

Mahvash Disease: Pancreatic Neuroendocrine Tumor Syndrome Caused by Inactivating Glucagon Receptor Mutation

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Abstract

Mahvash disease is a novel pancreatic neuroendocrine tumor syndrome caused by inactivating glucagon receptor mutations. Its discovery was triggered by comparison of a patient with pancreatic neuroendocrine tumors, pancreatic α cell hyperplasia, extreme hyperglucagonemia but without glucagonoma syndrome, and occasional hypoglycemia, with the glucagon receptor knockout mice which exhibit similar phenotype and ultimately prove to be a model of Mahvash disease. So far 6 cases have been reported. The inheritance, prevalence, pathogenesis, natural history, diagnosis, treatment, and long-term follow-up of Mahvash disease are discussed in this article. Although rare, Mahvash disease provides important insights into the pathogenesis of pancreatic neuroendocrine tumors, pancreatic α cell fate regulation by glucagon signaling, and safety of glucagon signaling inhibition for diabetes treatment.

Keywords: Mahvash disease; Pancreatic neuroendocrine tumor; Pancreatic α cell hyperplasia; Hyperglucagonemia; Glucagon receptor; Mutation

Introduction

Human tumor syndromes are usually caused by inherited or *de novo* mutations in tumor suppressor genes or oncogenes within the cells that give rise to the tumors [1,2]. In some occasions, the cells that give rise to tumors do not have intrinsic abnormalities in cell differentiation or proliferation but are forced into a hyperplastic state secondarily by neural or humoral factors. The latter phenomenon is especially relevant to certain endocrine tumors that arise in a hyperplastic background. Thyrotroph adenoma of the pituitary can arise from hyperplastic thyrotrophs in profound primary hypothyroidism; gastric carcinoids can arise from hyperplastic enterochromaffin-like cells in chronic atrophic gastritis or gastrinoma; and parathyroid adenoma can arise from hyperplastic parathyroid chief cells in chronic renal failure [3-5]. We here review a novel pancreatic neuroendocrine tumor syndrome arising in a background of pancreatic α cell hyperplasia caused by inactivating glucagon receptor mutation.

History of Discovery

One of us (R.Y.) first saw the index case of what we would eventually call Mahvash disease in 2007 [6]. Four years before the visit, the then 60-year-old female patient had presented with abdominal pain but without skin rash, hyperglycemia, weight loss, thromboembolism, or stomatitis, components of the glucagonoma syndrome which is an amino acid deficiency syndrome caused by high glucagon levels associated with glucagonoma [7]. She indeed sometimes had mild hypoglycemia. Her past medical history included gastroesophageal reflux, duodenal ulcer, diverticulosis, anemia, β thalassemia minor, anxiety, depression, and meningioma. She had no family history of endocrine tumors. Physical examination result was unremarkable. Abdominal CT identified a hypertrophic pancreas and a 3-cm mass in the uncinate process. Biopsy of the mass through endoscopic ultrasound revealed cytology consistent with neuroendocrine tumor. Screening hormone testing demonstrated extremely elevated glucagon levels of 59,284 pg/ml (normal <150) and mildly elevated pancreatic polypeptide of 735 pg/ml (normal <311). Levels of insulin, gastrin, vasoactive intestinal peptide, and calcitonin were all normal. There was no clinical, laboratory, or imaging evidence for hyperparathyroidism or pituitary

tumor. She was tentatively diagnosed with probable glucagonoma and underwent pylorus-sparing pancreaticoduodenectomy to remove this tumor. Glucagon levels, however, remained elevated postoperatively [6].

The histological examination of the resected surgical specimen revealed unexpected results. The gross tumor turned out to be a non-functioning neuroendocrine tumor that was negative for glucagon, insulin, somatostatin, or pancreatic polypeptide [6]. Most remarkably, in the tumor margin composed of apparently normal pancreas, there were numerous hyperplastic islets (Figure 1). Most of the islet cells were positive for glucagon but negative for insulin. Glucagon-positive cells were readily detected in the walls of pancreatic ducts, suggesting α cell neogenesis (Figure 1). Two microadenomas (1.6- and 3.0-mm) were also identified [6].

