Pediatric Diabetes

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Faculty Synopses

Synopsis #1

KATP-channel Hyperinulinism Variations

Charles A. Stanley, Children's Hospital of Philadelphia

Inactivating mutations of the ß-cell ATP-dependent potassium channel (KATP) comprise the most commonly found defects in children with diffuse HI, as well as focal HI (see Synopsis #7). The mutations can affect either of the KATP channel subunits, SUR1 and Kir6.2, which are encoded by two adjacent genes on 11p15.1, ABCC8 and KCNJ11. An important feature of the KATP HI mutations, not seen in other genetic forms of HI, is that they display a range of genotypes and phenotypes which has implications for clinical management. KATP HI mutations can be divided into three groups: (1) recessive KATP HI mutations which cause severe hypoglycemia that is unresponsive to treatment by the KATP channel agonist, diazoxide; (2) dominant KATP HI mutations which cause a severe hypoglycemia phenotype similar to recessive defects; and (3) dominant KATP HI mutations which cause a milder phenotype of hypoglycemia that is responsive to diazoxide therapy.

Patients with all three types of KATP HI defects show abnormal acute insulin responses (AIR) to secretogogues, as well as abnormal glucose and insulin responses to oral glucose and amino acids. Affecteds show increased AIR to calcium, but lack of AIR to tolbutamide; AIR to glucose is blunted. AIR to leucine is negative; however affecteds show hyper-sensitivity to protein-induced hypoglycemia (See Synopsis #4 and #16). Responses to oral glucose show glucose intolerance. Similar patterns of responses occur in SUR1-/- mice. Responses in carriers of recessive KATP HI mutations are normal. Carriers of dominant KATP HI mutations may appear asymptomatic; however, abnormalities of insulin regulation can be elicited by provocative tests, such as fasting or oral protein. These differences in genotypes and phenotypes correlate with in vitro expression studies of the mutations (discussed by Dr. Shyng) and are important for interpreting results of mutation analysis (discussed by Dr. Ganguly).

These observations indicate that KATP HI mutations impair beta-cell sensitivity to both low BS and high BS. The latter may play a role in the risk of developing diabetes in adolescent and adult years, which is of concern in patients that have had pancreatectomy, but also may be a risk in affected individuals who never had surgery.

Synopsis #2

Genotype-Phenotype of Expressed K_{ATP}-HI Mutations

Show-Ling Shyng, Oregon Health & Science University

The β-cell ATP-sensitive potassium (K_{ATP}) channel composed of the inwardly rectifying potassium channel Kir6.2 encoded by *KCNJ11* and the sulfonylurea receptor 1 (SUR1) encoded by *ABCC8*, plays a key role in insulin secretion by linking glucose metabolism to cell excitability. Mutations in *KCNJ11* and *ABCC8* that lead to loss of channel function are a major cause of congenital hyperinsulinism. Functional studies of recombinant mutant K_{ATP} channels over the past fifteen years have greatly advanced our knowledge of channel structure-function relationship, disease mechanisms and genotype-phenotype correlation. These studies show that mutations reduce or abolish channel function by disrupting channel trafficking to the cell surface, channel gating, or both. The predominant molecular defect seen in trafficking mutations is inability of the mutant protein to pass the endoplasmic reticulum quality control mechanisms. The most common gating defect is impaired response to the physiological stimulatory ligand, MgADP, due to mutations in the SUR1 subunit. In general, dominant mutations do not cause trafficking defects and are thus able to manifest their functional defects in the β-cell membrane. Patients with these mutations can be either diazoxide-responsive or unresponsive depending on the extent to which channel response to diazoxide is reduced. By contrast, recessive mutations tend to be trafficking mutations, and under heterozygous conditions the wild-type allele is sufficient to provide functional channels to prevent the disease.

In most cases *in vitro* functional data obtained in heterologous expression systems are well correlated with disease phenotype. There are however, exceptions. It is possible that a lack of genotype-phenotype correlation in some cases is due to the expression system or species of channel protein used. For example, certain

channel regulatory mechanisms involving protein phosphorylation and interaction with other proteins may occur only in β-cells. Some mutations seem to only cause disease in a subset of carriers or cause disease of variable severity. Insufficient clinical information for definitive diagnosis in some carriers could explain the apparent variability in mutation penetrance, highlighting the importance of complete clinical data. Possible role of epigenetic regulation or genetic modifiers in disease manifestation should also be explored. In conclusion, the wealth of information on expressed K_{ATP}-HI mutations facilitates disease diagnosis and treatment. In addition, it provides the knowledge base for future efforts aimed at identifying strategies that correct channel expression and/or gating defects to expand disease treatment options.

Synopsis #3

Glucokinase Hyperinsulinism

Charles A. Stanley, Children's Hospital of Philadelphia

Glucokinase (GK) mediates the first step in ß-cell oxidation of glucose and defines the threshold for glucose stimulated insulin secretion. Activating mutations of GK are the third most common defect found in children with HI. GK HI is inherited in dominant fashion, but de novo cases are increasingly detected with the availability of genetic mutation analysis.

Similar to patients with HI due to KATP channel mutations, birthweight is increased in GK HI neonates, reflecting the potent anabolic effect of insulin on fetal growth. Birthweight is reduced in unaffected infants born to mothers with GK HI mutations, reflecting reduced fetal insulin levels due to chronic low maternal blood glucose. Although GK HI is often assumed to be responsive to diazoxide suppression of insulin secretion, patients frequently cannot achieve normoglycemia, reflecting the profound effect of GK in setting the beta-cell threshold for insulin secretion. In 12 GK cases seen at CHOP, only 2 achieved good glycemic control on diazoxide alone; 2 required nocturnal continuous intragastric dextrose; and 3 required surgery. In GK HI, insulin and glucose responses to oral glucose appear normal; patients are not protein or leucine sensitive; and acute insulin responses to IV calcium are normal. Some investigators have suggested that GK HI is associated with increased diameter of pancreatic islets, implying that the activation of GK may stimulate islet growth; this has not been appreciated in other reports.

All of the 15 known disease-causing GK mutations are single amino acid changes, either substitutions or insertions, which increase enzyme affinity for glucose. Calculated glucose thresholds for insulin release for various mutations range from 4.2 mM to 1.2 mM. However, calculated thresholds do not correlate well with baseline pre-prandial blood glucose levels in patients, which generally range from 50–65 mg/dL. Thus, additional factors may influence the effects of mutations on glucose levels in vivo, so that affected individuals may require therapies in addition to diazoxide for control of hypoglycemia.

Synopsis #4

Amino Acid Stimulated Insulin Secretion in HI Mouse Models

Changhong Li, Children's Hospital of Philadelphia

Recent genetic studies of protein-sensitive hypoglycemia in children with congenital hyperinsulinism (HI) have highlighted the important role of amino acids in regulation of insulin secretion. Mouse models of three of these disorders have been investigated to elucidate the mechanisms of amino acid stimulated insulin secretion (AASIS), including activating mutations of glutamate dehydrogenase (GDH), inactivating mutations of short-chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD) and inactivating mutations of the KATP channel (SUR1 and Kir 6.2). All these three HI mouse models showed abnormal hyper-sensitivity to AASIS with two different mechanisms: a metabolic fuel mechanism and a metabolic signaling mechanism. Amino acids can serve as fuel for oxidation in islets to generate ATP to trigger insulin secretion; this occurs in GDH-HI and SCHAD-HI. Stable isotope tracing studies of the metabolic fluxes from glutamine to glutamate and to alpha-ketoglutarate confirm that leucine/glutamine stimulation of insulin secretion and the GDH gain of function mutation in GDH-HI both acts via the GDH reaction to increase glutamine oxidation and generate increased ATP production. A similar mechanism occurs in SCHAD-HI, due to loss of an inhibitory protein-protein interaction of SCHAD on GDH, which results in activation of GDH and increased glutamine oxidation. In contrast, AASIS in SUR1-HI appears to act via glutamine of distal cAMP-dependent stimulation steps in insulin release, which action requires beta-cell depolarization and elevation of cytosolic calcium. These mechanisms responsible for protein-sensitive hypoglycemia in genetic disorders of insulin secretion are likely also relevant to normal regulation of insulin secretion, where insulin responsiveness is required to protein meals (via the fuel mechanism) and is enhanced when protein is combined with carbohydrate meals (via the signaling mechanism).

Synopsis #5

Glutamate Dehydrogenase Hyperinsulinism

Andrea Kelly, Children's Hospital of Philadelphia

Amino acids have long been recognized to stimulate insulin secretion. In the 1980s, the specific mechanism by which leucine stimulates insulin secretion was clarified: Allosteric regulation of the mitochondrial enzyme glutamate dehydrogenase (GDH) by leucine stimulates insulin secretion. The roles of amino acids and GDH in insulin secretion received little attention until gain of function mutations in *GLUD1*, which encodes GDH, were identified in a form of congenital hyperinsulinism referred to as the hyperinsulinism/hyperammonemia syndrome (HI/HA). This disorder highlights the importance of GDH in regulating insulin secretion and has renewed interest in understanding amino acid-stimulated insulin secretion. This presentation will review both the clinical features of HI/HA and the patient-oriented studies that have provided insight into regulation of insulin secretion by GDH and amino acids. *In vitro* and animal model correlates are the subject of separate presentations. Finally, the role of GDH in the hyperammonemia and central nervous system manifestations of HI/HA will be explored.

Synopsis #6

Tissue Specificity of Glutamate Dehydrogenase as Illustrated in Pancreatic ß-Cells and Central Nervous System

Pierre Maechler, Geneva University Medical Centre, Switzerland

One of the genetic forms of hyperinsulinism highlights the role of glutamate dehydrogenase (GDH) in pancreatic ß-cells. GDH is a mitochondrial enzyme playing a pivotal role between carbohydrate and protein metabolisms, controlling production and consumption of the messenger molecule glutamate in neuroendocrine cells. GDH activity is under the control of several regulators, conferring to this enzyme energy-sensor property. Moreover, GDH is allosterically regulated by GTP and ADP. GDH is also regulated by ADP-ribosylation, mediated by a member of the energy-sensor family sirtuins, namely SIRT4. In the brain, GDH ensures the cycling of the neurotransmitter glutamate between neurons and astrocytes. GDH also controls ammonia metabolism and detoxification, mainly in the liver and kidney. In pancreatic ß-cells, the importance of GDH as a key enzyme in the regulation of insulin secretion is now well established. Inhibition of GDH activity decreases insulin release, while activating mutations are associated with a hyperinsulinism syndrome. Although GDH enzyme catalyzes the same reaction in every tissue, its function regarding metabolic homeostasis varies greatly according to specific organs. Newly generated mouse models lacking GDH in specific tissues offer the opportunity to decipher organ specificities of GDH regulation.

Synopsis #7

Focal K_{ATP} hyperinsulinism

Pascale de Lonlay, Hôpital Necker-Enfants Malades, Université Paris Descartes, France

One of the major advances in the care of patients with congenital hyperinsulinism (HI) was the discovery of focal adenomatous hyperplasia, since this mechanism accounts for close to half of all diazoxide-unresponsive HI, and patients can be definitively cured after a limited pancreatectomy. The histological features include a hyperplasia and an increased β-cells mass within the focal lesion. These findings were already reported in the pancreas of patients with Beckwith-Wiedemann Syndrome (BWS), a condition which is known to be associated with HI. However, the pathophysiology of focal hyperinsulinism (FoHI) is more complex and includes a ''two hits'' mechanism: i) first, a paternally inherited mutation in one of the genes of a K_{ATP} channel, both genes located on the 11p15 chromosome, ii) second, in a pancreatic endocrine progenitor, a deletion of the maternally inherited 11p15 chromosomal region, compensated by a paternal uniparental disomy (pUPD) as observed in some cases of BWS. The loss of heterozygosity in the imprinted 11p15.5 region bearing genes involved in tumor suppression and in cell proliferation explains the histopathological findings of FoHI, while the paternally inherited K_{ATP} channel mutation reduced to homozygosity within the β-cells of the focal lesion causes hyperinsulinemic hypoglycemia unresponsive to diazoxide. Patients with FoHI have usually one single and small-sized (2-5mm in diameter) pancreatic lesion. The area of abnormal pancreatic development can also be multilobular and can have satellites in the nearby pancreas that necessitates intraoperative margins analysis to ensure complete excision and avoid recurrence. However, some patients may have unusual focal lesions: multifocal lesions or large focal lesion which may involve a large portion of the pancreas, with one single and paternally inherited mutation of the *ABCC8* gene. Multifocal and ectopic FoHI were also reported, with a focus in the pancreas and another focus in ectopic pancreatic tissues of various localizations (jejunum and abdominal cavity) which were diagnosed on PET-scan imaging. Two hypotheses are to be discussed but are yet unanswered: the 2nd lesion

may be a satellite from the first one, or may be a second and independent FoHI in the same patient. The LOH is a rare sporadic event, as suggested by discordant identical twins, the absence of HI in the patients' fathers, and the negligible risk of recurrence of focal form among siblings. The latter was reported only once and the location of the focal lesions was different in both siblings (isthmus and head of the pancreas). Finally only one family was reported with the coexistence of both focal and diffuse neonatal HI in siblings. The first child carried a paternally inherited *ABCC8* mutation associated with a LOH for the maternal wild-type chromosome in the lesion. Its sibling presented also with hypoglycemia and was found homozygous for the same mutation in the peripheral blood. The genetic testing of the parents found they were heterozygous for the same *ABCC8* gene mutation and it appeared they were inbred.

