

**GLUCOSE HOMEOSTASIS AND GENOTYPE-PHENOTYPE INTERPLAY IN
CYSTIC FIBROSIS PATIENTS WITH GENE CFTR Δ F 508 MUTATION**

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Short running title: Gene CFTR Δ F 508 mutation in cystic fibrosis and glucose tolerance status

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Objective

To determine the clinical phenotype of adolescent/adult patients with cystic fibrosis (CF), according to heterozygosity or homozygosity for CFTR Δ F508 mutation, and to analyse their characteristics according to glucose tolerance status.

Design

76 CF patients with CFTR Δ F508 mutation (33 heterozygous, 43 homozygous) stratified according to normal (NGT, n=51) or abnormal glucose homeostasis (AGH; IFG-IGT or diabetes, n=25) had their HOMA β -cell function (B), insulin sensitivity (S), and [BxS] hyperbolic product measured. Pancreatic exocrine insufficiency was inferred from pancreatine requirements. Clinical effects of insulin therapy on weight and lung function were recorded.

Results

AGH was observed in 24 and 40% of heterozygous and homozygous subjects. AGH patients were older than NGT (29 \pm 10 vs. 23 \pm 8 years, p=0.006) and their B function was lower (93 \pm 49 vs. 125 \pm 51%, p=0.011). S values were comparable in NGT and AGH. A lower BxS product was observed in AGH, although non-significant when adjusted for error propagation. Pancreatic insufficiency was observed in 52 and 100% of hetero- and homozygous patients (p=0.001).

Conclusions

Prediabetes and diabetes represent frequent co-morbidities in CFTR gene Δ F 508 mutation in the homozygous or heterozygous states. Impairment of insulin secretion, as shown by HOMA, is an important determinant when compared with the magnitude of compensation from insulin sensitivity. Given the high prevalence of abnormal glucose tolerance, screening for (pre) diabetes is mandatory. Insulin supplementation in diabetic CFTR gene Δ F 508 subjects seems a rational therapy for consideration, although this does not preclude that therapy directed toward insulin resistance could also interact.

INTRODUCTION

Cystic fibrosis (CF) is one of the most common genetically-inherited autosomal recessive conditions (1-3). Mutations in the CF transmembrane regulator (CFTR) gene, located on the long arm of chromosome 7, have shown to impair fluid and electrolyte composition of secretions, in particular from the lung and the pancreas, leading to progressive obstruction and fibrosis of the organs (4). The CFTR gene $\Delta F 508$ mutation is most often involved, with identification rates up to 70% in CF Caucasian subjects (4,5).

Since the first description of CF, survival rates have markedly increased, due to optimised medical management (2,5,6). Such improvement underlies the subsequent rise in the prevalence of abnormal glucose homeostasis in later life, including a secondary form of diabetes mellitus (DM) which is becoming a major co-morbidity associated with CF (2,7,8). For some investigators, DM in CF is related to pancreatic exocrine-endocrine insufficiency, which itself correlates to CFTR mutations (9-12), but this link has not been confirmed by others (13-15). On the other hand, impaired insulin sensitivity has also been reported as an additional factor for developing DM (16).

In order to precisely estimate the relationship between genotype and phenotype in CF, we aimed at determining the clinical profile of young adult CF patients, according to heterozygosity or homozygosity for gene CFTR $\Delta F 508$ mutation. We also analysed their clinical phenotype (in particular insulin secretion and insulin sensitivity) according to glucose tolerance status defined as normal glucose tolerance [NGT] or abnormal glucose homeostasis [AGH], the latter representing either impaired fasting glycaemia [IFG] or

impaired glucose tolerance [IGT] or [DM], as recently defined (7,8,17).

RESEARCH DESIGN AND METHODS

We performed a retrospective study of all non-paediatric subjects (adolescents and adults, 14-42 years old), with identified CFTR gene $\Delta F 508$ mutation who attended UCL's Cystic Fibrosis Unit for regular follow-up over the course of 2005. 76 individuals were eligible for the study (age: 25 ± 9 years; mean \pm SD; male/female: 47/53%). The cohort included 33 patients who were heterozygous for the $\Delta F 508$ mutation while carrying various other corresponding alleles, collectively referred to as "other" ($\Delta F 508$ /other) and 43 homozygous individuals ($\Delta F 508$ /508). The reference population of non-mucoviscidotic subjects included consecutive diabetic outpatients with type 2 DM (n=450) or chronic alcoholic pancreatitis (n=35), followed in our centre over the study period by the same investigators (MH and MB) (*individual data not shown*).

