Riga Stradins University

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MOLECULAR MECHANISMS, IMMUNOGENETIC CHARACTERISTICS, CLINICAL AND THERAPEUTICAL CONSEQUENCES OF THE 21-HYDROXYLASE DEFICIENCY IN THE LATVIAN CHILDREN

(Speciality – paediatrics)

SUMMARY OF THE DOCTORAL DISSERTATION

Riga, 2010

The Doctorate work was prepared:

- Clinical part of the Doctoral program performed at the Children Clinical University Hospital in Riga;
- At the Riga Stradins University;
- Experimental part performed at the Children Endocrinology Centre and at the Endocrinology Scientific Laboratory of Karolinska Institute in Sweden, Stockholm.

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The Doctorate thesis can be acquainted at the Riga Stradins University Library.

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Abbreviations used in the paper

Numbers
11beta-HSD - 11-beta hydroxysteroid-dehydrogenase
17-HSD - 17- hydroxysteroid-dehydrogenase
17-OH-P - 17-hydroxy-progesterone
21-H - 21-hydroxylase
21-HD - 21-hydroxylase deficiency
3-beta-HSD - 3- hydroxysteroid-dehydrogenase
A
ACTH - adrenocorticotropic hormone
B
BKUS – Children Clinical University Hospital
C
cAMF - cyclic adenosine-monophosphate
CRH - corticotrophin-releasing hormone
CAH – congenital adrenal hyperplasia
D
ddNTF - 2,3-di-desoxinucleoside triphosphate
DHEA - dehydro-epi-androsterone
DHEA-S - dehydro-epi-androsterone sulphate
DNA – desoxinucleinic acid
DOC – desoxicorticosterone
E
ECG - electrocardiogram
ESPE - European Society of Pediatric Endocrinology
G
GC - glucocorticoids
GnRH - gonadotropin-releasing hormone
I
IGF - insulin-like growth factor
P
P - progesterone
PCR - polymerase chain reaction
PRA - plasma renin activity
S
StAR - acute regulating protein of steroidgenesis
SL – salt lost form of the CAH
T
TNF - tumour necrosis factor
V
SV - simple virilising form of the CAH

1. Introduction

Congenital adrenal hyperplasia (CAH) or the hereditary adrenogenital syndrome (AGS) is one of the most common congenital metabolic defects based on the deficiency of one or several steroid biosynthesis enzymes that is autosomal-recessive and monogenically determined. Deficiency of each of these enzymes results in particular, typical metabolic and clinical signs in CAH patients, but the common characteristic symptom in such a patient is the development of glucocorticoid insufficiency with the consequential compensatory, adrenocorticotropic hormone (ACTH)-hyperstimulated hyperplasia of the adrenal cortex.

1.1. Topicality of the research paper

Clinicians and scientists engaged in the medical research do pay more and more attention to the hormonal processes in critically ill patients trying to find out the factors the changes of which determine the disease outcome in such situations. Without doubt, adrenal cortex hormones are the one of most essential among these factors, therefore, individuals with hereditary secretional deficiency of these hormones promote special clinical and theoretical interest, since controlled adrenal cortex insufficiency may develop into uncontrolled in stress situations resulting in a life threatening acute adrenal insufficiency crisis symptoms. Since the incidence of milder forms of CAH in definite populations may be up to 1:100 [1, 5-7], the topicality of this problem should not be underestimated.

In Latvia, no CAH study in both the clinical and scientific direction has been performed up to now.

HLA III class genes studies in a specific population are urgent also due to the fact that the disease markers in different ethnic groups are not similar.

The Doctorate paper could give a development of a new CAH diagnostics option and a choice for a correct treatment strategy, including the prenatal diagnostics and treatment.

The main targets for a timely CAH diagnostics and treatment are:

- Reduction of neonatal mortality from undiagnosed acute adrenal insufficiency crisis in the case of CAH salt loss form,
- Establishment of a correct gender of the newborn in the case of IV - V level of virilisation (according to *Prader*), and avoidance of further medical, psychological and social problems caused by the gender change (according to the genetic gender),
- Reduction of the infertility problem.

The Doctorate paper is the first CAH study in children in Latvia.

1.2. Statement of the problem

Since CAH is a hereditary metabolic defect that may lead to the mortality of the newborn or the infant in the case of the salt loss form the direct cause of which is an acute adrenal insufficiency, the prenatal screening is strictly considered as justified for the establishment of this pathology and induction of the prenatal therapy. At present, this screening diagnostics is based mainly on the molecular genetic examinations, and it is an extensively introduced clinical routine method in many countries. According to the clinical experience up to now, steroid 21-hydroxilase deficiency may be caused

by many mutations of its gene (*CYP21* gene), besides, some of them are predominant in some populations [17,19,20,24,28,84].

Several unsolved problems exist clarification of which could improve the diagnostics of 21-hydroxilase deficiency and establishment of the disease risk and prognostic factors:

- Evaluation of the disease epidemiologic parameters dynamics to define precisely the role of CAH in the structure of pediatric chronic pathology, research of the disease initial versions development,
- Establishment of HLA III class genes and specific genes alleles mutations and relationship to CAH in order to identify immunogenetic markers of the disease and its clinical forms,
- Establishment of the risk factors (immunogenetic, clinical) for the disease in total and for its clinical forms,
- Establishment of predictive signs in patients with the simple virilizing form in order to develop timely algorithms for diagnostics of this form.

1.3. Purpose of the paper

Purpose of the Doctorate paper is to investigate molecular mechanisms, immunogenetic characteristics of the 21-hydroxilase deficiency and their clinical and therapeutic consequences in children in Latvia.

1.4. Work tasks

- To establish the potential relationship between the most common HLA haplotypes in Latvia related to CAH and the clinical forms of CAH, differences of CAH development HLA haplotypes in Latvia from the ones in Europe,
- To establish *CYP21* gene mutations predominant in Latvia,
- To establish differences of the predominant *CYP21* gene mutation range from the ones found in the European countries,
- Dynamic analyze of the course of CAH in included in the study children during the 5-years follow-up period.

1.5. Ideas posed for the presentation

- Predominant range of CYP21 gene mutations in Latvia is similar to the one found in European countries,
- CAH incidence in Latvia does not significantly differ from the one found in the Baltic and Nordic states region,
- CAH screening of newborns is an objective need to contribute to the reduction of infant mortality from timely undiagnosed acute adrenal insufficiency, to establish the correct gender of the newborn in the case of the girl's virilization, to reduce infertility problems in CAH patients during the fertile age.

1.6. Hypotheses

- The range of the predominant CYP21 gene mutations in CAH patients in Latvia is similar to the one found in European countries,

- HLA III class haplotypes in CAH patients in Latvia do not differ from the ones found in European countries.

2. Novelty of the results

1. For the first time, CAH epidemiologic data in Latvia have been collected and evaluated in progress. Since the data collection and analyses methodology used in the work has been chosen according to international standards, results obtained are comparable to results from another countries of the world. That, on its turn, has allowed to establish that the CAH morbidity in Latvia is lower compared to the one found in Northern European states.
2. CAH development risk factors in Latvia have been found not to differ significantly from those in the Northern European region, and they are related to the HLA-B14;DR1 haplotype and HLA-A3;Bw47;DR7 haplotype.
3. It has been established that Latvian patients with the CAH salt loss form predominantly have large deletions and conversions of *CYP21* gene, also point mutations (A / C to G) in the introne 2.
4. It has been established that Latvian patients with the CAH simple virilizing form predominantly have Ile-172 to Asn mutations.
5. It has been established that Latvian patients with the CAH classic form predominantly have Val281Leu and Pro30Leu mutations.

3. Structure and scope of the Doctorate paper

The Doctorate paper is written in Latvian. It consists of the Title page and 10 sections. The essence of the paper is given in 4 sections, but the rest 6 sections are devoted to the introduction, conclusions, novelty of results, synopsis of the work, literature references, and annexes, respectively. The paper is no 136 pages in typewriting. It has been illustrated by 22 tables and 52 figures. The paper has 27 annexes.

4. Materials and methods

4.1. Characteristics and selection of patients

Data on 53 patients with 21-hydroxilase deficiency used in the study have been aggregated in the Children Clinical University Hospital's (BKUS) Children and Adolescent Endocrinology Centre Congenital Adrenal Hyperplasia Patient Register. Register of the above mentioned disease patients thanks to the enthusiasm of physicians was started on 1989 based on the children endocrinology department.

Patients age was 0 to 18 years.

In the study, prospective and retrospective data analyses during the period from 1989 to 1992 was undertaken. In the retrospective study, all patients with CAH classic form were involved.

Patients were selected according to the IDC-10 diagnosis code E25.0.

Primary data source: BKUS endocrinology centre patients' hospital disease histories and out-patient records.

Secondary source: excerpts from out-patient records by family physicians.

53 disease cases of 21-hydroxilase deficiency (salt loss and simple virilizing forms) in Latvian children have been evaluated.

For each patient, a questionnaire "Initial information on CAH patient" and "Current information on CAH patient" was filled out and data were analysed.

Blood samples for immunogenetic and molecular genetic evaluations were taken during the period from 2000 to 2004 from all 53 patients following written consent from the parent or legal guardian of the child (consent and information forms on the study "Consent to the molecular genetic study" and "Information for the study subject's parents").

Prior to the involvement in the study, in all patients CAH diagnosis was confirmed based on diagnostic criteria developed by the ESPE (*European Society of Pediatric Endocrinology*).

Two control groups were arranged: for immunogenetic control: 20 healthy, non-relative children; for molecular genetic control: 10 healthy, non-relative children.

Venous blood samples were collected in cases not associated with an autoimmune, infectious or endocrine pathology. Control group children were matched to patients by the age and gender.

During the recent 5 years (year 2009 included) a follow-up of all 53 studied children with CAH was carried out and a course of their disease was dynamicaly analyzed using following criterias:

- clinical data – actual height, final height, age of puberty onset and age of menarche,
- biochemical findings – diagnostic levels of 17αOHPg, Na and K in blood and oscillations of the level of 17αOHPg during follow-up period,
- schedule of therapy,
- complications.

Children with CAH were under the care of BKUS Children and Adolescent Endocrinology Centre, therefore, data obtained in this research allow to a certain extent to judge on this problem in Latvia both in general and in some of its regions, as well as to compare the incidence of CAH in our country with the data in neirghbouring countries and world-wide.

Central Statistics Administration of the Republic of Latvia data on the annual birthrate and the number of children in Latvia have been used to calculate the epidemiologic parameters.

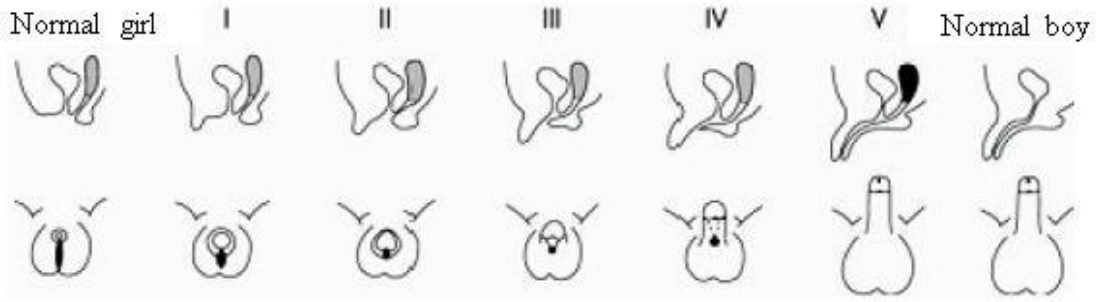
4.2. Study subjects

Data and clinical material from 53 paediatric patients with CAH were taken and analysed for this research. 30 patients were boys, but 23: girls; 4 of boys included in the study genetically were girls with CAH-related severe virilisation. In 29 patients, CAH salt loss clinical form was diagnosed, but in 24: simple virilizing form.