Synopsis #8

[18F]-DOPA PET Imaging: "GPS" for the Surgeon

Lisa J. States, Children's Hospital of Philadelphia

Molecular imaging with [18-F] -L 3, 4-dihydroxy phenylalanine ([18F]-DOPA) PET holds promise in becoming an integral part of the evaluation of patients with medically unresponsive hyperinsulinemic hypoglycemia and a suspected focal lesion. Compared with diagnostic interventional radiology techniques this procedure is less invasive, safer and easier to perform and interpret. Since 2003, [18F]-DOPA PET has been investigated for its ability to distinguish focal from diffuse disease and accurately localize a focal lesion. To date, the two largest published series of surgically proven focal disease reveal an [18F]-DOPA PET sensitivity of 75–100% for detection of a focal lesion with 100% accuracy in anatomic localization of detected lesions. (1,2) Recognition of patterns of both diffuse and focal disease is essential for the interpretation of the 18[F]-DOPA PET/CT. In our experience, failure to detect a focal lesion can be related to lesion size – small or excessively large, lesion shape – flat or irregular, or location – deep in the head or in the tail contiguous with the kidney. Measurement of radiotracer activity in a suspected focal lesion compared with the normal pancreatic tissue can be used to improve diagnostic confidence in an equivocal case. Advanced imaging techniques such as fusion with CT Angiography can be used to improve localization and provide a roadmap for the surgeon. Identification and anatomic localization of a focal lesion is used to create a more focused pre-operative plan, resulting in a limited pancreatic resection and decreased surgical morbidity. In combination with surgical expertise, [18F]-DOPA PET can offer an outcome with preservation of pancreatic function and cure of this devastating disease.

1) Hardy, O.T., et al. 2007. Accuracy of [18F]Fluorodopa Positron Emission Tomography for Diagnosing and Localizing Focal Congenital Hyperinsulinism. *J Clin Endocrinol Metab* 92:4706–4711.

2) Ribeiro, M.J., et al. 2007. The added value of [18F]fluoro-L-DOPA PET in the diagnosis of hyperinsulinism of infancy: a retrospective study involving 49 children. *Eur J Nucl Med Mol Imaging* 34:2120–2128.

Synopsis #9

A Multidisciplinary Approach to the Focal Form of Congenital Hyperinsulinism Leads to Successful Treatment by Partial Pancreatectomy

N. Scott Adzick, Children's Hospital of Philadelphia

Congenital Hyperinsulinism (HI) causes severe hypoglycemia in neonates and infants. Recessive mutations of the beta cell K-ATP channel genes cause diffuse HI, whereas loss of heterozygosity together with inheritance of a paternal mutation cause focal adenomatous HI. Although these two forms of HI are clinically identical, focal HI can be cured surgically. We reviewed our experience with pancreatectomy to treat focal HI.

From 12/1998 to 2/2012, 327 patients with HI underwent pancreatectomy: 169 for focal disease, 5 for focal disease/redo from elsewhere, 144 for diffuse disease, and 9 for insulinoma. The patients came from 47 of 50 states in the U.S. and from 11 other countries. The focal HI patients (ages 1 week to 14 months; median age = 7 weeks) were treated with partial pancreatectomy. At operation, the focal lesion was found using the preoperative localization data and multiple pancreatic biopsies with frozen section analysis, followed by partial pancreatectomy. A complete response at follow-up was defined as no requirement for glycemic medications and no diabetes mellitus.

The vast majority of pancreatectomies for focal HI were <50% (range 2%–98%). 56% of patients had involvement of the pancreatic head with the focal lesion. Twenty-seven lesions that required substantial resection of the pancreatic head underwent Roux-en-Y pancreaticojejunostomy (including 2 Whipple procedures) to preserve the normal body and tail. Lesions of the body or tail were treated with local resection or partial distal pancreatectomy. Some tail lesions underwent laparoscopic resection. >95% of patients had a complete response to surgery and are cured. Seven patients have required glycemic medications.

No patient is diabetic. Surgical complications included repeat resection for residual disease (7 of 8 cured), and adhesive small bowel obstruction (2) or small bowel-to-small bowel intussussception requiring laparotomy (4). We conclude that a multidisciplinary approach (pediatric endocrinology, radiology, pathology, and surgery) to patients with the focal form of congenital hyperinsulinism can distinguish focal from diffuse disease, localize focal lesions, and permit partial pancreatectomy with cure in most patients.

Synopsis #10

Congenital Hyperinsulinism: Genetic Testing and Genotype-Phenotype Correlations

Arupa Ganguly, University of Pennsylvania Perelman School of Medicine

Congenital hyperinsulinism (HI) represents a heterogeneous group of insulin regulation disorders. Over the last 15 years the CHOP HI Center has collected genotype information on 440 children with HI for the 4 genes commonly associated with HI (*ABCC8*, *KCNJ11*, *GLUD1*, *GCK*) followed by limited sequencing of the HNF4a, UCP2 and SCAHD genes in diazoxide-responsive (DZR) cases. Mutations in the KATP channel genes were found in 288 children: 265 diazoxide unresponsive (DZUR) HI and 23 DZR HI. Mutations of GCK resulted exclusively in DZUR HI. Mutations in *GLUD1*, HNF4a, UCP2 and SCHAD resulted exclusively in DZR disease.

A total of 184 unique mutations were identified in the KATP channel genes: 161 *ABCC8*, 23 *KCNJ11*. Approximately 70% of these mutations were 'private' mutations. The phenotypic variability in KATP mutations cases was high, because missense mutations can vary in functional consequences (recessive DZUR, dominant DZUR, dominant DZR). Expression studies in 53 cases revealed: 22 did not traffic to the plasma membrane and behaved as recessive mutations; 31 mutations trafficked normally, but had impaired channel function acting as dominant negative mutations.

Of the dominant mutations in GLUD1, GCK and ABCC8, ~60% were de novo; in contrast, a very small fraction (~10%) of recessive mutations in any of the genes tested were de novo. Two HI cases had post-zygotic mutations. In addition to the 440 cases of congenital HI, 23 children had syndromic HI without mutations in the HI genes: Beckwith-Wiedemann Syndrome (6), Turners Syndrome (6), Kabuki Syndrome (1), and acquired Insulinoma (10).

Only 9% of DZUR cases were negative for mutations; 55% of DZR cases were negative for mutations in the 4 common HI genes and 89% of these were also negative for mutations of HNF4a, UCP2 and SCHAD. This suggests the possible presence of either post-zygotic mutations or possible novel loci associated with DZR HI not yet discovered.

The sensitivity of predicting diffuse HI in cases with two recessive KATP mutations is 100%; inheritance of a single paternally derived recessive KATP mutation predicts focal-HI with 97% accuracy. Genotype-phenotype correlations in HI remain imperfect as a result of: a) many private mutations, b) difficulty predicting functional consequence, c) possible post-zygotic mutations, d) likelihood of additional loci. Genetic testing has proven to be important for counseling families with respect to the possible outcomes of surgery and future risk of having another child with the same disease.

Synopsis #11

HNF4a – Hyperinsulinism

Khalid Hussain, Institute of Child Health & Great Ormond Street Hospital for Children, England

Hepatocyte nuclear factor 4 alpha (HNF-4 encoded by the HNF4A gene) is a transcription factor that plays a key role in pancreatic development, maintenance of β-cell mass and regulation of insulin secretion. Mutations in the HNF4A gene cause young onset autosomal dominant diabetes mellitus (known as maturity-onset diabetes of the young type 1 or MODY1). Paradoxically HNF4A gene mutations also can cause hyperinsulinaemic hypoglycaemia (HH) and macrosomia in the neonatal period. The HH due to HNF4A mutations typically presents in the first few days of life, can be mild and transient or severe and persistent, requiring prolonged medical treatment. The hypoglycaemia in this condition responds to treatment with the KATP channel agonist, diazoxide. These observations suggest that HNF-4 might have different roles in regulating pancreatic development, maintenance of β-cell mass and insulin secretion during the foetal, neonatal and adult periods. The exact mechanism/s of HH due to mutations in HNF4A is yet to be elucidated.

Synopsis #12

SCHAD hyperinsulinism

Anders Molven, The Gade Institute, Haukeland University Hospital, University of Bergen, Norway

Deficiency of the metabolic enzyme short-chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD) is a rare, autosomal recessive form of congenital

hyperinsulinism of infancy. It is caused by inactivating mutations in the *HADH* gene on chromosome 4q. Most cases reported to date are from ethnicities where consanguineous marriages are relatively frequent. SCHAD-deficient patients have normal or slightly elevated birth weights. Their hypoglycemia is diazoxide-sensitive. The time of disease onset may vary from immediately after birth until at least one year of age, but is typically within the first six months of life. Although SCHAD participates in the degradation of fatty acids, the patients usually do NOT present with hepatic dysfunction, cardiomyopathy or skeletal muscle affection, features recognized in patients suffering from a fatty acid oxidation disorder. Metabolic profiling may, however, reveal increased level of 3-hydroxybutyryl-carnitine in blood and the presence of 3-hydroxyglutaric acid in urine. Recently, it was demonstrated that SCHAD serves to inhibit the activity of glutamate dehydrogenase in the pancreatic beta-cells, a finding that also may explain why SCHAD-deficient patients tend to be protein-intolerant. The SCHAD protein may therefore serve two functions in the body: One is exerted in all or most cells, relates directly to fatty acid oxidation, but is not absolutely critical for this process. The other function is specific for the pancreatic beta-cell, is involved in the regulation of insulin secretion and works through an inhibitory effect on the glutamate dehydrogenase enzyme.

Synopsis #13

MCT1-Hyperinsulinism

Timo Otonkoski, Children's Hospital & Biomedicum Stem Cell Center, University of Helsinki, Finland

Exercise-induced hyperinsulinism (EIHI) is a rare disorder so far described in two families as a dominantly inherited trait and in some isolated individuals. It is characterized by hypoglycemia which is triggered most prominently by anaerobic exercise. Blood glucose levels fall as blood pyruvate and lactate levels increase and, typically, hypoglycemia develops immediately following exhaustive exercise. The insulin secretion of these individuals is responsive to intravenous pyruvate unlike in healthy controls. In all familial patients, mutations have been found in the regulatory regions of the *SLC16A1* gene, which encodes the monocarboxylate transporter 1 (MCT-1) protein. MCT-1 transports lactate and pyruvate into the cell and is expressed in practically all cell types, except the pancreatic β- cell where it is transcriptionally silenced. The pathogenesis of EIHI is based on the failure of MCT-1 silencing, leading to inappropriate insulin secretion triggered by anaerobic exercise. Although the patients are responsive to diazoxide in terms of a general increase of blood glucose, diazoxide does not completely prevent exercise-induced hypoglycemia. Most patients do not need specific treatment because they do not have significant symptoms if they avoid strenuous exercise.

