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001

tRNAGln hypomethylation and fragmentation in patient iPSC-derived β-like cells mediates apoptosis in TRMT10A diabetes

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Background and aim
Loss-of-function mutations in *TRMT10A*, a transfer RNA (tRNA) methyltransferase, cause early onset diabetes and microcephaly. tRNAs play a crucial role in cellular homeostasis and post-transcriptional modifications modulate tRNA function and fragmentation. tRNA-derived halves (tiRNAs, 29-50 nt) and fragments (tRFs, 14-30 nt) are a new class of functional small noncoding RNAs, involved in cellular stress responses. Here we set out to investigate the molecular mechanisms underlying β-cell demise in TRMT10A deficiency.
Methods
Fibroblasts from a TRMT10A-deficient patient and 2 healthy controls were reprogrammed into induced pluripotent stem cells (iPSCs). iPSCs were differentiated into β- like cells using a 7-stage protocol. TRMT10A expression was silenced in human insulinproducing EndoC-βH1 cells by siRNAs. Reactive oxygen species (ROS) were measured using HPF fluorescent probe and mitochondrial function was assessed by Seahorse. qRT-PCR was used to detect guanine-9 methylation (m1G9) in tRNAs. tRNA fragmentation was assessed by Northern blot and qRT-PCR. Synthetic tRNA fragments and tRF inhibitors were transfected by lipofection. Apoptosis was examined by nuclear dyes, Western blot and immunocytochemistry.
Results
iPSCs from controls and TRMT10A diabetic patients were successfully differentiated into β-like cells. The β-like cells expressed insulin mRNA at levels comparable to EndoC-βH1 cells and human islets. In iPSC-β-like cells and TRMT10A-depleted EndoC-βH1 cells ($\geq$70% knockdown, $P<0.001$) m1G9 methylation was reduced in a subset of cytosolic tRNAs, including tRNAGln ($P<0.05$, $n=6$–12). Hypomethylation of tRNAGln resulted in fragmentation and increased 5'-tiRNAGln and 5'-tRFGln in patient-derived cells (1.5 ± 0.5 fold increase vs controls, $P<0.05$ $n=3$–6). Transfection of TRMT10A-competent EndoC-βH1 cells with synthetic 5'-tiRNAGln and 5'-tRFGln induced apoptosis. Conversely, transfection of antisense oligonucleotides targeting 5'-tiRNAGln and 5'-tRFGln protected TRMT10A-deficient β-cells from apoptosis (23 ± 2% apoptosis in TRMT10A-silenced cells vs 17 ± 2% following antisense transfection, $P<0.05$). TRMT10A deficiency induced oxidative stress and mitochondrial dysfunction in β-cells ($P<0.05$, $n=5$), triggering the intrinsic pathway of apoptosis. The ROS scavengers Tiron (25 μM) and NAC (1 mM) protected TRMT10Adeficient β-cells from apoptosis (20 ± 2% apoptosis without NAC vs 14 ± 2% with NAC, $P<0.05$, $n=4$).
Conclusion
Establishing a novel experimental model based on patient's iPSC-derived primary β cells, we demonstrated that TRMT10A deficiency induces β-cell demise via oxidative stress, mitochondrial dysfunction and activation of the intrinsic pathway of apoptosis. TRMT10A deficiency leads to hypomethylation and fragmentation of tRNAs, and the 5'-tRNAGln fragments are key mediators of β-cell death. These observations provide unequivocal evidence for the importance of tRNA modifications in human pancreatic β-cells and identify tRNA hypomethylation and fragmentation as a novel mechanism of β-cell demise in human diabetes.

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002

Physiological relevance of LBD-dimerization of the androgen receptor

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Most mutations found in the androgen receptor (AR) are associated with some form of androgen insensitivity syndrome (AIS), and correlate with a loss of either hormone, DNA or coactivator binding. For some mutations in the ligand binding domain (LBD) of the AR, it is unclear what the underlying mechanism of the AIS could be. In the beginning of 2017, a new dimerization interface was uncovered in a crystal structure of the ligand-binding domain (LBD) which provides a possible mechanism for several unexplained cases of AIS.[1] We now want to uncover the physiological relevance of this newly uncovered dimerization method as well as its role in AR functioning. We generated a mouse model based on a W752R mutation located in the LBD dimerization interface, described in two siblings with androgen insensitivity. *In vitro*, AR W752R is well expressed, binds androgens with high affinity and has proven an impaired LBD dimerization potential. Although at lower androgen concentrations AR W752R is less potent on androgen reporter genes, at higher concentrations it has strong transactivating potential similar to wild type AR. Via the CRISPR/Cas9 technique, we introduced the W to R mutation into C57BL/6J mice. This model is called AR Lmon (for monomeric mutation in the LBD). To exclude effects caused by off target mutations that may be incorporated during the CRISPR/Cas method, we performed backcrossing of the mice until at least the third generation. The male AR Lmon mice show an external female phenotype with female ano-genital distance and nipple development. Testes are present, smaller than wild type, but not as small as seen in our complete androgen knockout mice (ARKO). Analogous to complete AIS (CAIS), the Lmon mice did not developed epididymis, seminal vesicle or musculus levator ani. These observations suggest that the interruption of the dimerization of the AR via the LBD leads to a severe androgen insensitivity phenotype. The growth curve of the Lmon mice is comparable to the wild type females and lower than the one of normal males. However, surprisingly, these mice start to gain weight after the age of 12 weeks. Dissection at 16 weeks showed an increase in subcutaneous, retroperitoneal and gonadal fat comparing to wild type and ARKO males. Preliminary measurement of testosterone and LH concentrations in circulation of two animals uncovered that the production of both is highly elevated in Lmon mice, indicating a disrupted hypothalamus-pituitary-gonadal axis similar to what is observed in CAIS patients. In conclusion, a mouse model of the W752R mutation phenocopies the CAIS, despite the fact that *in vitro* the AR still can transactivate reporter genes. To uncover the exact place of LBD dimerization in the normal AR functioning and male development, further investigations are necessary.

Reference
1. Nadal, M. *et al*. Structure of the homodimeric androgen receptor ligand-binding domain. Nat. comm. 8, 14388 (2017).

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003

Use of 3D culture systems to generate human induced pluripotent stem cell-derived β-cells *in vitro*

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Background and aims
Diabetes currently affects 425 million people worldwide. Pancreatic β-cell failure is central in the development and progression of type 1 and type 2 diabetes. Diabetes research is slowed by the difficulty to study the diseased tissue, i.e. human islet bcells: these are only available in a few donor organ transplantation centers worldwide. β-cells differentiated from human induced pluripotent stem cells (hiPSCs) represent a novel cell source. Current *in vitro* differentiation strategies enable to generate immature β-cells. The traditional suspension culture used for β-cell differentiation is technically challenging and results in heterogeneously sized aggregates. Smaller and more homogeneously sized aggregates may increase the maturity and function of hiPSC-β-cells. Here we aimed to implement 3D culture in microwells (Aggrewells®, Stem Cells) to control the size of islet-like aggregates. We compared the *in vitro* characteristics of hiPSC-β-cells produced either in suspension or in microwells.
Methods
We used a published 7-step protocol with slight modifications that sequentially provides hiPSCs with differentiation signals important for pancreatic β-cell development. Until the pancreatic progenitor stage, cells were cultured on Matrigel-coated culture plates, after which cells were transferred either in rotating suspension culture or in microwells. Key markers of b-cell development were assessed across the differentiation by qPCR, immunocytochemistry and FACS. The function of hiPSC-β-cells was assessed by glucose- and forskolin-stimulated insulin secretion.

Results
The transfer of cells into suspension often resulted in the formation of large clumps of cells, leading to loss of 40% of the experiments. The seeding into microwells, in contrast, was always (100%) successful. The successfully transferred hiPSCs differentiated with similar efficiency in suspension and microwells, based on their protein and mRNA expression. The suspension aggregates were bigger and more heterogeneous in size compared to microwell aggregates. The yield of insulin-positive β-cells was the same in microwell (36%) and suspension (38%) aggregates, but there were fewer glucagon-positive α-cells in microwell aggregates (7% vs 19% in suspension). In both conditions 6% of cells were polyhormonal (insulin- and glucagon-positive). Aggregates from both culture systems did not induce insulin secretion when stimulated 16,7 mM glucose. However, they similarly increased insulin secretion by 4-5-fold when stimulated with glucose plus forskolin.
Conclusions
Compared to suspension culture, the microwells have a significantly higher experimental success rate, thereby saving significant costs. hiPSCs differentiate with equal efficiency into b-cells in microwells compared to traditional suspension, but the α-cell differentiation is reduced in the microwell system. Microwell aggregates are smaller and equally sized, which might be advantageous for their further maturation. More research is needed to optimize *in vitro* hiPSC-β-cell function. Even at the current stage of development, the technology provides us with an unlimited supply of human β-cells that is and will be instrumental to study β-cell demise in diabetes.

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004

GLP-1 analogs protect beta cells and prevent diabetes in models of Wolfram syndrome

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Background and aims
Wolfram syndrome is a rare autosomal recessive orphan disease. The clinical manifestations are young onset diabetes, optic nerve atrophy and deafness. Most Wolfram patients carry mutations in *WFS1*. WFS1 deficiency results in endoplasmic reticulum (ER) stress, leading to neurodegeneration and pancreatic β-cell dysfunction and death. Glucagon-like peptide-1 (GLP-1) analogs and the cAMP inducer forskolin have been shown to protect β-cells from ER stress. Our aim is to evaluate the therapeutic potential of GLP-1 analogs for Wolfram syndrome.
Materials and methods
WFS1 was silenced in clonal human EndoC-βH1 β-cells and human islets by RNA interference. Wolfram syndrome patients' induced pluripotent stem cells (iPSCs) were differentiated into β-like cells. Synthetic ER stress was induced by tunicamycin (5 μg/ml). β-cell apoptosis was evaluated by Hoechst 33342/propidium iodide staining. Expression of ER stress markers was examined by qPCR. Whole body *wfs1* knockout (KO) mice (homozygous exon 8 deletion on 129S background) were treated for 8 weeks with dulaglutide (1 mg/kg) by intraperitoneal injection every 4 days. Glucose tolerance was evaluated before, during and at the end of treatment by intraperitoneal glucose tolerance tests.
Results
WFS1 silencing (>70% knockdown, $n=6$, $P<0.001$) sensitized EndoC-βH1 cells to tunicamycin-induced apoptosis (29±3% apoptosis in WFS1-deficient cells vs 12±1% apoptosis in control cells, $n=5$, $P<0.01$) and increased mRNA expression of the ER stress marker CHOP ($P<0.001$). Exendin and forskolin protected WFS1-deficient EndoC-βH1 cells from ER stress (29±3% apoptosis with tunicamycin alone vs 22±1% with tunicamycin + exendin or 10±0.3% with tunicamycin + forskolin, $n=5$, $P<0.01$). iPSCs from 4 Wolfram syndrome patients were successfully differentiated *in vitro* into β-like cells using a 7-stage protocol. Forskolin protected Wolfram iPSC-β-like cells from tunicamycin-induced apoptosis ($n=4$, $P<0.001$) and increased expression of the ER chaperone BiP. Wfs1 KO and wild type mice had comparable glucose tolerance at weaning (4 weeks of age). By age 6 weeks *wfs1* KO mice developed glucose intolerance ($n=17$, $P<0.01$) and by age 10 weeks they developed diabetes. Dulaglutide administration, started at weaning, fully prevented the development of glucose intolerance in *wfs1* KO mice after 4 and 8 weeks of treatment ($n=3$ per group, $P<0.05$).
Conclusion
cAMP induction by exendin and forskolin protects WFS1-deficient β-cells from ER stress-induced apoptosis. *In vivo*, dulaglutide treatment prevented diabetes development in *wfs1* KO mice. These findings provide further evidence for the protective properties of GLP-1 analogs in the context of β-cell ER stress, and suggest that GLP-1 analogs hold preventive and therapeutic potential for Wolfram syndrome-related diabetes.

