low levels of knowledge and lack of coordination of care, this may be very difficult and for the health professional, a challenge. Such reality may present many difficulties to provide the adequate and desirable care.

In some situations, parents ask for some time to think, in other cases they sign the consent form during the first meeting. Nevertheless, a well-prepared consent form, with clear, accurate information and in appropriate language for parents and children/adolescents, regardless of their level of education, is an obligation of the professional and his/her team. In addition to being useful for family members, it is a safeguard for those involved in the care of these patients.

Historic medical violations of patients' rights are well known in the DSD area [43]. 'Corrective' surgery and sex reassignment are among the most impacting aspects. In this respect there are great conflicts and dilemmas that have been modified as the paradigms of care and approach also have changed.

In recent years with better knowledge and genetic advances on DSD, the care of these patients has gone from the techno-centred and paternalistic model to that of patient-centred which, in this case, would be the best approach. Also, of great contribution has been society and patients' opening to the discussion [43,45].

The urgency to treat the condition is justified precisely in the face of patient's vulnerability. This is the case of some life-threatening and highly impacting situations such as major genitourinary malformations and adrenal crisis or precocious puberty in CAH. The last two are exemplified in this report by Ana and Roberto.

Genitoplasty is not an urgent treatment for genital ambiguity itself because it is not a surgical urgency. According to Marsh (2006) [46], there is no argument to postpone corrective surgery when there is a threat to life; however professional paternalism cannot

prevail when it is possible to postpone, so that the person can participate in the process, as the protagonist of the decision making.

If the patient is a child or an adolescent, to feel, see and know oneself as different from sisters and brothers, schoolmates, and friends, brings significant discomfort. The impossibility or difficulty in understanding his/her condition and the fact that someone else will decide for him/her which is the best approach or treatment, brings about many concerns and may lead to other problems [47].

Wiesemann et al. (2010) [48], when addressing the autonomy of patients with DSD, point to the moratorium on early surgery (unless medical emergency). They establish three important ethical principles: to focus on child welfare and the future adult, to defend the child's rights, and to respect family and the relationship between parents and children. For these authors, the child should actively participate in decisions about his/her condition, as they consider that they are fit and even entitled to veto. They also comment on the incongruities of establishing limited ages for assent/consent of the child/adolescent for such serious and irreversible decisions.

An appropriate care for patients with DSD must include complete information for parents, obtaining consent on multiple occasions over an extended time period and ensuring that parents are appropriately informed by a multidisciplinary team [15].

Reis (2019) [44] comments on cases of surgeries performed on patients with DSD and emphasizes that the care of these patients needs an improvement and that the abstract ideals of bioethics alone might not be sufficient to secure that improvement.

In Dickens (2018) [18] there is an in-depth review of some of the ELSI aspects related to patients with DSD as well as a statement of the Children's Convention

> *"…that in all actions concerning children, whether undertaken in public or private social welfare institutions, which include public*

and private healthcare facilities, the best interests of the child shall be a primary consideration"

This author also describes how a growing number of jurisdictions and government agencies have accommodated non-binary sex and gender classifications.

'Corrective' surgery was not discussed in the three cases herein reported. Nevertheless, the dilemmas of sex reassignment have emerged in the very beginning of the follow up, when diagnosis still were a hypothesis for parents. Based on this observation, Authors agree with Reis (2019) [44] and highlight the complexity of psychological, social, cultural, and religious issues crossing (bio)ethical statements.

Ana's parents, the newborn infant patient, made the decision to redefine her rearing sex. Parents have decided "on the best interest of the child". However, this decision may not be the best option for her in the future. Roberto's mother says she will continue raising her son as a boy but admits to rethinking if he decides to reassign his sex. Antonio, the adolescent, decided to change his social name and behaviour after the acceptance of his social environment.

In none of these cases the possibility of non-binary sex or gender was raised by the assisted families.

The role of parents is fundamental in supporting and minimizing the discomforts and stigmas that these children face. However, they also need support and education to understand the "genetic lottery" that offered these children with different and unexpected characteristics.

Caring for families in the DSD clinic includes to shelter parent's solutions and trying to help them from their defences against the real as something impossible to symbolize. The three reported cases are exemplary. Ana's father silence on the diagnosis depicts at the same time the real impossible to bear [32] and his own subjective elaboration process. This

real impossible to bear also comes up against ‘not thinking’ to ‘look after’ her child in the words of Roberto’s mother, while Antonio’s mother says she ‘always knew’ he was a boy.