Otonkoski T, et al.: Physical exercise-induced hyperinsulinemic hypoglycemia is an autosomal dominant trait characterized by abnormal pyruvate-induced insulin release. Diabetes 52:199–204, 2003

Otonkoski T, et al.: Physical exercise-induced hypoglycemia caused by failed silencing of monocarboxylate transporter 1 in pancreatic beta cells. Am J Hum Genet 81:467–74, 2007

Synopsis #14

UCP2 and hyperinsulinism

Daniel Ricquier, Paris Descartes University & Necker-Enfants Malades Hospital, France

Although the most common mechanism underlying congenital hyperinsulinism is dysfunction of the pancreatic ATP-sensitive potassium channel, the pathogenesis and genetic origins of this disease remains unexplained in more than half of all patients. Since ATP controls insulin secretion, mechanisms regulating ATP level should be examined in hyperinsulinic patients. The largest part of ATP production comes from mitochondria through ADP phosphorylation that is tightly coupled to the respiratory activity of mitochondria and any alteration of this coupling should alter ATP synthesis and insulin secretion. Besides UCP1 (uncoupling protein 1), a natural mitochondrial and membranous uncoupler of respiration unique to the thermogenic brown adipocytes, we identified UCP2 as an homologue of UCP1. Whether UCP2 is also a true uncoupler of respiration is uncertain, but it was shown that this mitochondrial carrier is an inhibitor of insulin secretion : Ucp2 knockout mice exhibit an hyperinsulinemic hypoglycemia (Zhang et al. 2001). Therefore, to study a possible pathogenic role for UCP2 protein in the development of human congenital hyperinsulinism, in collaboration with Dr de Lonlay (Necker-Enfants Malades Hospital, Paris), we studied children exhibiting congenital hyperinsulinism, without detectable mutations in the known congenital hyperinsulinism-causing genes. Parental inherited heterozygous UCP2 variants encoding amino-acid changes were found in two unrelated children with congenital hyperinsulinism. Functional assays in yeast and in insulin-secreting cells revealed an impaired activity of UCP2 mutants. Therefore, we reported the finding of UCP2 coding variants in human congenital hyperinsulinism, suggesting a role for this gene in the regulation of insulin secretion and glucose metabolism in humans (Gonzalez-Barroso et al. 2008).

These results show for the first time a direct association between UCP2 amino acid alteration and human disease. These novel findings may contribute to define the pathogenesis and genetic origins of congenital hyperinsulinism which remains in part unexplained in patients and could have wider implications for unexplained hypoglycaemia and impaired glucose sensitivity. These data demonstrate that UCP2 should be added to the growing list of genes in which mutations can lead to congenital hyperinsulinism, and introduce UCP2 as a new player in the control of insulin secretion and glucose sensing by ß-cells in humans. In addition, these results also reinforce the critical role played by mitochondria in the control of insulin secretion.

Synopsis #15

Perinatal Stress Hyperinsulinism

Charles A. Stanley, Children's Hospital of Philadelphia

Neonates with a variety of conditions associated with perinatal stress (SGA birthweight, birth asphyxia, maternal pre-eclampsia) are known to have especially high risk of hypoglycemia after birth. For example, 20–60% of SGA neonates have plasma glucose levels <20mg/dL, if not fed during first 8 hr fasting post-delivery. In some of these newborns, hypoglycemia may persist for beyond a few days of age and last for several weeks to months before resolving. Studies of this disorder from several groups over the past 25 years suggest that this may be a form of prolonged hyperinsulinemic hypoglycemia.

In the CHOP series, perinatal stress HI accounted for 26% of infants with persistent neonatal hypoglycemia compared to 51% who had congenital HI. Since the latter cases were referred from across the US, whereas the former were predominantly local cases, it appeared that perinatal stress HI was not uncommon. This was confirmed in a separate survey of SGA neonates in a large local nursery which revealed that 10% had hypoglycemia persisting beyond 10 days after delivery.

The CHOP experience with perinatal stress hyperinsulinism was reported by in Hoe, et al. J Pediatr, 2005. Over a 3 year period, 36 neonates were seen with persistent hyperinsulinemic hypoglycemia that subsequently resolved spontaneously. Follow-up tests to document complete resolution were obtained in 26. Most of these 26 cases were male (81%), 62% were delivered by C-section, 27% were SGA, 23% were premature, and 12% had a history of maternal hypertension (only 19% had no obvious evidence of perinatal stress). Glucose utilization was usually increased (median GIR 12 mg/kg/min, range 0-19 mg/kg/min). Diazoxide treatment successfully controlled hypoglycemia in 19/21 cases; one had to be discontinued due to fluid retention; one failed to respond to either diazoxide or octreotide. Therapy was withdrawn by 18–400 days of age and follow-up fasting tests showed complete resolution of the hyperinsulinism, as demonstrated by appropriate suppression of insulin and activation of ketogenesis. Acute insulin response tests in 11 of the infants showed no response to calcium or leucine, and normal responses to glucose and tolbutamide. This pattern did not resemble that seen in KATP HI or GDH HI, but was similar to that seen in patients with HI due to activating mutations of glucokinase.

The phenomenon of perinatal stress HI appears to be very common in neonates that have been exposed to a variety of physiologic stresses prior to the time of delivery. The mechanism of the hyperinsulinism is uncertain, but it shares some features of glucokinase HI which causes a lowering of the beta-cell glucose threshold for insulin release. Since SGA infants are known to be at risk for later development of insulin resistance, obesity, and diabetes, it is tempting to speculate that a common pathophysiology underlies the neonatal insulin dysregulation and the subsequent islet impairment.

Synopsis #16

GLP-1 Receptor: A Target for Treating Hyperinsulinism

Diva D. De León, Children's Hospital of Philadelphia

The incretin effect refers to the observation that insulin secretion in response to oral glucose is significantly higher than the response to an isoglycemic intravenous glucose infusion. The recognition of this phenomenon led to the discovery of incretin hormones, gut-derived peptides secreted by enteroendocrine cells in response to a meal that make a significant contribution to post-prandial insulin release. Two peptides account for approximately 90% of the incretin effect, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). Both incretins contribute to post-prandial glucose regulation, but only GLP-1 is essential for the control of fasting glycemia. In addition to its insulinotropic actions, GLP-1 has other glucose lowering effects including, inhibition of glucagon secretion, hepatic glucose production, gastric emptying and appetite. The current premise is that GLP-1 insulinotropic effects are glucose-dependent, but there is evidence that exogenous or endogenous GLP-1 could contribute to overt hypoglycemia by stimulating an exaggerated insulin response. Particularly, increased GLP-1 secretion and/or actions may play a role in the pathophysiology of acquired and congenital forms of hyperinsulinemic hypoglycemia.

In a mouse model of congenital hyperinsulinism due to inactivating mutations in the ATP-sensitive potassium channel we evaluated the role of endogenous GLP-1 in the dysregulated insulin secretion. In *SUR1*$^{-/-}$ mouse islets, exendin-(9-39) decreases basal cellular cAMP levels and insulin secretion. These findings suggest that there is a tonic effect of GLP-1 receptor signaling in these islets. In addition, exendin-(9-39) inhibits amino acid-stimulated insulin secretion in *SUR1*$^{-/-}$ islets and in wild-type islets that had been depolarized by high glucose. This suggests that the hypersensitivity to amino acids under depolarized conditions is mediated by GLP-1 receptor signaling. Further, *in vivo* treatment with exendin-(9-39) decreases the abnormally elevated fasting insulin/glucose ratio in *SUR1*$^{-/-}$ mice and corrects the fasting hypoglycemia. More recently, in a pilot clinical study we have shown that exendin-(9-39) elevates fasting blood glucose levels in human subjects with hyperinsulinism due to inactivating mutations in the ATP-sensitive potassium channel. Taken all together, these findings suggest that GLP-1 and its receptor play a role in the pathophysiology of this condition.

The recognition of the importance of incretin actions on the control of glucose homeostasis has proven very useful in the development of new targets for the treatment of diabetes mellitus. New evidence suggests that the GLP-1 receptor may also be a useful therapeutic target for the management of individuals both with congenital K_{ATP}-HI and with post-gastric surgery hyperinsulinemic hypoglycemia.

Synopsis #17

The Genetics of Diabetes Mellitus

Graeme I. Bell, University of Chicago

The American Diabetes Association defines diabetes as "a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both." They recognize four "etiologic types": type 1, type 2, other specific types, and gestational diabetes. Genetic studies (primarily genome-wide association studies) have found that genetic variation in more than 40 loci affects risk of type 1 diabetes and more than 50 loci are implicated in the development of type 2 diabetes. However, more than 50% of the genetic risk for type 1 diabetes is conferred by the HLA region on chromosome 6 and probably by more than one gene in this region. By contrast, each of the various type 2 diabetes susceptibility genes has only a modest effect on overall risk. Many of the genes associated with type 2 diabetes are also associated with gestational diabetes. Studies of "other specfic types" of diabetes have highlighted the role of genetics in the development of diabetes and shown how a genetic diagnosis can impact treatment. Genetic forms of diabetes were once thought to be very rare and in often cases part of a syndrome. We now know that they are quite common representing perhaps 2% or more of all cases and most frequently present as diabetes in isolation that is misdiagnosed as type 1 diabetes or more commonly as type 2 diabetes. Mutations in three genes (*GCK*, *HNF1A*, *HNF4A*) account for more than 50% of all cases of genetic forms of diabetes. In this presentation, I will review the genetics of diabetes including comparative effectiveness research studies on the use of genetic testing in diagnosis and treatment of diabetes.

Synopsis #18

Transcriptional Regulation of Pancreas Development and Function

Doris A. Stoffers, University of Pennsylvania Perelman School of Medicine

We believe that the molecular mechanisms that govern pancreatic islet ß cell development and regeneration hold an important key to novel therapeutic approaches for this devastating disease. We have focused over the last 15 years on the transcriptional regulation of pancreatic islet ß cell development and regeneration, specifically on the homeodomain transcription factor Pdx1 that we and others have discovered to be mutated in congenital pancreatic agenesis, neonatal diabetes as well as in early and late onset forms of human type 2 diabetes. Pdx1 regulates key aspects of pancreas development and adult ß cell growth and function. We have found that during embryonic development Pdx1 regulates the formation of endocrine progenitors in part by synergizing with the onecut transcription factor Hnf6. During adulthood, Pdx1 regulates islet compensation for genetic and environmentally induced insulin resistance, failure of which underlies the progression of type 2 diabetes. In the adult beta cell Pdx1 directly regulates a number of genes involved in maintaining homeostasis of the endoplasmic reticulum whose optimal function is critical for highly secretory cells such as the beta cell. We are currently utilizing high throughput approaches to identify new direct Pdx1 targets relevant to its developmental and adult roles. By performing global location analysis (chromatin immunoprecipitation sequencing) of Pdx1 occupancy in adult human and mouse primary islets and evaluating for evolutionary conservation of target genes, we found that the conserved target set is highly enriched for genes annotated to function in endocrine system and metabolic disorders, various signaling pathways, and cell survival, providing a molecular

explanation for many of the phenotypes resulting from Pdx1 deficiency. These and other new targets will be discussed.

Synopsis #19

KATP Neonatal Diabetes

Siri Atma W. Greeley, Kovler Diabetes Center, University of Chicago

Permanent neonatal diabetes had been treated indistinguishably from type 1 diabetes until the discovery that nearly half of cases were due to activating mutations in either of the genes (*KCNJ11* and *ABCC8*) encoding the two subunits (Kir6.2 and SUR1) of the ATP-sensitive potassium (KATP) channel. This discovery led to the radically different and successful approach of oral sulfonylureas in lieu of insulin. Numerous case reports and series have confirmed the largest early report in which sulfonylureas strikingly improved metabolic control in 44/49 (90%) of patients. Treatment with oral agents is not only significantly easier for families, but our data suggest that many years later most patients continue to maintain near normal hemoglobin A1c levels. Since treating our first case in 2006, the establishment of our US Neonatal Diabetes Registry has led to steady recruitment of patients diagnosed with diabetes under a year of age, now including over 80 with KATP channel mutations. Our design allows for longitudinal monitoring of the stability of diabetes treatment in these patients, as well as characterization of possible associated features, whether related to specific mutations, long-term high dose sulfonylurea treatment, or diabetes related complications. In this regard, approximately 25% of patients with KATP mutations have an associated spectrum of neurodevelopmental disability, with recent mouse data strongly implicating brain expression of mutated channels as being causal. The V59M mutation in *KCNJ11* is the most common cause of a phenotype characterized by a moderate degree of speech, motor, and cognitive impairment. Sulfonylurea therapy has been suggested to improve these problems in a few reports utilizing inconsistent measures. We characterized visuo-motor function using the same well-standardized VMI assessment in a relatively large group of *KCNJ11* patients. V59M subjects exhibited significant impairment while those with the R201H mutation causing isolated diabetes had scores in the normal range. Importantly, age of sulfonylurea initiation was significantly inversely correlated with VMI scores. Another feature associated with these patients has been poor sleep, revealed only through our family support email discussion group. Preliminary data suggests that cases with a variety of mutations score poorly on sleep surveys and exhibit increased wake time after sleep onset. Further study of the underlying pathophysiology of beta cell dysfunction and extra-pancreatic features in these patients will not only lead to more effective treatment but is also likely to provide insight more broadly regarding common forms of diabetes as well as other disorders.