Development of external genitals was evaluated according to *Prader* virilisation scale (see figure 1).

Figure 1

Prader virilization scale [235].



Prader 0: Normal female external genitals.

Prader I: Female external genitals with enlarged clitoris: clitoromegalia.

Prader II: Clitoromegalia with partial merging of labia that form a funneled urogenital sinus; vagina and urethra opens in this funnelled formation.

Prader III: Clitoromegalia reaches the size of phallus; complete merging of labia that form an urogenital sinus where vagina and urethra opens; the urogenital sinus has one external opening.

Prader IV: External genitals are composed of phallus and completely merged labia making a scrotum-like formation; vagina and urethra opens in a common opening at the base of phallus.

Prader V: External genitals look like normal male external genitals; vagina and urethra opens in a common opening at the end of phallus.

Growth of patients was assessed according to charts of standard deviations (SDS).

Puberty was assessed according to Tanner criterias for puberty stages regarding the onset of puberty at testis volume 4 ml for boys, and breast enlargment for girls [237].

Level of 17αOHPg in blood was analyzed according to criterias of appropriate laboratory reference.

Genetic gender of the patient was established by a routine karyotype analysis at the State Genetics Centre.

4.3. Inclusion criteria

The study involved patients with clinically confirmed classic CAH form according to diagnostic criteria elaborated by ESPE [215] according to the IDC-10 diagnosis code E25.0.

Control group included BKUS patients with acute respiratory disease diagnosis without clinical and laboratory signs of any other health disorder.

4.4. Exclusion criteria

Patients who parents did not agree participating in this research study (theoretically). We did not receive any disagreement.

4.5. Diagnostics and classification

CAH is diagnosed by clinical, hormonal and molecular genetic criteria based on CAH diagnostic standards elaborated by ESPE and *Lawson* Wilkins Endocrine Society in collaboration in 2002 [3,4,31,209,210,213,215,218].

4.6. Applied methods

4.6.1. Primers used for *CYP21/CYP21P* molecular analysis

For *CYP21 / CYP21P* molecular analysis, primers manufactured by *Metabion International AG* (Germany) were used.

4.6.2. DNA extraction from blood

For extraction of chromosomal DNA (chrDNA), 2 to 3 ml of blood are used. Blood is collected in vacutainer tubes containing EDTA or citrate buffer, thus preventing blood clotting.

Blood is stored at - 20°C - 70°C. Repeated unfreezing of blood is not recommended.

chrDNA is extracted from leucocytes with the help of commercial *Qiagen* kit for DNA extraction from blood (*QIAamp® DNA Blood Mini Kit*) according to the technology approved by the manufacturer.

4.6.3. Detection of *CYP21* gene mutations with *PCR*

Polymerase chain reaction (*PCR*) is being extensively used in various biology and medicine fields, diagnostics, reproduction of DNA fragments, mutagenesis and sequencing.

The method is based on the DNA amplification performed by a thermostable polymerase (*Taq* polymerase). *PCR* is based on 20 to 40 times repeated DNA synthesis cycles. Each cycle of the synthesis consists of three stages of reaction: DNA denaturation, primer hybridization and DNA synthesis.

Material for testing 10 μL was suspended in 200 μL of normal saline, and 2 μL were used as a matrix for the amplification with specific primers (see Table 1). For the preparation of *PCR* amplification reaction of one sample, 10 mM dNTP mixture – 0.1 mM was used, corresponding primers 200 mM, MgCl2 – 1.5 mM, *Taq* polymerase - 1 V, distilled water added up to 12.5 ml.

PCR amplification product was rectified with kits according to the manufacturer's instructions. *PCR* amplification product was divided in 2% agarose gel for the use for DNA sequencing, and it was visualized with ethidium bromide.

4.6.4. DNA sequencing

For DNA sequencing, *Big Dye* kit (*Biosystems, UK*) was used. Reaction was carried out according to the manufacturer's instructions. Reaction was analysed with the help of the same primers like in the *PCR* reaction.

4.6.5. Haplotyping of HLA gene

1. HLA A,B,C genes amplification

Genome DNA have been obtained from patients' and control group's peripheral blood leucocytes. HLA gene 2nd polymorphous exon also underwent the amplification. Amplification *PCR* took place in the programmed terminal cycler (*Perkin Elmer, Cetus, Connecticut, USA*). HLA B amplification was done in 30 cycles: denaturation at the temperature 95^{o} C for 1 minute, hibridization at the temperature 55^{o} C for 1 minute, synthesis at the temperature 72^{o} C for 2 minutes. 35 cycles were used for HLA A amplification: denaturation at the temperature 94^{o} C for 1 minute, hibridization at the temperature 62^{o} C for 1 minute, and synthesis at the temperature 72^{o} C for 2 minutes. For the amplification of the second HLA exon, primers from *Erlich, Bugawan* were used.

2. HLA B gene typing

HLA B gene typing was performed with a low resolution polymerase chain reaction (*low resolution PCR-SSP*) using sequence-specific primers according to the methods specified by the manufacturer (*BA Gene; BAG Lich*, Germany). The main types of HLA B alleles have been identified.

4.7. Statistical analysis of the obtained results

SPSS program was used for the data treatment, T-test for the data comparison, but for the data analysis: descriptive and conclusive statistical methods.

According to the proposed tasks and kind of data, parametrical and non-parametrical statistical methods were used.

For the statistical treatment of the obtained results, the following methods were used:

- Central trend values with the calculation of the arithmetical mean,
- Dispersion for the data characterised by the standard deviation,
- Chi - square test (c2 test),
- CI analysis: (*Wilson* method) for the calculation of one sample data interval,
- *Newcombe* method: proportions and their differences,
- linear regression method.

As statistically reliable, significance level $p < 0.05$ was accepted.

For the evaluation of hypotheses, parameters differences were evaluated with the confidence 95% that corresponds to the significance level p=0.05.

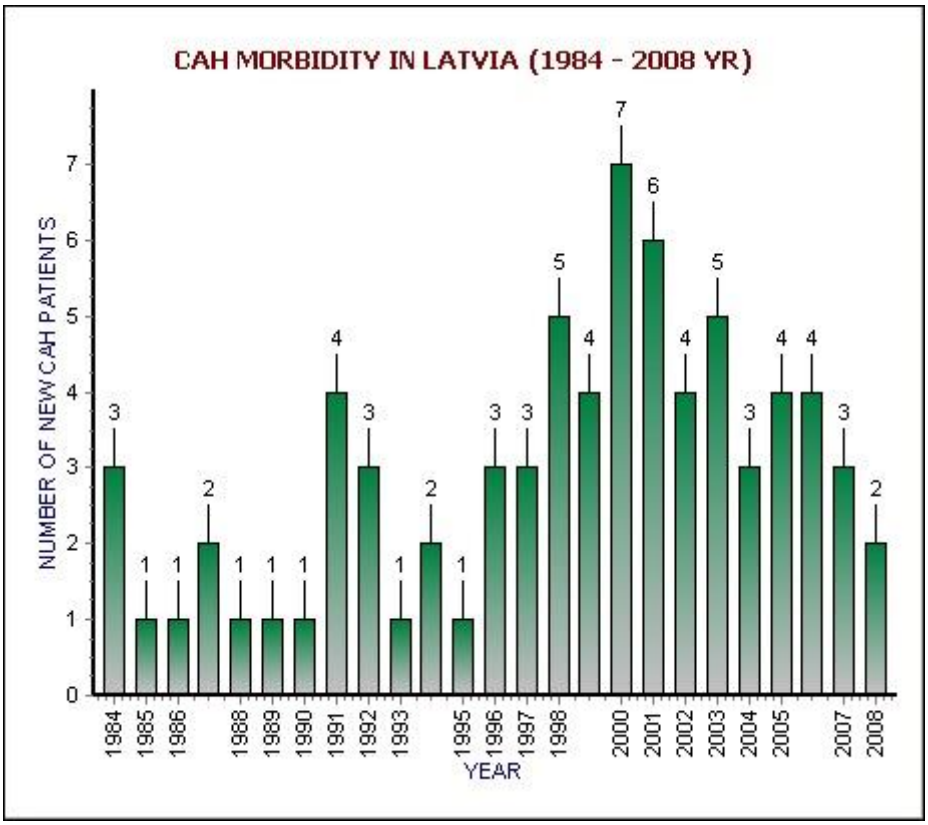
5. Results

5.1. Epidemiological research finding

The number of new CAH patients tends to increase during the last years. Namely, if the median number of new CAH patients is 1.6 per year during the first 5 years included in the research (from 1984 to 1988), then during the next 5 years (1989 - 1993): 2 per year, from 1994 till 1998: 2.8 per year, but during the last years of the research (i.e. 1999 - 2008) 5 new CAH patients per year on average have been diagnosed (see Figure 2).

Figure 2

CAH morbidity in Latvia (1984 - 2008)

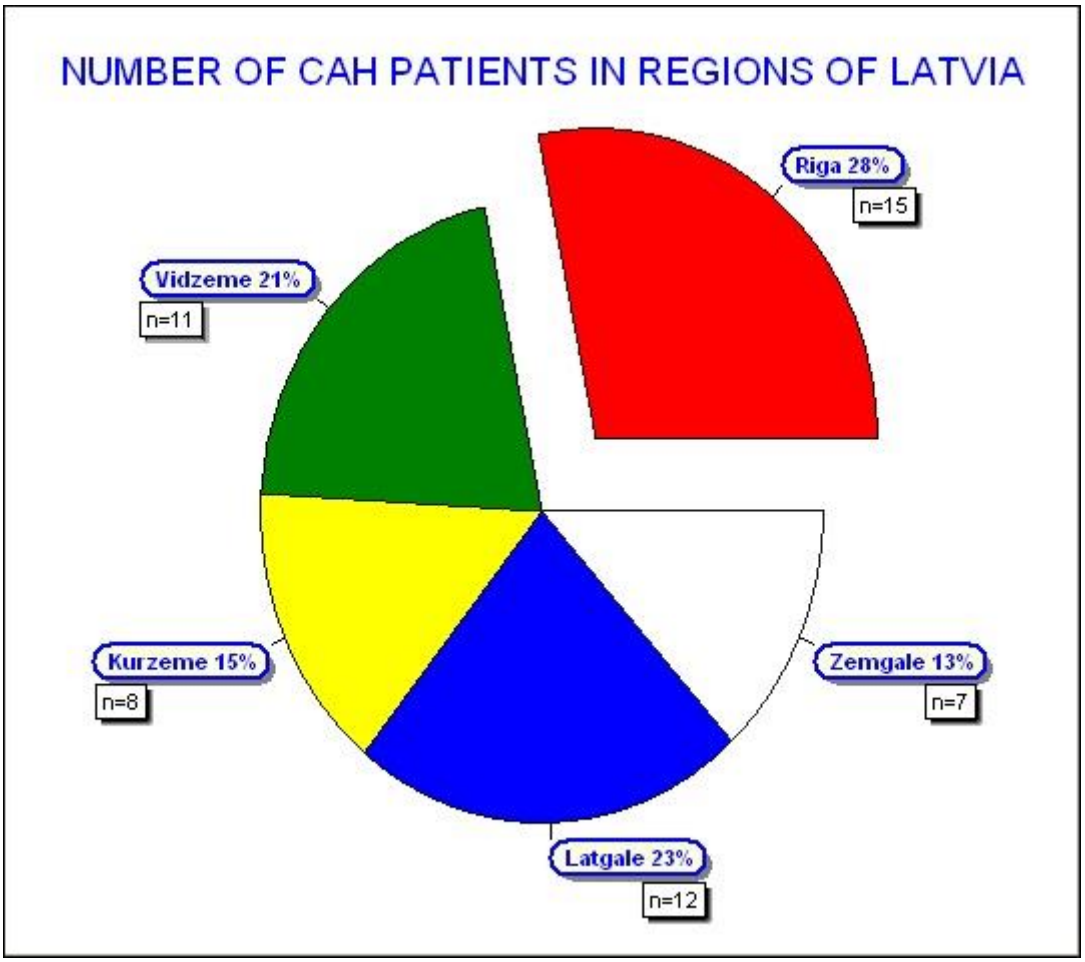


Almost one third of CAH patients included in the study live in Riga. Since the absolute number of patients is listed here, and the relation of the number of patients to the number of inhabitants is not considered, the result could be explained by the fact that almost half of the Latvian population live in Riga.