Nutritional status was evaluated by body mass index (BMI; weight (kg)/height² (m)). The presence of clinically-significant overt pancreatic exocrine insufficiency was indirectly inferred from chronic requirements for oral pancreatine supplementation (on basis of daily prescribed intakes of pancreatic enzyme capsules). Lung status was assessed from the means of function tests, which included measurement of forced vital capacity (FVC) as well as forced expiratory volume over one second (FEV1).

The total group of heterozygous and homozygous individuals was subdivided according to glucose tolerance status. Glucose tolerance was estimated according to criteria for IFG, IGT and DM based on the reports of

the Expert Committee on the Diagnosis and Classification of Diabetes and of the Cystic Fibrosis Consensus Conference (7,8,17,18). As recommended by the Consensus Conference (8), DM was also sub-stratified according to the presence or absence of fasting hyperglycaemia. Due to the small number of patients with IFG and IGT (prediabetes), and the transient nature of these conditions towards full-grown diabetes, those patients were combined with DM patients for modelling analysis, the group being thereafter referred to as abnormal glucose homeostasis (AGH).

β -cell function and insulin sensitivity were assessed by the Homeostasis Model Assessment (HOMA) in patients with and without AGH at a time when patients were not on active steroid therapy (18,19). In normoglycemic subjects, HOMA was calculated from fasting glucose and insulin levels obtained at the last outpatient clinic before data collecting. HOMA testing in AGH subjects was performed an average of 3 years earlier than in NGT patients, since it usually corresponded to the fasting measurements that led to DM diagnosis, performed prior to implementation of lifelong glucose lowering therapy. Values of HOMA-B were plotted as a function of HOMA-S, defining a HOMA-product $[B(\%) \times S(\%)]$; (normal value: 100%). Such a product corresponds to the true underlying β -cell function adjusted for individual insulin sensitivity, and therefore underlies the need for successive therapies in any type of diabetes. Kahn *et al* previously reported that these two variables followed a hyperbolic relationship in individuals with NGT (20). Thus, if insulin secretory capacity decreases but HOMA-S also increases, according to this relationship, a NGT status should be maintained albeit with a $[B \times S]$ function exhibiting different geometry. AGH develops when one variable or the other does not compensate, the individual then departing

from the normal hyperbolic relationship (21). In order to evaluate the magnitude of impairment of glucose homeostasis in CF patients, the cohort's results were plotted against a series of HOMA products means calculated from two local reference populations with common types of diabetes (type 2 DM and DM secondary to chronic alcoholic pancreatitis).

BMI and lung function were assessed, before initiation and after a mean 4.5 years ($n=21$) of insulin therapy. For genotype analysis, genomic DNA was isolated from whole blood samples to investigate the presence of the CFTR ΔF 508 mutation by polymerase chain reaction using allele specific primers (INNOLIPA, Innogenetics, Gent, Belgium). Insulin and/or C-peptide concentrations were measured with conventional radioimmunoassay. Glycated haemoglobin (HbA_{1C}) was measured by ion-exchange high performance liquid chromatography. Islet-cell cytoplasmic antibodies, as well as anti-glutamic acid decarboxylase (GAD65) and anti-tyrosine phosphatase (IA2) were determined by radioimmunoassay (CISBIO, Diegem, Belgium) in eight diabetic patients.

Results are presented as means \pm 1 SD, medians or proportions. The significance of difference between groups was assessed by Student's two-tailed paired or unpaired t or Welch's tests (22) for parametric and non-parametric data distributions. Differences between respective proportions were evaluated using chi-squared test. Since uncertainty in both HOMA parameters, B and S, could propagate error in their hyperbolic product, each measured value and its standard error of estimate were used to obtain after error propagation a z expression and its standard error, that was used to compare groups. Differences in means or proportions were considered statistically significant at p values <0.05 .

RESULTS

1. Gene CFTR ΔF 508/other vs. ΔF 508/508 patients

Main clinical data are described in Table 1. Age at diagnosis of CF was lower in homozygous than in heterozygous subjects ($p=0.001$), while age, sex ratio and BMI were comparable.