Synopsis #20

Mutations in the Insulin Gene and Diabetes

Louis H. Philipson, The Kovler Diabetes Center, The University of Chicago

Permanent neonatal diabetes is caused by a variety of monogenic forms of diabetes resulting from mutations in a number of different genes encoding proteins that play a key role in the normal function of the pancreatic beta-cell. Our group and others identified mutations in the insulin gene (INS) itself as an important cause of neonatal diabetes. Our first report described 10 heterozygous mutations in the human *INS* gene in 16 probands with neonatal diabetes. A combination of linkage and a candidate gene approach in a Chicago family with four diabetic members led to the identification of the initial *INS* gene mutation. The mutations are inherited in an autosomal dominant manner in this and two other small families whereas the mutations in the other 13 patients are *de novo*. Subsequent studies with additional probands have identified recessive mutations that are associated with lower birth weight. Diabetes presented in the initial probands at a median age of 9 weeks (although onset of *INS* diabetes can be later in life), usually with diabetic ketoacidosis or marked hyperglycemia, was not associated with beta cell autoantibodies, and was treated from diagnosis with insulin. The mutations are in critical regions of the preproinsulin molecule, and they prevent normal folding and progression of proinsulin in the insulin secretory pathway. The abnormally folded proinsulin molecule induces the unfolded protein response and undergoes degradation in the endoplasmic reticulum, leading to severe endoplasmic reticulum stress and beta cell death. This process has been described in both the Akita and Munich mouse models that have dominant-acting missense mutations in the *Ins2* gene, leading to loss of beta cell function and mass. One of the human mutations is identical to that in the Akita mouse. To characterize some of the human mutations, several groups expressed proinsulin-GFP fusion proteins in mouse insulinoma cells or fibroblasts. These studies suggest mutations can result in complete retention of proinsulin in the endoplasmic reticulum (ER); or have partial ER retention, and partial recruitment to granules; with differences in attenuation of wild-type insulin secretion. *INS* mutations are the second most common cause of permanent

neonatal diabetes and a rare cause of MODY and type 1B diabetes. Insulin gene mutation screening is recommended for all diabetic patients diagnosed before 1 year of age. The identification of insulin mutations as a cause of neonatal diabetes will facilitate the diagnosis, genetic counseling and possibly, treatment of this disorder.

Synopsis #21

Beyond the Beta-Cell in Diabetes: CEL and Other Forms of Diabetes Involving the Exocrine Pancreas

Pål R. Njølstad, University of Bergen & Haukeland University Hospital, Norway

Advances in molecular genetics have led to identification of monogenic forms of diabetes (1). We have been interested in monogenic forms of diabetes originating in other cells than the beta-cell.

Two MODYX families had diabetes and pancreatic exocrine dysfunction. By genome-wide marker analysis and sequencing, we found that mutations in the carboxyl-ester lipase (CEL) gene (CEL-MODY or MODY8) co-segregated with diabetes and fecal elastase deficiency (2). CEL is expressed only in pancreatic acinar tissue. Clinical investigations revealed that the patients had primary beta-cell failure together with exocrine dysfunction including pancreatic lipomatosis. In a KO-mouse model with targeted deletion of CEL, we observed mild glucose intolerance in female KO mice, but the full phenotype of human CEL-MODY was not reproduced, suggesting that the pathogenic mechanisms involved are more complex than loss-of-function. The CEL mutant tandem-repeat domain has different physico-chemical properties. In transfected cells, mutant CEL recovered in the large granule fraction upon subcellular fractionation and were seen as punctuate structures by confocal immunofluorescence aggregating in lysosomes and on the surface of cell membranes by immuno-EM (3). Thus, haploinsufficiency is unlikely to explain the disease in our families. Instead, we propose that CEL-MODY is a protein misfolding disease caused by a negative gain-of-function effect of the mutant proteins in pancreatic tissues, with the syndrome starting in the acinar tissue and diabetes being secondary.

HNF1B is expressed in many tissues including the liver, pancreas and urogenital system. Subjects with mutations in HNF1B (HNF1B-MODY or MODY5) suffer from dysfunction related to congenital malformations due to HNF1B mutations such as renal dysfunction, diabetes, exocrine dysfunction and congenital malformations (1). Using ultrasound and MRI, we have demonstrated that most of the HNF1B mutations carriers have a dorsal pancreatic aplasia explaining the pancreatic exocrine dysfunction and insulin-dependent diabetes (4). Using endoscopy, we have investigated the quantity and quality of the pancreatic juice after secretin stimulation. Mutation carriers had a reduced amount of juice with reduced activity of lipase and bicarbonate, although the reduction was not as pronounced as in CEL-MODY. It is important to evaluate also the exocrine pancreatic function in HNF1B mutation carriers to supplement with pancreatic enzymes and vitamin E if need.

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Synopsis #22

Case Studies in the Many Causes of Neonatal Diabetes

Siri Atma W. Greeley, Kovler Diabetes Center, The University of Chicago

Neonatal diabetes mellitus occurs in approximately 1 out of every 100,000 live births and can be either permanent or transient. There has been major progress in recent years uncovering the genetic causes of diabetes presenting in the first year of life, with over twenty genes now having been identified. An underlying monogenic cause is especially likely in those diagnosed before 6 months of age, but a small fraction of cases diagnosed at later ages will also have a similar genetic etiology. The most common causes accounting for the majority of cases are heterozygous mutations in the genes encoding the two subunits of the ATP-sensitive potassium channel (KATP), *KCNJ11* and *ABCC8*, the insulin gene (*INS*), and *GATA6* causing pancreatic agenesis, as well as the most common cause of transient neonatal diabetes due to over-expression of paternally imprinted genes at chromosome 6q24. The remaining genes are associated with very rare recessive syndromes. The main mechanistic categories for neonatal diabetes include: 1) disruption of the function of genes critical for pancreatic and/or beta cell development (*PDX1*, *PTF1A*, *RFX6*, *NEUROD1*, *NEUROG3*, *GATA6*, *HNF1B*, *GLIS3*); 2) alteration of signals for beta-cell insulin secretion (*KCNJ11*, *ABCC8*, *GCK*), 3) increased beta-cell death, either through disruption of proteins important for cellular homeostasis

(*INS*, *EIF2AK3*, *WFS1*), or alteration of immune regulation promoting autoimmune targeting (*FOXP3*), as well as 4) causes in which the pathogenesis remains unclear (6q24, *PAX6*, *IER3IP1*, *SLC19A2*, *SLC2A2*). Patients with activating mutations in *KCNJ11* and *ABCC8* respond dramatically to treatment with oral sulfonylureas in lieu of insulin injections. It remains to be determined what is the most appropriate treatment of other causes, though our preliminary data challenges the assumption that insulin replacement is the only option. Our US Neonatal Diabetes Registry now includes over 250 subjects diagnosed with diabetes under one year of age, with over 100 of these so far found to have one of ten different known causes. Our multifaceted efforts include capture of detailed longitudinal phenotypic data allowing for clear delineation of genotype/phenotype associations. We are developing methodology for efficient screening for monogenic causes utilizing next-generation sequencing approaches while we continue clinical studies to explore mechanisms of beta cell dysfunction and most effective treatment approaches. Neonatal diabetes represents a compelling example of personalized genetic medicine leading to improved glucose regulation and quality of life in many cases that may – with continued research – be repeated for other forms in the future.

Oral Abstracts

Oral Abstract #1

Restrictive Surgery for Non-Focal (Not 11p15 LOH) Congenital Hyperinsulinism

Barthlen W, Mohnike K, Müller C, Wildbrett P, Bahlmann H, Rahier J, Vogelgesang S, University Medicine Greifswald, Germany and University of Louvain, Brussels

Surgical therapy for non-focal (not 11p15 LOH) congenital hyperinsulinism (CHI) is up for debate because of the high risk of diabetes after an extended 95% resection. Objectives: To evaluate the efficacy of restrictive surgery (up tp 40%) in non-focal (not 11p15 LOH) congenital hyperinsulinism. Methods: Five children (mean age 17 months) and a 30 year-old woman with severe non-focal (not 11p15 LOH) CHI resistant to medical therapy underwent genetic analysis, 18F-DOPA-PET-CT and laparoscopic pancreatic biopsies with frozen section analysis. A laparoscopic pancreatic tail resection removing 25–40% of the gland was carried out in all patients. Results: Classical diffuse and segmental mosaic CHI were found in three patients each. After surgery, one child and the adult got cured. Three children are off any medication today and manage their glucose levels by frequent feeds and starch-enriched meals. One child relapsed after pancreatic tail resection, but got manageable with low dose diazoxide after a 2nd surgery with additional resection of the uncinate process. There were no surgical complications and all recovered quickly from the minimal invasive approach. At the time of this report the follow-up varies between 4 and 10 months. Conclusions The encouraging results of this innovative approach show that in non-focal (not 11p15 LOH) CHI a restrictive surgical resection might suffice in selected patients to get them manageable with starch-enriched nutrition. Further work is necessary to evaluate the mechanism how this limited approach works and to identify the patients who will benefit in advance.

Oral Abstract #2

Intraoperative Sonography: A Technique for Localizing Focal Forms of CHI

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The precise localisation and extension of the focus in Congenital Hyperinsulinism are essential for successful surgical intervention. Intra-operative inspection and palpation are unsuitable for a correct focus localisation. Based on initial experience with transabdominal pancreatic imaging intraoperative ultrasonography was proved. Patients and methods: 8 patients, aged 3 $^1/_2$ to 14 months, with focal CHI were operated between 06/2008 and 11/2011. Partial pancreatic resection was conducted 3 to 20 days after diagnosis by 18F-DOPA-PET/CT. Physiological saline solution was used to create a fluid start-up to the pancreas surface. Imaging was undertaken with high-end-ultrasound equipment, using high frequency linear transducers (9 to 15 MHz). The pancreas was displayed by ultrasound in skewed axial, sagittal and planes adapted to the operation site. Results: Focus presented as hypoechogenic area. Tiny, less than one mm measuring lobular, rope-shaped and island-shaped legs of a focus can be separated in real time and be considered with the resection. During the waiting time for the histological investigation, a resection control by ultrasound is possible. Problems of the procedure are the restricted insight into strongly segmented, unfavourably positioned and voluminous foci. Conclusions: (1) intra-operative ultrasound navigation permits a repeatable focus imaging, according to the surgical progress. (2) Important anatomical leading structures, like the intra-pancreatic cystic duct,

pancreatic main duct and surrounding blood vessels can be visualized. (3) Intra-operative high resolution sonography can be seen as an independent modality beside 18F-DOPA-PET/CT. (4) Of importance is the application during the operation process, it occurs in time and without X-ray exposure.

Oral Abstract #3

Long-Term Treatment with Long Acting Release Octreotide in Congenital Hyperinsulinism

Arnoux JB, Mamoune A, Brassier A, Barbier V, Valayannopoulos V, Broissand C, Le Quan Sang KH, de Lonlay P, Necker-Enfants Malades Hospital, Paris

Congenital hyperinsulinism (HI) is a common cause of hypoglycemia in infancy. The medical treatment of diazoxide-unresponsive HI is challenging, based on a somatostatin analogue. This study evaluates the long-term efficiency and tolerance of long-acting release (LAR) octreotide (Sandostatine LAR®, Novartis) administered as an intramuscular (IM) injection every 4 to 6 weeks in HI patients. Method: Twenty-five diazoxide-unresponsive HI patients (mean age 3.2 years) treated with subcutaneous (SC) octreotide given in 3 daily injections (mean dose 37.2 µg/Kg/d) were changed to LAR octreotide. Glycaemia, HbA1c, IGF1, height, weight and satisfaction were monitored under this new treatment with a mean follow-up of 17 months (min. 4 months, max. 4.3 years). The data collected just before the first LAR octreotide injection and at the latest visit were compared using the Wilcoxon test. Results (mean ± SD before LAR treatment vs. most recent visit): In all 25 patients, glycaemia were maintained in their usual range and HbA1c remained stable (5.34% ± 0.57 vs. 5.35% ± 0.53, $p = 0.22$). Because of a perfect glycemic control, in 5 patients, the IM injections frequency was expanded to every 5 weeks and even to every 6 weeks in 9 patients, using the same dose. Thus, the equivalent daily dose of octreotide was decreased by an average of 17.2% ± 14, $p = 0.0003$. Patients' growth followed their usual curves (variation from their initial curve: −0,04 SD ±1.05, $p = 0.94$). IGF1 levels improved, $p = 0,01$. No severe side effect was noted. Two patients with syndromic HI were treated with growth hormone because of a preexisting partial GH deficiency. One patient experienced transiently mild stomach pains and steatorrhea. Parents' questionnaire of satisfaction for LAR octreotide was highly positive. Conclusion: LAR octreotide is efficient and well tolerated in diazoxide-unresponsive HI patients and contributes to a clear simplification of their medical care.

