Genetics and Development (including Gene Regulation)

FROM BENCH TO BEDSIDE: GENETICS, DEVELOPMENT AND CELL SIGNALING IN ENDOCRINOLOGY

VDR, CYP2R1 and CYP27B1 Genes Are Differentially Expressed in Male`s and Females Tissues of Wistar Rats

Maria Oczkowicz, Asst. Prof., Beata Szymczyk, Prof., Małgorzata Świątkiewicz, Prof., Iwona Furgał-Dzierżuk, phd, Alicja Wierzbicka, Msc, Anna Steg, Msc.
National Research Institute of Animal production, Balice, Poland.

Effect of vitamin D on gene expression has been widely investigated in vitro; however, in vivo action of vitamin D supplementation has been much less analysed in this context. Moreover, recent findings suggest that the effect of vitamin D supplementation may be sex-specific. The aim of our study was to investigate how vitamin D supplementation affects the expression of vdr, cyp2r1 and cyp27b1 genes in the liver, fat, brain and kidney of male and female rats. 36 adult Wistars rats (18 male and 18 females) were divided into 3 groups obtaining the same standard diet, differing only in the dose of vitamin D (group I - no supplementation, group II 1000 IU/kg of vitamin D, group III 5000 IU/kg of vitamin D). After 3 months of the experiment, animals were euthanised, and samples of tissues were collected from all animals. RNA was isolated using the Purelink RNA isolation kit (Thermofisher). RNA was reverse transcribed using High-Capacity cDNA Reverse Transcription Kit (Thermofisher). qPCR was performed using TaqMan™ assays and TaqMan™ Fast Advanced Master Mix on Quant Studio 7 instrument (Thermofisher). Data were analysed using SAS software. In the liver, we observed that the expression of the vdr gene was ~5 fold higher and cyp2r1 was ~2 fold higher in females compared to males ($p<0.0003$) ($p<0.0002$) respectively. In adipose tissue expression of cyp27b1 was ~1.8 fold higher ($p<0.014$) in females compared to males. In the kidney expression of vdr and cyp2r1 was slightly higher in females (fold change-(fc) 1.18 $p<0.03$) (fc=1.37, $p<0.0001$), while expression of cyp27b1 was 1.7 fold higher in males ($p<0.013$). In the brain, we observed slightly increased expression of cyp2r1 and cyp27b1 genes in females when compared to males (fc=1.14 $p<0.0001$), fc=1.23 $p<0.01$). Supplementation with 5000 IU/kg of vitamin D resulted in the decrease in the expression of vdr, cyp2r1 and cyp27b1 in the kidney when both sexes were analysed together($p<0.0127$, $p<0.0326$, $p<0.0326$) but this decline was only statistically significant for vdr in males when sexes were analysed separately ($p<0,0403$). In the liver of males expression of vdr gene tended to increase and expression of cyp27b1 tended to decrease after vitamin D supplementation ($p<0.06$) ($p<0.0501$). In the adipose of males expression of cyp27b1 was downregulated by the high dose of vitamin D ($p<0.04$) but not in females. In the brain of males, we observed a slight decrease in the expression of cyp2r1 in a group obtaining 5000 IU/kg of vitamin D when compared to animals obtaining 1000 IU/kg ($p<0.025$). Concluding, our study shows that in a number of tissues of Wistar rats, expression of vdr, cyp27b1, and cyp2r1 is higher in females when compared to males. The only exception is the expression of cyp27b1 in the kidney, where it is higher in males. Moreover, the effect of vitamin D supplementation on the expression of these genes is weak and more evident in males and when high doses (5000 IU/kg) of vitamin D was used.

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What Every Internist Should Know About Rare Genetic Syndromes in Order to Prevent Needless Diagnostics, Missed Diagnoses and Medical Complications: Five-Year Experience of Internal Medicine for Complex Rare Genetic Syndromes