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005

The effect of androgens on renal calcium and phosphate handling, independent of bone and in circumstances of low dietary calcium

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Aims
We have previously shown that orchidectomy (ORX) in adult mice induces hypercalciuria and upregulation of renal Ca^{2+} and PO_4^{3-} transporters, but also bone loss already after 2 weeks. Pretreatment with risedronate inhibited bone resorption and hypercalciuria but confirmed the upregulation of renal Ca^{2+} and PO_4^{3-} transporters, suggesting bone-independent renal effects of androgens. We hypothesize that androgens regulate renal Ca^{2+} and PO_4^{3-} homeostasis, independent of the dietary Ca^{2+} content as well.
Methods
We performed ORX on 18-week-old male mice (vs SHAM) and pretreated all animals with risedronate. Mice were fed either a normal Ca^{2+} diet (NCD, 1%) or a low Ca^{2+} diet (LCD, 0.02%), while PO_4^{3-} was unchanged (0.6%). At 1 and 2 weeks post-ORX, 24 hr urine (metabolic cages) and serum was collected. After sacrifice (2 w post-ORX), kidneys were taken for qPCR of Ca^{2+} and PO_4^{3-} transporters, trabecular bone was analyzed by microCT (L5 vertebra), and femoral bone was analyzed for Ca^{2+} content by ashing.
Results
Serum Ca^{2+} and PO_4^{3-}, and urinary Ca^{2+} excretion were similar under both diets, but PO_4^{3-} excretion was significantly increased in both the SHAM and ORX groups (239 and 268%, $P<0.0001$) under LCD diet. Under LCD, increased serum levels of 1,25-dihydroxyvitamin D and PTH were observed. While risedronate efficiently blocked ORX-induced bone loss under the NCD, microCT analysis revealed that bone resorption was not fully blocked under the LCD, with a decrease in bone volume density of 14% compared to the NCD fed SHAM mice ($P<0.05$). Also the femur Ca^{2+} content was significantly reduced in the LCD group (7.2 vs 8.8 mg in the NCD fed SHAM mice, $P<0.0001$). Under the NCD, an increase of renal Ca^{2+} and PO_4^{3-} transporter expression after ORX was confirmed. In the LCD group a small but significant additional increase of renal Ca^{2+} transporter expression was observed, with a 1.7-fold increase for TRPV5, a 2.1-fold increase for CaBP9K and a 1.5-fold increase for PMCA (vs respectively a 1.6-, 1.5- and 1.3-fold increase for the NCD). Expression of PO_4^{3-} transporters (NaPi-2c, PiT1, PiT2) was similar in both diet groups.
Conclusions
Low dietary Ca^{2+} results in secondary hyperparathyroidism with LCD-induced bone loss despite treatment with bisphosphonates, and an additional increase in renal Ca^{2+} transporters. These findings underline the importance of adequate dietary Ca^{2+} intake along with anti-resorptive drugs in circumstances of bone loss post-androgen deprivation.

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006

TAOK3 as a regulator of glucose tolerance in obesity

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Aims or Objectives
We here describe a role for the kinase thousand and one kinase (TAOK) 3 as a modulator of insulin resistance. TAOK3 is one of three members of the TAOK family of kinases, which are classified within the Ste20-like kinases as mitogen activated protein kinase 4 kinases. TAOK3 was identified in a RNAi library screening in *C. elegans* as a target molecule that could revert an insulin resistance-like state induced by a daf-2 mutation (Devgen, Ghent). Moreover, TAOK3 has been linked to childhood obesity in an epigenetic study.
Methods
Using the Sanger Gene Trap ES cell platform, TAOK3$^{-/-}$ mice were generated in collaboration with Prof. Jody Haigh (IRC, UGhent). Importantly, the gene trap is floxed, implicating that TAOK3 expression can be rescued in a certain tissue if crossed to a specific mouse '*cre*' line. TAOK3$^{-/-}$ and TAOK3$^{+/+}$ control mice were placed on a 60% (high fat diet, HFD) or a 10% (standard diet, SD) fat diet.
Results
On the HFD, TAOK3$^{-/-}$ mice were a bit leaner than their WT littermates. The HFD fed TAOK3$^{-/-}$ mice had significantly lower glucose and insulin levels, as assessed by IP GTT (intra-peritoneal glucose tolerance test), PTT (pyruvate tolerance test) and ITT (insulin tolerance test). This was also true, although to a lesser extent, for the SD fed mice, despite no differences in body weight in this group. We then crossed Adipoq-cre and TAOK3 mice to generate mice with a gene trap reversal specifically in adipose tissue (KO/Tg+). Interestingly, this led to a partial reversal of the phenotype in mice on HFD (Fig. 1).

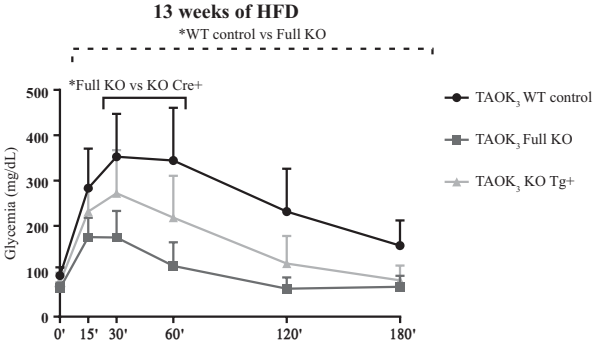

Figure 1

Conclusions or Summary
Our data demonstrate that TAOK3 is implicated in glucose tolerance and insulin sensitivity in mice, at least in part through adipose tissue. Being a kinase, TAOK3 is druggable and our data sparked interest of drug companies for targeting this kinase. Therefore, we hope that our work will lead to a novel target for the treatment of metabolic disease.

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007

A heterozygous splice-site mutation in PTHLH causes autosomal dominant shorting of metacarpals and -tarsals

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Short metacarpals and/or -tarsals are observed in pseudohypoparathyroidism (PHP) type Ia (PHP1A) or pseudo-PHP (PPHP) caused by inactivating *GNAS* mutations involving exons encoding the stimulatory G protein alpha-subunit (Gαs). Skeletal abnormalities indistinguishable from those caused by Gαs mutations were present in five members of an extended Belgian family, who showed no evidence for abnormal regulation of calcium and phosphate homeostasis. Direct nucleotide sequencing of the proband's (190/II-1) genomic DNA excluded mutations involving *GNAS* exons 1-13, but whole exome analysis revealed a heterozygous A>G change at nucleotide -3 of *PTHLH* exon 3 that encodes the last two amino acids of the Pre-Pro sequence of PTHrP. The same nucleotide change was also found in the proband's affected sister 190/II-2 and three of this sister's descendants (190/III-1, 190/IV-1, and 190/IV-2), but not in two unaffected family members and not in public databases. Complementary DNA derived from immortalized lymphoblastoid cells of the affected male 190/IV-2 and his unaffected sister 190/IV-3 was PCR-amplified using forward primers located either in *PTHLH* exon 1 or 2, and reverse primers located in the 3'-non-coding regions of exons 3 or 4. Nucleotide sequence analysis of these amplicons revealed for 190/IV-2, but not for 190/IV-3, a heterozygous insertion of genomic nucleotides -2 and -1, causing a frame-shift after residue 34 of the PrePro sequence and thus 29 novel amino acids without homology to PTHrP or any other protein. Our findings extend previous reports indicating that PTHrP haploinsufficiency causes AHO-like features, which are similar to those observed with *GNAS* mutations.

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008

Vegf-A mRNA transfection as a novel approach to improve mouse and human islet graft revascularization

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Aims/hypothesis
The initial avascular period following islet transplantation seriously compromises graft function and survival. Enhancing graft revascularization to improve engraftment has been attempted through virus-based delivery of angiogenic triggers, but risks associated with viral vectors have hampered clinical translation. *In vitro* transcribed mRNA transfection circumvents these risks and may be used for improving islet engraftment.
Methods
Mouse and human pancreatic islet cells were transfected with mRNA encoding the angiogenic growth factor *Vegf-A* before transplantation under the kidney capsule in mouse.
Results
At day 7 posttransplantation (PT), revascularization of *Vegf-A* mRNA transfected grafts was significantly higher compared to respectively non-transfected or *Gfp* mRNA transfected controls in both mouse islet grafts (1.87- and 2.11-fold), EndoC-bH3 (2.85- and 2.48-fold), and human islet grafts (3.17- and 3.80-fold). At day 30 PT, human islet grafts maintained a vascularization benefit (1.70- and 1.82-fold) and a higher beta cell volume (1.64- and 2.26-fold).
Conclusions/interpretation
Vegf-A mRNA transfection before transplantation provides a promising and safe strategy to improve engraftment of islets and other cell-based implants.

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009

The accuracy of self-reported fractures among a Belgian cohort of postmenopausal women: The FRISBEE study

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In large population-based epidemiological studies of osteoporotic fractures, self-report is an important way of obtaining information. However, this method is

subject to errors of recall and may result in misclassification of fracture status. Surprisingly, the accuracy of self-reported fractures has only rarely been assessed. The purpose of our study was to assess the accuracy of self-reported fractures in the FRISBEE cohort (Brussels, Belgium) of 3560 postmenopausal, aged 60–85 years. Baseline assessment parameters were collected during an interview by trained nurses. Participants were followed yearly by phone call for the occurrence of incident fragility fractures. From 967 reported fractures, 79.3% ($n=767$) were radiologically confirmed. Among the 20.7% ($n=200$) unconfirmed fractures, 56.5% ($n=113$) had no fracture (true false positive; the radiology report indicated that the area was investigated but no fracture was found), for 21% ($n=42$) no radiology report was available (no x-ray was taken or not enough information was given to find the record), 16% ($n=32$) reported an existing fracture (the x-ray at the time the subject reported the fracture showed an old fracture), and 6.5% ($n=13$) of fractures were unconfirmed because of an equivocal radiology report or wrong declared area. Based on the fracture site, among the 56.5% ($n=113$) of true false positive, we found a percentage of 2.7% ($n=3$) for hip, 9.7% ($n=11$) for wrist, 9.7% ($n=11$) for humerus, 23% ($n=26$) for spine, 10.6% ($n=12$) for ankle, 5.3% ($n=6$) for pelvis and 38.9% ($n=44$) for 'minor' fractures (face/skull, ribs, knee, carpal/metacarpal bones, tarsal/metatarsal bones). Further, we investigated the characteristics of individuals who gave a 'wrong information' by using a multivariate analysis - covariates - age, BMI, fracture site, ethnicity, education, smoking, alcohol intake, history of fracture, falls, insomnia, physical activity, calcium and vitamin D intake. We found that subjects with a higher BMI (>25), with fractures on other site than hip, a lower education level, sedentarity and subjects taking calcium supplements were more likely to report unvalidated fractures. In conclusion, the inaccuracy of self-reported fractures is far from being negligible for wrist, humerus, ankle and spine and is inacceptably high for fractures considered as minor.

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010

Comparison of overnight and 48 h low dose dexamethasone suppression tests in volunteers using oral contraceptives

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Introduction
Measurements of midnight salivary cortisol, 24-hour urinary free cortisol (UFC) and 1mg overnight Dexamethasone (DXM) suppression test (short DST) represent classical first line screening tests to diagnose Cushing syndrome. In women using combined Estrogen–Progestogen contraception, cortisol response during short DST is often decreased, leading to false positive (FP = morning plasma cortisol post-DST $>$ 50 nmol/l).
Purpose
The main purpose of our work is to compare overnight (1 mg at midnight) and 48 h (0.5 mg/6 h for 48 h) low dose dexamethasone suppression tests (long DST) in volunteers using oral contraceptives. If the long DST shows less false positive than the short DST, it could be an alternative to stopping contraception in patients with a suspicion of Cushing syndrome.
Method
Thirty healthy volunteers using combined Estrogen–Progestogen contraception participated to the study. Plasma cortisol, ACTH and CBG were measured at baseline (first visit), after short DST (second visit) and after long DST (third visit). Measurements were performed by a 2nd generation Cobas E immunoassay. Results to DST were divided into three categories, the two last corresponding to false positive:
- morning plasma cortisol $\leq$ 50 nmol/l (normal)
- morning plasma cortisol 51 - 138 nmol/l (doubtful)
- morning plasma cortisol $>$ 138 nmol/l (abnormal)

Results
Plasma cortisol levels decreased to a median of 69 nmol/l (percentiles 10–90: 31.8–162.6 nmol/l) after short DST and to a median of 36.5 nmol/l (percentiles 10–90: 18.7–68.7 nmol/l) after long DST. The results showed 63% and 27% of false positive (FP) after respectively short and long DST (significant difference: $P=0.008$ [χ^2]). When we focused on categories of response, no value was higher than 138 nmol/l after the long DST compared to 11% after the short DST, 27% of the values were in the doubtful zone after the long DST compared to 52% after the short DST. In this population, the higher specificity of the long DST was confirmed by a repeated measure ANOVA ($P=0.001$). Finally, when we divided the population into tertiles on the base of their initial plasma cortisol level we observed:
- in the highest tertile ($>$ 900 nmol/l): the short test had 89% FP vs 66% FP for the long test;
- in the average tertile (between 700 and 900 nmol/l): the short test had 66% FP versus 11% FP for the long test;
- in the lowest tertile ($<$ 700 nmol/l), the short test showed still 33% FP vs 0% for the long test.

After multivariate analysis, we showed that the 2 major factors determining a participant's risk of FP were: the basal plasma cortisol level followed by the type of Estrogen–Progestogen contraception. In conclusion, our results suggest that, if a DST is required, the long-DST may be a better option than the short one in a population using an oral contraception and that the cessation of oral contraception is probably not mandatory to interpret the results. These conclusions must be confirmed in a population suspected of hypercortisolism.