On the other turn, the number of CAH patients living in the Eastern part of Latvia (Vidzeme and Latgale) significantly exceed the number living in the Southern (Zemgale) and Western part (Kurzeme) of Latvia.

Figure 3

Number of CAH patients in the regions of Latvia



It can be seen that, out of the Latvian administrative units, more CAH patients live in the large towns: Riga, Daugavpils, Liepaja, Jurmala, Valmiera and Jelgava, but less in the southern and western regions of Latvia (see Figure 3).

The latest trend of increased number of new CAH patients can be attributed also to the new patients with CAH classic type salt loss form, i.e., if the number of these patients at the beginning of the monitoring period was on average 1 per year, then during the last years it is about 3 per year.

5.2. Evaluation of the clinical material

5.2.1. Patient population structure

The patient population structure shows that patient groups by the genetic gender are almost the same by numbers, and that patients with the salt loss form are more than patients with the simple virilizing form.

Figure 4 shows one of the main CAH problems: severe virilization of a girl when the gender is defined incorrectly at birth, i.e., 4 of boys included in the research turned out to be severely virilized girls with incorrectly defined gender at birth.

Figure 4

General characteristics of CAH patients

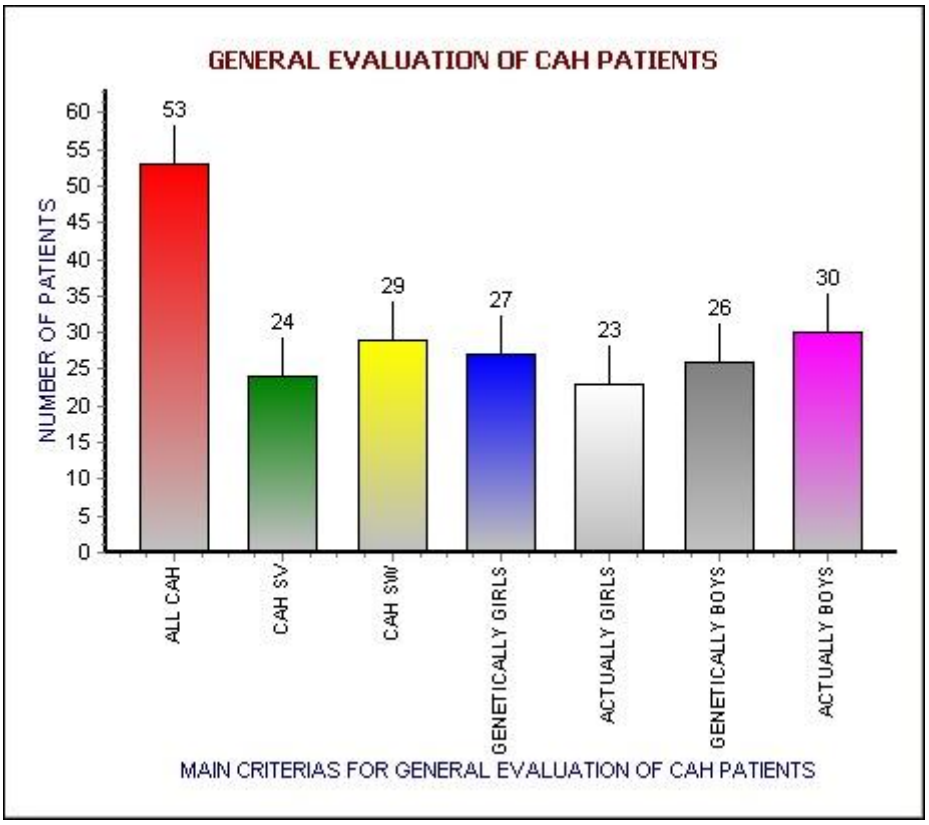
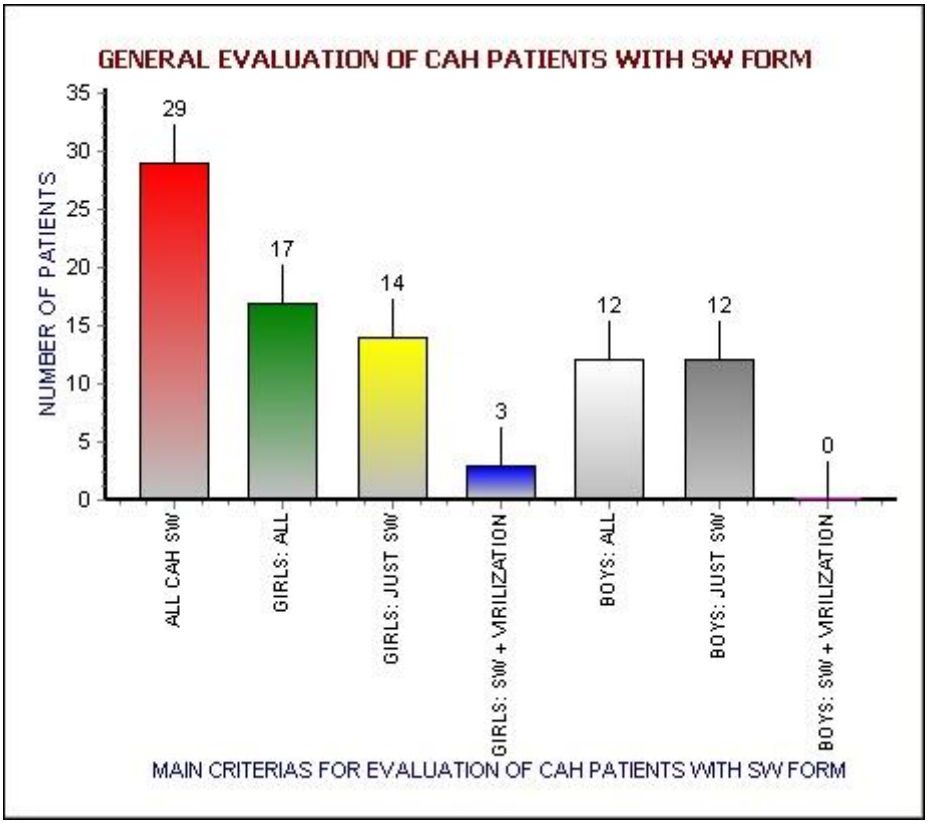


Figure 5

General characteristics of CAH patients with the salt loss form



Among CAH patients with the salt loss form included in the research, most are girls. Obtained results, as can be seen in the Figure 5, show that a severe virilization is not the leading symptom of this form.

On the other turn, in the group of patients with CAH simple virilizing form, boys prevail (which is difficult to explain, since the virilization in boys can be diagnosed only in very severe cases).

Four girls of genetic gender in whom the gender was diagnosed incorrectly at birth have CAH simple virilizing form.

Figure 6 shows that the virilization is the leading symptom of CAH simple virilizing form.

Virilization stages according to *Prader* III - V causes problems with a correct establishment of girl's gender at the obstetrician department. Commonly this mistakes is made, because external genitals are not palpated and the absence of testes is not established, and the phenotypical gender, boy, is established that does not correspond to the genetic gender, girl.

5.2.2. Patient characteristics by birth parameters related to the age at CAH diagnosis

CAH salt loss form in 20% of patients is diagnosed during the first 2 weeks of life, in about 40% of patients within 1 month, in about more 30% in the 2nd month, but when the age of 3 months is reached, the diagnosis has been established in all patients of this group (see Figure 7).

However, previously the CAH simple virilizing form in patients has been diagnosed within first 6 months and first 2 years (see Figure 8). It has been established by a noticed virilization. Another rise in a more active diagnostics is about the beginning of puberty. This is, however, established by a premature puberty onset in boys or delay in girls.