Conclusions

To establish any degree of sensitivity or a value on the vulnerabilities that affect patients and parents under these conditions is not an easy task. In a scenario of poverty, lack of education and non-coordination of the health system, a satisfactory approach and care may be hard to achieve.

It is expected from health professionals the duty to mitigate the anguish, discomfort and uncertainties of patients and their families. However, in the clinical area of DSD it is equally important to consider that they may also be faced with vulnerability. A survey on health care of patients with genital ambiguity revealed the anguish and difficulties faced by professionals in their clinical practices notably marked by impasses, by the issue of human sexuality and the real of sex and the enigma of sexual difference [49].

As a standard of care to patients the DSD team has opted on a case-by-case agreement strategy. Decisions on what conduct to adopt and how to approach the diagnosis, therapeutic options and their implications are agreed upon in monthly clinical meetings. Moreover, there is the team’s effort to assure patients and family members that the different professionals are equally committed to patient care. As such, authors consider this is the way better care can be offered to the patients.

Recent advances in social and biological sciences have led to new understandings that the categorization of sex and gender is not simply binary, but much more complex, which has resulted in reflections and led health professionals to consider that persons with DSD are poorly understood.

With the knowledge available today, the concept of normality also deserves a reflection when a person's characteristics differ from what would be expected.

DSD cannot be addressed as a problem to be solved but as a condition to be addressed in such a way as to cause minimal damage and trauma to the affected person in a social context not yet prepared to accept such situation. This is true for patients themselves, parents, community, and professionals, and nowadays still leads to the error of urgency to correct or adjust to normality. However, in the face of psychic and genetic variability, what would be a normal pattern?

Understanding diversity requires knowledge and altruism; otherwise, persons with DSD are bullied, and often, there will be those who will want to adjust them to normality without taking into account their autonomy, the risks and benefits, thus breaking with the fundamental principles of bioethics – autonomy, beneficence, non-maleficence.

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APPENDICE

Original Portuguese transcriptions of patients and families' speeches:

"Until then he was a little boy and received a male civil registry"

"Até então era um menininho, ela foi registrada com nome de home".

"it did not matter whether it was a boy or a girl, for me it was a child and that's it"

"tanto fazia ser home ou mulher, pra mim era uma criança e pronto**".**

"but I think parents saw the baby in a different way. I do not understand it, because the father does not say anything about it"

"mas pra eles eu acho que eles via... hum... com um jeito diferente né. Eu não entendo não, porque ele num fala né".

"When her hormone levels are high changes can be seen in her body"

"No corpo dela, quando os hormônio dela tá alto, ela muda um pouquinho".

"a change in this anatomical site"

uma mudança 'disso aqui dela'

"this is a child and that is it"

"uma criança e pronto"

"I keep on thinking it's because of the hormone, which is masculine."

"Eu acho... eu fico pensando que é por causa dos hormônio, que é masculino, essas coisa"

"... I was already told that her preference for boy's toys has nothing to do with the hormone levels. But I keep on thinking whether it is due to the masculine hormone or not. But they said it has nothing to do with it. I know, but I am not sure"

"... já dissero que não tem nada a ver não, né, os hormônio com ela gostar de brinquedo de homem. Mas eu tenho... essa parte assim eu fico pensando, será que é por causa do hormônio ou não. Mas já dissero que não tem anda a ver não. Aí eu num sei, certeza não"

"we, once saw the tiny small penis, thought it was a boy, but for us it was normal ..."

"a gente por ver aquele... negocinho, paviuzinho, pensou que fosse um menino, mas para a gente era normal..."

"tiny small penis"

"paviuzinho"

"until the age of 7 for us he was a boy, even with that difference, he was a boy"

"até 7 anos para a gente era um menino, mesmo assim com aquele defeito, era um menino"

"And until today we see him as a boy. And we were shocked to realize that inside he is a girl"

"E até hoje a gente tá como um menino. E a gente tomou aquele choque de saber que por dentro ele é uma menina"

"inside he is a girl"

"por dentro ele é uma menina"

“We even talk at home, but we cannot accept what he biologically is, what he was born like”

“A gente até conversa em casa, mas não tem condições de querer ser o que ele é, o que ele nasceu”

"something from another world, no one believes it. It is so much trouble that we live day by day. I want to see him as a boy, as he sees himself, but if by any chance he chooses to be what he was born, we will see it in the future”

“coisa de outro mundo, ninguém acredita nisso. É tantos problemas que… assim, a gente vai levando. Vivendo um dia de cada vez. (...) Quero ver ele como um menino, como ele quer e ele tá vivendo, mas por acaso se ele optar por ser o que ele nasceu, aí a gente vê”

"Keep going."