The patient's pancreatic neuroendocrine tumors (PNETs), pancreatic α cell hyperplasia, and extreme hyperglucagonemia without glucagonoma syndrome constitute a unique syndrome which had not been reported by 2007 to our knowledge after extensive and exhaustive search of clinical literature. We published the clinical and pathological observations on this case in early 2008 [6]. Due to the unique features of this case and our research interests in PNET pathogenesis, we continued to search the literature on PNETs, pancreatic α cell hyperplasia, and hyperglucagonemia and we subsequently expanded the search to the literature on animals. In the middle of 2008, we stumbled upon 2 papers on glucagon receptor knockout (*Gcgr*^{-/-}) mice published in 2002 and 2003, respectively [8,9]. Both papers demonstrate extreme hyperglucagonemia in the *Gcgr*^{-/-} mice and much higher glucagon

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content in the *Gcgr*^{-/-} pancreas; one of the papers further demonstrates extensive pancreatic α cell hyperplasia [9]. Because the phenotype of the *Gcgr*^{-/-} mice closely resembles the clinical and pathological features of our patient, we hypothesized that our patient may harbor glucagon receptor mutations. We sequenced the 13 coding exons and intron-exon borders of the glucagon receptor gene (*GCGR*) and identified a homozygous, missense C-to-T mutation in exon 4, resulting in replacement of proline with serine at amino acid residue 86 in the *GCGR* protein. We published the details of the P86S mutation in the index patient in 2009 [10].

We characterized the P86S mutant *GCGR* extensively and firmly established that the P86S mutant is much less active than the wild type *GCGR* [10,11]. The P86S mutant is primarily localized in the endoplasmic reticulum while the wild type *GCGR* is predominantly localized on the plasma membrane, indicating abnormal trafficking of the P86S mutant. Cells expressing the P86S mutant bind much less radiolabeled glucagon (~4%) than the wild type *GCGR* and make much less cyclic adenosine monophosphate (cAMP) after stimulation by physiological concentrations of glucagon (~15%). Since the index patient's condition has novel etiology (inactivating *GCGR* mutation), novel pathology (PNETs and pancreatic α cell hyperplasia), and novel clinical presentation (pancreatic mass and extreme hyperglucagonemia without glucagonoma syndrome), we named the condition "Mahvash disease" after the index patient's first name per her wish [12]. Mahvash is a common Persian name and does not divulge identifying information of the index patient.

We have since continued to search the literature and identified 2 additional cases of Mahvash disease in the literature based on clinical diagnosis [13,14]. Recently, another group identified 3 patients with Mahvash disease based on pathology and *GCGR* mutation [15,16]. Thus 6 patients to date have been identified as having Mahvash disease

(Table 1). As all 6 patients do not have parathyroid or pituitary tumor or family history of multiple endocrine neoplasia syndrome type 1 (MEN1), MEN1 is excluded clinically from the patients [6,13-15]. MEN1 mutations are tested in 3 of the 6 patients and no mutations are found [15]; however, MEN1 mutations have not been tested in the index patient.

Animal Model

The remarkable similarities between the patients with Mahvash disease and the *Gcgr*^{-/-} mice make the *Gcgr*^{-/-} mice a compelling model for Mahvash disease. We have characterized the *Gcgr*^{-/-} mice in 3 publications [17-19]. The wild type and heterozygous mice do not exhibit any noticeable differences except for slightly elevated glucagon levels in the latter [17]. In contrast to their wild type and heterozygous littermates, the *Gcgr*^{-/-} mice do not gain weight after 3 months old, and exhibit progressive hypoglycemia [17,18]. Endocrine and exocrine hyperplasia is evident in adult *Gcgr*^{-/-} mice [17-19]. The endocrine hyperplasia is predominantly caused by α cell neogenesis but the exocrine hyperplasia is mostly due to increased acinar cell proliferation [17,19]. Glucagonomas and other PNETs continuously develop in a background of α cell hyperplasia [17,18]. Exocrine tumors are not detectable even in very old *Gcgr*^{-/-} mice despite exocrine hyperplasia [19].