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011

Radioiodine treatment for euthyroid goiter: The evolution of thyroid volume, tests and occurrence of autoimmunity

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Background
In Belgium, a land with borderline iodine intake, the prevalence of goiter is 6 to 40%. This raises the question of an economically affordable treatment with few side effects. In euthyroid goiters, the effect of radioiodine on volume reduction is debated. Most data were described last century and do not take into account recent thyroid hormone measurements, newer ultrasonography techniques and changes in iodine supplementation. Doses administered in the past and elsewhere in Europe exceed doses used in Belgium on ambulatory basis.
Patients and methods
Between 2010 and 2017, 211 patients with a euthyroid or a subclinical hyperthyroid multinodular goiter were treated with ^{131}I. Patients had a baseline echography and thyroid function tests. After one, three, six and twelve months, a blood test was carried out. Control ultrasound was scheduled after six and twelve months.
Results
The median volume reduction was 24% after six and 42% after twelve months. An overall tendency indicates a decreasing fT4 and an increase in TSH after a one year follow-up. 24 patients developed transient hyperthyroidism one to three months after treatment and 11 received antithyroid agents. Two patients had emergence of TSI one month after treatment. Definite hypothyroidism developed in 13 patients, necessitating substitution. Both dose of radioiodine and the baseline volume have a significant influence on volume reduction six months post-treatment. Baseline volume also influences volume reduction after twelve months.
Conclusion
A significant decrease in thyroid volume was observed six and twelve months after treatment with radioactive iodine for a non-toxic multinodular goiter.

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012

Immune checkpoint inhibitors and type 1 diabetes mellitus: a case report and systematic review of the literature

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Introduction
Immune checkpoint inhibitors have revolutionized the treatment of advanced malignancies. These monoclonal antibodies target cytotoxic T-lymphocyte antigen-4 (CTLA-4), programmed cell death 1 (PD-1) or its ligand (PD-L1). Endocrine side effects are common, and while hypophysitis and thyroid disorders are predominant, checkpoint-blockade associated diabetes mellitus deserves further notice.
Methods
We present the case of a 61-year old male with metastatic non-small cell lung carcinoma, who was treated with pembrolizumab (anti-PD-1). He was admitted one week before his third cycle with a clinical manifestation of diabetic ketoacidosis (DKA). Blood analysis confirmed marked hyperglycemia (1194 mg/dl), capillary ketones and metabolic acidosis. Glutamic acid decarboxylase autoantibodies (GAD) were detectable, along with a low c-peptide. We subsequently reviewed the literature to identify similar cases. PubMed was searched through August 2018, by two reviewers, working independently (J.d.F. and A.V.K.).
Results
Aside from our patient, we identified 64 additional cases (56% male; aged 22–84 y/o). Half of these were treated for advanced melanoma (52%). Most patients developed diabetes mellitus while being treated with anti-PD-1 in monotherapy (45/64, 70%). On average, patients were diagnosed after 5.7 cycles (range 1–42), while this appears to be earlier for the combination of anti-CTLA-4 and PD-1 blockade (2.9 cycles). Three-quarter of patients presented with DKA, with an average glycaemia of 601 mg/dl and glycated haemoglobin of 7.6%. C-peptide levels at diagnosis were low in 85% of cases. Pancreatic autoantibodies were found positive in 50% of cases, with GAD being the most reported (47%, 28/59 analysed patients). Other endocrine adverse events were mentioned in 25% of cases, of which the thyroid was clearly the most frequently affected.
Conclusions
Checkpoint-blockade associated diabetes mellitus is a rare, but potentially lethal complication, as DKA is often the first presentation. Health-care workers should be aware and patients need to be educated. This is of utmost importance as immune checkpoint therapy is increasingly used, affecting patients in earlier stages and for a longer period of time. Routine measurement of blood glucose and glycated haemoglobin seems to be the most practical and feasible options. Predisposing factors, such as HLA haplotype, may explain why some individuals are at greater risk, though further exploration (e.g. identifying those individuals carrying an increased risk) is required.

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013

No correlation between serum testosterone levels and aggression or anger intensity in transgender people: Results from five European Centres

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Aim
Anger is a state of emotions ranging from irritation to intense rage. Aggression is the externalization of anger through destructive/punitive behaviour. The World Professional Association for Transgender Health (WPATH) Standards of Care, Edition 7 (SOC7) guidelines warn about aggression in transgender men (TM) on testosterone treatment. We aimed to assess whether aggression and anger intensity increase in TM and decrease in transgender women (TW) after initiation of gender affirming hormone therapy and to identify predictors for anger intensity in transgender people, including levels of sex steroids as well as psychological measurements.
Method
This work is a collaboration between the Nottingham Centre for Transgender Health, who assessed aggression, and the European Network for the Investigation of Gender Incongruence (ENIGI), who assessed anger intensity. Prospective changes in aggression were measured at baseline and after one year of gender affirming hormones in 155 transgender persons (64 TM, 91 TW), using the Inventory of Interpersonal Problems (IIP-32) factor 'too aggressive'. Anger intensity was prospectively assessed in 898 participants (440 TM, 468 TW) by the STAXI-2 (State-Trait Anger Expression Inventory-2) State Anger (S-Anger) questionnaire during a three-year follow-up period, starting at the initiation of hormone treatment (testosterone in TM, oestrogens plus anti-androgens in TW). At baseline, psychological questionnaires were administered. Data were analysed cross-sectionally and prospectively.
Results
No prospective changes were reported in 'too aggressive' scores (after one year of hormone therapy) and S-Anger scores (over 3, 12 and 36 months of hormone therapy) in TM and TW. 'Too aggressive' scores were positively correlated to increasing anxiety scores in the entire study population and with lower support from friends in TW. At three, twelve and thirty-six months of gender affirming hormone therapy, anger intensity was not correlated to serum testosterone levels, although there was a correlation with various psychological measures after three and twelve months. TM experiencing menstrual spotting after three months had higher S-Anger scores compared to those without (median 26.5 [18.0 – 29.8] versus 15.0 [15.0 – 17.0], $P=0.020$). Changes in STAXI-2 S-Anger scores were not correlated to changes in serum testosterone levels after three, twelve and thirty-six months in TM or TW.
Conclusion
Aggression and anger intensity are associated with psychological and/or psychiatric vulnerability or the persistence of menstruation in TM, but not with exogenous testosterone therapy in TM or serum testosterone levels in both TM and TW.

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014

Changing practice in the management of differentiated thyroid carcinoma - experience at Brugmann Hospital

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Aim of the work
In patients with differentiated thyroid cancer, the basic goal of the therapy is to improve overall and disease-specific survival, reduce the risk of persistent/recurrent disease while minimizing treatment-related morbidity and unnecessary therapy. In 2015, the American Thyroid Association (ATA) published evidence-based guidelines for the staging and management of differentiated thyroid cancer, including the possibility of avoiding systematic complementary 131-iodine therapy in low-risk patients. Based on these new recommendations, we have modified our management, treatment and monitoring of thyroid carcinoma. The current study aimed at evaluating the influence of these modifications on the therapeutic efficacy in patients with thyroid cancer before and after 2015.
Methods
We conducted a retrospective study in a cohort of patients diagnosed and treated at the Brugmann Hospital between 2007 and 2017. A few patients with metastatic disease were treated several times. Patients were divided into 2 groups: before (Group 1) and after 2015 (Group 2). We compared the two groups in terms of general characteristics, risk of recurrence (based on the 2015 ATA recommendations), cumulative administered 131-iodine activity and biological and morphological response to therapy. Due to the repeated treatment in some patients, the distribution of cumulative activity was not Gaussian.
Results
A total of 98 patients were included: 53 in Group 1 and 48 in Group 2, with a mean age of 50 vs 43 years. Both groups were different in terms of risk stratification: in Group 1, 37.7% were classified as low risk, 45.3% as intermediate risk and 17% as high risk. These figures were respectively 16.7, 54.2 and 29.2% in Group 2 ($P=0.048$). The median cumulative activity of 131-iodine was significantly higher in group 1 (3700MBq, range 1110–14800 MBq) than in group 2 (1110MBq, range 1110–20350 MBq), $P=0.000012$. Excellent response, meaning no clinical, biological or morphological evidence of residual/recurrent disease, was found in 90.5% in Group 1 vs 89.5% in Group 2 ($P=0.347$).

Conclusions
The publication of the ATA evidence-based guidelines for the staging and management of differentiated thyroid cancer in 2015 modified our therapeutic management. After 2015, although the number of patients with a high risk of recurrence was greater and the median administered 131-iodine activity significantly lower, including no systematic iodine administration in low-risk patients, the rate of excellent therapeutic response remained unchanged.

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015

A novel syndrome of neonatal diabetes, microcephaly and epilepsy caused by homozygous mutations in *YIPF5*

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Background and aims
Neonatal diabetes diagnosed before 6 months is caused by mutations that reduce. β cell number (reduced formation or increased destruction) or impair β cell function. We investigated the genetic cause of a syndrome characterised by neonatal diabetes, microcephaly and epilepsy.
Materials and methods
We performed whole genome sequencing for two unrelated patients with neonatal diabetes, epilepsy and microcephaly. Replication studies were performed in 394 patients with neonatal diabetes, using a targeted next generation sequencing assay. YIPF5 was silenced in the human β cell line EndoC-βH1 using RNA interference. Cells were exposed to the endoplasmic reticulum (ER) stressors thapsigargin and brefeldin A. Apoptosis was evaluated by staining with DNA-binding dyes or real-time annexin V binding assay. mRNA expression was assessed by qPCR and *in situ* hybridization.
Results
The two patients were of Turkish and Indian origin, both born to consanguineous parents. They were diagnosed with diabetes at the age of 9 and 15 weeks and were treated with insulin. Genetic testing revealed homozygous likely deleterious mutations (missense and in-frame deletion) in *YIPF5*. Replication studies identified 2 homozygous *YIPF5* mutations in 3 patients (2 siblings) with insulin-treated diabetes diagnosed below age 12 months. All patients had epilepsy and microcephaly. Based on the patients' consistent phenotype, we examined YIPF5 mRNA expression. YIPF5 was abundantly expressed in human pancreatic islets and brain. *In situ* hybridization in foetal brain showed ubiquitous expression of YIPF5 throughout the developing brain, in neurons, progenitor cells and the choroid plexus. We next examined the impact of YIPF5 depletion in β cells. As YIPF5 is thought to play a role in trafficking between ER and Golgi compartments, we examined β cell survival during ER stress, a stress response activated by the accumulation of proteins in the ER. YIPF5 silencing did not affect basal β cell survival, but it increased apoptosis in ER stress conditions. When exposed to chemical ER stressors, YIPF5-depleted cells showed increased expression of the ER stress markers CHOP, sXBP1 and BiP, indicating an exaggerated activation of the ER stress response. YIPF5 silencing enhanced the expression of the pro-apoptotic proteins PUMA and DP5.Silencing the pro-apoptotic transcription factor CHOP protected YIPF5-depleted cells from apoptosis.
Conclusions
We identified homozygous *YIPF5* mutations as a novel cause of neonatal diabetes associated with microcephaly and epilepsy. YIPF5 was abundantly expressed in islets and brain. Functional studies show that YIPF5 deficiency reduces human β cell survival. This is the first form of neonatal diabetes caused by dysregulated ER-to-Golgi trafficking, resulting in increased β cell ER stress and apoptosis.

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016

Retrospective analysis in a patient population with 'EMPTY SELLA TURCICA' based on neuroimaging
Study of the population subgroups, clinical presentation and hormonal function

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Background
An "empty sella" is characterised by a sella turcica filled with cerebrospinal fluid. It is called primary when the empty sella is not linked to any pathological process of the pituitary itself. The prevalence of empty sella has been reported in up to 38% of imaging studies. Some patients may present with endocrine, neurological and ophthalmological symptoms due to the aberrant anatomy of the sellar region. The aim of this study was to analyse clinical presentation, hormonal function and subgroups of the studied primary empty sella population. A second goal was to examine brain imaging and investigate if an association exists between the degree of severity of empty sella and hormonal dysfunction in the examined patients.
Methods
A search-query for "empty sella" was executed throughout the medical records of the university hospital of Brussels for the period from January 2007 to September 2017. Patients with primary empty sella (PES) were selected and basic patient characteristics, hormonal laboratory results and brain imaging were retrospectively analysed. Patients were divided into a moderate and severe PES group, according to pituitary height loss.
Results
One-hundred-thirty-six patients with PES were included. Hormonal dysfunction was observed in 14.7% of the studied population. Growth hormone deficiency and secondary hypogonadism were the most prevalent hormonal dysfunctions in the studied population. These hormonal abnormalities were more frequent in patients with a severe PES compared to patients with a moderate PES. A statistically significant difference was not demonstrated.
Conclusion
A trend was observed between the degree of severity of PES and the occurrence of some hormonal abnormalities. Since hormonal dysfunction did not exclusively occur in patients with severe PES, we suggest that all PES patients should be clinically evaluated and assessed for hormonal dysfunction by an endocrinologist. Further research is needed to define a clear radiological cut-off for empty sella.