Figure 6

Characteristics of patients with CAH simple virilizing form

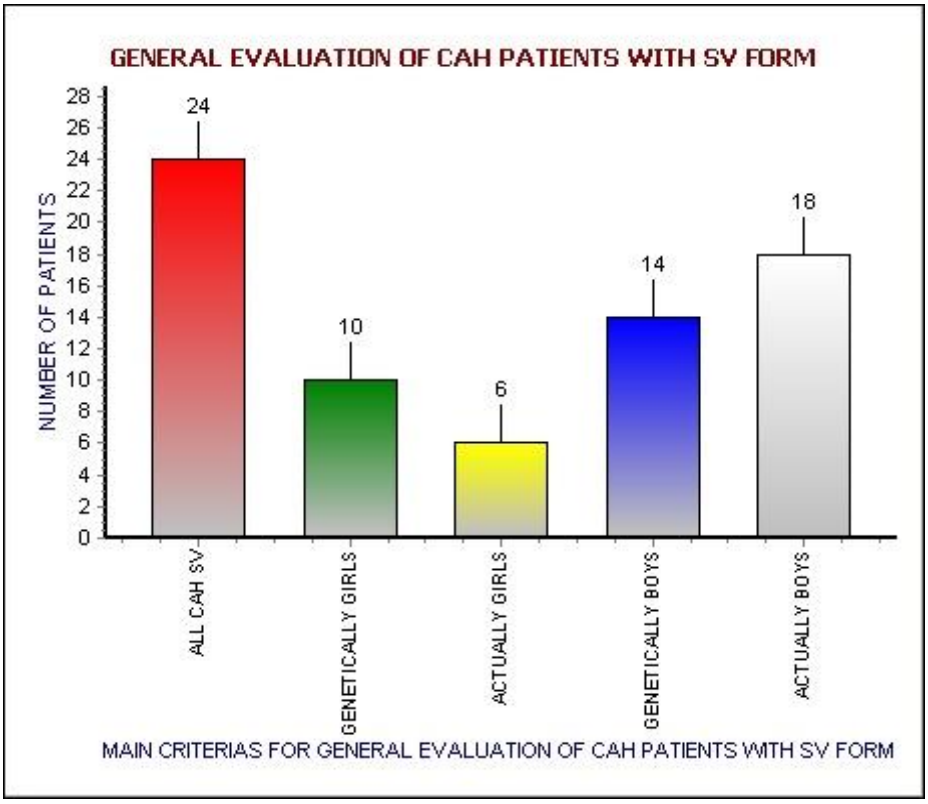


Figure 7

Age of CAH diagnostics in patients with the salt loss form

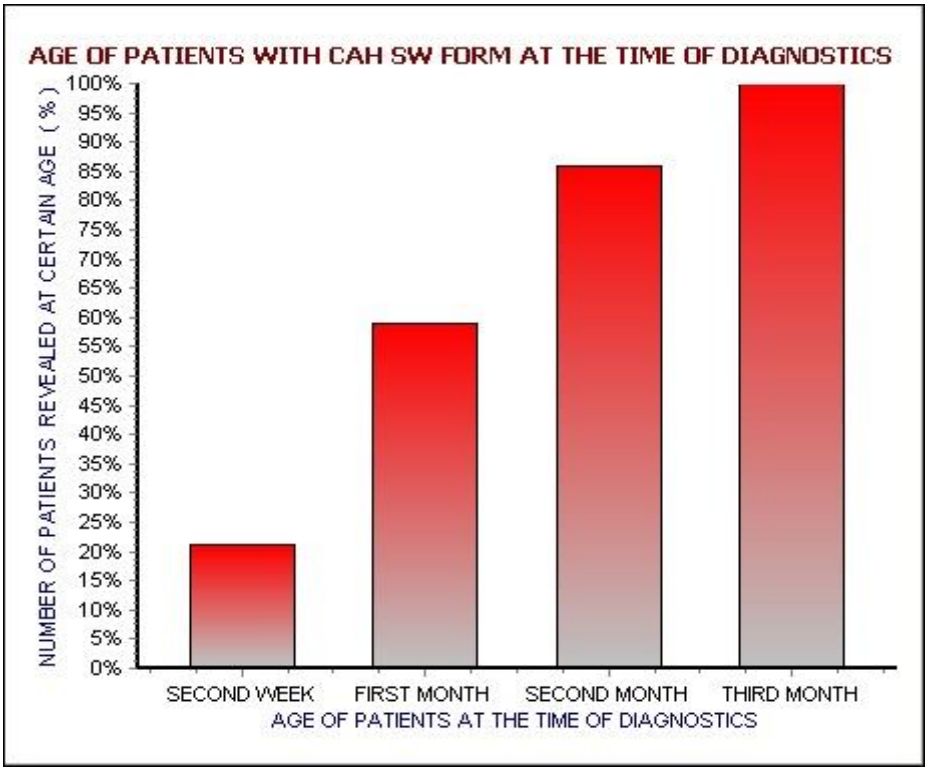


Figure 8

Age of CAH diagnostics in patients with the simple virilizing form

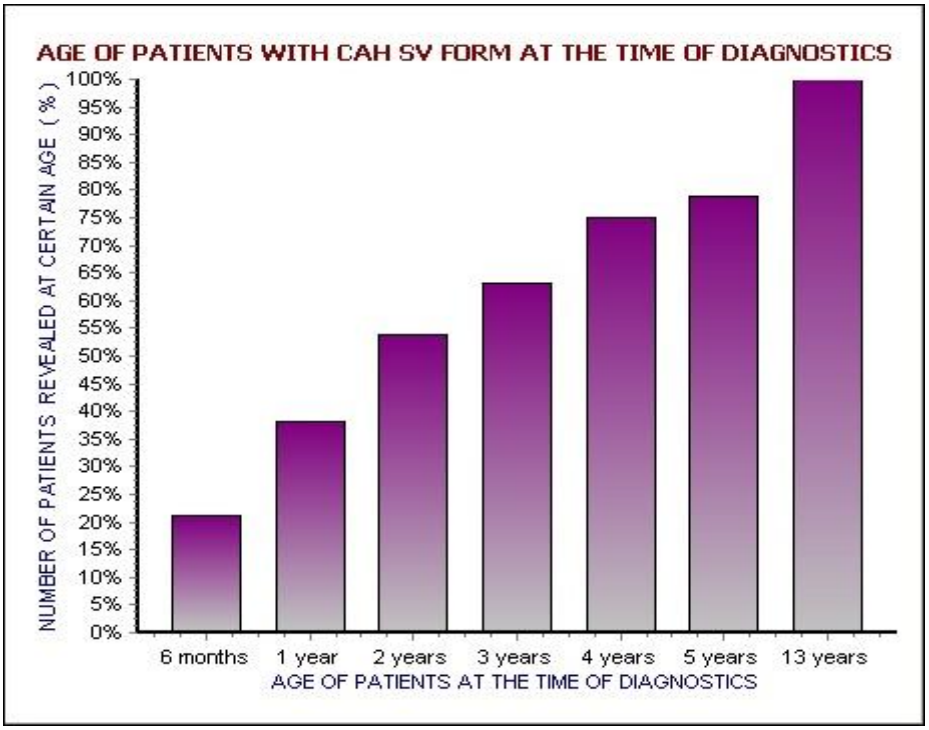
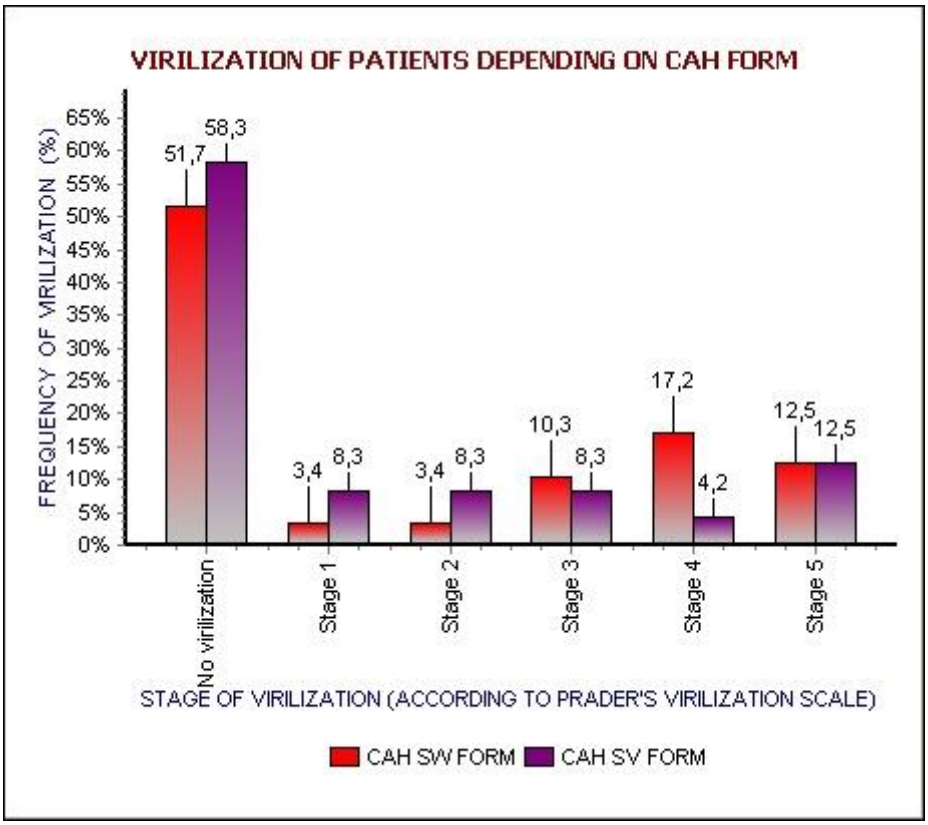


Figure 9

The level of virilization in CAH patients depending on the disease form



5.2.3. Patients characteristics by the virilization stage

Out of all 53 CAH patients involved in the study, virilization was observed in 24, i.e., 45.3%. In one third CAH patients involved in the study a severe virilization (Stage III according to *Prader* or higher) was diagnosed. At the same time in more than a half of patients no virilization was observed. This mainly applies to boys in whom the virilization can be noticed only in very severe cases. Besides, as can be seen in the Figure 9, disproportionally low seems the incidence of a mild virilization. It is possible that a mild virilization in these patients was considered as normal. So the biggest part of CAH patients without virilization are boys and virilization underdiagnostics.

In patients with CAH simple virilization form have all stages of virilization equally, but in patients with the salt loss form mild stage of virilization is less common, but more severe stages of virilization are more common.

In the case of CAH salt loss form, the severe virilization diagnosed should be attributed to the total deficiency of 21-hydroxilase that results in a high hyperandrogenemia already during the intrauterine period, and determines the development of gender differentiation disorders that may require surgery to be eliminated.

Totally, 35 genital plastic surgeries have been performed. In 11 patients genitals two-stage surgical corrections have been performed. In 4 cases, phenotypic gender does not correspond to the genetic one.

Most commonly, clitoris correction was required due to its enlargement.

Vaginal plastic surgery was mostly needed to remove the urogenital sinus.

In 3 cases, complex measures to change the gender were performed.

Genetic gender change (severe medical, legal and psychosocial problem) was performed in 3 cases, i.e. in 10% of the total number of surgeries (95% CI 3.5 - 25.6%).

Vaginal plastic surgery in CAH female patients due to severe virilization was performed within the first two years of life, clitoris correction up to the age of 4, but the gender change was performed at the age of 8 to 10 years (see Figure 10 and Figure 11).