Natural History

As Mahvash disease has only been recognized for 5 years and only 6 patients are known so far, its natural history is not entirely understood. Based on available data from case reports, patients with Mahvash disease develop gross PNETs in their middle age [6,13-16]. They exhibit severe hyperglucagonemia before and after tumor resection. After tumor resection, recurrence is not evident in 2-9 years. The multiple microadenomas in the resected pancreas highly suggest

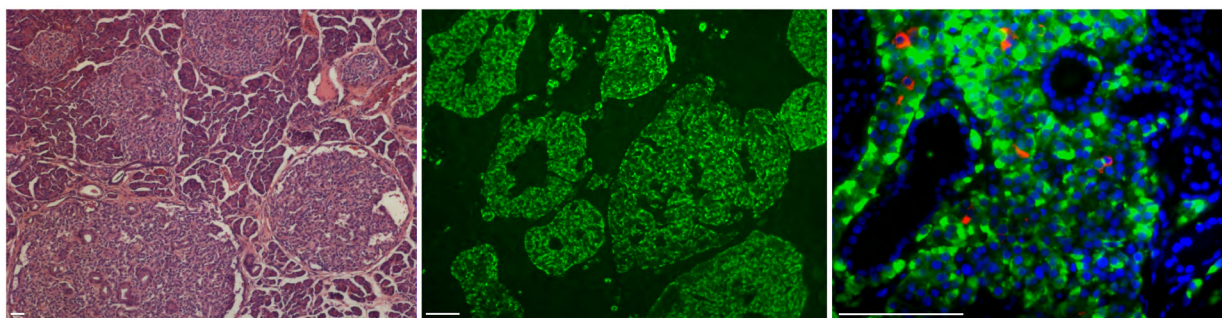


Figure 1: Pancreas histology and hormone expression in a patient with Mahvash disease. (Left) Low-power view of pancreas stained with hematoxylin and eosin. (Middle) Intermediate-power view of pancreas immunostained with glucagon antibody (green). (Right) High-power view of pancreas double stained with antibodies to glucagon (green) and to insulin (red), and counterstained with nuclear dye (blue). Bar, 100 μ m.

	Index patient [6,10]	Brown et al. [13]	Azemoto et al. [14]	Henopp et al. [15,16]
Age	60	48	59	25-44
Sex	Female	Male	Male	Not described
Ethnicity	Persian	Not described	Asian	Not described
Presentation	Abdominal pain	Diabetes	Non-specific	Non-specific
Glucagon (pg/mL), preoperative	59,284	4,200	54,405	Not described
Glucagon (pg/mL), postoperative	5,000-10,000	5,700	11,322	25-fold
Gross PNET	Non-functioning	Glucagonoma	Glucagonoma	Glucagonoma
Follow-up (months)	108	26	24	Up to 48
Recurrence of gross PNET	No	No	No	No

Table 1: Summary of 6 cases of Mahvash disease. PNET, pancreatic neuroendocrine tumor.

similar adenomas are also present in the remnant pancreas so that lifetime recurrence risk appears high.

The natural history of Mahvash disease is more completely characterized in the *Gcgr*^{-/-} mice, the murine model of Mahvash disease. At birth, the *Gcgr*^{-/-} pancreas is similar in gross appearance and islet morphology [9]. By 2-3 months, the *Gcgr*^{-/-} pancreas develops a cell hyperplasia without obvious dysplasia [17]. By 5-7 months, these mice have evident dysplastic islet growth. By 12 months, microscopic PNETs are found in all the *Gcgr*^{-/-} pancreata, and gross tumor are found in the majority. In older animals, the PNETs can grow very large and constitute 15% of body weight [18]. The PNETs in the *Gcgr*^{-/-} mice are well-differentiated and occasionally metastasize to the liver [17,18]. Meanwhile, the islet morphology and composition remain normal and no PNETs are found in any WT or heterozygous pancreata. The gross characteristics of the *Gcgr*^{-/-} mice are also subject to certain abnormalities. At 12 months, the average weight of the *Gcgr*^{-/-} mice is 40% lower than that of the WT and heterozygous mice, the average pancreas size is around 2.5-fold larger in the *Gcgr*^{-/-} mice than in the control animals, and random glucose levels are lower in the *Gcgr*^{-/-} mice over the entire course of 24 months [17,18]. Most importantly, the survival of the *Gcgr*^{-/-} mice is significantly reduced. The Kaplan-Meier survival probability of the *Gcgr*^{-/-} mice at 18 months is only 17% while that of the wild type or heterozygous at the same age is about 80% (18). Other abnormalities of the *Gcgr*^{-/-} mice include decreased fetal survival and vision loss [20,21]. Although the pancreas pathology of the *Gcgr*^{-/-} mice is identical to that of humans with Mahvash disease, whether the abnormal overall survival, fetal survival, and vision are present in humans with Mahvash disease remains to be tested.