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017

Evaluation of knowledge regarding gestational diabetes and evaluation of (group) education for gestational diabetes: the ELENA study

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Background and aims
The prevalence of gestational diabetes (GDM) increases worldwide with rates between 9 and 35%. The management of GDM is a labor-intensive discipline, in which the global increase in GDM prevalence poses challenges to maintain high-quality care. A valuable solution could be the organization of group education. The ELENA study therefore aimed to evaluate women's satisfaction about (group) education and treatment, their knowledge about GDM and whether the diagnosis is associated with feelings of depression and anxiety.
Methods
This monocentric prospective and observational cohort study enrolled 175 women with a recent diagnosis of GDM. GDM was diagnosed by a universal two-step screening strategy with a glucose challenge test and diagnosis of GDM was based on the 2013 WHO criteria. Participants attended two education sessions, with the first session offered as a group education (max. 6 participants) for Dutch-speaking women. An individual follow-up session was planned within two weeks. Participants completed questionnaires before and after the education measuring sociodemographic characteristics, knowledge about GDM, satisfaction about education and treatment, and feelings of depression and anxiety.
Main results
Of all participants, 86 received their first education session in group and 89 received an individual session. Patients were overall satisfied with the content and

duration of both the first and second session and this was generally not different between women who received education in group or individually. 97.7% was very confident in the given advice and 59.1% thought the advice was not too strict. Moreover, knowledge of participants about their condition considerably improved after education was given and this was generally not different between women who received education in group or individually. Feelings of depression were apparent in 27.1% of all participants prior to the education, but declined to 20.2% afterwards ($P=0.124$). With regard to the STAI-6 questionnaire on anxiety, the median total score decreased significantly from 12 (10–14) at the start of the first education session to 11 (8–13) at the end of the second education session ($P<0.0001$). 90.5% of all women receiving group education were satisfied with the group size and 77.4% found that group education fulfilled their expectations. The most frequently reported advantages of group education were 'learning from the questions of others' (77.4%) and 'learning from the experience of others' (52.4%).

Conclusion

Women diagnosed with GDM were overall satisfied with (group) education and had a better understanding of their condition after education. Group education could therefore be a valuable alternative to offset many practical problems associated with the increase in GDM prevalence and it appears to be an added value in the treatment of women with GDM.

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018

Peripheral arterial disease and neuroischemic ulcers in diabetic Charcot foot: a criminal conspiracy?

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Aims

The aims of our study were to compare 1) the prevalence of peripheral arterial disease (PAD) and neuroischemic foot ulcers (NFU) in diabetic patients with and without Charcot foot (CF), 2) the characteristics of PAD in these 2 groups, 3) the major outcomes of NFU including the one year limb salvage rate and one year survival rate in patients with and without CF.

Methods

We retrospectively reviewed the medical records of 172 diabetic patients hospitalized in our unit between 2010 and 2014 for diabetic foot problems, identified using the ICD-9-CM classification. The CF group included 56 patients and the diabetic foot (DF) group included 116 patients.

Main Results

Patients in the CF group were younger (61 ± 10 years vs 65 ± 11 years, P 0.01), had a higher BMI (31 ± 7 kg vs 28 ± 5 kg) and reported more often alcohol abuse than those in the DF group (46.4% vs 19.8%, $P<0.001$). The prevalence of PAD (66.1% vs 66.4%, P 0.97) and neuroischemic FU (69.5% vs 65.8%, P 0.73) were similar between the groups. PAD mostly affected the infrapopliteal arteries alone in the CF group (59.4% vs 26.7%, P 0.005) and both the infrapopliteal arteries and the femoral axis in the DF group (34.4% vs 65.3%, P 0.005). The need for revascularization was lower in the CF group (34.4% vs 78.7%, $P<0.001$). The one year limb salvage rate was similar between the groups (90.6% vs 89.3%, P 0.96) while the one year survival rate was lower in the CF group (93.8% vs 100.0%, P 0.03).

Conclusions

Clinicians should no longer consider CF as free from PAD and NFU despite their association is rarely reported. Interestingly, PAD in CF has specificities as it seems less severe and mostly involves the infrapopliteal arteries alone. The prognosis of NFU is poorer in patients with CF whose one year survival rate was lower. Thus, while NFU and PAD are excessively common in diabetic patients, their association with CF remains silent such as a criminal conspiracy, potentially harmful for both the limb and the patient.

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019

Adrenocortical function during prolonged critical illness and beyond: a prospective observational study

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Aim of the work

The time courses of ACTH, (free) cortisol and cortisol metabolites beyond the first week of critical illness and upon recovery have not been documented. We studied adrenocortical function over time in relation to critical illness duration and presence of sepsis/septic shock, to initiation of glucocorticoid treatment, and to recovery/death.

Methods

Patients still in ICU on day 7 ($N=392$) and 20 matched healthy subjects were included. Morning blood and 24h-urine were collected daily and cosyntropin tests (Synacthen®, 250 μg) performed weekly, repeated 7 days after ICU-discharge.

Main results

In patients who remained free of glucocorticoid treatment until ICU-day 28 ($N=347$), plasma ACTH remained low/normal, whereas free cortisol remained high ($P\leq0.002$) explained by reduced binding proteins ($P\leq0.02$) and suppressed cortisol breakdown ($P\leq0.001$). Beyond ICU-day 28 ($N=64$ long-stayers), plasma ACTH and (free)cortisol became comparable to healthy subjects. Long-stay-patients always showed low incremental total ($P\leq0.001$), but normal incremental free, cortisol responses to weekly cosyntropin-tests, explained by low binding proteins whereby increased cortisol distribution volume. Sepsis/septic shock patients were not different from others, patients subsequently receiving glucocorticoids ($N=45$) were not different from those who did not, and non-survivors were distinguishable from survivors only by higher (free) cortisol. One week after ICU-discharge, plasma ACTH and (free)cortisol increased to supra-normal levels ($P\leq0.006$).

Conclusions

Low plasma binding proteins and suppressed cortisol breakdown in prolonged critical illness confound interpretation of cosyntropin-test results. The absence of elevated ACTH and free cortisol beyond ICU-day 28, followed by a clear rise after ICU-discharge, suggests central adrenocortical suppression that could predispose long-stay ICU patients to adrenal insufficiency.

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020

Epidemiological, biochemical, genetical aspects of pheochromocytomas and paragangliomas followed in CHU of Liege between 1993 and 2017

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Object

Pheochromocytomas and paragangliomas (PPGLs) are rare tumors that are responsible for an inappropriate production of catecholamines. They are heritable in 40% of cases and can integrate different genetic syndroms. Thus, there are several interests to detect them precociously: avoid comorbidities due to the catecholamine hypersecretion, search syndroms and manage their related manifestations and screen the families. The objectives of this study were to separate PPGLs into 3 groups (pheochromocytomas, sympathic and parasympathic paragangliomas paragangliomas) and determinate for each group the proportion of germinal mutations. We were also interested in the type of catecholamine secretions and the correlation between the catecholamine secretion and the tumor size. Finally, we describe the relapsed and malignant forms and searched factors that had promoted them.

Design of the study
It's a retrospective study, based on 80 patients, operated in CHU of Liege between 1993 and 2017.
Methods
We had collected datas about age at diagnosis, genetic results, the presence of hypertension and the necessary number of anti-hypertensive medications before and after surgery. We classified the size of the tumors, their location and their features (bilateral tumors at diagnosis, relapsed and malignant tumors). We had also indicated the datas of 24-hour urinary excretion of catecholamines.
Results
Regarding pheochromocytomas ($n=56$), median age is 51 years (minimum: 11; maximum: 79). Three cases were bilateral at diagnosis (5.37%), three cases relapsed (5.37%) and two were malignant (3.57%). We observed a correlation between secretion and tumor size for metanephrin ($P=0.0423$) and normetanephrin ($P=6.18\times10^{-5}$). Finally, we found 13 germinal mutations for 6 different genes among the 40 patients which had profited of a genetic screening (32.50%). Regarding sympathic paragangliomas ($n=8$), median age is 43.5 years (minimum: 28; maximum: 71). There were two relapsed cases (25%) and four malignant cases (50%). We did not observed correlation between secretion and tumor size, neither for metanephrin ($P=0.121$) nor normetanephrin ($P=0.579$). Among 6 patients which profited of a genetic screening, we found 2 germinal mutations into 2 different genes (33.33%). Regarding parasympathic paragangliomas ($n=17$), median age is 54 years (minimum:20; maximum: 88). Two cases had relapsed (11.76%) and one case was malignant (5.88%). Their location concerned tympanic glomus (52.94%), carotid body (35.29%), jugular glomus (5.88%) and vagal nerve (5.88%). A genetic screening had been realised in one patient and was negative.
Conclusions
With our study, we have shown the correlation between tumor size and catecholaminergic secretion in our pheochromocytoma group. We have also illustrated the link between genetics and development of bilateral/relapsed PPGLs, expecially in young patients. A genetic screening had been done among 47 patients and shown germinal mutations in 29.78% of cases. Finally, one of these patients had a syndrome described for the first time few months ago, characterized by a pheochromocytoma and a pituitary adenoma due to a deletion in *MAX* gene.

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021

Cardiovascular complications in pituitary gigantism (results of an international study)

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Background
Cardiovascular disease is an important cause of morbidity/mortality in chronic GH hypersecretion.
Aim
To evaluate cardiovascular system in a large series of patients with pituitary gigantism. Standard case report forms were used with height assessments related to local country norms. Results: 151pts (123 male) with GH-excess and abnormal growth velocity for age or final height >2SD over local norms had complete data on cardiac assessments at baseline or on median 7,5 yr [3;17] of follow-up. Median ages at first symptoms and at diagnosis of pituitary adenoma (PA) were 14yr [11;16] and 21yr [16;27], respectively; latency was 6yrs [2.9;12]. Overall, cardiovascular disorders were reported in 38,3% during the period of follow-up. Clinical evaluation of cardiovascular system appeared normal in 65 pts who had no instrumental examination, 83 pts were evaluated by echocardiography either as routine examination or in aligned patients with cardiovascular symptomology, another 3 pts were reported as with heart disease without further details. Cardiovascular disorders prevalences in the group of 83 pts assessed by echocardiography: left ventricular hypertrophy- 56%; concentric biventricular hypertrophy- 9%; tachycardia- 7%; bradycardia- 3.6%; diastolic dysfunction- 23.6%); systolic dysfunction- 16.4%; heart failure- 7.3%; dilatative cardiomyopathy- 18.2%; arrhythmia- 18.2%; valvular disease- 27.3%; stroke- 1.8%; coronary heart disease- 3.6%; aortic dilatation- 3.6%. Cardiovascular disorders were related to older age at diagnosis of pituitary adenoma ($P=0.04$), longer latency period ($P=0.02$), delayed treatment ($P=0.04$) and disease control ($P=0.001$), but not to hormonal (GH/IGF-1) levels. In those patients with hormonal control achived before age of 20 yr, cardiovascular disease occurred less frequently ($P=0.03$). Impact of gender and gonadal status was not significant, whereas cardiovascular disorders were associated with more frequent impaired glucose metabolism (48% vs. 19%).
Conclusions
This first large, specific study of pituitary gigantism shows a high prevalence of cardiovascular disorders, mainly influenced by uncontrolled pituitary disease duration. Given their young age, particularly careful attention should be given to this complication and its adequate early management.

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022

Non-alcoholic fatty liver disease and its association with insulin resistance in an obese population

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Background
Obesity induced inflammation is a key component in the pathogenesis of insulin resistance (IR). In addition, obesity-related non-alcoholic fatty liver disease (NAFLD) also seems to contribute to IR development. Until now, however, it is unclear which, if any component of NAFLD specifically associates with IR. Therefore, the aim is to assess if individual components of NAFLD contribute to IR in obese patients undergoing gastric bypass surgery (GBS).
Subjects and methods
This cross-sectional study included 73 obese patients (mean age 45 ± 11 years; BMI 42.2 ± 4.8 kg/m^2) undergoing GBS. Glucose levels were analysed by hexokinase method and insulin levels with electrochemiluminescence. Homeostasis model assessment-estimated insulin resistance (HOMA-IR) was calculated with following formula

$$\text{HOMA} - \text{IR} = \frac{\text{fasting glucose}\left(\frac{\text{mmol}}{\text{l}}\right) * \text{fasting insulin}\left(\frac{\mu\text{U}}{\text{ml}}\right)}{22.5}.$$

Liver biopsies taken during GBS were evaluated using Steatosis, Activity and Fibrosis scoring (SAF score).
Main results
Among GBS patients, according to the SAF score, patients with non-alcoholic steatohepatitis (NASH) had higher glucose levels compared to those without. Besides, patients with a higher grade of inflammation had higher HOMA-IR and insulin levels compared to those with a lower inflammation grade ($P < 0.05$), an association that was independent from age and BMI ($F(2,67) = 5517$; $P = 0.006$). Patients with no fibrosis had lower glucose levels ($P = 0.037$) and a trend towards lower insulin ($P = 0.079$) and HOMA-IR ($P = 0.088$) levels compared to patients with a higher grade of fibrosis. Ballooning and steatosis grade were not associated with HOMA-IR.
Conclusion
This study shows that within an insulin resistant group of obese patients, the level of IR correlates with histopathologic subcomponents of NAFLD. Specifically, whereas steatosis and ballooning are not associated with HOMA-IR, a higher grade of hepatic inflammation is associated with higher IR. For fibrosis, a trend towards higher IR with higher grade of fibrosis is found. Whether this finding reflects a subgroup of patients with more severe adiposity-related consequences, such as whole-body systemic inflammation, or whether this results from a direct effect of hepatic inflammation (and fibrosis) on IR needs to be further investigated.