“Ir levando”

"Even when I was pregnant, I thought it was a boy. I was sure it was a boy"

“Até mesmo quando eu tava grávida eu achava que era um menino, tinha certeza que era um menino”

"they said he was a girl. I just never believed it because I saw a boyish way"

“falaram que era uma menina. Só que eu nunca acreditei que era uma menina porque eu via jeito de menino”

"Then God gave me a dream showing that I was not a woman, that I was truly a man"

“Aí Deus me deu um sonho mostrando que eu não era mulher, que eu verdadeiramente era homem”

“The clergyman knew the truth this was not gender reassignment, but a genetic disease. Then I talked, the brothers accepted when I changed, cut my hair, changed clothes. They truly accepted it as something from God and I am going on since then"

“Ele soube da verdade isso não foi troca de gênero, mas foi uma doença genética. Ai eu conversei, os irmãos aceitaram quando eu mudei, cortei o cabelo, troquei de roupa. Eles aceitaram verdadeiramente de Deus e eu tô caminhando até hoje”.

Table 1. Laboratory data

Test	**Case 1** (neonate)	**Case 2** (six years old)	**Case 3** (15 years old)
17-OHP	1,211.8 ng/mL (RV: < 2.5 ng/mL)	2,000 ng/dL (RV: 8 – 258 ng/dL)	> 2,000 ng/dL (RV: < 130 ng/dL)
Testosterone	NP	198 ng/dL (RV: < 40 ng/dL)	490.51 ng/dL (RV: 10 – 75 ng/dL)
Abdominal US	Enlarged adrenal glands with modified texture. Normal uterus. Ovaries not visualized.	Normal uterus and ovaries.	Hypoplastic uterus. Ovaries not visualized.
Karyotype	46,XX[40]	46,XX[40]	46,XX[40]
CYP21A2 variant	p.Gln318*	c.290-13A/C>G	p.Ile172Asn

17-OHP: Hydroyprogesterone; US: Ultrasonography; RV: reference value; NP: not performed.

Apêndice B – Ambiguidade genital em paciente com distúrbio do desenvolvimento do sexo ovário-testicular (DDS OT) atendido em serviço de referência em genética clínica no SUS em Alagoas

36º CONGRESSO BRASILEIRO DE PEDIATRIA

CERTIFICADO

PONTUAÇÃO CNA
Protocolos 93829/93833

Certificamos que

Promoção:

Realização:

Apoio:

Nestlé

ILANNA FRAGOSO PEIXOTO GAZZANEO; CAMILA MAIA COSTA DE QUEIROZ;
RAFAELLA LIMA BORGES DE MENDONÇA; MARIA EDUARDA BAÍA CORREIA DE OLIVEIRA;
RICARDO LUIZ SIMÕES HOULY; ROSEMARY BARBOSA MARINHO;
WALTER FERREIRA DE ARAÚJO FILHO; REINALDO LUNA DE OMENA FILHO;
ISABELLA LOPES MONLLÉO

participou do **36º CONGRESSO BRASILEIRO DE PEDIATRIA** realizado no período de 08 a 12 de outubro de 2013 em Curitiba - PR, na qualidade de autores do POSTER:
AMBIGUIDADE GENITAL EM PACIENTE COM DISTÚRBIO DO DESENVOLVIMENTO DO SEXO OVÁRIO-TESTICULAR (DDS OT) ATENDIDO EM SERVIÇO DE REFERÊNCIA EM GENÉTICA CLÍNICA NO SUS EM ALAGOAS

Curitiba, 12 de outubro de 2013

Dr. Eduardo da Silva Vaz
Presidente da Sociedade Brasileira de Pediatria

Dra. Darci Vieira da Silva Bonetto
Presidente do 36º Congresso Brasileiro de Pediatria