Figure 10

Types of surgical plastics in virilized CAH girls

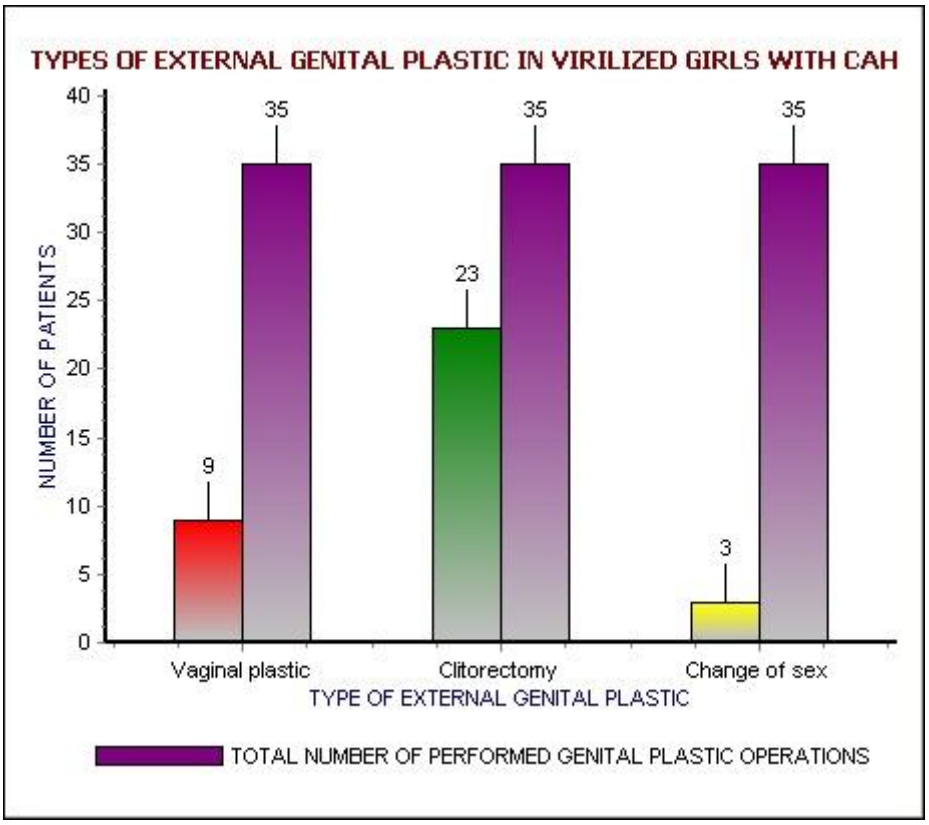
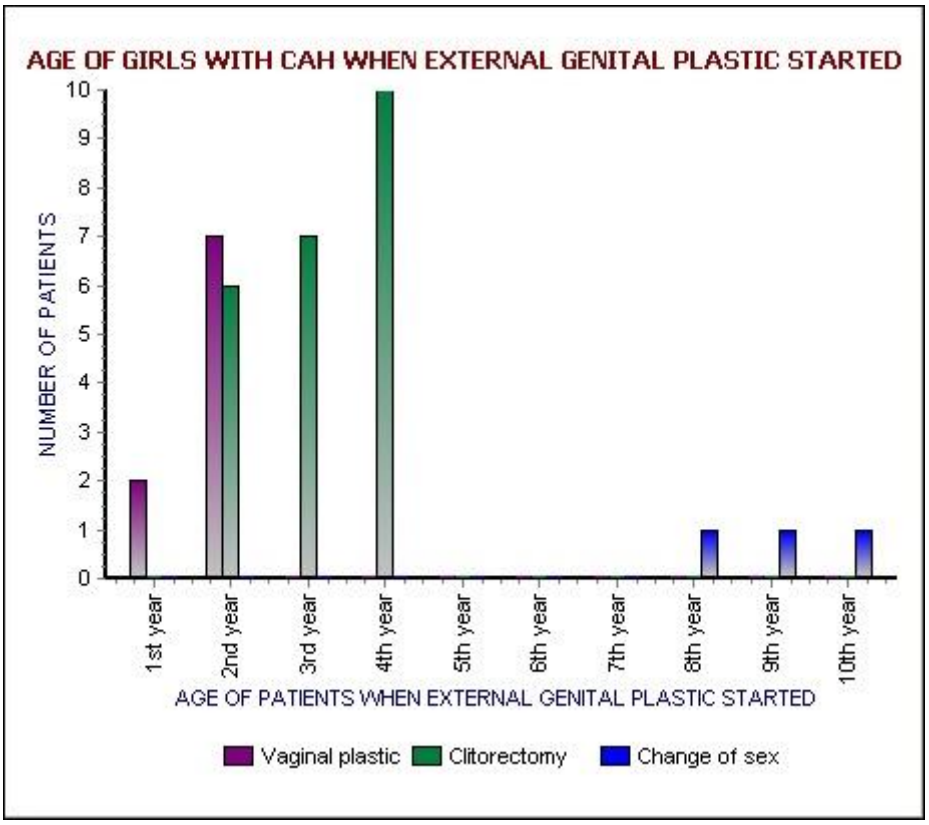


Figure 11

Age at the surgical plastics



5.3. Molecular genetic analysis of the clinical material

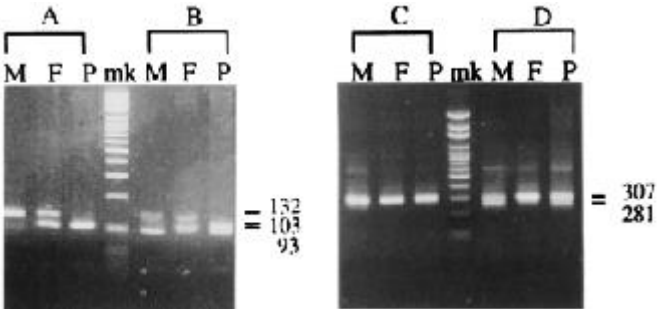
5.3.1. *CYP21A2* gene amplification with *PCR* method

During the work, chromosomal DNA was extracted from the patients' blood, then *CYP21A2* gene amplification with *PCR* method was performed, *CYP21A2* gene sequenced, and sequencing results were analysed.

Figure 12 shows an example of *CYP21P/CYP21* gene analysis performed in the study. A,B - *CYP21P/CYP21* molecule analysis following secondary *PCR* product (132-bp) – amplification with the help of Sacl restriction enzyme. C,D - "size-different" *PCR* products analysis by the amplification with mixed primers B1/2HP/IN3R. Primers B1/IN3R and 2HP/IN3R in a secondary *PCR* amplification activate 307- and 281-bp fragments. Primary *PCR* amplification with primers BF1/21BR is analyzed in A and B. Primary *PCR* amplification with mixed primers BF1/AF1/21BR is analyzed in C and D. Column "mk" - 100-bp molecular marker.

Figure 12

CYP 21P/CYP21 gene analysis following secondary PCR product (with the same type of primers)



5.3.2. *CYP21A2* gene sequencing and results obtained

For detection of mutations that can't be detected with the *PCR* methodology, sequencing was used.

All 10 gene *CYP21A2* exons were sequenced, since it is not long (only 3336bp). Results in exons 1, 2, 3 were convincing, since it was possible to sequence 847bp (P1+P48) *PCR* fragment precisely, however, the quality of sequences obtained in sequencing of 2508bp (P55+P4) *PCR* fragments is unsatisfying unfortunately.

For the development of a complete gene *CYP21A2* sequencing, it would be necessary to change the PCR strategy for *CYP21A2* gene exons 4-10 amplification, taking into consideration the existence of *CYP21A2P* pseudogene.

5.3.3. HLA locus finding in CAH patients

The research finding shows the relationship between HLA locuses A1, Bn47 and B60 with the development of CAH salt loss form, but HLA locuses B14 and Bw51 are related to the development of CAH simple virilizing form. HLA locus A3 is related to the development of both CAH salt loss and simple virilizing form (see Figure 13 and Figure 14).

Figure 13

Relationship of HLA locus finding in CAH patients with SL and SV forms

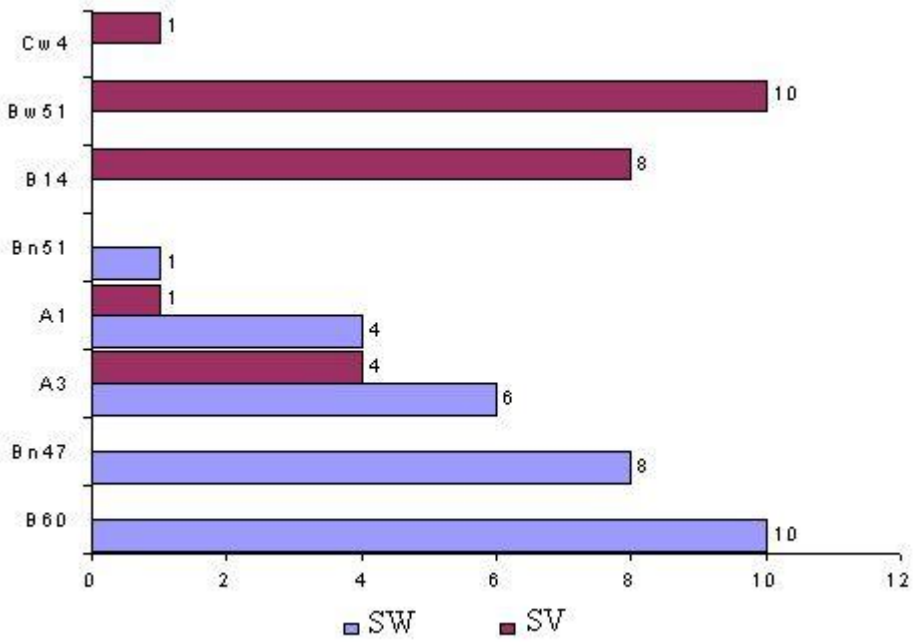
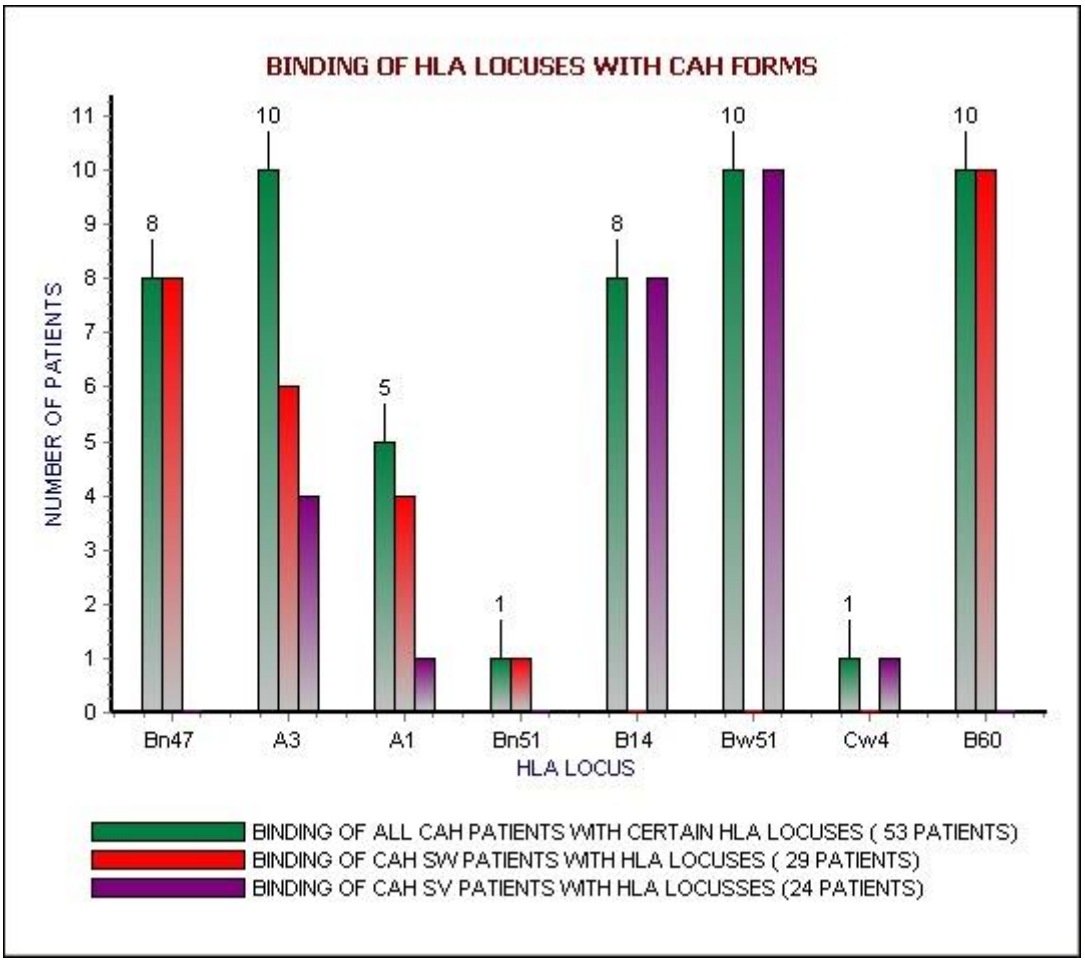