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023

Endogenous testosterone supports spermatogenesis even in the absence of gonadotrophins: evidence from a case report

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Background
In patients with testicular dysgenesis syndrome, reduced semen quality and testicular cancer are common. We report a case of a testicular tumour in a patient with a history of cryptorchidism and oligoasthenospermia. He had an unusual hormonal profile, which was not fully explained by the pathological findings.
Case
A 31-year-old man was referred to our tertiary care andrology unit for primary infertility with a history of bilateral orchidopexy during childhood. Testes were small (12 cc). Gynaecomastia was absent. Semen analysis repeatedly showed oligoasthenospermia (2.4 to 7.1 million/mL, 85 to 92% immotile). Gonadotropins (LH and FSH < 0.1 U/L) were undetectable, but testosterone and estradiol were normal (850.7 ng/dL and 38.6 ng/L). Prolactin, other pituitary hormones, DHEAS, AFP, HCG and inhibin B were also normal. He denied using anabolic steroids. Suppressed gonadotrophins suggested a sex steroid producing testicular tumour. However, scrotal ultrasound only showed diffuse microcalcifications and three millimetric hypolucent lesions in the left testis, but no intratesticular mass. There were no suspicious lesions nor microcalcifications in the right testis. To further investigate the possibility of increased testicular sex steroid production, selective testicular venous sampling was performed. In the left spermatic vein, testosterone and estradiol levels were very high (3744 ng/dL and 378 ng/L), with a testis-to-periphery gradient of 4.4 and 9.0 respectively. There was no gradient in the right spermatic vein. These results confirmed increased sex steroid producing in the left testis. However, histopathological examination after orchidectomy revealed a multifocal seminoma (largest diameter 3 mm) and profuse germ cell neoplasia in situ. There were neither isolated syncytiotrophoblastic cells, nor choriocarcinoma. Leydig cell hyperplasia was present without Leydig cell tumour. HCG was remeasured with three different methods, all showing very low HCG between 0.6 and 1.1 IU/L. After orchidectomy gonadotrophin levels increased (LH 24.3 U/L, FSH 10.3 U/L), with normal total testosterone and estradiol, indicating recovery of suppression of the hypothalamic-pituitary-testis axis. Sperm concentration increased (10 million/mL.)
Key messages
1. Our case shows that endogenous testosterone may support spermatogenesis even without gonadotropins.
2. In patients with suppressed gonadotropins, normal sex steroid levels and no testicular mass, selective testicular venous sampling can be useful in identifying the site of hormonal overproduction.
3. Thus far, the pathology findings cannot explain the hormonal profile. Further investigations are therefore ongoing.

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A rare cause of polycythemia in an octogenarian

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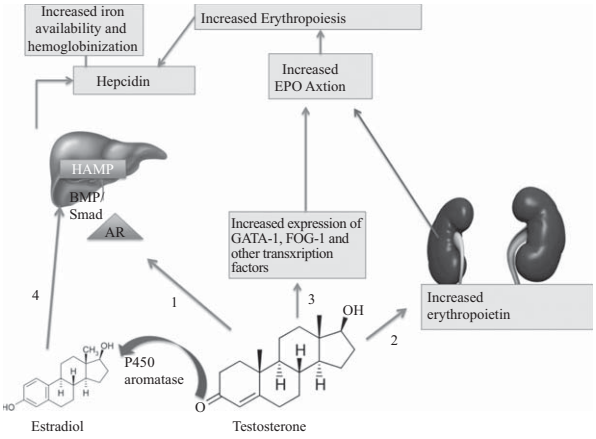
An 82-year-old woman with a history of breast carcinoma and hypertension underwent right laparoscopic adrenalectomy for a large mass (7×5 cm) of high density without contrast (> 30 UH), non-secreting. Anatomapathological examination concluded to an adrenocortical adenoma without signs of malignancy: Weiss score of 2 and Ki67 $< 2\%$. Two years later, she developed polycythemia (Hb: 17.9 g/dl (N: 11.8–15.5); hematocrit: 53.5% (N: 35.3–46.1)) without involvement of other hematopoietic lineages. EPO level was in the normal range (13.5 U/l (N: 3.1–17)) and no JAK2 mutation was detected. A few months later, she complained of unusual hair on arms and face and weight gain. Clinical examination revealed signs of hypercortisolism: fatty supraclavicular fullness, dorsal fat pad, ecchymosis and muscular atrophy. Hormonal evaluation demonstrated severe hyperandrogenemia (total testosterone: 28.3 nmol/l (N: 0.50–1.67); DHEAs $>$ 27.0 µmol/l (N: 0.33–4.18); androstenedione: 18.0 ng/ml (N: 0.1–3.0)) and hypercortisolism (no inhibition of plasma cortisol by 1 mg overnightdexamethasone test and urinary free cortisol (UFC) at three-fold the upper limit of reference range). Abdominal imaging showed local tumour recurrence in right adrenalectomy sitewith distant peritoneal metastases. Ketoconazole therapy was initiated to control excessive cortisol secretion. Four months after diagnosis, despite Ketoconazole therapy, UFC increased to 3400 nmol/24 h (N: 100–379). Because of uncontrolled hypercortisolism and patient's choice, surgical resection was finally performed after 7 days of continuous intravenous infusion of Etomidate at the dose of 0.3 mg/kg per hour. Androgens levels and UFC returned to normal after a large debulking. Remarkably, Ki67 was also $< 2\%$ in the resected tumour but many criteria of malignancy scoring systems were observed: marked atypical mitoses, confluent necrosis, venous invasion. While polycythemia is a well-known side effect of excessive exogenous androgens or anabolic steroids intake, it is an unusual manifestation in adrenal carcinoma secreting androgens.Testosterone-induced increase in hemoglobin and hematocrit is associated with stimulation of erythropoietin (EPO) secretion and reduced hepcidin concentrations. In our case, EPO level was inappropriately normal-high in the presence of a secondary erythrocytosis suggesting a possible EPO-producing tumour. However, it is impossible to distinguish an increase in EPO due to a tumor production or due to an increase renal production induced by testosterone excess. This case illustrates



well the limits of clinical and prognostic value of malignancy scoring system such Weiss score and the need to identify new markers of aggressiveness (maybe EPO-producing by the tumour?) and therefore the importance of a complete anatomo-clinical characterization and the need multidisciplinary management for rare tumours such as adrenal carcinoma.

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025

Rare cause of 1,25-dihydroxy vitamin D mediated hypercalcemia: A case report and literature study

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Background

1,25-dihydroxy vitamin D mediated hypercalcemia resulting from an inflammatory reaction caused by a foreign body such as a textiloma has never been described.

Clinical case

A 61 year-old man with a medical history of Alport syndrome and in need of chronic dialysis after transplant kidney resection because of relapsing severe urinary tract infections, presented with recurrent and progressive hypercalcemia two years after parathyroidectomy (C and B) because of tertiary hyperparathyroidism. There was no intake of calcium supplements or diuretics. Laboratory evaluations showed a normal 25-hydroxyvitamin D but an elevated 1,25-dihydroxyvitamin D and an inadequately normal PTH (Table 1). A ^{99}mTc parathyroid scintigraphy could not show any adenoma. In order to localize a possible inflammatory process, an FDG-PET-CT was performed and revealed an abdominal mass with a high metabolic captive border. Four days later the patient arrived at dialysis and showed signs of fatigue. Inflammatory values were high. He was admitted to the hospital and empirical antibiotics were started. Despite the antibiotics, inflammatory parameters raised and the patient complained of progressive abdominal pain. A diagnostic laparotomy was performed and revealed a textiloma (a surgical gauze). After removal of the textiloma a complete normalization of the hypercalcemia and 1,25-dihydroxyvitamin D was observed.

Table 1 Laboratory results.

Lab	Value	Value after surgery	Reference value
Calcium	2.88 mmol/l	2.50 mmol/l	2.2–2.55 mmol/l
Phosphorous	1.80 mmol/l	1.08 mmol/l	0.81–1.45 mmol/l
PTH	23 ng/l	218 ng/l	15–65 ng/l
25-hydroxyvitamin D	47 ng/ml	53.3 ng/ml	>30 ng/ml
1,25-dihydroxyvitamin D	172,6 pmol/l	45.9 pmol/l	<5–42 pmol/l
Serum creatinine	13.6 mg/dl	8.8 mg/dl	0.6–1.2 mg/dl

Conclusion

Hypercalcemia mediated by 1,25-dihydroxy vitamin D is rather uncommon. Well-recognized etiologies are sarcoidosis and lymphomas. Less than 10 case reports were found where the underlying cause was a foreign body exposure, no case was found where the foreign body was a textiloma. The underlying mechanism remains unclear, but the literature mainly suggests that a foreign body reaction results in formation of foreign body granulomas. Activated macrophages express 1-α hydroxylase which transforms 25-hydroxyvitamin D into 1,25-dihydroxyvitamin D, leading to hypercalcemia.

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026

Secondary amenorrhea reveals a polyglandular auto-immune syndrome of type II

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Introduction

Auto-immune polyglandular syndrome type 2 (APS-II) is an autoimmune condition which combines Addison's disease (primary adrenal insufficiency) with another autoimmune pathology like thyroiditis, diabete mellitus, primary hypogonadism, vitiligo, Biermer anemia, etc. Patients can develop those pathologies concurrently or many years after the first manifestation. Prevalence of APS-II is 1 or 2 cases/100.000/year and the sex ratio is 3 women for 1 man. Genetic transmission is polygenic and multifactorial, suggesting an impact from the environment. Polymorphism of genes HLA, CTLA-4 and PTPN22 could explain this complex transmission. Different polyglandular autoimmune syndromes exist, each one distinguished by the genetics and the organs it affects.

Case

We report a case of a 37-year's old woman addressed at our Endocrinology department for a secondary amenorrhea. After stopping her contraception, menstruations did not come back for six months and she lost nine kilograms in nearly six months. Nauseas, loss of appetite and flushes were present. Hashimoto thyroiditis has been diagnosed six years ago and therefore the thyroid function was substituted by 75 micrograms thyroxine. Explorations consisted in hormonal blood test, karyotype, ACTH test and renin activity measurement. High levels of gonadotrophins and ACTH in combination with low levels of estrogens, cortisol, aldosterone and DHEA suggested a deficit of hormonal secretion. Anti-adrenal and anti-ovary antibodies were detected in the blood test. Karyotype was 46XX. The ACTH test revealed an incapacity for the adrenal glands to produce and deliver cortisol (glucocorticoids). The aldosterone/renin activity measurements showed a deficit in mineralocorticoid secretion. The diagnosis of autoimmune oophoritis and Addison's disease was considered. Glucocorticoid (hydrocortisone) and mineralocorticoid (9-α-fluorohydrocortisone) substitution treatment was initiated.

Discussion

One percent of women beneath 40 years suffer from autoimmune oophoritis which could also be one of the manifestations of APS. Therefore this diagnosis should be kept in mind when exploring secondary amenorrhea in young females. Antibodies attack steroid synthesis enzymes as 21-hydroxylase, 17-alpha hydroxylase and P450scc cytochrome. Autoimmune oophoritis results in a specific aggression of thecal cells causing a lack of estrogens but sparing preantral follicles. Preantral and antral follicules produce antimullerian hormone (AMH), which is a biological marker of the reserve of follicules. The AMH level remains high in autoimmune oophoritis. According to those findings, the pool of follicules seems to be preserved in autoimmune ovarian insufficiency cases. Immunomodulators could reduce the inflammation and protect the ovarian function. Glucocorticoid administration may partly restore the ovarian activity and two months after the intake, ovulation can occur but menstrual cycles remain fluctuant.