As shown in Figure 1a, an abnormal glucose homeostasis was observed in 24% of ΔF 508/other patients ($n=8$) and in 40% of ΔF 508/508 group ($n=17$) ($p=0.24$). One heterozygous patient had IFG/IGT while 7 (21%) had DM. Seven percent of homozygous subjects ($n=3$) had IFG/IGT and 33% ($n=14$) had DM (NS). Five patients (71%) in the ΔF 508/other group had fasting hyperglycaemia when compared with 6 subjects (43%) in the ΔF 508/508 cohort (NS).

There was a trend for more impaired lung function, as reflected by absolute FEV1 ($p=0.084$) and higher colonisation rate with *Pseudomonas aeruginosa*, in ΔF 508/508 subjects ($p=0.074$). Pancreatic exocrine insufficiency was present in 52 and 100% of hetero- and homozygous individuals, respectively ($p=0.001$). Patients without oral pancreatine supplementation were older than those receiving daily substitution (29 ± 10 vs. 24 ± 8 years, $p < 0.05$). They were also older at the time of CF diagnosis than those receiving pancreatine (19 ± 12 vs. 3 ± 6 years, $p < 0.05$). BMI was 22 ± 4 and 21 ± 3 kg/m² in subjects without and with supplementation (NS).

2. Normal vs. abnormal glucose homeostasis patients

The clinical and biological characteristics of CF patients with NGT and AGH are described

in Table 1. The prevalence of gene CFTR ΔF 508/508 mutation was 51% in NGT and 68% in AGH subjects respectively ($p=0.20$; Fig 1b). Patients with NGT were younger than those with AGH (23 ± 8 vs. 29 ± 10 years, $p=0.006$). Age at CF diagnosis as well as sex ratio and BMI were comparable in subjects with NGT or AGH.

AGH was diagnosed at a mean age of 23 ± 8 years. The diagnosis of IFG/IGT was based upon impaired fasting glycemia in one subject and upon an abnormal OGTT in three subjects. DM was diagnosed as a result of fasting hyperglycaemia (>126 mg/dl) in five patients, of a random glycemia higher than 200 mg/dl with symptoms in five subjects, and of an abnormal OGTT in 10 individuals. One patient attended the Mucoviscidosis Unit after diagnosis of DM was made in another hospital.

We observed a lower HOMA-B in patients with AGH than in normoglycemic subjects (93 ± 49 vs. $125 \pm 51\%$, $p=0.011$). In contrast, HOMA-S was comparable in subjects with NGT and AGH. Adjusting for potential confounders such as age, sex and BMI did not affect HOMA values distribution between groups. As a result, the mean HOMA product was lower in hyperglycemic than in normoglycemic individuals (89 ± 50 vs. $116 \pm 40\%$, $p=0.013$ prior to adjustment for error propagation). Thus, AGH patients had a marked reduction in β -cell function explaining the bulk of hyperbolic loss, despite a small increase in insulin sensitivity (Figure 2). The difference between HOMA products did not however reach statistical difference once propagation of error in product terms was taken into account. Comparison between HOMA products obtained in other common types of diabetes showed on the other hand significant differences in hyperbolic values between all CF groups and T2DM or chronic pancreatitis patients, with the two latter

groups lying close to the 25% hyperbole, hence displaying a much more severe deficit in beta-cell function adjusted for insulin sensitivity (Figure 2).

HbA_{1C} at diagnosis in AGH subjects was $7.3 \pm 1.3\%$ (5.8 ± 0.4 and $7.0 \pm 1.2\%$ in patients with IFG/IGT and DM, respectively, $p < 0.01$). Eighty-two percent of patients with AGH were treated with insulin (0.52 ± 0.38 units/kg/day). Current HbA_{1C} levels were 5.5 ± 0.5 and $6.8 \pm 1.2\%$ in patients with normal and abnormal glucose homeostasis ($p < 0.0001$). Pancreatic exocrine insufficiency was present in 73% of patients with NGT and 92% of those with abnormal glucose metabolism, a difference close to statistical significance ($p = 0.098$).

There was a higher rate of (past) resorting to systemic use of steroid therapy in patients with AGH than in normoglycemic subjects (40 vs. 16%, $p = 0.04$), Liver cirrhosis was more frequent in patients with AGH (24 vs. 8%) but the difference did not reach the level of statistical significance ($p = 0.11$). There was a trend for FVC ($p = 0.059$) and FEV1 ($p = 0.071$) to be lower in patients with AGH than in individuals with NGT (Table 1). The higher prevalence of *Pseudomonas* colonisation in AGH patients was not significant.