Oral Abstract #4

Pharmacological Rescue of Trafficking-Impaired KAP Channels

Zhou Q, Chen PC, Shyng SL, OHSU, Portland

ATP-sensitive potassium (KATP) channels mediate glucose-induced insulin secretion by coupling metabolic signals to beta-cell membrane potential. Reduced KATP channel expression caused by mutations in sulfonylurea receptor 1 (SUR1) or Kir6.2 results in loss of channel function and congenital hyperinsulinism. The objective of this study is to identify small molecules that act as pharmacological chaperones to rescue trafficking impaired mutant KATP channels. Specifically, we wish to test compounds which have been shown to rescue the trafficking defect of a CFTR mutant deltaF508, based on the rationale that CFTR shares structural similarity with SUR1. COS cells were used to transiently express Kir6.2 and several SUR1 mutants known to cause trafficking defects: A116P, G716V and deltaF1388. Cells were treated with CFTR correctors. Processing and surface expression of channels were assessed by western blots, immunostaining and surface chemiluminescence assays. The function of rescued channels was assessed by Rb efflux assays and patch clamp recording. Promising compounds were further tested in the rat insulinoma cell line INS-1 and human islets infected with recombinant adenoviruses of mutant channel subunits. All seven CFTR correctors tested enhanced surface expression of wild-type KATP channels. Of these, several also rescued the three trafficking impaired mutants. One of these compounds, carbamazepine (CBZ), was further characterized in INS-1 cells and human islets transduced with the A116P mutant SUR1 and Kir6.2 adenoviruses. CBZ showed rescue effects in both INS-1 cells and human islets. Importantly, CBZ did not disrupt the function of mutant channels rescued to the cell surface. Our results identify novel reagents that rescue trafficking impaired KATP channels in in vitro expression systems. These reagents may be further developed as a potential treatment for CHI patients carrying KATP channel trafficking mutations.

Oral Abstract #5

A Novel Syndrome of Hypoinsulinaemic Hypoglycaemia and Hemi-Hypertrophy due to an Activating Mutation of AKT2

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Hypoglycaemia is one of the most common biochemical findings in the childhood period. A large number of endocrine and metabolic conditions lead to hypoglycaemia. We studied three unrelated children with persistent, severe fasting hypoglycaemia associated with accelerated and asymmetrical growth in whom known causes of hypoglycaemia had been excluded. The clinical and biochemical picture resembled hyperinsulinism (suppressed fatty acids and ketone bodies) but no serum insulin, c-peptide or pro-insulin were ever detectable when the patients were hypoglycaemic. All patients were found to carry the same de novo mutation, p.Glu17Lys, in the serine/threonine kinase AKT2, in two cases as heterozygotes and, in one case, in mosaic form. In heterologous cells, the mutant AKT2 was constitutively recruited to the plasma membrane, leading to insulin-independent activation of downstream signalling. This AKT2 mutation represents a novel mechanism of severe hypoglycaemia, characterised by the constitutive, cell-autonomous activation of signal transduction pathways normally controlled by insulin.

Oral Abstract #6

Novel Form of Autosomal Dominant Hyperinsulinism Maps to Chromosome 10q21

Pinney SE, Ganapathy K, Bradfield J, Givler S, Becker S, Hughes N, Stokes D, Stanley CA, The Children's Hospital of Philadelphia

Genetic forms of hyperinsulinism (HI) are the most frequent cause of persistent hypoglycemia in infants and children. We studied the genetic locus for HI in the original family with ''Idiopathic Hypoglycemia of Infancy'' described by Irvine McQuarrie, M.D. in 1954. Objectives: To identify the genetic locus and clinical phenotype of this novel form of HI. Methods: We surveyed 24 affected individuals by questionnaire or interview. Seven affecteds participated in phenotyping tests of insulin regulation, including 24 hr fasting test, oral protein and glucose tolerance tests (OGTT). Linkage analysis was performed using data from Illumina SNP chips. Whole transcriptome sequencing, gene capture, whole genome sequencing and next generation sequencing were performed to identify novel pathogenic variants. Results: Most affecteds had normal birth weights and were diagnosed with HI before age one. One patient had severe cognitive impairment due to hypoglycemia and forty percent presented with seizure. All affecteds responded well to diazoxide. Affecteds showed a failure to adequately suppress insulin secretion following OGTT or prolonged fasting; none had protein-sensitive hypoglycemia. By linkage analysis, the HI mapped to an 8.2Mb region on Chr 10q21 containing 49 genes with a LOD score of 6.802. We identified several novel variants present in all affecteds. Three were variants in hexokinase 1(HK1); two upstream of the transcription start site and one in intron 2. There was also 1 missense variant in the coding region of DNA2, a mitochondrial DNA replicase. Conclusion: This historically-important dominant form of diazoxide-responsive congenital hyperinsulinism maps to a novel HI genetic locus on Chr 10q21. HK1 is a stronger candidate disease gene than DNA2, since mutations leading to abnormal expression of the HK1 enzyme in beta-cells could result in higher affinity for glucose, thus explaining a lowered glucose threshold for suppressing insulin secretion.

Oral Abstract #7

Leucine-Sensitive Hyperinsulinaemic Hypoglycaemia in Patients with Loss of Function Mutations in 3-Hydroxyacyl-CoA Dehydrogenase

Heslegrave AJ, Kapoor RR, Eaton S, Chadefaux B, Ackay T, Simsek E, Flanagan SE, Ellard S, Hussain K, Institute of Child Health, University College London

Loss of function mutations in HADH cause protein sensitive hyperinsulinaemic hypoglycaemia (HH). HADH encodes short chain 3-hydroxacyl-CoA dehydrogenase, an enzyme that catalyses the penultimate reaction in mitochondrial β-oxidation of fatty acids. Mutations in GLUD1 encoding glutamate dehydrogenase, also cause protein sensitive HH (due to leucine sensitivity). Reports suggest a protein-protein interaction between HADH and GDH. In order to understand the mechanism of protein sensitivity in patients with HADH mutations an oral leucine tolerance test was conducted in nine patients with HADH mutations. Basal GDH activity and the effect of GTP were determined in lymphoblast homogenates from 4 patients. Immunoprecipitation was conducted in patient and control lymphoblasts to investigate protein interactions. Patients demonstrated severe HH (glucose range 1.7–3.2 mmol/l; insulin range 4.8–63.8 mU/l) in response to the oral leucine load, this HH was not seen in control patients subjected to the same leucine load. Basal GDH activity and half maximal inhibitory concentration of GTP was similar in patients and controls. HADH protein could be co-immunoprecipitated with GDH protein in control samples but not patient samples. We conclude that GDH and HADH have a direct protein-protein interaction, lost in patients with HADH mutations causing leucine induced

HH. This is not associated with loss of inhibitory effect of GTP on GDH (as in patients with GLUD1 mutations).

Oral Abstract #8

The Spectrum of Hyperinsulinism in Mosaic Beckwith-Wiedemann Syndrome: Dissection of 11p15 Paternal Uniparental Isodisomy

Kalish JM, Kara S, Conlin LK, Bhatti T, Ganguly A, Stanley CA, Deardorff M, The Children's Hospital of Philadelphia

Hyperinsulinism (HI) and Beckwith-Wiedemann Syndrome (BWS) both lead to neonatal hypoglycemia and are linked to genetic and epigenetic alterations on 11p15. Hypoglycemia in BWS is associated with mosaic 11p15 paternal uniparental isodisomy (pUPD). Additionally, subunits of the β-cell plasma membrane KATP channel (KATP) are located on 11p15. Paternally inherited mutations of KATP genes and somatic 11p15 pUPD are seen in focal KATP HI. Diffuse KATP HI is due to autosomal recessive mutations. Uncovering mutations by pUPD is a common mechanism of pathogenesis between HI and BWS. Objectives: To correlate the range of phenotypic and molecular findings in 12 patients with HI and BWS. Methods: Clinical BWS criteria included macrosomia and hemihypertrophy. SNP array analysis, mutation analysis of KATP genes, and pancreatic histology were performed. Results: 11p15 pUPD was found in 10 patients in lymphocytes and in 2 patients in skin fibroblasts. 9 patients were diazoxide unresponsive. Of these, 4 patients required escalated treatment and pancreatic samples showed 11p15 pUPD. Notably, the islet cell histology of these BWS patients showed pancreatic endocrine tissue expansion in a distinct pattern compared to focal and diffuse KATP HI. 1 patient had a paternal KCNJ11 mutation, required a GIR of >50 and died. 2 patients had >90% pancreatectomies and one patient required a GIR of 5. 5 patients were treated with octreotide and those over age 2 years no longer require therapy. Conclusions: Mosaic BWS caused by 11p15 pUPD was present in HI patients with a range of disease severity. BWS patients with HI requiring escalated treatment showed higher percentages of mosaic 11p15 pUPD in pancreas compared to blood. The mechanism of HI in BWS may be similar to the mechanism of focal KATP HI with pUPD and an undetected KATP mutation or a mutation in another gene within the pUPD region. SNP array analysis for 11p15 pUPD is recommended for HI patients without KATP mutations.

Oral Abstract #9

Common Clinical Features among Small-for-Gestational-Age Infants who Develop Hyperinsulinemic Hypoglycemia

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Some small-for-gestational-age (SGA) infants manifest transient hyperinsulinemic hypoglycemia (HI). Objective: To test our hypothesis that we can predict HI in SGA infants at the time of NICU admission, and that they experience common complications and a similar response to treatment. The medical records of all SGA infants admitted to our NICU between 2009 and 2011 were reviewed retrospectively (n = 64). We excluded six extremely-low-birth-weight infants, who had received early parenteral nutrition and indomethacin, both of which are known to increase glucose requirements. We also excluded infants born after 39 weeks gestation and infants with chromosomal abnormalities or multiple organ anomalies. Infants who experienced repeated hypoglycemia with hyperinsulinemia beyond day 1 and those who required a glucose infusion of >8 mg/kg/min to maintain normoglycemia were categorized as the HI group (n = 12), and the others as the non-HI group (n = 52). Results: In the HI group, the reticulocyte and erythroblast counts and the rate of mechanical ventilation were significantly higher, and the white blood cell and platelet counts, fibrinogen, and blood glucose values were significantly lower than in the non-HI group. These common findings in the HI group may be interpreted as the consequences of fetal chronic hypoxia. We had to increase the glucose infusion rate to avoid hypoglycemia, and the maximal treatment occurred a few days after birth. Diazoxide was effective and could be discontinued in all cases several months after birth. Respiratory problems were common and many cases required mechanical ventilation. Thrombocytopenia and coagulation abnormalities were attributed to decreased synthesis, and responded well to transfusion. Conclusion: HI in SGA infants could be predicted by particular data obtained on NICU admission. HI may be most severe several days after birth, and close monitoring is necessary in high-risk infants.

Oral Abstract #10

GATA6 Mutations Cause a Broad Phenotypic Spectrum of Diabetes and Congenital Heart Defects

Ellard S, De Franco E, Shaw-Smith C, Allen HL, Flanagan SE, Hattersley AT, Peninsula Medical School, Exeter

We recently reported 15 patients with de novo heterozygous GATA6 mutations causing pancreatic agenesis (insulin-treated neonatal diabetes and exocrine insufficiency requiring replacement therapy). This established GATA6 mutations as the most common cause of pancreatic agenesis. Extra-pancreatic features included cardiac malformations, neurocognitive deficit, biliary tract anomalies and gut developmental abnormalities. Objectives: To determine whether GATA6 mutations also cause neonatal diabetes in patients not reported to have exocrine pancreatic insufficiency. Methods We sequenced the GATA6 gene in 132 probands with permanent and 21 with transient diabetes diagnosed before 6 months of age. Mutations in KCNJ11, ABCC8 and INS were excluded by sequencing and 6q24 analysis for TNDM cases was normal. Results: We identified GATA6 mutations in 9/153 probands (5.9%), 8 PNDM (8/132) and one TNDM (1/21). All mutations were novel; 3 missense, 3 splicing, two deletions and one nonsense. Parental samples were available for 7 cases; 3 mutations were de novo and 4 inherited from a parent. Clinical details were available for 3 transmitting parents diagnosed with diabetes at the ages of 12, 30 and 46 years. The probands were diagnosed between 1 day and 8 weeks. Follow-up data revealed pancreatic insufficiency in 6/9 probands and 0/3 parents. Extra-pancreatic features were present in 11/12 mutation carriers and included congenital heart malformation (n = 9), developmental delay (4), hypothyroidism (3), hepatobiliary anomalies (2) and hernia (2). Conclusions: GATA6 mutations cause a variable phenotype of diabetes diagnosed aged 1 day to 46 years with exocrine insufficiency in most, but not all, patients. The most common extra-pancreatic feature is a structural cardiac malformation. Genetic testing for GATA6 mutations is recommended in all patients with permanent neonatal diabetes and exocrine insufficiency, especially if a structural cardiac malformation is present.