Apêndice C – Diagnóstico tardio de Síndrome de Klinefelter 46,XX/47,XXY com distúrbio da diferenciação do sexo ovário-testicular: relato de caso

Evento Online
CBGM 2021
XXXII Congresso Brasileiro de
GENÉTICA MÉDICA
Genética: olhares e ações

Realização:
SBGM
Nº 132884

CERTIFICADO

Certificamos que

REINALDO LUNA DE OMENA FILHO, MARSHALL ITALO BARROS FONTES, DIOGO LUCAS LIMA DO NASCIMENTO, MICHEL ALVES DO NASCIMENTO, WALTER FERREIRA DE ARAÚJO FILHO, RAYANE FERREIRA DA SILVA, REGINALDO JOSÉ PETROLI, ISABELLA LOPES MONLLEO

Participou(aram) do XXXII Congresso Brasileiro de Genética Médica, realizado totalmente ONLINE nos dias 30 de abril e 01 de maio de 2021
Na qualidade de autor(es) do Trabalho Científico - Poster Eletrônico: DIAGNÓSTICO TARDIO DE SÍNDROME DE KLINEFELTER 46,XX/47,XXY COM DISTÚRBIO DA DIFERENCIAÇÃO DO SEXO OVÁRIO-TESTICULAR: RELATO DE CASO

01 de Maio de 2021.

Código de Autenticação: 9QJH7Y

TÊMIS MARIA FÉLIX
PRESIDENTE DA SBGM

SALMO RASKIN
PRESIDENTE DO CONGRESSO

Verifique a autenticidade em www.e-certif.com.br

Evento Online
CBGM 2021
XXXII Congresso Brasileiro de
GENÉTICA MÉDICA
Genética: olhares e ações

ANAIS

TEMAS LIVRES APRESENTAÇÃO POSTER ELETRÔNICPO

PE-121 - DIAGNÓSTICO TARDIO DE SÍNDROME DE KLINEFELTER 46,XX/47,XXY COM DISTÚRBIO DA DIFERENCIAÇÃO DO SEXO OVÁRIO-TESTICULAR: RELATO DE CASO

REINALDO LUNA DE OMENA FILHO (UNIVERSIDADE FEDERAL DE ALAGOAS / UNIVERSIDADE ESTADUAL DE CIÊNCIAS DA SAÚDE DE ALAGOAS), MARSHALL ITALO BARROS FONTES (UNIVERSIDADE FEDERAL DE ALAGOAS / UNIVERSIDADE ESTADUAL DE CIÊNCIAS DA SAÚDE DE ALAGOAS), DIOGO LUCAS LIMA DO NASCIMENTO (UNIVERSIDADE FEDERAL DE ALAGOAS / UNIVERSIDADE ESTADUAL DE CIÊNCIAS DA SAÚDE DE ALAGOAS), MICHEL ALVES DO NASCIMENTO (UNIVERSIDADE ESTADUAL DE CIÊNCIAS DA SAÚDE DE ALAGOAS), WALTER FERREIRA DE ARAÚJO FILHO (UNIVERSIDADE FEDERAL DE ALAGOAS), RAYANE FERREIRA DA SILVA (UNIVERSIDADE FEDERAL DE ALAGOAS), REGINALDO JOSÉ PETROLI (UNIVERSIDADE FEDERAL DE ALAGOAS), ISABELLA LOPES MONLLEÓ (UNIVERSIDADE FEDERAL DE ALAGOAS)

Introdução: A Síndrome de Klinefelter (SK) clássica tem ocorre em 1:500 nascimentos masculinos, manifesta-se como hipogonadismo hipergonadotrófico, sem ambiguidade genital, com cariótipo 47,XXY em mais de 80% dos casos. O Distúrbio da Diferenciação do Sexo Ovário-testicular (DDS OT) é uma condição rara, na qual coexistem tecidos ovariano e testicular, com ou sem derivados mullerianos. A genitália pode ser masculina, feminina ou ambígua. Os cariótipos compreendem 46,XX (70%), seguido por 46,XX/46,XY e 46,XY. Relato do caso: Adolescente com 4 genitoplastias masculinizantes, apresenta-se para a avaliação genética aos 10 anos de idade. Nega hematúria e dor escrotal. Desenvolvimento sexual G1/P2 (Tanner), ausência de ginecomastia, falo com 57 mm, hipospádia, gônada esquerda no escroto (com 2 cm3). A ultrassonografia de abdome não identificou derivados mullerianos. Não se identificou útero através da videolaparoscopia e a biópsia gonadal mostrou: tecido ovariano com cistos foliculares à direita, tuba uterina congesta e tecido testicular atrófico à esquerda. FSH: 2,06 mUI/mL, LH: 0,49 mUI/mL e Testosterona: 25,40 ng/dL, caracterizando um hipogonadismo hipergonadotrófico. Cariótipo de sangue periférico: 46,XX[56]/47,XXY[4], com