After initiation of insulin therapy, there was a BMI increase of $1.8 \pm 2.0\%$ ($p = 0.03$). We observed a trend toward improvement in absolute FVC (0.3 ± 0.6 l, $p = 0.06$). A significant increment in predicted FVC was nevertheless observed ($5.6 \pm 9.1\%$, $p = 0.046$). As far as absolute and predicted FEV1 were concerned, the small increases assessed following insulin therapy (respectively, 0.1 ± 0.6 l.sec. and $1.5 \pm 4.4\%$ l.sec⁻¹) did not reach the level of significance.

DISCUSSION

Our data show that gene CFTR ΔF 508 homozygous subjects differ from heterozygous individuals, in particular in terms of age at CF diagnosis as well as regarding the presence of pancreatic exocrine deficiency, as also reported by Kerem *et al.* (5). As far as IFG, IGT or DM are concerned, overall data from homo- and heterozygous ΔF 508 subjects show prevalences of abnormal glucose homeostasis close to figures reported in previous studies (1,17,23,24). In this line, it is worth mentioning that Moran *et al* observed a 35% DM prevalence in a cohort of 105 individuals aged 20-29 years (8). As life expectancy is slowly increasing in CF subjects (e.a as a result of more rationale use of targeted antibiotics), it is not unexpected to witness an ever increasing prevalence of DM, a rather late-occurring complication of CF (6,7,23,25). In our study, mean age at DM diagnosis was 23 years, in keeping with previous reports (23,25).

Concurrent genetic factors could contribute to the development of CF-related diabetes, as also indicated by Derbel *et al* (26) who mentioned the role of SNP-19 polymorphism in the calpain-10 gene. Additional factors, such as genetic modifiers, could eventually modulate phenotypic expression of the disease (27). In our patients with ΔF 508/508 mutation who all had marked exocrine pancreatic insufficiency, only a mere 40% developed AGH. This is in line with previous data showing that the gene CFTR ΔF 508 genotype-phenotype association with DM was not very strong, although genotype is considered to be a potential predictor of pancreatic exocrine status (1, 5,9-11,13-15). In our subgroup of heterozygous individuals, in whom half had pancreatic insufficiency, the prevalence of AGH was 24%, a figure somewhat lower when compared with

homozygous subjects although the difference did not reach significance. This could be due to the relatively small size of the groups. However, our data are keeping with the report of Marshall *et al.* (24) and imply that even in individuals who carry one single copy of ΔF 508 and who could have milder CF disease, in particular pancreatic exocrine insufficiency, than homozygous patients, screening for abnormal glucose tolerance is mandatory, a point suggested in several other reports (7,23). It is likely though that increasing life expectancy will be associated with a higher prevalence of AGH in all subgroups of patients, the phenotype of older CF patients being only sketchily known so far.

The precise pathogenesis of IFG/IGT and DM in CF remains quite controversial. No difference in sex ratio, as reported by Mackie *et al.* (1) and Marshall *et al.* (24) but not by others (12), was observed between our CF patients with and without NGT. Moreover in the present study, in scope with other data (25), autoantibodies against B-cell were absent at the time of diagnosis of DM when measured. Cross sectional studies using hyperinsulinemic euglycemic glucose clamp have documented peripheral (28-33) or hepatic insulin resistance (34) which could contribute to abnormal glucose tolerance in patients with CF. As recently reported, elevation of TNF- α and impaired translocation of GLUT-4 were proposed as contributive mechanism(s) (16). By contrast, several studies also underscored the paramount importance of insulin deficiency (28,35,36,37). These observations are in keeping with morphological data published by Couce *et al.* (38).

In the present study, we observe in our patients with AGH a significant β -cell defect in the presence of rather normal insulin sensitivity indices, as compared with a common type 2 diabetes cohort. The

observed decrease in [BxS] product is not significant but is trending toward insufficient compensation for decreased insulin secretory capacity, as suggested by departure from the normal hyperbolic relationship illustrated on Figure 2. A larger cohort size and patient's parity in comparison groups on steroid therapy will be necessary to more accurately evaluate between insulin secretory and sensitivity compensation effects among CF patients with AGH.