Oral Abstract #11

Clec16a, a T1DM Susceptibility Gene and Novel Pdx1 Target, Regulates Endosomal and Mitochondrial Function In Min6 ß-Cells

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The homeodomain transcription factor Pdx1 is a master regulator of pancreatic β-cell mass and function. Pdx1 regulates β-cell function through direct regulation of the insulin gene and of genes involved in mitochondrial metabolism, homeostasis of the endoplasmic reticulum and insulin secretion. By high throughput analysis of Pdx1 regulated gene expression and occupancy in isolated mouse islets and mouse insulinoma cells, we identified a novel Pdx1 target, C-lectin domain family 16, member A (Clec16a), whose expression is reduced in the islets of Pdx1+/− mice compared to littermate controls and whose promoter is occupied by Pdx1 in Min6 cells and primary islets. Genome-wide association studies have identified Clec16a as a gene associated with type 1 diabetes mellitus (T1DM) in humans. We find that Clec16a is expressed in both human and mouse islets at levels similar to immune cells. By alkaline carbonate extraction and co-IP assays, Clec16a is a membrane-associated protein that directly interacts with members of the Class C-Vps HOPS complex, which regulates endosomal maturation and mitochondrial function in S. cerevisiae. Utilizing RNAi, loss of Clec16a function causes a 46% reduction in glucose-stimulated insulin secretion in Min6 cells that is not due to changes in total insulin content ($p < 0.05$). Correspondingly we observe a significant reduction in both basal (47% of control) and maximal uncoupled (55% of control) oxygen consumption following loss of Clec16a function in Min6 cells ($p < 0.05$). Consequently, we also observe a 37% reduction in intracellular ATP levels following Clec16a shRNA treatment ($p < 0.01$). Finally, we observe an increase in Rab7-labeled late endosomes following Clec16a loss-of-function, suggesting a defect in endosomal maturation. Taken together, we identify Clec16a as a novel Pdx1 target whose effects on insulin secretion and on endosomal and mitochondrial function that may mediate critical actions of Pdx1 in the pancreatic β-cell.

Poster Abstracts

Poster Abstract #1

"Missing" Mutations: Post-Zygotic Mosaicism in Congenital Hyperinsulinism

Lord K, Snider K, MacMullen C, Becker S, Ganguly A, Stanley CA, Children's Hospital of Philadelphia

Genetic analysis of peripheral blood fails to find mutations in 50% of children with congenital hyperinsulinism (HI). Some children with "missing" mutations may have post-zygotic mutations of genes associated with dominant forms of HI in the pancreas. Objective: To determine if post-zygotic mutations in the pancreas are a cause of HI. Methods: Direct sequencing of dominant HI genes was performed on pancreatic

DNA from 3 of 4 children with HI whose blood lacked mutations in SUR1, Kir6.2, glutamate dehydrogenase (GDH), and glucokinase (GK). Results: Patient 1 had diazoxide-responsive HI, protein-induced hypoglycemia and elevated ammonias. She was diagnosed with hyperinsulinism/hyperammonemia syndrome (HI/HA) and had a 95% pancreatectomy due to family concerns about diazoxide side effects. Pancreatic DNA identified a known missense mutation (p.S445L) in GDH. Patient 2 had diazoxide-unresponsive HI that clinically was not consistent with a mutation in SUR1 or Kir6.2. GK-HI was suspected and she underwent a 92% pancreatectomy. Pancreatic DNA identified a low-level mutation (p.454dupAla) in GK. Patient 3, born at 27 weeks, had presumed perinatal-stress induced HI that failed to resolve. A fasting test was characteristic of GK-HI. She had a 10% pancreatectomy for histologic and genetic diagnosis. Analysis of the pancreas failed to identify a mutation in GK. Patient 4 was diagnosed with HI/HA after presenting with hypoglycemia and elevated ammonias. She demonstrated protein-induced hypoglycemia. She was well controlled on diazoxide and did not require surgical intervention. Conclusions: These four patients had clinical phenotypes suggestive of dominant forms of HI. In 2 of 4, suspected GDH and GK mutations were confirmed in pancreatic DNA. The third patient may have a mosaic GK mutation below the limit of detection by conventional sequencing. Post-zygotic mutations should be considered in children with the appropriate phenotype who lack mutations in the blood.

Poster Abstract #2

Novel Presentations of Congenital Hyperinsulinism due to Mutations in the Mody Genes: Hnf1a and Hnf4a

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Mutations in HNF1A and HNF4A cause familial monogenic diabetes. Individuals carrying HNF4A mutations that result in familial monogenic diabetes later in life can present in early infancy with hyperinsulinism. Objective: To describe two unusual cases of hyperinsulinism associated with mutations in HNF1A and HNF4A. Methods: Clinical data were obtained from chart review. Gene sequencing of HNF1A, HNF4A and SLC2A2 were performed. Results: Case #1 presented at 20 months with persistent hypoglycemia due to hyperinsulinism. She was later found to have a MODY3 HNF1A mutation also carried by her father who had diabetes. Case #2 presented as a newborn with diazoxide-responsive hyperinsulinism and later developed renal Fanconi syndrome, hypophosphatemic rickets and hepatomegaly with increased hepatic glycogen. Although clinically suggestive of Fanconi-Bickel syndrome, sequencing of the SLC2A2 gene was normal. She was found to have a known MODY1 mutation in HNF4A. In both cases, the hyperinsulinism improved with age. Conclusions: These cases demonstrate that mutations in both HNF1A can cause hyperinsulinism early in life and diabetes later, similar to the well-established phenotype of the HNF4A (MODY 1) mutations. Moreover HNF4A mutations may affect the liver and kidney, producing a non-progressive disease phenotype similar to GLUT2 deficiency, and thereby expanding the genetic causes of Fanconi syndrome.

Poster Abstract #3

Permanent Neonatal Diabetes Mellitus in Monozygotic Twins with KCNJ11 Mutations

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KCNJ11 mutations are associated with permanent neonatal diabetes. Objective: To report monozygotic twins diagnosed with neonatal diabetes, discovered to have KCNJ11 mutations, and controlled with sulfonylurea therapy for four years. Case: 2 month old female monozygotic twins, formerly 36 week IUGR neonates with birth weights of 1.48 kg and 1.8 kg, were brought to a county emergency center for fever evaluation. Both twins were incidentally discovered to be hyperglycemic. Transfer was initiated to a nearby children's tertiary care center. Twin A demonstrated tonic clonic movement with serum glucose of 989 mg/dl, serum ketones of 10 mmol/L, and pH of 7.19. HbA1c was 10.9%. Electrographic seizures were recorded, and imaging revealed diffuse cerebral edema. Twin B had a serum glucose of 605 mg/dl, serum ketones of 6.10 mmol/L, and pH of 7.27. HbA1c was 10.7%. Twin B did not have a seizure focus or cerebral edema. Both received continuous insulin infusion and transitioned to subcutaneous glargine at 6.5 units and 7 units daily, respectively. Family history was significant for consanguinity in a distant generation and a paternal aunt with type 1 diabetes. Genetic testing for uniparental disomy of chromosome 6 was negative. Further testing revealed KCNJ11 mutations at c.602G>A p.Arg201His heterozygous. At 4 months of age, both transitioned to sulfonylurea therapy. They have remained on glyburide at minimal doses of 0.06 mg/kg/day with HbA1c levels $< 6\%$ for four years. Twin A has not had further seizures, and both have achieved normal developmental milestones. Both children follow the 75–90% for height and the 50% for weight

while on sulfonylurea therapy. Conclusion: No known reports of monozygotic twins with permanent neonatal diabetes exist in the literature to our knowledge. Our data suggests a genotype-phenotype correlation in monozygotic twins with KCNJ11 mutations. The clinical course suggests marked sensitivity to sulfonylurea therapy.

Poster Abstract #4

Hyperinsulinemic Hypoglycemia in Tube Fed Preterm Infants

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Hyperinsulinemic hypoglycemia is of particular concern in newborns. Recurrent hyperinsulinemic hypoglycemia occurs in some preterm infants when they receive intermittent tube feeding. Although little is known about its etiology and treatment, we believe that it is different from perinatal-stress induced hyperinsulinism. Objectives: To identify the daily glycemic patterns in preterm infants with late onset hypoglycemia by continuous glucose monitoring (CGM). Patients and Methods: Late-onset hypoglycemia (Day 12 to 16, blood glucose <50 mg/dL) was detected in four preterm infants (birth weight 998–1780g; gestational age 27 to 30 weeks) by routine screening. All infants showed high serum insulin levels and extremely low ketone levels at the time of hypoglycemia. They had no known perinatal-stress factors such as intrauterine growth restriction and/or birth asphyxia. CGM was conducted at 30 to 34 weeks post-conceptual age when they received intermittent gastric tube feeding (every 3 hours via a slow (over 1 hour) injection from a syringe with no intravenous glucose infusion). CGM was repeated after the infants began to orally ingested milk formula. Results: In all infants, the first CGM showed characteristic postprandial glucose rises followed by subsequent sharp declines along with many hypo- and hyperglycemic events. In one case, diazoxide caused worsening of the glycemic fluctuations. In 3/4 cases, the fluctuations were stabilized by feeding via continuous infusion. Because hyperglycemia preceded hypoglycemia, the fluctuating glycemic change could be attributed to immature control of insulin secretion (''delayed and prolonged'' according to each feed). This fluctuating pattern disappeared at 38 to 40 weeks post-conceptual age. Conclusions: It is difficult to differentiate this type of hyperinsulinism by limited blood sampling alone. CGM is warranted and continuous infusion may be beneficial for glycemic stability in some preterm infants.

Poster Abstract #5

Hypoglycemia Hyperinsulinemic of Infancy: Clinical Characteristics of Brazilian Patients

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Hyperinsulinemic hypoglycemia (HH) is a very serious situation, especiallyin the neonatal period. The congenital forms (HHC) are the most frequent and are associated with high morbidity and mortality. These congenital cases are the result of mutations in seven different genes. AIM: Review the clinical and laboratory data of cases from different regions of Brazil in order to set up a HHI in Brazil. Methods: All pediatric endocrinology services in the country were invited. Medical records were retrieved and the characteristics of birth, age at onset of hypoglycemia, laboratory data collected in the ''critical sample'' and the treatment used. Results: 56 cases of HCC were recovered, three of those children had been born by vaginal delivery. Birth weights ranged from 2075 to 5240 grams (M:3370g). The onset of hypoglycemia ranged from 1 to 240 days, and in 7 cases, age at onset was greater than 60 days. Glycaemia at diagnosis ranged from 1 to 48 mg/dl (24.7). The rate of glucose infusion ranged from 11 to 40 mg/kg/min (19.1) and concomitant insulin ranged from 3 to 147 mIU/ml (26.36). In 25 cases glucocorticoid were used as treatment, in 2 glucagon, octreotide in 20, nifedipine in 2, diazoxide in 15 and growth hormone in 13. 17 cases needed pancreatectomy to control glycemic level. Discussion: The clinical and laboratory data retrieved in this series proved to be similar to the reported literature. The therapeutic options, however, were different, probably because it involves different routines. In a great number of cases glucocorticoids were used and the low use of glucagon is different from other series. In less than half the cases we used diazoxide. In almost half the cases the choice was pancratectomy, with all the inherent risks. An increased in sample size will allow a genetic evaluation and its correlation with clinical data.

Poster Abstract #6

Next Generation Sequencing to Identify Recurrent Cryptic Abcc8 and HADH Splice Site Mutations in Patients with Congenital Hyperinsulinaemic Hypoglycaemia

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Inactivating ABCC8 or KCNJ11 mutations are found by Sanger sequencing in 80% of patients with focal or diffuse forms of diazoxide unresponsive hyperinsulinaemic hypoglycaemia (HH). Some patients with a normal result have partial gene deletions. The mutational mechanism for focal HH suggests all patients will have an ABCC8/KCNJ11 mutation. Sequence analysis of the exons, intron/exon boundaries, minimal promoter and alternative transcripts previously detected mutations in only 3/6 consanguineous probands with diazoxide-responsive HH who were homozygous across HADH (Flanagan JCEM 2011). Objectives: To use next generation sequencing (NGS) to search for non-coding ABCC8 mutations in 2 probands with focal HH and non-coding HADH mutations in the 3 consanguineous probands linked to HADH. Methods Long range PCR was used to amplify 117kb genomic DNA encompassing ABCC8/KCNJ11 or 94kb encompassing HADH. The products were sequenced on an Illumina GAIIx. Additional patients were tested by Sanger sequencing for novel mutations, including 23 diazoxide-unresponsive cases (for ABCC8) and 47 consanguineous diazoxide-responsive cases (for HADH). Results: We identified a paternal ABCC8 intronic A>G variant in the 2 probands with focal HH and a homozygous HADH G>T mutation in the 3 consanguineous probands. Sanger sequencing detected the ABCC8 mutation in a further 3 patients and the HADH mutation in 5 additional consanguineous probands. In silico analysis predicts that both variants create a cryptic splice donor site which is likely to result in the incorporation of an out of frame pseudoexon. RNA studies are in progress. Conclusion: We identified a novel intronic ABCC8 mutation in 5/25 (20%) probands with diazoxide unresponsive HH and a homozygous intronic HADH mutation in 8/50 (16%) consanguineous probands without a genetic diagnosis. This study highlights the importance of non-coding variants in the aetiology of HH and demonstrates the utility of NGS to identify these mutations.