presença dos marcadores de cromossomo Y (DYZ1, DYZ3, SRY e ZFY) confirmada através da PCR. Discussão: A SK 46,XX/47,XXY é uma condição ultrarrara, com 17 casos descritos nos últimos 50 anos. A despeito da ambiguidade genital reconhecida ao nascimento e de diversas intervenções cirúrgicas prévias, o presente caso foi diagnosticado tardiamente, mediante avaliação especializada com médico geneticista. A utilização conjunta de recursos da citogenética, genética molecular e histopatologia gonadal foi fundamental para o estabelecimento do diagnóstico. Conclusão: Embora tardio, o reconhecimento do diagnóstico permitirá o acompanhamento da puberdade, a prevenção de comorbidades, especialmente neoplasias, e a orientação e suporte às decisões reprodutivas do indivíduo. Este caso exemplifica a importância da atuação multidisciplinar nos DDS e o papel do médico geneticista na equipe.

ANEXOS

ANEXO A – Protocolo genético-clínico

SERVIÇO DE GENÉTICA CLÍNICA

AMBULATÓRIO DDS - PROTOCOLO GENÉTICO-CLÍNICO

REG DDS: **REG HUPAA:** **DATA:**

I – IDENTIFICAÇÃO

Nome:

Data de nascimento: Idade:

Sexo atribuído pela família: ( ) Masculino ( ) Feminino ( ) Nenhum

Registro civil: ( ) Não ( ) Sim

Naturalidade (cidade e UF):

Procedência (cidade e UF):

Forma de acesso ao Serviço:

( ) Demanda espontânea. Encaminhado por (especialidade):

( ) Outra. Especifique:

Nome da mãe, data de nascimento e idade atual:

Nome do pai data de nascimento e idade atual:

Endereço completo e telefones para contato:

II – QUEIXA(S)

( ) Ambiguidade genital

( ) Hipospadia ( ) Micropênis ( ) Criptorquidia ( ) Ginecomastia

( ) Clitoromegalia ( ) Fusão labial ( ) Virilização ( ) Puberdade precoce

( ) Infertilidade ( ) Amenorréia primária ( ) Hipodesenvolvimento sexual secundário

( ) Gônadas palpáveis em pessoa aparentemente do sexo feminino

( ) Outro(s). Especifique:

III – ANTECEDENTES GESTACIONAIS E DO PARTO

Usou Testosterona e derivados (Danazol); progesterona e derivados (19-nortestoterona, noretindrona, estirona e o noretinodrel); Tamoxifeno; Clomifeno; Dietilestilbestrol:

( ) Não ( ) SI ( ) Sim. Especifique:

Gestação: ( ) Pré-Termo (<37 sem) ( ) Termo (37-42 sem) ( ) Pós-termo (>42 sem)

Peso: Comprimento: PC:

IV - RECORRÊNCIA FAMILIAL DE:

( ) Sem recorrência ( ) Ambiguidade genital

( ) Hipospadia ( ) Micropênis ( ) Criptorquidia ( ) Ginecomastia

( ) Clitoromegalia ( ) Fusão labial ( ) Virilização ( ) Puberdade precoce

( ) Infertilidade ( ) Atraso puberal ( ) Amenorréia primária

( ) Hipodesenvolvimento sexual secundário

( ) Gônadas palpáveis em pessoa aparentemente do sexo feminino

V - HEREDOGRAMA (mínimo 3 gerações – pais, irmãos, avós - em todos os casos)

VI – SUMÁRIO DOS FATORES DE RISCO

Consanguinidade parental: ( ) Não ( )SI ( ) Sim. Especifique:

Mãe ≥ 35 anos (no momento da concepção): ( ) Não ( )SI ( ) Sim

Pai ≥ 40 anos (no momento da concepção): ( ) Não ( )SI ( ) Sim

Recorrência familial do distúrbio: ( ) Não ( )SI/Duvidoso ( ) Sim

Ocorrência familial de outro DC: ( ) Não ( )SI/Duvidoso ( ) Sim

Exposição teratogênica: ( ) Não ( )SI/Duvidoso ( ) Sim

VII – EXAME FÍSICO GERAL:

PC: Altura: Envergadura: Peso:

VIII - EXAME FÍSICO DIRIGIDO:

Manifestações Genitais (PRADER): ( ) P1 ( ) P2 ( ) P3 ( ) P4 ( ) P5

(QUIGLEY): ( ) Q1 ( ) Q2 ( ) Q3 ( ) Q4 ( ) Q5 ( ) Q6/7

( ) Genitália Feminina ( ) Genitália Masculina ( ) Micropênis ( ) Hipoplasia Genital

( ) Hipospádia glandar ( ) Fusão labial posterior ( ) Malformada ( ) Criptorquidia bilateral

Manifestações Associadas:

( ) Hipodesenvolvimento Sexual Secundário ( ) Ginecomastia ( ) Amenorréia Primária ( ) Baixa Estatura ( ) Virilização ( ) Heterocromia de Íris ( ) Hiposmia/Anosmia

( ) Surdez ( ) Sincinesia bimanual ( ) Sem anormalidades

Gônada palpável: ( ) Não ( )Bilateral ( ) Unilateral (E ou D)

Volume: Localização:

Localização do meato uretral:
Tamanho do falo/clitóris/pênis esticado (mm): Diâmetro do falo/clitóris/pênis esticado (mm):
Hiperpigmentação em genitália: ( ) Não ( ) Sim
Desenvolvimento mamário (TANNER): ( ) M1 ( ) M2 ( ) M3 ( ) M4 ( ) M5
Desenvolvimento genital (TANNER): ( ) G1 ( ) G2 ( ) G3 ( ) G4 ( ) G5
Desenvolvimento pelos (TANNER): ( ) P1 ( ) P2 ( ) P3 ( ) P4 ( ) P5

IX – Registro fotográfico: ( ) Não ( ) Sim . Número das fotos:
X – Exames laboratoriais prévios ou solicitados na 1ª consulta:

XI – Manifestações associadas detectadas em exames complementares:
( ) Derivados Mullerianos ( ) Agenesia/Malformações do TGU ( ) Agenesia de gônadas

( ) Coarctação de aorta

XII – Tipo ou subtipo do distúrbio: ( ) DDG ( ) DDS XX ( ) DDS XY ( ) DGU
XIII – Diagnóstico nosológico:
XIV – Situação do diagnóstico nosológico: ( ) Suspeito ( ) Conclusivo
XV – Se o diagnóstico nosológico for conclusivo, qual a evidência?
( ) Cariótipo ( ) Histopatológico ( ) Exames de imagens
( ) Testes moleculares ( ) Outro. Qual?

XVI – Diagnóstico etiológico:

XVII – Tipo de acompanhamento
É paciente índice? ( ) Sim ( ) Não. Qual relação com índice?
É caso familial? ( ) Não ( ) Sim. Quem?
É casal? ( ) Não ( ) Sim. Quem?
OUTROS DADOS RELEVANTES:

Responsável pelo preenchimento

 # AMBULATÓRIO INTEGRADO DE DISTÚRBIOS DO DESENVOLVIMENTO DO SEXO#

NOME:

MÃE:

PAI:

REG / HUPAA:

REG/PROJETO:

DATA DA 1ª CONSULTA:

DATA DE NASCIMENTO:

PESO AO NASCER:

FORMA DE ACESSO AO SERVIÇO:

FATORES DE RISCO:

SUMÁRIO DO CASO:

EXAMES COMPLEMENTARES:

Exame	Data da Solicitação	Data do Resultado	Resultado

HIPÓTESES DIAGNÓSTICAS:

SEGUIMENTO:

ANEXO B – Protocolo de aprovação do CEP – UFAL

UNIVERSIDADE FEDERAL DE ALAGOAS - UFAL - Comissão de BIOÉTICA

UNIVERSIDADE FEDERAL DE ALAGOAS
COMITÊ DE ÉTICA EM PESQUISA

Maceió – AL, 25/09/2009

Senhor (a) Pesquisador (a), Suzane Vasconcelos Zanotti
Isabella Lopes Monlléo
Carlos Guilerme Gaelzer Porciuncula

O Comitê de Ética em Pesquisa (CEP), em 25/09/2009 e com base no parecer emitido pelo (a) relator (a) do processo nº **010367/2009-29** sob o título **Atenção integrada em saúde a pacientes com ambiguidade genital em hospital terciário do SUS em Alagoas**, vem por meio deste instrumento comunicar a aprovação do processo supra citado, com base no item VIII.13, b, da Resolução nº 196/96.