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027

A curious case of hypercalcemia, hypercalciuria and recurrent nephrolithiases

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Background
Most patients consulting an endocrinologist for hypercalcemia suffer from primary hyperparathyroidism. Rarely, toxic vitamin D levels can also induce hypercalcemia while suppressing parathyroid hormone (PTH) levels. Infantile hypercalcemia can be caused by mutations in CYP24A1 (24-hydroxylase), which are inherited in an autosomal recessive manner (1). This enzyme is responsible for the inactivation of 25-OH-vitaminD (25-OHD) and $1,25(OH)_2$vitaminD_3 ($1,25(OH)_2D_3$). A loss of function in CYP24A1 can result in toxic vitamin D levels causing hypercalcemia and hypercalciuria. Several cases have been described in which hypercalcemia due to often more mild CYP24A1 mutation has not been discovered until adult age, and these patients usually present with recurrent nephrolithiases (2–4).
Case
A 44-year old male was first referred to the outpatient endocrinology clinic in the year 2016. He had a medical history of recurrent nephrolithiasis, with a first episode occurring at the age of 22. At the age of 33, a blood work-up showed hypercalcemia (2.8 mmol/l (ref 2.1–2.65)) with normophosphatemia (1.03 mmol/l (ref 0.75–1.45)) and suppression of PTH (5 ng/l (ref 12–72)). Calciuria was increased (9.2 mmol/24h (ref $<$7.5 mmol/24h)). 25-OHD was in the high normal range (66.2 µg/l (ref 11–60)) and $1,25(OH)_2D_3$ was normal (44.6 ng/l (ref 29–65)). Thyroid function was normal and there was no evidence of cortisol deficiency. Granulomatous and malignant diseases were excluded by negative imaging (FDG-PET, bone scintigraphy), protein electrophoresis and Mantoux test. The diagnosis was a non-PTH mediated hypercalcemia of unknown etiology. Kidney function at that time was at the lower limit of normal (serum creatinine: 1.2 mg/dl (ref 0.7–1.2)), corresponding to an eGFR (CKD-EPI) of 79 ml/min per 1.73 m^2. Eleven years later at the age of 44, hypercalcemia was confirmed (2.90 mmol/l), but PTH was no longer suppressed (31.6 ng/l). Neck ultrasound and parathyroid scintigraphy were normal. Choline-PET scintigraphy suggested two hotspots possibly suggestive for parathyroid adenomas. Further biochemical analysis showed elevated 25-OHD (67 µg/l), high-normal $1,25(OH)_2D_3$ (65.2 ng/l), but very low levels of $24,25(OH)_2D_3$ ($<$0.2 ng/ml), suggestive for reduced breakdown of 25-OHD and $1,25(OH)_2D_3$. Genetic analysis revealed a homozygous inactivating mutation in the CYP24A1 gene (c.1186C$>$T (p.Arg396Trp) confirming the diagnosis of congenital 24-hydroxylase deficiency. A high phosphate diet (to lower the fractional gastrointestinal calcium absorption), bisphosphonates (to lower the calcium flux from the bone), itraconazole (P450 inhibitor) and cinacalcet (to suppress PTH and to directly increase calciuria) either failed to lower serum calcium levels or were not tolerated. Hypercalcemia persisted and kidney function deteriorated (eGFR nadir 41 ml/min per 1.73 m^2). Ultrasound of the kidneys confirmed bilateral nephro-ureterolithiasis. A kidney biopsy showed extensive interstitial fibrosis, tubular atrophy and interstitial calcium-phosphate deposits (nephrocalcinosis). Given the severity of the condition and the failure of other treatment options, a subtotal parathyroidectomy was performed. Two out of four glands showed hyperplasia on pathological examination. PTH levels decreased from 48 to 12 ng/l two months after surgery, along with normalization of the serum calcium levels and calciuria (resp. 2.28 mmol/l and 3.1 mmol/24 h. 25-OHD and $1,25(OH)_2D_3$ levels also diminished (resp. 53.9 µg/l and 38,3 ng/l). Kidney function partially recovered (eGFR 51 ml/min per 1.73 m^2).
Discussion
In patients with recurrent episodes of nephrolithiasis at early adult age, hypercalcemia and hypercalciuria with low PTH levels, a problem of CYP24A1 inactivation needs to be ruled out. $25\text{-OHD}/24,25(OH)_2D_3$ ratio should be measured, followed by confirmatory genetic analysis when the ratio is high. These patients should also carefully be monitored for kidney dysfunction, which may occur as a consequence of nephrocalcinosis. Effective treatment of this condition is however challenging. To the best of our knowledge this is the first case of patient with a CYP24A1 mutation where a subtotal parathyroidectomy resulted in normal serum calcium levels, along with an improved kidney function.
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028

A delayed diagnosis of endocrine hypertension

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A 43-year old woman was referred to the endocrinologist due to resistant arterial hypertension with a history of bilateral adrenal hypertrophy. The first medical contact with the patient dates back to 2012 when she was admitted to the urology department. A CT scan of the abdomen revealed a bilateral adrenal mass, hypodens lesions in the liver and a small left kidney. Further imaging with MRI showed similar findings. The liver lesions were described as hemangiomas. Because of high tensions during hospitalisation the patient was send to nephrology. Hormone balance showed a high to normal serum cortisol, low ACTH and an increased PAC/ARC ratio. A 24-hour urine collection showed no arguments for a pheochromocytoma. An oral salt loading test did not match with hyperaldosteronism. Six months after first imaging, a CT angiography of the renal arteries revealed a mass at the right kidney lower pole. At first the enlarged mass was described as a hemorrhagic cyst, but after further MRI imaging, the mass was suspected of malignancy A lesion in the fourth liver lobe, previously described as focal hepatic steatosis, was also suspected of malignancy. The patient never presented for consultation and was lost in follow-up. Untill four years later the family doctor send the patient to cardiology due to resistant arterial hypertension. Redo CT abdomen showed a new renal mass next to the known mass of the right kidney under pole and new bilateral adrenal nodules next to the known bilateral nodules as earlier described. Five years after the first contact, the patient was seen by an endocrinologist. A new hormonal balance showed an increased serum cortisol with a low ACTH. Control blood sample also revealed a detoriation of the kidney function and an elevated HbA1C (8.4%). Patient was admitted to the endocrinology department. A 48-hour low dose dexamethasone suppression test showed no

suppression of serum cortisol. In summary, this case contains three main problems. The first problem being resistant hypertension. Adrenocortical causes of hypertension are primary hyperaldestoronism, hyperdeoxycorticosteronism and Cushing syndrome. Primary hyperaldosteronism was excluded by an oral salt loading test. Cushing syndrome was confirmed by an elevated free cortisol on a 24-hour urine collection, a 48-hour low dose dexamethasone suppression test and an elevated late night salivary cortisol. CT and MRI abdomen revealed bilateral macronodular adenoma which lead us to the diagnosis of macronodular hyperplasia. Secondly the patient has chronic renal failure stage four. Metabolic, we see a high to normal calcium with an elevated PTH. Thirdly, two renal masses and a liver lesion are suspected for malignancy wherefore a PET-CT was performed. This displayed an increased tracer uptake of the lesion in the fourth liver lobe. Liver biopsy withheld no arguments for malignancy. A partial right nephrectomy was proposed. Macronodular hyperplasia is an ACTH-independent Cushing syndrome with bilateral adrenal adenoma greater than one centimeter. It is a rare cause for Cushing syndrome. Macronodular hyperplasia occurs sporadically, however more than fifty percent of the patients are carrier of a mutation of the ARMC5 gene. Pathophysiology is not fully known but two important findings are: an aberrant expression of hormone receptors and a paracrine secretion of ACTH. Diagnosis is based on hormone balance and imaging. Screening for aberrant hormone receptors is indicated for luteinizing hormone (LH), human chorionic gonadotropin (hCG) and beta adrenergic receptors. Most patients have a mild form of Cushing syndrome, a unilateral adrenalectomy as treatment may be sufficient. Rather than surgery, drug treatment is an option for macronodular hyperplasia with aberrant LH, hCG or beta adrenergic receptors.

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029

Rara avis or recurrence of papillary thyroid carcinoma

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A 21-year-old woman underwent total thyroidectomy and unilateral central neck dissection for papillary thyroid carcinoma. Four of the removed lymph nodes tested positive for metastasis. Additional radioactive iodine treatment was given and the post I-131 therapy total body scan showed increased uptake of iodine-131 in a thyroid remnant but none in any of the lymph nodes. Postoperatively thyroglobulin levels decreased but remained elevated. During follow-up, a nodular enlargement was palpable in the patient's neck. Ultrasound revealed a suspicious lymph node with a diameter of $>$ 5 cm, localized posteriorly to the sternocleidomastoid muscle. Fine needle aspiration (FNAC) and biopsy showed no evidence for metastatic disease. However, FDG-PET/CT showed mild metabolic hyperactivity. Given this context, after multidisciplinary consultation, a neck dissection was performed. The result showed Castleman disease. Although this entity is rare, Castleman disease has to be considered in the differential diagnosis of cervical nodular lesions. This case demonstrates Castleman disease as a possible condition to be confused with recurrence of thyroid carcinoma and the potential use of FDG-PET/CT in patients with measurable thyroglobulin levels but negative post I-131 therapy total body scan in the followup of thyroid carcinoma.

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030

Preoperative treatment of benign insulinoma: diazoxide or lanreotide?

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Introduction

Insulinoma is a rare neuroendocrine tumour of the pancreas. Its resection is the only curative treatment. However, medical therapy may be needed to prevent severe preoperative hypoglycaemia, when surgery is contraindicated, delayed or refused and in case of unresectable metastatic disease. To our knowledge, a comparison between these two medications has never been performed.

Case report

We describe the case of a 27-year-old patient without specific past history who was referred for discomfort suggestive of hypoglycaemia. A diagnosis of insulinoma was made in April 2018 after a fasting test. The patient had a symptomatic hypoglycaemia after 12 hours with a blood glucose at 33 mg/dl, an insulin level at 74.8 pmol/l, a c-peptide at 1.38 pmol/ml and B-hydroxybutyrate at 0.102 mmol/l. We did not detect sulfonylurea in the 24-hour urine collection. Pancreatic magnetic resonance imaging confirmed a 17×20 mm lesion in the upper part of the pancreatic body, that was also visualized by indium-111 pentetreotide scintigraphy (OctreoScan). We therefore scheduled an operation that was possible 6 weeks later. Diazoxide treatment was then initiated at a dose of 50 mg three times a day which was gradually increased to 150 mg three times a day. On this treatment, the patient kept symptomatic hypoglycaemia twice a week. In addition, he had increasing dyspnoea. A cardiopulmonary assessment, including a chest radiograph, a spirometry and cardiac ultrasound, was normal. We assumed that dyspnoea could be favoured by water retention caused by diazoxide. So, after a month on diazoxide, the patient was admitted to hospital to shift treatment to lanreotide 120 mg under glucose monitoring. Thereafter, the patient did not experience hypoglycaemia and dyspnoea disappeared until operation. An EORTC Quality of Life Questionnaire-Core 30 (version 3.0) was filled in by the patient, without treatment, under diazoxide 450 mg a day, lanreotide 120 mg and a month after surgery. Summary scores were respectively 84.7, 73.3, 90.9 and 99.1. The operation, consisting in an enucleation was uneventful.

Conclusion

In our patient, diazoxide failed to demonstrate its effectiveness at the maximum tolerated dose and at the cost of side effects decreasing the quality of life. Studies have already demonstrated the presence of these side effects such as that of Gill *et al*. who tested diazoxide in 40 patients. 1) Forty-seven percent had side effects, mainly sodium-water retention and hirsutism for a total effectiveness rate of 59%. Cases of heart failure have also been reported under diazoxide. 2) Regarding somatostatin analogues, there are few reports available. A prospective study including 21 patients showed efficacy of octreotide in 67% of cases with a good tolerance. 3) OctreoScan does not seem to be a predictor of response to this treatment. In conclusion, if medical treatment is required, somatostatin analogues rather than diazoxide may be an effective and well-tolerated option in insulinoma.

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031

TSH receptor-blocking autoantibodies: the pregnancy paradigm

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Background

Graves' disease (GD) is an autoimmune disease caused by antibodies directed to the TSH receptor (TRAb), which can be activating (TSAb), neutral, or blocking (TBAb). This balance determines the final clinical result. Typically TSAbs dominate, resulting in hyperthyroidism. Rarely, TBAbs prevail and result in hypothyroidism (1). The TRAb assay routinely used in the clinic does not allow to differentiate between stimulating or blocking characteristics.

Case

A 28-year old female was initially referred to the thyroid outpatient clinic because of hyperthyroidism caused by typical GD. She was treated with block-replacement therapy for 36 months because of persistently high TRAb titers. Six weeks after drug cessation she developed an overt hypothyroidism. Further analysis showed highly blocking properties of the TRAbs. Levothyroxine (LT4)

substitution was initiated, and her thyroid function tests remained stable over the following months under a LT4 dose similar to athyreotic patients. Two years later she became spontaneously pregnant, after which the functional TRAb status was monitored meticulously and LT4 substitution in first instance was increased to ensure adequate fetal LT4 supply in the first trimester. During the course of the pregnancy, TRAbs remained high and blocking, so the maternal LT4 dose was kept high. The fetal development was normal, without evidence for fetal hypothyroidism (including absence of a fetal goiter) and thus without need for intra-amniotic LT4 treatment. A healthy newborn (male) was delivered on term. In the postpartum period the newborn developed transient mild hypothyroidism and was treated with LT4 substitution until disappearance of the maternal TRAbs. The maternal functional TRAb status remained unchanged in the postpartum period. Four years later a second pregnancy and postpartum followed an identical course.