Mode of Inheritance

Mahvash disease is very likely an autosomal recessive inherited disease. The index patient is homozygous for P86S mutation but her brother has two wild type *GCGR* alleles, strongly suggesting that their parents are heterozygous for P86S mutation [6,10]. Their parents' consanguinity (second cousins) also supports the assumption that the parents each harbor the same heterozygous *GCGR* mutation. Observation of the *Gcgr*^{-/-} mice further supports the autosomal recessive mode of inheritance of Mahvash disease, as discussed earlier [17-19].

Prevalence

As there have been only 6 cases of Mahvash disease reported so far, the prevalence of Mahvash disease can only be inferred from the estimated frequency of deleterious *GCGR* polymorphisms. Data from dbSNP and 1000 Genomes revealed 2 single nucleotide polymorphisms (SNPs) in the 5' untranslated region, 72 SNPs in introns which have unknown significance, 10 synonymous SNPs in exons, and 12 non-synonymous SNPs in exons [22]. Of the 12, 4 (Y84D, F303C, R111P, and V229L) are likely deleterious but their frequency is unknown. Two other potentially inactivating exonal SNPs (T76M and G442V) have a known frequency of 0.1%. Thus the frequency of inactive *GCGR* mutation may be ~0.2%. Therefore, the prevalence of Mahvash disease is perhaps ~4 per million and that of the heterozygous carrier state ~0.4%. The *GCGR* genotype of the index patient (P86S) may be very rare. Since there have been only 6 cases of Mahvash disease identified so far, the estimated prevalence may overestimate the true prevalence.

Etiology

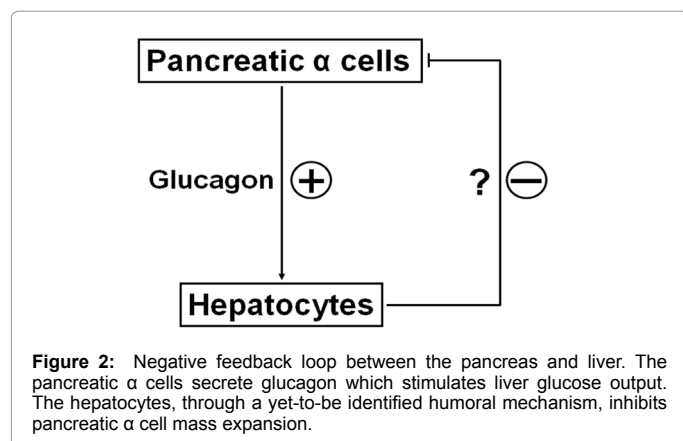
The identification of Mahvash disease in the index patient was through a candidate-gene approach [10]. The intrinsic weakness of

the candidate-gene approach is that it cannot reliably establish *a priori* a causal relationship between the mutations identified and the phenotype observed. One possibility is that the *GCGR* mutations and the phenotype are merely coincidental. The subsequent study of the *Gcgr*^{-/-} mice solidly establishes that inactivating *GCGR* mutations indeed cause Mahvash disease. As the PNETs and pancreatic α cell hyperplasia are only present in the *Gcgr*^{-/-} mice whereas otherwise genetically identical wild type and heterozygous littermates are entirely normal, the absence of functional *Gcgr* must be responsible for the observed pancreatic abnormalities [17-19].