Poster Abstract #7

Current Status of Congenital Hyperinsulinism in Japan

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Despite the recent progress in the diagnosis and management of congenital hyperinsulinism (CHI), until recently, the situation in Japan has been that of early 1990's. The epidemiology is unknown and state-of-the art management has not been available. Objectives: To investigate the epidemiology of CHI and establish a better treatment system in Japan. Methods: A nationwide survey was conducted on cases of CHI born between Oct 2007 and Sept 2009, which asked the number of the cases, clinical and laboratory presentation, treatment and the outcomes. Separately, molecular (Osaka City General Hospital) and 18F-DOPA PET (Kizawa Memorial Hospital) diagnostic systems were established and started service for nationwide patients since 2009. Results: In Japan, the incidence of persistent CHI was 1 in 35400 births whereas that of transient CHI was 1 in 17000. The gestational age and the birth weight were the only factors that could differentiate persistent versus transient forms. Pancreatectomy was performed on 11.5% of the persistent cases. Most of the surgeries were subtotal pancreatectomy or blindly performed distal pancreatectomy. Overall, 8% of the total cases presented with neurological sequelae. Molecular epidemiological study on a different cohort of 71 patients with persistent hyperinsulinism revealed that KATP CHI consisted of 46.4% of the total persistent cases and 78.8% of them had paternally-inherited monoallelic mutation suggesting the presence of a focal lesion. After 2009, clearly focal cases could be successfully cured by partial pancreatectomy. Since the surgeons were generally reluctant to perform pancreatojejunostomy, many of the cases with a focal lesion in the head of the pancreas were treated by long-term subcutaneous octreotide infusion aiming at eventual spontaneous remission. Conclusions: The epidemiology of CHI in Japan was investigated for the first time, and the molecular and PET diagnostic systems were established.

Poster Abstract #8

Predicting Mechanisms of CHI: Engineered KATP Channels with Increased ATP Sensitivity

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To date, mechanisms that cause KATP dysfunction in CHI include defects of trafficking and Mg•ADP-induced stimulation. Surprisingly, no case has been reported in which the fundamental defect is increased ATP-induced inhibition. Objectives: We set out to better understand how SUR1 and Kir6.2 – the two subunits composing KATP channels – work together to become sensitive to inhibition by ATP. We used Kir6.2 subunits with mutations at residue 52 that we found to have charge-dependent ATP-sensitivity as a starting point. Methods: ATP-sensitivity and cysteine cross-linking were assessed by inside-out patch voltage-clamp in transiently transfected mammalian COSm6 cells. Proximity between subunits was tested

by substituting select residues with cysteine then exposing mutant channels to oxidizing or reducing agents. 86Rb+−efflux was used to evaluate channel activity under conditions of metabolic inhibition (i.e., simulated low blood glucose) in intact cells. Results: We found that substituting Q52 in Kir6.2 with a negatively charged residue (Q52E) significantly increased ATP sensitivity, shifting the dose–response ~5-fold vs wild-type. Interestingly, substitution with a positively charged residue (Q52R) causes PNDM by significantly deceasing ATP-sensitivity; further, the effect of Q52R-Kir6.2 is known to depend on the presence of SUR1. Using Q52E-Kir6.2 to screen for potential sites of interaction with SUR1, we found that E203K-SUR1 – when co-expressed with Q52E-Kir6.2 – caused ~100-fold increase in ATP-sensitivity vs wild-type. Substitution at both sites with cysteine (Q52C//E203C) allowed for chemical cross-linking, verifying their close proximity. Both Q52-Kir6.2 and Q52-Kir6.2//E203K-SUR1 channels had significantly reduced activity under conditions of simulated low blood glucose, mimicking CHI-causing mutants. Conclusions: Our results are proof of principle that it is possible to increase KATP channel ATP-sensitivity such that a CHI phenotype would result.

Poster Abstract #9

[18F]-DOPA PET/CT Imaging in Congenital Hyperinsulinism – First 12 Months of Australian Experience

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In the southern hemisphere, [18F]-DOPA PET/CT (Positron Emission Tomography/Computed Tomography) became uniquely available in Brisbane, Australia in early 2010. This may enable pre-operative distinction of focal and diffuse forms of Congenital Hyperinsulinism (CH) without the challenges of travel to an overseas center. Objective: Review the cases of [18F]-DOPA PET/CT in CH since imaging became available. Methods: Case records reviewed for clinical details, metabolic and genetic investigations, PET/CT result, histology if surgery performed and clinical outcome. Results: Five PET/CT scans had been performed and all cases reviewed. In case 1, a scan was performed when the male infant (paternal mutation KCNJ11) had continuing hypoglycemia post partial pancreatectomy at 5mths. Diffuse disease was confirmed by PET/CT and a near-total resection at 7mths. He is well at 30mths with no medication. Case 2 (genetics not available) had a scan at 35mths in the context of high-dose diazoxide, with glucose instability. PET/CT suggested diffuse disease, confirmed by histology following pancreatic tail and body resection. Case 3, a female (paternal mutation ABCC8) was scanned at 6mths showing a focal lesion, confirmed at resection. She is well at 18mths with no medication. Case 4 (paternal mutation ABCC8), with a diazoxide requirement, had a scan at 4yrs showing diffuse uptake. Case 5 (no mutation in ABCC8 or KCNJ11) had a scan at 14mths of age, 10 days after presentation with seizures and a requirement for intensive medical management. The scan showed diffuse uptake. Conclusions: In Australia, PET/CT has been useful in planning surgery for infants and children with CH, correctly identifying focal and diffuse disease. It has allowed targeted resection of the focal lesion with confident extensive resection in the two infants with diffuse disease who required surgery. Acknowledgements: This work is partly supported by the Royal Children's Hospital Foundation, QLD.

Poster Abstract #10

[U-13C]-Glucose Metabolism in Islets from Infants with KATP Hyperinsulinism

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Loss of function mutations of beta-cell KATP channels cause elevation of cytosolic calcium leading to hyperinsulinemic hypoglycemia (HI). KATP-HI patients have impaired glucose stimulated insulin secretion, but are hyper-sensitive to amino acids. To investigate how this alteration of beta-cell fuel sensing reflected altered fuel metabolism we examined [U-13C]glucose metabolism by stable isotope tracing in islets from infants with KATP-HI. Islets were isolated from 3 cases of HI undergoing subtotal pancreatectomy and compared to normal human islets. Islets were incubated with 4 mM amino acid as basal condition and then with 5 or 25 mM [U-13C]glucose. Compared to controls, KATP-HI islets had 2-fold increase of alanine levels and 6-fold increase of 13C enrichment of alanine, suggesting increased rates of glycolysis. In contrast, HI islets had slower citric acid cycle flux rate, as evidenced by reduced levels of aspartate and its 13C enrichment, a lack of elevation of 13C-glutamate in response to glucose stimulation, and lowered rates of pyruvate cycling. HI islets also had higher glutamine levels and its 13C enrichment in response to 5 mM glucose. In control human islets, glucose stimulation resulted in a decrease of islet GABA levels and an increase in

its 13C enrichment, indicating an increase in GABA shunt activity in response to glucose. In contrast, KATP-HI islets had very low GABA levels and a lack of 13C incorporation into GABA in response to glucose stimulation, similar to what we had found in SUR1−/− mouse islets. This defect in GABA shunt activity in HI islets was due to reduced expression of glutamate decarboxylase. KATP-HI islets manifest increased glycolysis, but decreased citric acid cycle flux, impaired GABA shunt activity, and increased glutamine synthesis. These alterations in islet glucose metabolism may result from elevations in cytosolic calcium and may also be responsible for the switch in fuel sensing from glucose to amino acids in KATP-HI.

Poster Abstract #11

Genetic Testing for Maturity Onset Diabetes of the Young in Australia

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Maturity Onset Diabetes of the Young (MODY) is a group of disorders that affects about 2% of people with diabetes mellitus. The term MODY describes a heterogeneous group of disorders caused by mutations in genes important to beta cell development, function, regulation, glucose sensing, and in the insulin gene itself. To date, mutations in at least 12 different genes have been identified to cause MODY. Objectives: In 2005, molecular genetic testing for MODY was introduced into Australia by Mater Health Services, Brisbane. We aim to report our experience in the molecular diagnosis of MODY in Australia over the last seven years. Methods: Four genes which account for the majority of reported MODY phenotypes, HNF1A (MODY3), GCK (MODY2), HNF4A (MODY1) and HNF1B (MODY5) were tested for gene sequence variations and copy number changes. This was carried out by bidirectional DNA sequencing and Multiplex Ligation Dependent Probe Amplification (MLPA). Results: Of 159 families screened for MODY, the laboratory identified 53 mutations. This consisted of 27 GCK mutations (6 novel), 13 HNF1A mutations, 7 HNF4A mutations (2 novel) and 6 HNF1B mutations. In patients with a high index of suspicion (negative pancreatic auto-antibodies and strong family history), mutations were identified in 70% of cases. Conclusion: Our rate of detection of mutations was similar to other international studies of MODY. The proportions of each MODY subtype are similar to other centres, with 75% of our mutations identified as MODY 2 or 3. Given the important implications of confirming the clinical diagnosis of MODY and identifying the specific MODY subtype, genetic testing should be more widely utilized in patients with an autosomal dominant pedigree who are negative for pancreatic autoantibodies. A high index of suspicion coupled with availability of genetic testing should decrease the interval between clinical diagnosis of diabetes mellitus and confirmation of MODY subtype.

Poster Abstract #12

Anaerobic Exercise Resulting in Massive Insulin Secretion and Severe Hypoglycaemia: A New Case of Exercise-Induced Hyperinsulinism

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Exercise-induced hyperinsulinism (EIHI) is a rare dominantly inherited disorder characterised by recurrent hypoglycaemia caused by inappropriate insulin secretion during anaerobic exercise. Promoter-activating mutations inducing SLC16A1 expression in pancreatic beta-cells have been described in patients with EIHI. This gene is usually not transcribed in beta-cells and expression is supposed to result in pyruvate uptake and pyruvate-stimulated insulin release. We describe a patient with a history of hyperinsulinemic hypoglycaemia since age of 11 years. At the age of 16 years the patient was diagnosed and evaluated at our institution. Methods: Short exercise tests with frequent blood samples for glucose, lactate and insulin. Results: A short period of mildly anaerobic exercise on a bicycle ergometer resulted in an increase of lactate up to 4.0 mmol/l. This was accompanied by an increase of insulin from 18 to 180 μU/ml and a drop of glucose levels from 3.9 mmol/l to 2.3 mmol/l. The test was repeated with increased intensity of exercise. The patient was subsequently asked to exercise after 3 min of warm-up for 1 min at maximal intensity (400W). This resulted in a massive increase of lactate to 11.8 mmol/l. Insulin increased from 2.0 μU/ml to 585 μU/ml leading to severe hypoglycaemia, with glucose levels decreasing from 3.7 to 0.7 mmol/l. Conclusions: This seems to be a new unrelated case of EIHI. Up to now, EIHI has been reported in one patient from Germany and two families from Finland. Since this is the second case that was identified from our group we suspect that EIHI might be underdiagnosed due to the lack of awareness of this condition. We recommend anaerobic exercise testing in all individuals with documented hypoglycaemia related to physical exercise.