Discussion

GD dominated by TBAb is believed to be a very rare entity in the spectrum of autoimmune thyroid diseases. Although its treatment is rather straightforward (LT4 substitution), the challenge and message of this case is the safety of a pregnancy. Because TBAb, like TSAb, can readily transfer transplacentally, fetal hypothyroidism could develop (2) which if left untreated can cause severe and irreversible impairment in neurodevelopment. In utero, the clinical endpoint of high maternal TBAb is comparable with a status of congenital thyroid dysgenesis. The best treatment is to provide adequate maternal LT4 in order to ensure sufficient transfer to the fetus (3) along with intensive follow-up by an experienced gynecologist. The true challenge was however the evolution of the TRAb status, as pregnancy itself represents a state of physiologic immune modulation, which could potentially lead to an altered quantitative and qualitative maternal TRAb status. In that respect, in the best case scenario the patient could also have evolved towards a state of remission of her GD during pregnancy (as in the case of the majority of classical cases) with less (or no) need for LT4. However, in the worst case scenario an immune shift could have occurred towards high and dominating TSAbs, resulting in maternal and fetal hyperthyroidism, which would have been far more challenging with respect to medical treatment (4, 5). Because GD with TBAb is a rare disease, it is of utmost importance to detect the cases, especially women in childbearing age. This case shows the added value of functional analysis of TRAbs before and during pregnancy along with intensive clinical multidisciplinary follow-up (endocrinologist, gynaecologist, pediatrician). This case highlights that TRAb screening is indicated in every women with a history of GD, especially women under LT4 substitution (either after radioiodine treatment or thyroidectomy or a spontaneous evolution towards hypothyroidism). In case of high TRAb titers differentiation between stimulating and blocking antibodies is of added value in pregnancy counselling and clinical management during pregnancy. Although there is still a long way to go, recent breakthroughs have drawn more attention to this condition that could be more prevalent than currently known.

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032

Small stature with osteochondritis: a clinical report of a family with *ACAN* mutation and review of the literature

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Introduction

Thanks to advances in genetics, idiopatic short stature have, more frequently, a molecular diagnosis. Mutations in the *ACAN* gene are responsible for different forms of syndromic short stature but were also described in association with idiopathic short stature or with joint damage and advanced bone age. Transmission is autosomal dominant. Less than 25 mutations have been described since 2010 and are localized all along the protein. The *ACAN* gene located on chromosome 15q26 encodes a protein called the Aggrecan protein. It is a major component of the extra-cellular matrix of articular and growth cartilage. Some affected patients have been recently treated by growth hormone (GH) therapy and showed a variable increase in height. We report a 9-years-old girl with a familial history of autosomal dominant short stature and osteochondritis of the knee, where we found by clinical exome sequencing an heterozygous nonsense mutation c.2110 C> T (p.Gln704*) in the ACAN gene.

Case report

Lea consulted in 2013 for a short stature at 6 years old. She is the fourth in a family of four children from non-consanguinous parents. The father's height is 156 cm (−3.4 SDS) and the mother's heigt is 155 cm (−1.9 SDS). Her target height is therefore 150 cm (−2.8 SDS). Her 22 years old sister's height is 145 cm (−3.6 SDS) with a normal endocrinological assessment (IgF1, cortisol, prolactin, TSH-T4, glucagon stimulation test). The 2 other sisters (19 and 18 year old) have a normal height (160 cm; −1.1 SDS). In the family history, there were several short stature in the father's family associated with osteochondritis of the knee. Lea was born at term (39w) with a birth weight of 2680 gr (−1.7 SDS) and a birth length of 46 cm (−2 SDS). There was any neonatal problem. At the age of 6, her height was 100.8 cm (−3 SDS, −0.2 SDS below her target height) with a BMI of 0.7 SDS and a growth velocity of 6.5 cm per year (0.1 SDS). She had no symptoms, except transient knee pain without diagnose. The clinical examination was normal, prepuberal with normal proportion but a relative macrocrania (head circumference 52.8 cm +0.8 SDS). Endocrinological screening was normal (IGF-1 159 ng/ml – range 85–315 ng/ml), bone age (Greulich and Pyle) was 8 for a chronological age of 6 and genetic assessment was normal (CGH-array, *SHOX* and *FGFR3* genes). In 2017, she start entry into puberty (Tanner P2M2) with a height of 115.7 cm (−3.1 SDS, −0.3 SDS below her target height) and a growth rate of 3.5 cm/year (−2.6 SDS). Bone age was 10 years old with a height prediction of ∼ 147 cm. A mendelioma was done and showed a stop heterozygous C.2110 C> T mutation (p.Gln704 *) in the *ACAN* gene responsible for her familial short stature. GH treatment was started in combination with GH-RH analogs. Adult height is not yet reached.

Conclusion

We describe the case of a 9-year-old child with familial short stature associated with osteochondritis of the knee, advance bone age and the absence of growthspurt entering puberty in a context of heterozygous mutation in the *ACAN* gene. Paediatricians should accord importance to symptoms of osteochonditis associated with familial short stature as the association of small stature with advance bone age. According to some studies, GH treatment in this case could allow her to reach an adult height closer to her target height but more prospective studies are needed to clarify the height gain.

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033

The importance of post-resection histologic confirmation in the diagnosis and differentiation of neuro-endocrine tumors of the lung

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Introduction
First assessment of lung tumors is often performed by imaging combined with biopsy. This is necessary for immunohistochemical tumor typing, evaluation of treatment options and prognosis. It has been shown in literature reviews that Endobronchial ultrasound (EBUS) with fine needle aspiration cytology (FNAC) is not inferior in diagnostic quality, but much less invasive than mediastinoscopy of chest laparotomy (1–3). In this case report we will describe the cut-backs of fine needle biopsies in the differentiation of lung tumors.
Case
A 49-years old woman was referred to the pneumology department after incidental finding of a pulmonary nodule in the left lower lobe on a post-operative abdominal CT scan. Further imaging showed additional nodules in the left and right lung. Our patient had no concerning complaints nor did she present with any noticeable clinical red flags. Past medical history included a resected melanoma and auto-immune hypothyroidism, as well as an extensive oncological family history. *Bronchoscopy with EBUS-miniprobe guided transbronchial biopsy* was performed to assess the nodules. *Biopsy* using FNAC defined a epithelioid tumor which was classified as a small cell lung cancer. However, post-resection histology proved the tumor to be a carcinoid tumor. Literature review showed a recently published case report with very similar findings (4).
Discussion
Differential work-up of pulmonary malignancies is often made *with small biopsy or FNAC*. Especially in case of atypical presentation, it is of importance to re-evaluate the working diagnosis with histology of the resected tumor (5). The differentiation between small cell lung cancer and carcinoid tumors based *on small biopsy* seems often to be inaccurate. This has great implications in choice of therapy and follow-up (6). Through this case, we want to emphasize the shortcomings of FNAC in tumor typing and the importance of tumor type confirmation with post-resection histology.
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034

A family history of short stature

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Introduction
Short stature is a common cause of consultation in pediatric endocrinology. In 80% of cases, the etiology remains unknown[1] and classified as « idiopathic short stature. We report the case of a child with a heterozygote complete deletion of the IGF1 gene.
Case report
A 21 months old boy was referred in pediatric endocrinology because of his extreme short stature. The parents of Sicilian origin are not consanguineous. The father's height is 160.8 cm (−2.3 SD) and head circumference is 54.7 cm. The mother's height is 148.3 cm (−2.6 SD) and head circumference is 50.2 cm. The target height of our patient is 159.8 cm (−2.5 SD). He has a 9 years old brother with a normal height for his parents. The patient was born after a spontaneous normal pregnancy at 39.5 weeks by vaginal delivery. His weight was 2340 g (−2.9 SD), his length was 43 cm (−3.95 SD) and his head circumference was 32 cm (−2 SD). He had a left orchidopexy at the age of one year. This infant has a good appetite according to his parents and a correct psychomotor development. At physical examination the height is 72.7 cm (−4.2 SD), the weight is 6.9 kg (−5.6 SD) and the body mass index is 13.1 kg/m^2 (−3.8 SD). A microcephaly with a head circumference of 42.7 cm (−5.5 SD) is noticed. He presents a fifth finger clinodactyly and has no other dysmorphic feature. Hearing tests are normal. The physical examination is otherwise normal. The growth curve showed a failure-to-trive from the age of 4 month. Previous check-up excluded celiac disease, cystic fibrosis, cardiopathy, thyroid disturbances and other chronical disease. We are therefore in front of a small for gestational age (SGA) child with a severe failure-to-thrive and a microcephaly, in a context of a familial short stature. We suspect a defect in the GH/IGF1 axis. The IGF1 level is low (26 ng/ml) (range: 70.3–128.5 ng/ml)^2, the IGFPB3 level is normal and a glucagon test shows a normal response of growth hormone (GH peak at 7.4 mcg/l). The IGF1 generation test shows no increase of IGF1 after 7 days (day 0, IGF-1: 30 µg/l and day 7, IGF-1: 34 µg/l) of GH treatment (0.05 mg/kg/day GH). The diagnostic hypothesis is an anomaly of the IGF1 gene. Indeed, the cGH array shows a 12q23.1q23.3 deletion including the whole IGF1 gene. This same deletion is found in his mother, maternal grand-father (height:150 cm), maternal uncle (height:165 cm) and his son already treated with GH for SGA.
Discussion and conclusion
We report the second case of complete heterozygous IGF1 gene deletion in a young boy and his family with an autosomal dominant inheritance pattern. The IGF1-haploinsufficiency is the cause of the intra-uterine growth retardation and the short stature with microcephaly. To our knowledge, only one other case of complete heterozygote deletion of the IGF1 gene has been reported until now in the literature[3]. The phenotype of our patient with short stature associated to microcephaly and clinodactyly, is very similar to other patients with incomplete deletion of IGF1 gene[3]. The psychomotor development in our patient is normal unlike the other cases described in the literature[3]. The diagnostic trap is the diagnosis of familial short stature even though our patient is shorter than his target height. CGH array was helpful to confirm at the molecular level the clinical and biological diagnosis of IGF1 deficiency. This IGF1-happloinsufficiency with heterozygote complete deletion of the IGF1 gene may respond to GH therapy unlike those with homozygous complete loss of function variants in IGF1. The efficiency of GH or IGF1 treatment remains to be documented in this very short stature.
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035

Pituitary metastasis from follicular thyroid carcinoma – a case report

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A 64-year-old Algerian woman was transferred to our institution with a pituitary tumor causing severe visual impairment. Magnetic resonance imaging (MRI) showed a T1-isointense, T2-hyperintense sellar mass measuring 48 × 35 × 30 mm, with homogenous enhancement after gadolinium injection (Figure 1). This mass extended to the suprasellar region, compressing the optic chiasma, and to both cavernous sinus, completely surrounding and narrowing carotid arteries. Ophthalmological examination showed near complete ophtalmoplegia due to bilateral palsies of VI and III nerves, bitemporal hemianopsia and bilateral optic nerve atrophy. Biological testing demonstrated complete anterior hypopituitarism with low prolactinemia and normal natremia (Table1). Medical history revealed that (i) a thyroid nodule corresponding to a follicular adenoma with signs of necrosis and hemorrhage had been resected in 2008, and (ii) that a non-functional pituitary tumor had been already diagnosed in Algeria two years before admission and incompletely resected by a trans-sphenoidal approach. The patient had developed postoperative corticotrope and thyrotrope insufficiency requiring treatment with hydrocortisone 20mg/day and levothyroxine 125 µg/day.

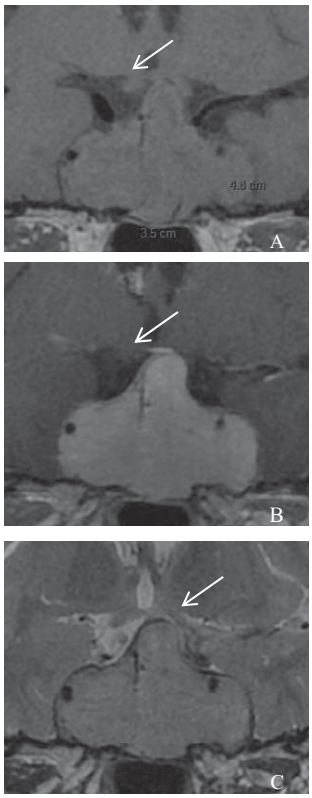

Figure 1 Magnetic resonance of the brain showing a large sellar and suprasellar tumor measuring 48×35×30 mm, iso-intense on T1-weighted images (A), enhancing after gadolinium injection (B), and moderately hyper-intense on T2-weighted images (C). The arrow shows the optic chiasma which is compressed on the left side. Both cavernous sinuses are largely invaded.