Anna G.W. Rosenberg, BSc[1], Minke R.A. Pater, BSc[1], Kirsten Davidse, MSc[2], Anja A. Kattentidt-Mouravieva, MD, PhD[3], Rogier Kersseboom, MD, PhD[3], Anja G. Bos-Roubos, MSc[4], Agnies van Eeghen, MD, PhD[5], Jiske J. van der Meulen, MSc[6], José M.C. Veen, BSc[7], Nina van Aalst, BSc[1], Franciska M.E. Hoekstra, MD, PhD[1], Aart J. Van der Lely, MD, PhD[1], Laura C.G. de Graaff, MD, PhD[1].
[1]Erasmus MC, University Medical Center, Rotterdam, Netherlands, [2]Erasmus University Medical Center, Rotterdam, Netherlands, [3]Stichting Zuidwester, Medical service, Middelharnis, Netherlands, [4]Center of Excellence for Neuropsychiatry, Vincent van Gogh, Venray, Netherlands, [5]Amsterdam University Medical Center, Department of Pediatrics, Amsterdam, Netherlands, [6]'s Heeren Loo, Care Providing Agency, Wekerom, Netherlands, [7]'s Heeren Loo, Care Providing Agency, Ermelo, Netherlands.

Introduction: Patients with complex rare genetic syndromes (CRGS) have, by definition, combined medical problems affecting multiple organ systems. Intellectual disability (ID) is often part of the syndrome. During childhood, most patients with CRGS receive multidisciplinary (MD) and specialized pediatric care in tertiary centers. As improvement of medical care has improved life expectancy, more and more patients are now reaching adult age. While the complexity of the syndromes persist into adulthood, adequate multidisciplinary syndrome-specific care is rarely available for adults with CRGS. Although multiple organ systems are usually affected, internists are rarely involved. Pediatricians have expressed the urgent need for adequate, syndrome-specific, MD tertiary healthcare for adults with CRGS.
Methods: In 2015 we have launched the Center for Adults with CRGS, a specialized MD outpatient clinic (MOPC) within the Endocrinology unit of the department of Internal Medicine. As adult manifestations are unknown for most CRGS, all CRGS patients who visit our MOPC undergo a systematic health screening (followed by treatment, if indicated). Before visiting the MOPC, caregivers fill out a medical questionnaire. We gathered the physical complaints, medication use and missed diagnoses of 726 adults with CRGS.
Results: Between 2015 and 2020, 256 males and 470 females with over 60 syndromes visited the Center for Adults with CRGS. The main features of this population, as compared with general internal medicine patients, were

intellectual disability, polypharmacy and use of psychotropic drugs. Missed diagnoses were common and many patients had undergone extensive diagnostic tests for symptoms that could actually be explained by their syndrome. Fatigue (52%), abdominal discomfort (23%) and hypertension (10%) were among the most frequent reasons for referral to Internal Medicine. Based on the literature and our clinical findings, 73% of the syndromes was associated with endocrine problems. We provide an algorithm for the clinical approach to CRGS adults, in order to prevent unnecessary diagnostics as well as missed diagnoses.
Conclusion: Our overview of 726 adults with CRGS shows that missed diagnosed and needless invasive tests are common in this patient population. As more and more CRGS patients are now reaching adult age and transfer to Internal Medicine, internists and endocrinologists should be aware of the special needs of adults with CRGS and of the medical pitfalls. Knowledge about syndrome-specific health problems and multidisciplinary expert care is crucial to prevent the personal and financial burden of unnecessary diagnostics and under- and overtreatment.

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Young Adult LEW.1WR1 Rats, a Model of Liver FAT10 Overexpression, Develop Insulin Resistance and Fatty Liver With Age

Madushika M. Wimalarathne, PhD student[1], Luis D. Mercado, BS[2], Quiana C. Wilkerson Vidal, MS[3], James Wolfsberger, MS[2], Victoria J. McConnell, MD[2], Bernhard Vogler, PhD[2], Sharifa Tahirah Love-Rutledge, PhD[2].
[1]University of Alabama in Huntsville, Huntsville, AL, USA, [2]University of Alabama at Huntsville, Huntsville, AL, USA, [3]University of Alabama Huntsville, Huntsville, AL, USA.