Poster Abstract #13

Inactivating Mutations in the ATP-Sensitive Potassium Channels Result in Increased Fractional ß-Cell Area

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Inactivating mutations in the ATP-sensitive potassium channel are the most common cause of congenital hyperinsulinism (KATPHI). In KATPHI, lack of functional KATP channels results in plasma membrane depolarization and dysregulated insulin secretion. Although increased rates of ß-cell proliferation were previously reported, it is not clear if increased ß-cell mass plays a role in the pathophysiology of KATPHI. Objective: To examine rates of proliferation and fractional area of ß- and α-cells in children with KATPHI. We hypothesize that in addition to the functional defect, increased ß-cell mass contributes to the phenotype of KATPHI. Methods: Pancreata obtained from partial pancreatectomy from 15 children with KATPHI were compared with pancreata from 5 children obtained during a splenectomy or a pancreatectomy after abdominal trauma. 5 μm sections were stained for insulin, glucagon, DAPI, and Ki67. Proliferation rates were determined by counting the number of cells co-stained for Ki67 and insulin (or glucagon) and related to the total number of insulin- (or glucagon) positive cells. Fractional area was calculated as the area of insulin (or glucagon)/total pancreas area. Results: β-cell replication rates were significantly increased in pancreata from children with KATPHI compared to controls (1.6 ± 0.9% vs. 0.2 ± 0.1%, $p = 0.003$). Accordingly, fractional ß-cell area was significantly increased in pancreata from children with KATPHI compared to controls (4.6 ± 2.2% vs. 2.2 ± 1.1%, $p = 0.03$). α-cell replication rates were also increased in children with KATPHI (1.1 ± 0.5% vs. 0.4 ± 0.4%, $p = 0.006$). Fractional α-cell area was increased in KATPHI pancreata but did not reach statistical significance (1.8 ± 1.5% vs. 0.9 ± 0.3%, $p = 0.2$). Conclusion: KATP channel defects result in increased replication rates of ß- and α- cells. Increased fractional β-cell area in addition to inappropriately increased insulin secretion may contribute to the pathophysiology of KATPHI.

Poster Abstract #14

Functional Evaluation of Islets from Infants with KATP Hyperinsulinism

Patel P, Li C, Givler S, Matschinsky F, Stanley CA, De León DD, Children's Hospital of Philadelphia

Loss-of-function mutations in the KATP channel are the most common cause of congenital hyperinsulinism (KATPHI). The clinical phenotype of KATPHI is characterized by severe fasting and protein-induced hypoglycemia. In contrast, glucose-stimulated insulin secretion is impaired. Islets from the KATPHI mouse model (SUR1−/−) have elevated cytosolic calcium and respond to amino acids but not glucose. Objective: To examine fuel responsiveness and cytosolic calcium changes in KATPHI human islets. We hypothesize KATPHI islets will have similar responses as SUR1−/− islets. Methods: Islets from 27 diffuse HI cases (age 5.9 ± 2.3 m) were compared to islets from cadaver donors. 21 HI cases have confirmed disease-causing mutations in ABCC8 ($n = 19$) or KCNJ11 ($n = 2$). 6 cases were negative for mutations in either gene. Islet fuel responsiveness and calcium influx were examined by perifusion and static incubations. Results: Basal insulin secretion in perifused KATPHI islets was greater than in controls (3.0 ± 0.5 vs. 0.4 ± 0.1 ng/150 islets/min, $p < 0.01$). Basal [Ca2+]i was higher compared to controls (1.68 ± 0.07 vs. 0.86 ± 0.03 −340/380 ratio, $p < 0.01$). Opposite to controls, [Ca2+]i and insulin secretion in KATPHI islets increased after stimulation with amino acids but not glucose. Exendin (9–39), a GLP-1 receptor antagonist, inhibited amino acids-stimulated insulin secretion in KATPHI islets. Islets from 3 cases with clinical phenotype of KATPHI but without confirmed mutations exhibited the same fuel responsiveness as islets from genetically confirmed KATPHI cases. Conclusions: KATPHI islets replicate islet phenotype of SUR1−/− mice and are congruent with the clinical phenotype. The stimulatory effect of amino acids in KATPHI islets may be mediated by the GLP-1 receptor. Overall, clinical and islet phenotype may help elucidate the pathophysiology in cases without confirmed mutations. Further studies may reveal specific genotype-phenotype correlations.

Poster Abstract #15

Medical Treatment of Congenital Hyperinsulinism: Possible Side Effects and Different Response to Treatment Depending on the Underlying Disease Causing Mutation

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Congenital hyperinsulinism (CHI) is a heterogeneous genetic disorder leading to an unregulated secretion of insulin from pancreatic beta cells. Although much is known about the mechanism and course of disease, medical treatment still remains challenging and keenly depends on the clinicians experience with CHI. Only few is known about the used medications. Especially in the diffuse type of CHI medical treatment is of specific

interest to avoid the risks of a subtotal pancreatectomy. Objectives: We were interested in dosage, efficacy and side effects of the used medications. In addition, we evaluated the underlying disease causing mutations such as mutations in the ABCC8, KCNJ11, GLUD1, GCK, HADH, HNF4A and UCP2 gene in view of the clinical phenotype of CHI and the response to conservative treatment. Methods: We searched MEDLINE (from 1947) and EMBASE (from 1988) using the OVID interface for relevant data. The last search was run in March 2011. Results: More than 1000 patients were evaluated of which 600 patients received long-term conservative treatment. Diazoxide constantly led to mild hypertrichosis, fluid retention and dyspeptic syndrome. Severe side effects such as encephalopathy and heart failure occurred in particular cases. Typical side effects of Octreotide include tachyphylaxis as well as abdominal discomfort and poor appetite, whereas gallstones, necrotizing enterocolitis and an impairment of growth velocity were less frequent. No side effects were observed during the rarely used therapy with calcium channel blocking agents. However, half of the patients treated with these agents required further medication. Clinical phenotype and response to medical treatment varied with regard to the underlying disease causing mutation. Conclusions: This review provides a basis to reconsider diagnostic and treatment strategies in children with congenital hyperinsulinism. Our data emphasizes the need for prospective studies on drug treatment in CHI.

Poster Abstract #16

Detection and Quantification of Mosaic Isodisomy in Beckwith-Wiedemann Patients Using SNP Arrays

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Historically, cytogenetic abnormalities, such as inversions and duplications involving 11p, have been reported in a small percentage (1–2%) of patients with Beckwith-Wiedemann syndrome (BWS). The majority of BWS patients present with methylation abnormalities, detectable by molecular methods. Objectives: With the advent of SNP array analysis in the cytogenetics laboratory, we have been able to expand our detection of pathogenic findings in BWS patients to include paternal uniparental isodisomy. Methods: To date, we have identified mosaic loss of heterozygosity (LOH) of 11p in 18 patients referred for BWS, hemihypertrophy, hypoglycemia, and/or congenital hyperinsulism. Mosaic LOH was detected by identification of abnormal genotype frequencies in combination with normal probe intensities, and was quantified using a mathematical model developed in our laboratory. Results: Our testing was more sensitive than methylation testing, as SNP array analysis allowed for quantification of the level of mosaicism to less than 5% UPD. In addition, the extent of the LOH along the chromosome was determined for each patient. Fourteen patients presented with LOH involving only 11p and one patient presented with whole chromosome LOH. Surprisingly, three patients were found to have mosaic genome-wide LOH and a 46,XX karyotype, indicating a subset of cells with complete uniparental paternal isodisomy. Conclusions: The use of SNP array technology in the cytogenetics laboratory has enormously enhanced our ability to detect isodisomy associated with BWS. This technology is useful for finding low levels of mosaicism and in determining parent-of-origin. The high resolution allows for identification of breakpoints and precise gene involvement in the isodisomy. In summary, the robustness of the genome-wide SNP assay provides the ability to diagnose unexpected etiologies and characterize uniparental disomy in BWS.

Poster Abstract #17

Mitochondrial Uncoupling Protein 2 (UCP2) Mutations in CHOP Hyperinsulinism Patients

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Among CHOP HI patients that are diazoxide-responsive, 53% have no mutations in the common HI loci (ABCC8, KCNJ11, GCK, GLUD1, SCHAD, HNF4A). This suggests that there are other disease-associated loci. A recent report by Ricquier et al., of inactivating UCP2 mutations in 2 of 10 HI patients responding to diazoxide indicates that this could be a candidate gene for screening. Objective: To screen a group of diazoxide-responsive patients who are negative for mutations in the common HI loci. Method: We screened 60 diazoxide-responsive patients for exonic mutations in UCP2 by direct sequencing. Results: Sequence analysis revealed two patients with UCP2 mutations. Patient 1 had an Ala268Gly paternal mutation previously reported by Ricquier et al., in 2008 as a disease-associated mutation that resulted in impaired activity of UCP2. Patient 2 had a Gly61Ser paternal mutation with no family history of hypoglycemia or diabetes. The Gly61Ser novel change was predicted to be damaging by SIFT and Polyphen. Both patients were successfully treated with diazoxide. HI resolved in Patient 1 at 6 years and in Patient 2 at 7 months. Conclusion: Haploinsufficiency for UCP2 is predicted to upregulate glucose stimulated

insulin secretion (GSIS) by increasing ATP yield. Our findings confirm that mutations in UCP2 may be a rare cause of diazoxide responsive HI. UCP2 HI appears to behave as a transient disorder that resolves in early childhood.

Poster Abstract #18

Pancreatic Islet Architecture in a Model of Neonatal Diabetes, Akita *INS2* (C96Y) Mouse

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Dominant mutations of the insulin gene in humans have been identified as a cause of permanent neonatal diabetes. A mouse model of this disease, the ''Akita'' mouse, contains a mutated form of the proinsulin 2 gene, which produces a substitution C96Y in the insulin sequence and disrupts an essential disulfide bond formation, leading to proinsulin misfolding and subsequent diabetes.

By double immunofluorescence staining with confocal imaging of mouse pancreatic sections and studies of purified islets of Langerhans in culture, we have defined the progression of insulin biosynthesis and early secretion defects in Akita mouse development, leading to diabetes. Our studies indicate a statistically significant decline and heterogeneity of insulin producing cells in Akita mouse islets. Functionally relevant insulin granule formation was affected in Akita cells as shown by decreased association with granule protein Rab3A. Mitochondrial structure and function in beta cells was disrupted at early stages of the disease. Additional statistically significant changes associated with Akita islet development, compared to age-matched littermate controls, included increased content of glucagon – producing alpha-cells and their altered intra-islet distribution. Results indicate that in Akita mice, generalized defects in ß-cell physiology stemming from mutant insulin expression include decreased insulin biosynthesis, reduced granule formation and altered islet cyto-architecture.

Poster Abstract #19

Continuous, Subcutaneous IGF1 Therapy via Insulin Pump in a Patient with Donohue Syndrome

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Donohue syndrome is the most severe end of a spectrum of syndromes of congenital insulin receptor (InsR) dysfunction, with a very limited life expectancy. Given the similarities in insulin and IGF1 receptors and signaling, IGF1 has been used to treat InsR mutation syndromes, and postulated to preserve β cell function. However, the shortened IGF1 half life due to low IGF-BP3 levels in these patients can be problematic. Clinical Case: Patient was IUGR at birth, weighing 1.6 kg, with dysmorphic facies, decreased SC fat, polycystic ovaries, hyperglycemia, and insulin of 4925 uU/mL. InsR sequencing revealed an insertion in exon 2 causing an immediate stop codon (Y87X). Management goals were glycemic control and weight gain. Pt did not require treatment until 15 mos, when she had diabetic ketosis. High-dose insulin suppressed ketones, but did not correct hyperglycemia. Metformin 30 mg/kg/day was initiated. At 19 mos, HbA1c peaked at 9.5%. IGF1 SC BID was started at 80 mcg/kg/day, and increased for hyperglycemia to 400 mcg/kg/day. HbA1c improved over 6 mos to 7.7%. When BID dosing was no longer effective, continuous IGF1 via insulin pump was started at 31 mos. IGF1 dose was gradually increased to 1200 mcg/kg/day for hyperglycemia. IGF1 was maintained at 40–50 degrees F. HbA1c decreased from 9.8 to 8.8% over 3 mos. Pre- and 1-hr post-feed c-peptide and insulin were measured to assess insulin resistance (IR) (fasting level) and β cell function (Δ change with feed). Prior to IGF1: fasting c-peptide (CPEP) = 37 ng/ml, Δ = 69; fasting insulin (INS) = 1953 uU/ml, Δ = 1172. On BID IGF1: After 7.5 mos CPEP = 44ng/ml, Δ = 83, INS = 1238 uU/ml, Δ = 2732; After 12 mos CPEP = 25.8 ng/ml, Δ = 58.9; INS = 3382 uU/ml, Δ = 1677. After 1 mos on IGF1 pump: CPEP = 26.5 ng/ml, Δ = 33.9. Enlargement of cystic ovaries accelerated from 30 to 35 mos, when pt had a 9.5 × 15 × 11.4 cm cystic abdominal mass, causing respiratory distress. Pathology showed juvenile granulosa β cell tumor. Conclusion: This is the first report of continuous SC IGF1 therapy via insulin pump in a pt with congenital InsR mutation, which may be advantageous given the decreased IGF1 half life. Glycemic control appeared improved, but effects on IR and cell function are difficult to interpret. It is unclear whether ovarian growth and tumor were due to high-dose IGF1, or to the natural history of disease given pt's prolonged survival. Continuous IGF1 to treat patients with InsR mutations has the potential for benefit and warrants further study.