Histopathological examination was compatible with a metastasis of a follicular variant of papillary thyroid carcinoma. A total thyroidectomy with central and laterocervical lymph node dissection was performed thereafter, confirming the presence of a 5 mm papillary microcarcinoma (pT1N0Mx). While thoraco-abdominal CT was negative, bone metastases of the left scapula and chondro-costal joints were demonstrated by a bone scintigraphy. Before planning a new pituitary surgery, a PET-CT was performed, showing a pituitary tumor without metabolic activity (SUV max 4.5) and without bone metastases. A transsphenoi-dal resection was then performed for biopsy and visual decompression but the intervention was complicated by abundant bleeding. The patient developed a transient diabetes insipidus post-operatively treated with desmopressin. Histo-pathological examination of the resected material revealed a metastasis of a follicular thyroid carcinoma. Tumoral cells were positive for TTF1, PAX8, thyroglobulin and TPO but negative for LH, FSH, GH, ACTH and TSH. The Ki67 proliferation index was estimated between 10% and 15%. Radioiodine ablation after recombinant human TSH administration was planned upon return of the patient in Algeria. Pituitary metastases from thyroid cancer are very rare. Less than 30 cases have been reported so far in the literature. In 11 cases, the thyroid cancer was a follicular thyroid carcinoma[1]. Most often, the metastases are part of a disseminated disease but in 9 cases it was the first sign of thyroid cancer[1], as observed in our patient. As pituitary metastases from thyroid cancer tend to become large lesions with mass effects, symptoms most commonly include oculomotor palsies and visual deficit[2]. On the other hand, pituitary hormone deficiencies as well as hyperprolactinemia and diabetes insipidus are less frequently reported[1] and seem to be more often associated with papillary and medullary rather than follicular thyroid carcinoma[2]. At MRI, pituitary metastases usually appear as iso- or hypo-intense lesions on T1-weighted images and hyper-intense on T2weighted image with marked enhancement after gadolinium injection[1]. Yet, these signs are not sufficient for diagnosis. As a consequence, neurosurgical biopsy or resection allows a definitive diagnosis as well as a primary treatment option especially when visual decompression is required. After neurosurgery and total thyroidectomy, if not performed earlier in the course of the disease, options include radioiodine (with or without recombinant human TSH) and electron beam radiotherapy[1,3].

Table 1 Results of biological testing at admission showing a complete anterior hypopituitarism associated to low prolactin and normal natremia.

Biological testing	Normal values	Results
ACTH	5.0–49.0 pg/ml	3.6
Cortisol	130.0–500.0 nmol/l	1.5
TSH	0.27–4.2 mU/l	<0.01
Free T4	12.0–22.0 pmol/l	28.8
GH	<4.0 ng/ml	<0.1
IGF-1	100.0–232.0 ng/ml	42.6
LH	7.7-15.5 UI/l[$]	0.3
FSH	25.8–134.8 UI/l[$]	0.9
Estradiol	<5.0–54.7 ng/l[$]	<12
Prolactin	5.0–23.0 µg/l	3.2
Natremia	135–145 mmol/l	143

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036

ACTH independent hypercorticism with normal adrenal imaging and negative genetic screening for micronodular adrenal disease in a female teenager: what to suspect?

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Introduction
Micronodular adrenocortical disease is a very rare cause of Cushing syndrome in children. This adrenocorticotropic hormone (ACTH)-independent form of Cushing syndrome is mostly a part of the Carney Complex, which is caused by mutations in the *PRKAR1A* gene (1). A young female with endogenous ACTH independent hypercorticism without the classical gene mutations in the pigmented and the non-pigmented form of micronodular adrenal disease is presented.
Case Report
An 11-year old girl presented with increasing adiposity despite a hypocaloric diet for several months. She was born after 38 weeks of an uneventful gestation with a birth weight of 3.23 kg (−0.25 SDS) and birth length 50 cm (0 SDS). Psychomotor development was normal. No previous health problems or use of medication were reported. Familial history was negative for endocrine and neoplastic problems and obesity. She had no other complaints, beside increasing acne. However, retrospective growth curve analysis showed a severely delayed linear growth (1.2 cm in the last 12 months). At physical examination, standing height was 136.7 cm (−1.9 SDS), BMI 20.28 kg/m^2 (+0.8 SDS), Tanner stage A1P2M1 and blood pressure 110/84 mmHg. No striae, lentigines or acanthosis nigricans were present, but slight facial acne and blushing cheeks were observed. Fasting glucose was 82 mg/dL, insulin 100 pmol/L, total cholesterol 267 mg/dL, HDL 79 mg/dL, LDL 177 mg/dL and triglycerides 52 mg/dL. Morning serum cortisol (187.2 µg/l) and DHEAS (0.44 mg/L), as well as IGF-1, FT4 and TSH were normal. Bone age was 9.48 years. Because of the clinical suspicion of Cushing syndrome, additional screening investigations were performed. Cortisoluria (586.7 µg/24h) and salivary night cortisol level (12.71 µg/L) were elevated. Repeated morning serum cortisol was slightly elevated (215.5 µg/L) with an undetectable ACTH concentration. The administration of a low dose of dexamethasone at 11 PM resulted in an overnight serum cortisol of 177.3 µg/L. An absent ACTH response to CRH was documented. Magnetic Resonance imaging, as well as additional Computed Tomography imaging of the adrenals were normal. The adrenal origin of the hypercorticism in combination with the absence of nodular findings on imaging raised suspicion for PPNAD. Ultrasound of the heart and thyroid was normal. Mutation analysis of the *PRKAR1A*, *PDE11A* and *PDE8B* gene were normal. Ketoconazole treatment resulted in a normal cortisoluria after 2 weeks. Diagnosis of isolated micronodular adrenal disease was made.
Discussion
Delayed growth and bone maturation, cutaneous changes (blushing cheeks and acne) raised the clinical suspicion of Cushing syndrome in the by a dietician

referred young female, complaining of persisting weight gain despite an extreme hypocaloric diet. The finding of hypercortisoluria, non-suppressed adrenal androgens and a normal adrenal size at imaging supported the diagnosis of an endogenous cause of hypercorticism, since in children the hidden administration of glucocorticoids should always be suspected in case of un unexplained ACTH independent Cushing syndrome presenting with normal adrenal imaging (2). The diagnosis of micronodular adrenal disease is often a challenge due to a variable clinical presentation (often insidious) with variable lesions at different imaging techniques, especially when a family history or other clinical signs of Carney Complex are lacking (3). Genetic testing might therefore be helpful in the diagnosis of this difficult form of Cushing syndrome. The absence of *PRKAR1A* gene mutation however does not fit with the diagnosis of Carney Complex or the pigmented form in our case.

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037

Euglycemic ketoacidosis in diabetes type 1

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Background

SGLT2i have shown promising results as an adjunct therapy in T1DM, resulting in better glucose control, weight loss and lower blood pressure. No increase in hypoglycemia risk, in particular severe hypoglycemia, was observed, but, an mild increased risk of (euglycemic) diabetic DKA was reported (Van den Mooter L. *et al*. Exp Opinion Pharm 2018).

Case report

A 49-year-old Caucasian female nurse with underlying type 1 diabetes was admitted to the emergency department with weakness, dyspnea, abdominal pain, nausea and vomiting. On examination, she was afebrile, she suffered from tachycardia, tachypnea and hypertension. She was dehydrated with dry oral mucosa and poor skin turgor. A clinical diagnosis of diabetic ketoacidosis was confirmed by arterial blood measurements showing severe metabolic acidosis with an elevated anion gap (pH 6.94, CO_2 17.3 mm Hg, HCO_3 3.7 mEq/L, anion gap 38.3 mEq/L), with hyperglycemia (364 mg/dL). Urine was positive (4+) for ketones, and serum lactate levels were mildly elevated (3.0 mg/dL). She was promptly admitted to the intensive care unit and treated for DKA through intravenous rehydration therapy with insulin infusion. Serial blood gas analyses showed gradual resolution of her ketoacidosis with normalized anion gap and clearance of ketones within $<$24 hours. These symptoms were preceded by diarrhea and poor oral intake for 4 days. She has type 1 diabetes for 30 years, treated with a bolus-basal regime (lispro-glargine) and struggles with achieving satisfactory glucose control (HbA1c values $>$8.5%) and weight gain (BMI 32.4 kg/m^2). Three months prior, empagliflozin 10mg, an SGLT2i, was added. Patient received intense education on the mechanisms of action of SGLT2i, in particular the potential for euglycemic DKA. She received an alert card with instructions in case of nausea, on ketone measurements and increasing insulin doses. Upon the start of gastrointestinal symptoms, patient stopped the SGLT2i, but failed to measure ketones and even stopped all meal-time insulin, as glucose levels below 250 mg/dl reassured her and her treating family doctor. Thus, although patient confirmed she was well informed about the risk of euglycemic DKA when combining insulin therapy with SGLT2i., had written instructions and material to measure ketones, she did not realize her symptoms could potentially be explained by DKA and declined ketone measurement. She was reassured by the 'normal' blood sugar level.

Conclusion

This case report shows that even in carefully selected and well trained patients combining SGLT2i with insulin is not without a risk. Caretakers should emphasize more on symptoms of DKA and repeatedly instruct patients to measure ketones when they feel ill. Diabetes type 1 (T1DM), sodium glucose cotransporter inhibitor 2 (SGLT2i); diabetic ketoacidosis (DKA)

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038

Pregnancy unmasking a pelvic sympathetic paraganglioma: a case report

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Introduction

Paraganglioma (adrenal or extra-adrenal) in pregnancy is a rare condition with a high maternal and foetal mortality rate. A multidisciplinary approach is important as it will influence the maternal and foetal outcome. This is illustrated in the following case report.

Case report and results

A 32-year-old woman was referred because of persistent hypertension at 33 weeks of pregnancy. There were no spontaneous complaints of headache, palpitations or sweating. She did mention presyncopes and tachycardia when making bowel movements. Biochemical evaluation was performed on a 24-hour urine collection, which showed elevated levels of adrenaline (204.5 μg/24h 0−20 μg/d, noradrenaline (2736 μg/24h 10−100 μg and normetanephrines (8694 μg/24h, 100−610 μg). Whole body MRI showed a pararectal mass (6 cm), suggestive of an extra-adrenal paraganglioma, explaining the specific occurrence of complaints. At diagnosis the patient was 33 weeks into her second pregnancy. The obstetric history showed one uncomplicated vaginal delivery. On clinical examination there were no syndromic features. No significant family history was noted. Since the patient had already reached her third trimester of pregnancy, resection of the paraganglioma was postponed until after delivery. She was pretreated with alpha-adrenergic receptor blockers, beta-adrenergic receptor blockers and calcium agonists, and responded well to medical treatment. An elective Caesarean section was performed at 34 weeks. The delivery was uneventful with stable hemodynamics. Both mother and child were admitted to the intensive care unit, but no hemodynamic complications occurred. One week before the second surgery she was put on a salt-rich diet and intravenous fluids to restore the intravascular volume. Because of the size of the tumour and its location close to the right internal iliac artery, surgery consisted of an open resection. Histologically, the tumour was confirmed to be a paraganglioma. Genetic analysis showed a complete loss of SDBH expression. Follow up was arranged four weeks after discharge from hospital, at which time biochemical re-evaluation by measuring plasma and urinary metanephrines will be performed.

Discussion

Paraganglioma during pregnancy is a rare condition. Establishing a correct diagnosis is important as it will influence both maternal and foetal outcome. High concentrations of catecholamines can result in a compromised uteroplacental circulation, leading to intrauterine growth restriction or intrauterine hypoxia (1). Paraganglioma during pregnancy can be harder to diagnose as its most common presenting feature is hypertension. In this situation it can be difficult to differentiate between a paraganglioma and pregnancy related hypertension. The latter usually develops after 20 weeks of pregnancy. Hypertension due to paraganglioma can present at any time during pregnancy, though it is more paroxysmal in nature and hypotensive episodes can also occur. Diagnosis of a paraganglioma is similar in pregnant and non-pregnant patients and includes measuring plasma and urinary fractionated metanephrines (1). There are no specific recommendations for the treatment of paraganglioma during pregnancy and each case should be evaluated by a multidisciplinary team at centres with appropriate expertise (2).

Conclusion

Currently, optimal treatment of paraganglioma during pregnancy remains debatable. This case illustrates the importance of a timely diagnosis as well as the need for a multidisciplinary team that will make decisions concerning the timing and method of delivery and the treatment for the underlying paraganglioma.

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