Figure 14

HLA locus relationship to the development of a definite disease form

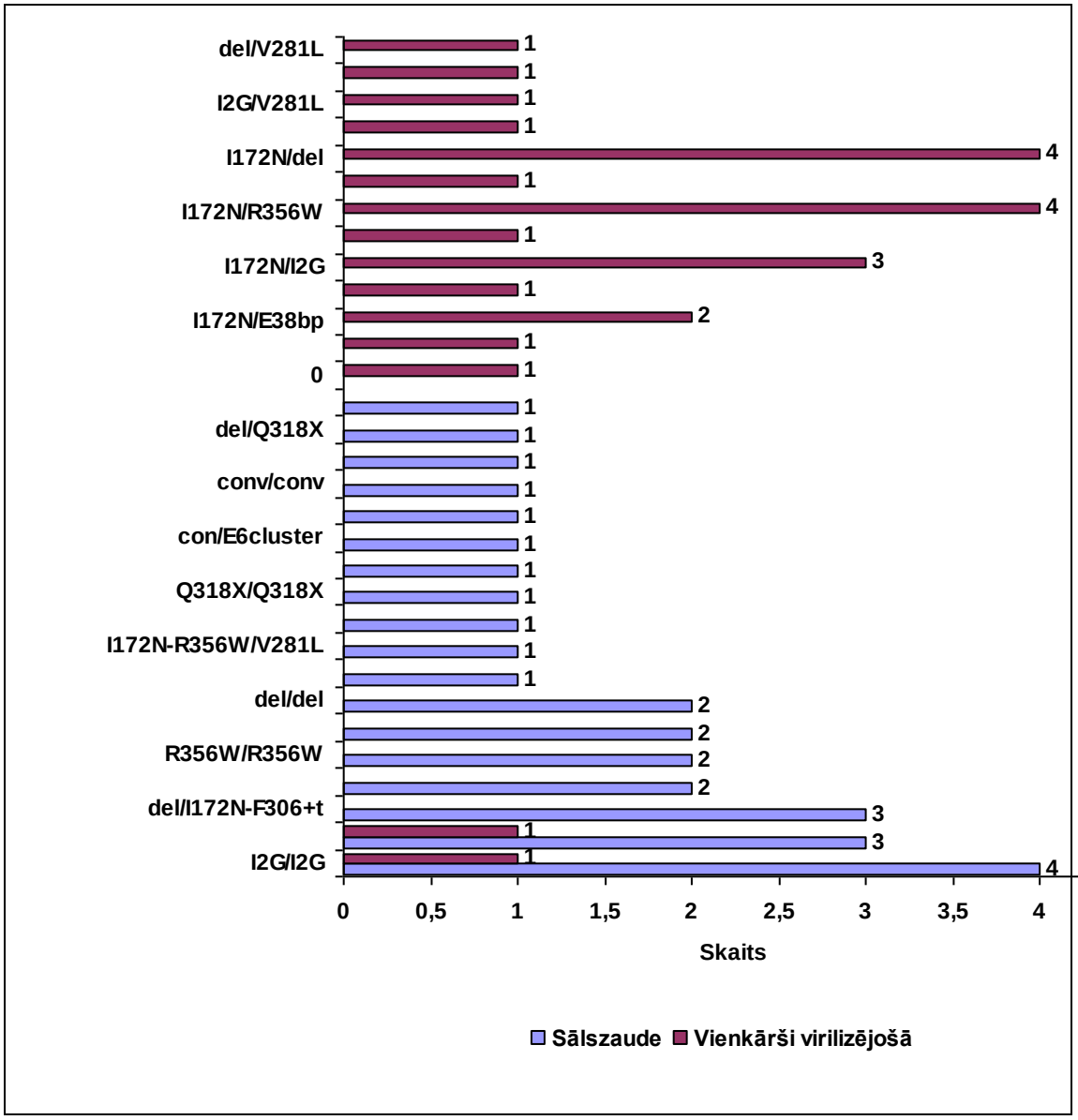


According to the research data, HLA locuses Bn47, A1 and B60 are related to the development of CAH salt loss form, but HLA locuses B14 and Bw51: to the development of the simple virilizing form.

5.3.4. *CYP21* gene mutations finding in CAH patients

Figure 15

Summary of CYP21 gene mutations finding in CAH patients.



The most common finded CYP21 gene mutations in CAH patients are 12G/12G mutations intron 2, also - Ile172Asn and 8-bp deletions in exon 3 (see figure 15).

CYP21 gene 12G/12G mutations intron 2 and other 12G depending mutations (12G/del, 12G/E38bp, 12G/R356W) involving with CAH salt loss form development in boys and also in girls.

CYP21 gene Ile172Asn depending mutations (I172N/del, I172N/E38bp, I172N/12G I172N/R356W) involving with CAH simple virilizing form development, more in boys than girls.

CYP21 gene Val281Leu and Pro30Leu mutations involving with CAH simple virilizing form development.

In CAH patients 12G/V281L mutation was found in girl, but del/V281L and P30L mutations – in boys.

CYP21 gene del/I172N-F306+t mutation involving in virilization of CAH salt loss form.

12G/del, 12G12G and 12G/R356W mutations involving in virilization of CAH salt loss form and simple virilizing form also. CYP21 gene I172N/del, I172N/R356W and I172N/E38bp mutations involving with CAH simple virilizing form development.

5.4. Dynamic analyze of the course of CAH included in the study children during the 5-years follow-up period

During the recent 5 years (year 2009 included) a follow-up of all 53 studied children with CAH was carried out. Number of outpatient visits during the first year of life was 3±1 (recommended – 4), but after the age of 1 year – 2±1 (recommended – 3).

During the follow-up period 14 patients (8 patients with salt wasting form and 6 patients with simple virilizing form) born in years 1984 – 1991 have reached the age of 18 years. The rest 37 patients (19 patients with salt wasting form and 18 patients with simple virilizing form) are growing up. 2 patients with salt wasting form have had exitus letalis.

Patients reached the age of 18 years during follow-up period have grown-up to their final height as follows:

- girls: -1.9 ± 0.4 SDS (among them, girls with salt wasting form have grown-up to -1.7 ± 0.4 SDS, but girls with simple virilizing form have grown-up to -2.0 ± 0.4),
- boys: -1.7 ± 0.3 SDS (among them, boys with salt wasting form have grown-up to -1.5 ± 0.2 SDS, but boys with simple virilizing form have grown-up to -1.9 ± 0.2 SDS).

Analyze of gene mutations in the group of grown-up 14 patients has revealed that existance of salt wasting form is connected with 12G/12G and 8-bp deletion in exon 3 mutations, but existance of simple virilizing form is connected with Pro30Leu, Val281Leu, Ile172Asn mutations.

Analyze of growth pattern in the group of growing-up 39 patients has revealed growth dependency on age as follows:

- from birth to 6 months: - 0.5 ± 0.3 SDS
- 6 months to 1 year: 0.1 ± 0.2 SDS
- 1 year to 2 years: 0.7 ± 0.4 SDS
- 2 years to 5 years: 0.9 ± 0.3 SDS
- 5 years to 10 years: 0.6 ± 0.4 SDS
- 10 years to 15 years: boys – - 1.1 ± 0.5 SDS, girls – - 0.9 ± 0.4 SDS.

Onset of puberty for girls (n = 16) has been at age of 10.1 ± 0.6 years, menarche (n = 12) at age of 13.4 ± 0.4 years, while onset of puberty for boys (n = 20) has been at age of 9.2 ± 0.5 years.

Levels of electrolytes in the blood at the time of CAH diagnostics were as follows:

- Natrium 125 – 134 mmol/l found in 35 patients (66 %),
- Natrium below 125 mmol/l found in 13 patients (24.5%),
- Potassium 5 – 7 mmol/l found in 21 patients (39.6%),
- Potassium above 7 mmol/l found i 29 patients (54.7%).

29 patients have documented simptoms of acute suprarenal insufficiency as clinical signs of their salt wasting form of the CAH.

Level of 17αOHPg in the blood as basic criteria for CAH diagnostics is used in 45 patients (85% of all patients). In 8 patients at the time of CAH diagnostics is estimated the level of 17-ketosteroids in urine.
Diagnostic level of 17αOHPg was found between 15.9 ng/ml to 200.7 ng/ml, in average – 40.08 ng/ml (n = 45, $p<0.05$).
Oscillations of the level of 17αOHPg during the follow-up period of patients was found between 0.3 ng/ml to 121.4 ng/ml, in average – 24.716 ng/ml (n = 1060, $p<0.05$). In the group of patients at age of 10 – 15 years the mean value of 17αOHPg was 45.76 ng/ml (n = 486, $p<0.05$). No statistically safe difference was revealed between oscillations of 17αOHPg in salt wasting and simple virilizing forms.

Analyze of the schedule of therapy revealed following:

- Regardless of the form of CAH all patients have received treatment with glucocorticosteroids:
  - 16 patients (30.2%) regularly and 12 patients periodicaly (independently on CAH form) have received Prednisolone in doses as follows:
    - up to age of 1 year – 3.4 ± 1.2 mg/m²,
    - from 1 year to 10 years age – 6.2 ± 1.3 mg/m²,
    - from 10 to 18 years age – 7.9 ± 1.2 mg/m².
  - 25 patients (47.2%) regularly and 12 patients periodicaly have received Hydrocortisonum in doses as follows:
    - in case of salt wasting form: up to age of 6 months – 23.4 ± 1.9 mg/m², from 6 months to 1 year age– 19.2 ± 2.1 mg/m², from 1 year to 10 years age – 17.4 ± 1.5 mg/m², from 10 to 18 yeras age – 20.5 ± 1.4 mg/m²,
    - in case of simple virilizing form: up to age of 1 year – 18.4 ± 2.3 mg/m², from 1 year to 10 years age – 13.8 ± 1.3 mg/m², from 10 to 18 years age – 24.8 ± 1.3 mg/m².
- 29 patients with salt wasting form have received mineralocorticoids - 9α fludrocortisonum as a single daily dose according to their age as follows:
  - up to age of 3 months – 150 μg/m², from 3 months to 1 year age – 100 μg/m², from 1 year to 2 years age – 50 to 100 μg/m², after age of 2 years – 50 μg/m².
- All 29 patients suffering from salt wasting form of CAH additionally have received NaCl 1 – 2 g/day up to age of 1 year.

Severe complications of CAH among patients included in the study have documented 15 times in 12 patients (22.6%), all suffering from salt wasting form and having at least one of the following mutations – I2G/I2G, I2G/R356W, I2G/del, Q318X/R356W, R356W/R356W. There were 2 lethal cases (1.06%) among patients studied, both in boys, one case – pneumonia at age of 8 months, another case – meningococcemia at age of 2 years. In 3 patients observed repeated acute adrenal crises. All cases (100%) of acute suprarenal insufficiency developed in patients during acute intercurrent illnesses, mainly (in 10 cases) during gastrointestinal infections. Hypoglycemia observed in 9 cases (7.8%) at age from 8 months to 3 years. Salt wasting crisis with hyponatriemia have been documented in group of patients at age 1.8 to 4.6 years.