As human lifespan increases, comorbid conditions that impact quality of life have become a serious problem. FAT10 has been identified as a gene that when knocked out, improves age associated metabolic dysfunctions and increased longevity in mice (1). There is increased *Fat10* expression in the liver in obesity (2,5). Providing evidence that fat10 expression may be important for triggering the transition to metabolic dysfunction in aging. Adult LEW.1WR1(1WR1) rats have increased body mass without excess abdominal fat mass compared to control rats (3). Yet, it was unclear where the excess mass was stored. We hypothesized that the 1WR1 rat would develop increased liver fat mass as a product of increased insulin resistance in response to increased liver fat10 expression over time. To test this hypothesis, we did insulin tolerance tests(7 weeks & 15 weeks), triglyceride assays, and histological analysis of the liver(23 weeks), in 1WR1 rats(n=7) and Wistar Furth (WF) rats(n=7) on control diets. We analyzed images using histological scoring for nonalcoholic fatty liver disease from the literature (4). We also assessed the slides for Mallory Denk bodies (MBs). The body mass of 1WR1 rats were increased compared to WF rat groups starting from the age of 7 weeks (391.4∓8.572g vs. 271.8∓11.62g; p <0.0006). 1WR1 rats became more insulin resistant with age, the 1WR1 rat group has increased AUC of 7 and 15 week Insulin Tolerance Tests (401.5∓23.54 vs. 245.3∓10.20 7w ITT1; p= 0.0728, 15w ITT2 328.2∓14.86 vs. 217.8∓9.; p <0.0003) compared to WF rats. 1WR1 rats have increased liver mass (11.85g∓0.7699g vs. 7.235g∓0.3864g; p=0.0006) liver triglyceride levels compared to WF rats (192.8∓21.8 mg/mL vs. 130.1∓13.075 mg/mL; p=0.0728). 1WR1 rats have increased steatosis scores(1.857∓0.2608 vs. 1.143∓0.1429;p= 0.0862) yet significantly reduced inflammatory foci level (2∓0.8165 vs. 3∓0;p= 0.007), most 1WR1 hepatocytes are enlarged (ballooned) and contained MBs compared to WF rats suggesting 1WR1 rats have already passed the early inflammation stage. Adult 1WR1 rats developed reduced insulin sensitivity and lipid accumulation in the liver. These data support our hypothesis that 1WR1 rats would develop increased liver fat and impaired insulin resistance in response to aging and show that this process may be inflammation driven.
(1) Canaan et al.,PNAS. April 2014; 111 (14): 5313-5318.**(2)**Vidal et al., FASEB.2020, 34: 1-1,**(3)** Collins et al., J Endocr Soc. 2019;3(1),**(4)**.Kleiner et al., Hepatology, 2005; 41 (6): 1313–1321,**(5)**.Dali-Youcef et al., Hepatol Commun. 2019;3(9):1205-1220.

Neuroendocrinology and Pituitary

CLINICAL ADVANCES IN PITUITARY DISEASES

GLP1 Receptor Agonists Reduce Fluid Intake in Primary Polydipsia

Bettina Felicitas Winzeler, MD[1], Clara Odilia Sailer, PhD[1], David Coynel, PhD[1], Vogt Deborah, PhD[1], Zanchi Davide, PhD[1], Sandrine Urwyler, MD[2], Julie Refardt, MD[3], Mirjam Christ-Crain, Prof[4].
[1]University of Basel, Basel, Switzerland, [2]University Hospital Basel, Rheinfelden, Switzerland, [3]Erasmus MC, Rotterdam, Netherlands, [4]Universitaetsspital Basel, Basel, Switzerland.

Background Primary polydipsia, characterized by excessive fluid intake, carries the risk of water intoxication and hyponatremia, but treatment options are scarce. Glucagon-like peptide-1 (GLP-1) reduces appetite and food intake. In experimental models, they also play a role in thirst and drinking behavior in. The aim of this trial was to investigate whether GLP-1 receptor agonists reduce fluid intake in patients with primary polydipsia. **Methods:** In this randomized, double-blind, placebo-controlled, 3-week crossover-trial, 34 patients with primary polydipsia received weekly dulaglutide (Trulicity®) 1.5mg and placebo (0.9% sodium chloride). During the last treatment week, patients attended an 8-hour evaluation visit with free water access. The primary endpoint was total fluid intake during the evaluation visits. The treatment effect was estimated using a linear mixed-effects model. In a subset of 15 patients and matched controls, thirst perception and neuronal activity in response to beverage pictures were assessed by functional MRI. **Results** Median [IQR] total fluid intake was 2250ml [1600-2600] on dulaglutide versus 2400ml [1850-3400] on placebo. Patients on dulaglutide reduced fluid intake by 490ml [95%-CI -780, -199], p=0.002, corresponding to a relative reduction of 17%. 24-hour urinary output was reduced by -943ml [95%-CI -1473, -413]. Thirst perception in response to beverage pictures