O CEP deve ser informado de todos os efeitos adversos ou fatos relevantes que alterem o curso normal do estudo (Res. CNS 196/96, item V.4).

É papel do(a) pesquisador(a) assegurar medidas imediatas adequadas frente a evento grave ocorrido (mesmo que tenha sido em outro centro) e enviar notificação ao CEP e à Agência Nacional de Vigilância Sanitária – ANVISA – junto com seu posicionamento.

Eventuais modificações ou emendas ao protocolo devem ser apresentadas ao CEP de forma clara e sucinta, identificando a parte do protocolo a ser modificada e sua justificativa. Em caso de projeto do Grupo I ou II apresentados anteriormente à ANVISA, o(a) pesquisador(a) ou patrocinador(a) deve enviá-los à mesma junto com o parecer aprovatório do CEP, para serem incluídas ao protocolo inicial (Res. 251/97, item IV. 2.e).

Relatórios parciais e finais devem ser apresentados ao CEP, de acordo com os prazos estabelecidos no Cronograma do Protocolo e na Res. CNS, 196/96.

Na eventualidade de esclarecimentos adicionais, este Comitê coloca-se a disposição dos interessados para o acompanhamento da pesquisa em seus dilemas éticos e exigências contidas nas Resoluções supra - referidas.

(*) Áreas temáticas especiais

ANEXO C – Protocolo de aprovação do CEP – UFAL

UNIVERSIDADE FEDERAL DE ALAGOAS
COMITÊ DE ÉTICA EM PESQUISA

Maceió – AL, 05/11/2013

Senhor(a) Pesquisador(a), SUSANE VASCONCELOS ZANOTTI
ISABELLA LOPES MONLLEO
REINALDO LUNA DE OMENA FILHO

O Comitê de Ética em Pesquisa (CEP), em 29/10/2013 e com base no parecer emitido pelo (a) relator (a) do processo nº **19144013.5.0000.5013** sob o título, **DISTÚRBIOS DA DIFERENCIAÇÃO DO SEXO EM ALAGOAS: ABORDAGEM CLÍNICA NO SUS,** vem por meio deste instrumento, comunicar a APROVAÇÃO do processo supra citado, com base no artigo X, parágrafo X.2, alínea 5.a, da Resolução nº 466/12.

O CEP deve ser informado de todos os efeitos adversos ou fatos relevantes que alterem o curso normal do estudo (Res. CNS 466/12, item V.3).

É papel do(a) pesquisador(a) assegurar medidas imediatas adequadas frente a evento grave ocorrido (mesmo que tenha sido em outro centro) e enviar notificação ao CEP e à Agência Nacional de Vigilância Sanitária – ANVISA – junto com seu posicionamento.

ANEXO D – Protocolo de aprovação do CEP – UFAL

UNIVERSIDADE FEDERAL DE ALAGOAS

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Caracterização de Distúrbios da Diferenciação do Sexo em Alagoas: uma abordagem multidisciplinar no SUS

Pesquisador: SUSANE VASCONCELOS ZANOTTI

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);

Versão: 1

CAAE: 59929716.8.0000.5013

Instituição Proponente: Universidade Federal de Alagoas

Patrocinador Principal: Financiamento Próprio

ANEXO E – Protocolo de aprovação do CEP – UFAL

UNIVERSIDADE FEDERAL DE ALAGOAS

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: CARACTERIZAÇÃO MOLECULAR DE FAMÍLIAS COM HIPERPLASIA ADRENAL CONGÊNITA EM ALAGOAS: ESTUDO PILOTO

Pesquisador: Reginaldo José Petroli

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);

Versão: 1

CAAE: 59931616.6.0000.5013

Instituição Proponente: UNIVERSIDADE FEDERAL DE ALAGOAS

Patrocinador Principal: Financiamento Próprio