Pathogenesis

GCGR is a G protein-coupled receptor found principally in the liver and kidneys [23]. Upon glucagon binding, *GCGR* activates the stimulatory G protein and triggers cAMP production, leading to upregulated glycogenolysis and gluconeogenesis and ultimately increasing hepatic and renal glucose output [24]. How does *GCGR* inactivation lead to PNETs? The human pancreas histology and the progression from hyperplasia to PNETs in *Gcgr*^{-/-} mice over time both indicate a multi-step pathogenesis of the PNETs in Mahvash disease [6,13-16,17-19]. Based on the temporal sequence of hyperplasia, dysplasia, and PNETs in the *Gcgr*^{-/-} mice and the pathogenesis of sporadic PNETs and PNETs in (MEN1) [17-19,25-27], we posit that the pancreatic α cell hyperplasia is a direct result of *GCGR* inactivation while PNET tumorigenesis from the hyperplastic background may be secondary and due to additional, stochastic mutations.

Unlike the hyperplastic islet cells in MEN1 which harbor heterozygous loss of *menin* in the islet cells [26,27], the hyperplastic α cells in Mahvash disease may not have intrinsic abnormalities based on the following lines of arguments although direct genetic evidence is still lacking. Reconstitution of liver *Gcgr* in *Gcgr*^{-/-} mice quickly decreases glucagon levels, suggesting the hyperplastic α cells are still under liver control [28]. Furthermore, similar α cell hyperplasia in prohormone convertase 2 knockout mice (with diminished glucagon production) is reversible upon glucagon infusion [29]. More likely, extrinsic factors are responsible for the α cell hyperplasia in Mahvash disease. From an endocrine point of view, the pancreatic α cell hyperplasia after *GCGR* inactivation is readily comprehensible because the hyperplasia of endocrine cells is often observed when the cognate hormone target organ is hypofunctioning. For example, thyrotroph hyperplasia is well known to occur when the thyrotropin target organ, the thyroid, is hypofunctioning (hypothyroidism) [3]. The main target organ of glucagon is the liver which has the highest concentration of *GCGR* [24]. Liver-specific deletion of *Gcgr* or *Gsa* produces similar pancreatic α cell hyperplasia as global *Gcgr* deletion does, confirming that hypofunctioning liver leads to a cell hyperplasia [30,31]. How the hypofunctioning liver sends the signal to promote a cell hyperplasia is being actively studied by other researchers. They have shown that the wild type α cells transplanted under the renal capsule in *Gcgr*^{-/-} and *Gcgr*^{hep-/-} (liver-specific *Gcgr* knockout) mice exhibit the same α cell hyperplasia, suggesting the existence of one or more humoral factors capable of promoting α cell hyperplasia in those mice [31]. Whether the hypofunctioning liver releases a stimulatory or down regulates an inhibitory humoral factor is not clear. It is evident now that there is a pancreas-liver negative feedback loop regulating pancreatic α cell mass and liver glucose output (Figure 2). The nature of the additional, stochastic mutations that switch the hyperplasia-to-neoplasia transition remains to be investigated. There have been no experimental studies addressing the transition besides the abnormal cytoplasmic *menin* localization in the PNETs in *Gcgr*^{-/-} mice [17]. We hypothesize



that there may be recurring mutations in the individual PNETs as those in sporadic human PNETs [25]. A candidate-gene or exomic approach can be used to identify those mutations.

Clinical Presentation

Despite a limited number of cases, clinical hallmarks of Mahvash disease are easily identified, owing both to consistency among the patients in those cases, and the complete characterization of the $Gcgr^{-/-}$ mice as a model for the human disease [6,9,13-16,17-21]. Patients without previous genetic testing present as non-functioning PNET. The initial symptoms are usually abdominal pain and discomfort. The murine model suggests visual loss as another clinical presentation, but visual loss has not been clearly encountered in humans. Patients with Mahvash disease do not exhibit glucagonoma syndrome because their glucagon signaling is defective due to the inactivating $GCGR$ mutations even though they have very high glucagon levels. Patients whose Mahvash disease is diagnosed by genetic screening may be asymptomatic but exhibit very high glucagon levels.

Diagnosis

The common distinct clinical features of Mahvash disease make the diagnosis straightforward [6,10,13-16]. Mahvash disease should be in the differential diagnosis of PNETs, hyperglucagonemia, pancreatic hypertrophy, and possibly hypoglycemia. PNETs and remarkable hyperglucagonemia without glucagonoma syndrome are the clinical hallmarks of Mahvash disease. Pancreas histology or $GCGR$ sequencing confirms the diagnosis. Biopsy-proven PNETs in a hypertrophic pancreas and remarkable hyperglucagonemia without glucagonoma syndrome establish the diagnosis of Mahvash disease without the need of surgical histology or $GCGR$ sequencing.