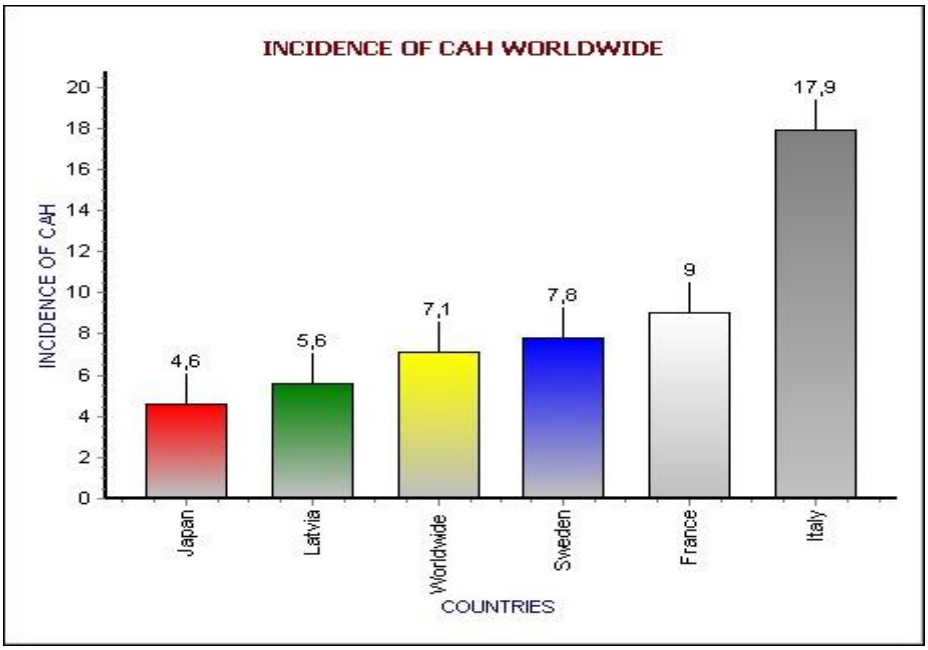
6. Discussion

Epidemiologic data

When congenital adrenal hyperplasia research was started, at first it seemed important to evaluate the incidence of this congenital metabolic disease in Latvia and to compare it with similar research data in neighboring countries and world-wide (see Figure 16). Such comparison of data shows that the CAH morbidity in Latvia is on average by 5000 lower than in Northern European countries. It means that there is one CAH patient per 17 795 newborns in Latvia, while in Sweden this value is 1:12578, but world-wide: 1:14000. However, when overall study data are analyzed, it can be concluded that there is an underdiagnostics of CAH in Latvia, and the number of CAH patients in our country could be greater.

Figure 16

CAH incidence world-wide



Although the number of patients involved in our research is not high, the data collected give some insight into the incidence of CAH in our country, and may serve as a basis for discussion on the need for CAH neonatal screening in Latvia as well.

The research revealed that one third of CAH patients involved in the study live in Riga. Since the absolute number of patients was counted, not the proportion of patients against the population, such result can be explained by the fact that almost one half of the Latvian population live in Riga. However, the number of CAH patients who live in the Eastern part of Latvia (Vidzeme and Latgale) significantly exceeds the number of patients living in the Southern (Zemgale) and Western part (Kurzeme).

Collected data show that CAH patients outside Riga are located mainly in the large towns: Daugavpils, Liepaja, Jurmala, Valmiera and Jelgava, less in Southern and Western regions of Latvia. Part of CAH patients in rural areas remain undiagnosed, since, unless it is a salt loss form, this disease is not life-threatening in an everyday life. At the same time, with severe stress, infections, injuries or similar situations CAH patient may develop an acute adrenal insufficiency or the adrenal crisis that is life-threatening. In particular, this applies to pediatric patients with this pathology, and it is understandable why clinical pediatricians world-wide pay more attention to the CAH problem. Namely, the question that is being asked and discussed within the pediatric endocrinologists' society is: "How much lives can be saved by prenatal CAH diagnostics or neonatal CAH screening?". Up to now world-wide, more than 6.5 millions of newborns have undergone the CAH screening. In many countries, this has become a clinical routine, and this research is a step to promote such a need in Latvia as well.

Evaluation of data collected during the study on CAH morbidity in Latvia shows that a trend to an increased number of new CAH patients can be observed during the last years. Namely, if the number of new CAH patients within the first 5 years included in the research (from 1984 to 1988) is 1.6 per year on average, within the next 5 years (1989 - 1993): 2 per year, within the period from 1994 to 1998: 2.8 per year, then during the last 4 years of the research (1999 - 2002) 5 new CAH patients were diagnosed per year on average. The mentioned above is equally applicable both to the CAH salt loss and the simple virilizing form. Knowing that the incidence of the simple virilizing form is higher compared to the salt loss form, data collected in the study probably do not show the real number of patients with CAH simple virilizing form in Latvia (which should be significantly higher compared to the number of patients with the salt loss form). The Reason could be the relatively mild clinical course of this form that, to the contrary to the salt loss form, does not force to seek medical advice.

Clinical data

CAH patients group included in the study is composed of 30 boys and 23 girls; 4 of these boys genetically are girls with a severe virilization; therefore, 26 boys and 27 girls have been evaluated during this research which shows that no gender difference is being observed in the development of CAH: this observation conforms to the literature data.

CAH salt loss form is found in 55% of cases, but the simple virilizing form: in 45% of cases. The incidence of both CAH forms has a gender difference: salt loss form has been diagnosed in 40% of boys and 74% of girls, but the simple virilizing form in 60% of boys and 26% girls. These data could probably show the

acknowledged clinical problem, i.e. CAH underdiagnostics in boys, since the virilization signs in newborns / infant boys usually are unnoticeable.

When these study data are evaluated, it becomes clear that there is a need for additional educational information for parents, children and also doctors on CAH simple virilizing form and problems caused by it, since there are certainly more girls suffering in Latvia compared to the number revealed by the study.

CAH salt loss form in 20% of patients was diagnosed during the first 2 weeks of life, in about 40% of patients in 1 month, in another 30% in 2 months, but when the age reaches 3 months, the diagnose has been established in every patient of this group.

Patients with the CAH simple virilizing form were diagnosed earlier, within first 6 months and first 2 years of life. This is due to the noticed virilization. The other rise in a more active diagnostics has been around the onset of puberty. That, on its turn, is caused by a premature puberty onset in boys or delay in girls.

The mean age for diagnostics of the simple virilizing form is 39 weeks (9.7 months), but for the salt loss form: 1.4 months. In general, the mean age for CAH diagnostics in Latvia is 5.2 months, while in Europe such diagnostics usually takes place by the age of 3 months.

Regarding clinical manifestation of virilization, a certain polarisation of its clinical signs exist. From one side, most of evaluated patients (i.e., 54.7% of all CAH patients or separately: 51.7% with the salt loss form and 58.3% with the simple virilizing form) did not have any virilization signs at the time of CAH diagnostics. Virilization signs probably were not looked for sufficiently enough. From the other side, severe (degree III to V) virilization has been found in 40% of patients with the salt loss form and 25% of patients with the simple virilizing form. These data also could reveal the clinical practice that sometimes exists, i.e., less severe virilization signs remain unnoticed.

Like regarding the incidence of CAH forms, also regarding the incidence of virilization, there are big differences between genders in the study results. Namely, in 86% of boys and 13% of girls no virilization was observed at the time CAH was diagnosed, but degree III to V virilization in girls has been found in 77% of cases, while in boys it has not been found at all.

Since the virilization in girls is one of the leading CAH symptoms, the need to perform genitoplastic surgery was analysed during the study. It turned out that 35 genitoplastic surgeries were performed in total in female patients with CAH included in the study. Most commonly, i.e., in 23 cases, there was a need to perform a clitoris correction due to the enlargement. Vaginal plastics was performed in 9 cases, mainly for removing of urogenital sinus. Genetic gender change (severe medical, legal and psychosocial problem) was performed in 3 cases, i.e., in 10% of the total number of surgeries (95% CI 3.5 to 25.6%).

Vaginal plastic surgery in female CAH patients due to severe virilization was performed during the first two years of life, clitoris correction: up to the age of 4 years, but the gender change performed at the age of 8 to 10 years.

Laboratory data (immunogenetic and molecular genetic findings)

One part of the study's laboratory work determined the relationship of CAH and its clinical forms with certain HLA locuses in the material of involved patients. It was established that most related to the development of CAH are HLA locuses A3, B60 and Bw51 (18.9% cases each), as well as locuses B14 and Bn47 (15% cases each). When the potential relationship between the above mentioned HLA locuses and the

development of some definite clinical form of CAH was evaluated, there was an impression that the study finding presents an evidence on the possible relationship between HLA locus A1, Bn47 and B60, and the development of the CAH salt loss form, but HLA locuses B14 and Bw51, on their turn, show some relation to the development of the CAH simple virilizing form. HLA locus A3 could be related to the development of both clinical forms of CAH. Potential relationship between the simple virilizing form of CAH and the locus Cw4 can not be excluded as well.

CYP21 gene mutations were tested in the material from patients involved in the second part of the study's laboratory work. Obtained results show that in the evaluated CAH patients group (represented by all Latvian regions) the following *CYP21* gene mutations prevailed:

8-bp deletion in exon 3 and 12G/12G mutation in intron 2, causing development of CAH salt loss form and

Pro30Leu, Val281Leu, as well as Ile172Asn mutations that in their turn cause development of CAH simple virilizing form; in addition it is known that Pro30Leu is related to the moderate prenatal hypertrophy of clitoris, Val281Leu – to classic CAH form, but Ile172Asn – to the severe prenatal virilization without salt loss.

These observations match to the literature data on the predominant *CYP21* gene mutations in Europe [13, 15, 92, 93] and on the genotype / phenotype correlation in CAH patients.

Pro453Ser, 8bpdel, as well as point type mutations in intron 2 prevail in Central Europe (Austria, Check Republic, Hungary, Slovakia, Slovenia) [20,85,208].

Pro453Ser, 8bpdel and Val281Leu mutations prevail in Northern European region (Sweden, Finland, Denmark) [17,155].

8bpdel and Val281Leu mutations prevail in Sothern Europe (Spain, Italy) (18,19), but in Western Europe (Germany, France, United Kingdom) – in addition to the above mentioned mutations, point type mutations in intron 2 are quite common [73,87,133].

5 years follow-up of clinical course in studied children with CAH

During the longlasting 5 years follow-up of all 53 studied children with CAH connection between clinical manifestation of CAH, laboratory findings and genetical characteristics of CYP21 gene mutations has been clearly eluciated. Respectively, marked disbalance of electrolytes, elevated level of 17αOHPg, development of acute suprarenal insufficiency as a complication observed in patients with salt wasting form of CAH, and typical for this disorder CYP21 gene mutations – I2G/I2G, I2G/R356W, I2G/del, Q318X/R356W, R356W/R356W, make a certain clinical, laboratory and genetical complex or whole entity.

In the course of study an analyze of data and making conclusions were bothered by several substantial circumstances, respectively, it was not possible to detect a level of 17αOHPg in years 1989 – 1992 and there was not available Hydrocortisonum untill year 1993 in Latvia. Although allowed for use thereafter, Hydrocortisonum periodically was unavailable for patients with CAH because of bad supply and problems to include it in the list of compensated pharmaceuticals. As a result, there were certain difficulties to set appropriate treatment for these patients.

Outpatient controls less frequent than recommended indicate that cooperation of patients in the process of treatment and follow-up was inadequate low.

Analyze of growth allows to conclude that a mean final height in these patients is substantially lower than in average for Latvian population. Although it concerns to both clinical forms of CAH, growth retardation in patients with simple virilizing form

is more pronounced than in patients with salt wasting form, because of later diagnostics of CAH, more longlasting influence of hyperandrogenia on bone epiphyseal growth plates and earlier closure of growth zones.

Analyzing dynamics of growth some following tendencies can be observed. Respectively, during the first year of life growth velocity of CAH affected children is slightly lower (-0.5 ± 0.3 SDS) than that of their healthy peers. Thereafter, up to age of 10 years, growth velocity of children with CAH (regardless the form of CAH or sex of child) uses to be higher than in average for population (0.6 ± 0.3 SDS). During puberty, instead, a progressive growth retardation in case of CAH can be observed and it is more expressed in boys (-1.1 ± 0.5 SDS) than in girls (-0.9 ± 0.4 SDS).

Short final or adult height is one of the most actual problem during treatment of CAH in childhood. In clinical trials and studies there are different attempts to find solution of this problem using combined glucocorticoid treatment with somatotropin, GnRH agonists, antiandrogens, inhibitors of aromataze or bilateral adrenalectomia in case if it is very difficult to reach appropriate CAH compensation [239].

Onset of puberty in boys with CAH usually is earlier than in girls. It means, that in boys undiagnosed simple virilizing form of CAH clinically can manifest as pubertas precox.

Level of 17αOHPg in blood is regarded as one of the basic criteria of CAH compensation. During patients follow-up in this study it is checked that the mean level of 17αOHPg exceeds the interval accepted as compensation (1.0 – 10.0 ng/ml) [239, 241]. Mainly it concerns to the period of puberty what allows to conclude, that because of physiological hypersecretion of gonadal androgens an appropriate compensation of CAH at this age it is difficult to reach. Additional factor influencing compensation of this disorder is patients and parents cooperation.

Dosage of glucocorticoids at the age from 1 to 10 years use to be higher in case of salt wasting form, because of elevated risk of hypoglycemia or acute CAH decompensation in early childhood. During puberty, instead, higher dosage of glucocorticoids use to be necessary in case of simple virilizing form because of pubertas precox, hypersecretion of gonadal androgens and increased cortisol clearens at this age [238].

Dosage of mineralocorticoids use to be higher during infacy and up to 2 years of age, because of elevated acute salt wasting risk. The highest dosage of mineralocorticoids usually is necessary up to 3 months of age with gradual decreasing of the dose thereafter.

Acute suprarenal insufficiency as a complication of CAH in studied patients has developed on the background of acute severe intercurrent illnesses at the age of elevated risk for children with CAH. The leading symptom observed in these cases was hypoglycemia. Eventual reasons of lethal cases probably are in delaying to increase the dose of glucocorticoids at home situation and in delaying to start parenteral treatment with glucocorticoids at hospital as well. Certain influence on results in these cases have had an insufficient education of parents to act in critical situations and inappropriate evaluation of child's condition. Compared with published data where stated lethality in patients with salt wasting form of CAH in 4.6%, but severe hypoglycemia in 9% cases [240], lethality observed during follow-up of studied patients is considerably lower (1.06%), but episodes of hypoglycemia – fewer (7.8%).

Early diagnostics of CAH, optimal pharmaceutical and surgical treatment, appropriate and repeated education of parents, regular dynamic follow-up, patients

and parents cooperation help to reach adequate compensation of CAH, avoid acute decompensation of CAH and its complications, provide normal growth, preserve fertility and improve a quality of patients life.

Practical considerations

1. There are different DNA diagnostic methods for determination of mutations that differ by the accuracy, costs of the method and availability of equipment. Known mutations can be established by restriction polymorphism analysis, genotyping methods. For detection of new mutations the most appropriate is sequencing or one strain polymorphism analysis. Method for sequencing of *CYP21* gene has been developed, but the work should be continued on the use of *CYP21* exon sequencing development in routine diagnostics.

2. Complete gene sequencing is expensive method for use in routine diagnostics, however with it help rare and unknown mutations can be found, with exception of large deletions and inversions, which not always can be detected by sequencing. For needs of routine diagnostics less expensive method should be used, which would be capable to detect common mutations, e.g. – genotyping.

3. If it has been found that both mutations are heterozygote, then for confirmation of 21-hydroxylase deficit diagnosis it must be proved that each of these mutations is localized in a separate allele. It could be proved by analysis of parent DNA samples. If these mutations will not be found only in one of parents, then we could consider that they are placed in different alleles and cause CAH.

4. Presence of CAH salt loss form should be excluded in all newborns [208, 209, 216, 91] with:

- Changed external genitals (including severe hyperpigmentation and/or visual cryptorchidism in boys) or unclear gender of the newborn,
- Severe eating disorders, repeated vomiting, weight loss, dehydratation,
- Broncho-pulmonal dysplasias, considering possibility of CAH also in case of other type respiratory insufficiency in neonatal period,
- Clinical picture of septicemia without any infectious cause,
- Laboratory finding of adrenocortical insufficiency (first of all – hyperkaliemia and hyponatriemia "scissors" in routine biochemical examinations).

5. Research data on diagnostics age clearly show that CAH diagnostics in Latvia is on the age of approximately 5.2 months, but in Europe on average by the age of 3 months. Substantiated impression occurs that the cause of such delayed diagnostics is CAH hypodiagnostics. In addition, it is not related only to CAH diagnostics in boys (that is a well known problem in pediatric practice world-wide, as virilization can be noticed in boys only in very severe cases), but also in girls with less severe virilisation signs. It marks the future job – development of wider and deeper information for children, parents and also physicians on CAH, its signs and possible health problems both in childhood and later periods of life of these patients.

6. On the bases of recommendations elaborated by ESPE and *Lawson Wilkins Pediatric Endocrine Society* [214] appropriate CAH diagnostic algorithms have been widely introduced in the world pediatric practice [33, 219]. The following algorithms have been adopted for conditions of Latvia:

'Congenital adrenal hyperplasia manifested during newborn period' and

'Congenital adrenal hyperplasia manifested following newborn period'.

These algorithms are used also in the Endocrinology center of BKUS as everyday routine, they are used in this research work and can be recommended for any pediatric practice in the country.

7. Conclusions

1. Laboratory finding of HLA locus research indicates:
   - the association of HLA locuses A1, Bn47 and B60 to the development of CAH salt loss form,
   - the association of HLA locuses B14 and Bw51 to the development of CAH simply virilizing form,
   - the association of HLA locus A3 to the development of CAH salt loss and simply virilizing form,
   - the possible association of HLA Cw4 to the CAH simply virilizing form.
2. CAH development HLA types in Latvia do not differ significantly from those in Europe [11,13,91,92].
3. Laboratory finding of CYP21 gene mutation research indicates that the following *CYP21* gene mutations prevail in Latvia:
   - 8-bp deletions in exon 3 and 12G/12G mutations in intron 2 causing development of CAH salt loss form,
   - Pro30Leu, Val281Leu and Ile172Asn mutations causing development of CAH simply virilizing form.
4. Range of *CYP21* gene predominant mutations in CAH patients in Latvia do not differ from that established in the European countries [11,13,91,92].

5. Dynamic analyze of the course of CAH in included in the study children during the 5-years follow-up period up to year 2009 shows that:

- there is a relevance between clinical and laboratory parameters, development of CAH complications and characteristics of CYP21 gene mutations,
- adult or final height in patients suffering from any of both CAH forms is considerably shorter than average statistical norm for population; growth retardation ir more pronounced in patients with simple virilizing form of CAH,
- there is an early pubertal development in boys,
- it is difficult to reach a satisfactory level of CAH compensation during period of puberty,
- dosage of glucocorticoids at age from 1 to 10 years are higher in case of salt wasting form, but during period of puberty higher doses of glucocorticoids are necessary for patients with simple virilizing form,
- dosage of mineralocorticoids are highest from birth up to 3 months of age, gradually decreasing thereafter,
- acute suprarenal insufficiency in studied patients has developed as complication of CAH at high-risk early childhood age on the background of acute severe intercurrent illnesses; the leading symptom observed in these cases was hypoglycemia,

6. Lethality observed during follow-up of studied patients (1.06%) was considerably lower than reported in literature (4.6%); episodes of hypoglycemia observed in CAH patients during this study (7.8%) were fewer compared with published data (9%) as well.

Paper approbation

Paper approbation has been performed in the united meeting of RSU Department of Pediatrics, Latvian Association of Endocrinologists and Latvian Association of Pediatric Endocrinologists on March 4, 2009.

Publications reflecting the Doctorate paper

1.I. Dzīvīte. Iedzimtas virsnieru garozas hiperplāzijas raksturojums Latvijā

1. konference "Bērnu un pusaudžu ginekoloģijas medicīniskie un sociālie aspekti Latvijā un Austrumeiropā". Tēzes, 4-5,1996. X, Vaivari

2. I. Dzīvīte. Adrenogenitālais sindroms. Latvijas Ārsts 10, 24 – 27, 1996.

3. I. Dzīvīte. Review of Latvian patients with CAH. Hormone Research 48 (suppl 2), 856-857, 1997.

4. LBPEC uzskaitē esošo iedzimta AGS slimnieku dažu klīnisko un laboratoro datu analīze. Trešā Pasaules Latviešu Ārstu kongresa tēzes, 6, 1997.

5. I. Dzīvīte. 21-hidroksilāzes deficīta molekulārie mehānismi un tā klīniskās un terapeitiskās sekas. Latvijas Ārstu Žurnāls 11, 15 – 19, 1999.

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1. Characteristics of hereditary adrenocortical hyperplasia in Latvia. 1st conference "Medical and social aspects of children and adolescents gynecology in Latvia and Eastern Europe". October 28, 1996, Vaivari.

2. Characteristic of CAH patients. ESPE Winter School, January, 1997, Lezno, Poland.

3. Analysis of some clinical and laboratory data of hereditary AGS patients listed in LBPEC. 3rd Congress of World Latvian Physicians, June 20, 1997, Riga.

4. Review of Latvian patients with CAH. European Society for Pediatric Endocrinology (ESPE) / Lawson Wilkins Pediatric Endocrine Society (LWPES) 5th Joint Meeting. June 4, 1997, Stockholm, Sweden (poster).

5. Characteristics of hereditary adrenocortical hyperplasia in Latvian children. AML BS conference, December, 1999, Riga.

6. Molecular genetic characteristics of Latvian CAH patients. Congress of Swedish Society of Endocrinologists, October 15, 2001, Goteborg, Sweden.

7. Immunogenetic analysis of CAH patients in Latvia. Conference of Pediatrics clinics of the Free University of Amsterdam, April 10, 2002, Amsterdam, the Netherlands.

8. Hereditary hyperplasia of adrenal cortex, Conference of Latvian Society of Endocrinologists, November, 2002, Riga.

9. Molecular mechanisms of 21-hidroxylase deficiency and its clinical consequences in Latvian children. Conference of Baltic Pediatric Endocrinologists, May 3, 2005, Vaivari.

10. 21-hidroxylase deficiency in Latvia. Conference of Association of Pediatric Endocrinologists, September 26, 2006, Kaunas, Lithuania.

11. CAH as a cause of short stature. 3rd Postgraduate Training Courses of the European Society of Pediatric Endocrinology on March 24, 2007 in Ohrid, Macedonia.

12. I.Dzivite-Krisane. Molecular mechanisms, immunogenetic characteristics, clinical and therapeutical consequences of the 21-hydroxylase deficiency in the Latvian children. RSU Scientific conference. 19 March, 2010. Poster presentation.

13. I.Dzivite-Krisane. Molecular mechanisms, immunogenetic characteristics, clinical and therapeutical consequences of the 21-hydroxylase deficiency in the Latvian children. European Society for Paediatric Endocrinology (ESPE). 22 - 25 September, 2010, Prague. Poster presentation.

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