Based on the autosomal recessive mode of inheritance (discussed earlier), siblings of patients with Mahvash disease have a 25% probability of having Mahvash disease and they should be screened by glucagon measurement. The children of patients with Mahvash have a very small probability of having Mahvash disease. We suggest measuring glucagon levels once for each child at young adulthood to confirm their heterozygous state.

Treatment

Due to the small number of patients identified so far, the optimal treatment of Mahvash disease is not clear. We provide some thoughts here but emphasize that they are mostly theoretical. As the gross PNETs in Mahvash disease are either glucagonomas in patients

without functional GCGR or true non-functioning tumors, they behave clinically as non-functioning tumors (6, 10, 13-16). The management strategy of non-functioning PNETs in MEN1 provides some guidance to the management of gross PNETs in Mahvash disease [32,33]. Tumors smaller than 2-3 cm may be monitored and tumors larger than 3 cm should be surgically resected. Although somatostatin analogs decrease glucagon levels, the clinical utility of somatostatin analogs in patients without gross tumors is not clear [6,34]. Pharmacological chaperones have been shown *in vitro* to be able to traffick endoplasmic reticulum-stranded mutant GCGR to the plasma membrane and restore GCGR functionality, but clinically feasible chaperones are still in development [35].

Follow-up

Follow-up of patients with Mahvash disease can be inferred from that of those with other endocrine tumor syndromes such as MEN1 [32]. Patients should undergo regular (every 2-3 years?), lifelong imaging surveillance in order to detect any recurrent tumors at an early stage. It may be prudent to measure glucagon levels yearly.

Implications and Significance

Although rare, Mahvash disease, by virtue of being the result of an experiment by nature, suggests important insights into the pathogenesis of PNETs, pancreatic α cell fate regulation by glucagon signaling, and safety of therapies inhibiting glucagon signaling for diabetes treatment. The pancreatic endocrine cells in patients with other genetic syndromes such as MEN1 and von Hippel-Lindau disease (VHL) are intrinsically abnormal and proliferate more than those in normal subjects [36,37]. In contrast, the pancreatic α cells in patients with Mahvash disease probably are intrinsically normal (without direct genetic evidence) and do not necessarily proliferate more; the α cell hyperplasia is the result of hormonally-driven α cell neogenesis rather than proliferation [17]. Therefore, stochastic secondary mutations in the hyperplastic α cells may initiate tumorigenesis.

Mahvash disease highlights a strong and conserved mechanism to regulate glucagon signaling in rodents and humans (Figure 2). As virtually any means of inhibiting glucagon signaling results in a cell hyperplasia in rodents and inactivating $GCGR$ mutations result in a cell hyperplasia in humans [6,10,15-18,30,31,38,39], it is reasonable to assume that other means of inhibiting glucagon signaling in humans would result in a cell hyperplasia that can potentially lead to PNETs. In fact, glucagon antagonism as a pharmacological therapy for diabetes in humans causes moderate hyperglucagonemia, suggesting underlying a cell hyperplasia [40-43]. The recent controversy over the safety of incretins raises the crucial question of whether partial inhibition of glucagon release by incretins also causes α cell hyperplasia and even glucagonomas [44,45].

Summary

Mahvash disease is a novel pancreatic neuroendocrine tumor syndrome caused by inactivating glucagon receptor mutations. The authenticity of Mahvash disease has been confirmed by 5 more cases, including 3 who are known to harbor glucagon receptor mutations. The understanding of clinical characteristics and pathogenesis of Mahvash disease is greatly enhanced by a murine model, the $Gcgr^{-/-}$ mice. Mahvash disease is one of the few autosomal recessive tumor syndromes and involves the development of pancreatic neuroendocrine tumors in middle age. Mahvash disease should be in the differential diagnosis of pancreatic neuroendocrine tumors and hyperglucagonemia. This rare

disease has already provided insights into more common scenarios such as pathogenesis of pancreatic neuroendocrine tumors and safety of diabetes treatments that target glucagon signaling. Furthermore, Mahvash disease highlights a conserved pancreas-liver negative feedback loop regulating glucagon signaling.

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