HOMA testing was no longer performed subsequently to formal diagnosis of DM for obvious medical and ethical grounds in these otherwise frail patients. This could also have contributed to the lack of segregation between groups regarding hyperbolic product, since HOMA-B was likely to keep on decreasing with time in patients with AGH. In contrast and as predicted in NGT patients with CF, the [BxS] product was higher. It is however interesting to note that the [BxS] product in CF patients with AGH was not as altered as that observed in our type 2 DM cohort, nor as that of DM subjects with chronic pancreatitis, yet 80% of AGH patients had a formal diagnosis of DM. This difference in secretory function adjusted for individual insulin sensitivity could account for the relative mild clinical expression of AGH in CF (13). In keeping with this, only five of our subjects had diabetes-related clinical symptoms at diabetes diagnosis.

Our results are in scope with, and expand, previously published data trending toward a B-cell defect rather than classical insulin resistance in CF patients with IFG/IGT and diabetes (28,35-37). In this view, it appears rational, from a therapeutic prospect, to consider that most CF patients with AGH will at some time be considered eligible for insulin supplementation, especially since their lean body mass was usually low, in accordance with CF phenotype. In agreement with this

observation, most of our diabetic CF patients indeed received daily insulin injections, and may potentially have benefited to a certain degree from the anabolic and anti-catabolic effects of insulin on lean body mass.

In summary, impaired glucose metabolism, either IFG/IGT or diabetes, is a frequent comorbidity in young adults who carry the ΔF 508 mutation in the homozygous or heterozygous state. The interplay between genetic status and combined endocrine-exocrine pancreatic deficiencies seems to play the major role in the development of glycemic abnormalities. Impairment in insulin secretion is an important determinant of this condition, and as HOMA modelling suggests, considerably contributes to glycemic dysregulation when compared to the magnitude of compensation from insulin

resistance, as documented by HOMA-S. Given the high prevalence of abnormal glucose tolerance in CF ΔF 508 patients, regular screening for (pre)diabetes is mandatory. On a pathophysiological basis, insulin supplementation seems a rational therapy for consideration in ΔF 508 CF patients with diabetes, although this does not preclude that therapy directed toward a component of insulin resistance could also interact.

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TABLE 1. CLINICAL CHARACTERISTICS OF CYSTIC FIBROSIS (CF) PATIENTS WITH GENE CFTR Δ F 508 MUTATION *

	Heterozygous (Δ F 508/other)	Homozygous (Δ F 508/508)	P ₁	Normal glucose tolerance	Abnormal glucose homeostasis**	P ₂
N	33	43	-	51	25	-
Age (years)	27 \pm 9*	24 \pm 8	0.129	23 \pm 8	29 \pm 10	0.006
Sex ratio (M/F) (%)	42/58	51/49	0.600	41/59	60/40	0.194
Age at diagnosis of CF (%)	11 \pm 12	3 \pm 6	0.001	6 \pm 10	8 \pm 11	0.431
BMI (kg/m ²)	22 \pm 4	21 \pm 3	0.235	21 \pm 4	21 \pm 4	1.000
Exocrine pancreatic insufficiency (%)	52	100	0.001	73	92	0.098
HbA _{1c} (%)	-	-	-	5.5 \pm 0.5	7.3 \pm 1.3	0.001
HOMA-B (%)	-	-	-	125 \pm 51	93 \pm 49	0.011
HOMA-S (%)	-	-	-	123 \pm 105	137 \pm 117	0.601
Lung Function						
FVC (l)	3.8 \pm 1.1	3.5 \pm 1.0	0.219	3.6 \pm 1.1	3.5 \pm 1.2	0.719
FVC (%) ⁺	93 \pm 17	89 \pm 20	0.360	94 \pm 16	84 \pm 23	0.059
FEV1 (l/sec ⁻¹)	2.8 \pm 1.1	2.4 \pm 0.8	0.084	2.6 \pm 0.9	2.3 \pm 1.0	0.192
FEV1 (%) ⁺	79 \pm 23	71 \pm 24	0.147	78 \pm 20	66 \pm 29	0.071
Corticosteroids (%)	19	26	0.624	16	40	0.040
Liver cirrhosis (%)	5	19	0.207	8	24	0.110
<i>Pseudomonas aeruginosa</i> carriage (%)	30	53	0.074	37	56	0.193
Allergic bronchopulmonary aspergillosis (%)	15	14	0.883	14	16	0.791

* expressed as mean \pm 1SD or proportions

** either impaired fasting glycemia, impaired glucose tolerance or diabetes mellitus

+ expressed as percentage of predicted value

Student's t or Welch tests were used for parametric and non parametric data distributions and chi-squared for differences between proportions
P₁: heterozygous vs. homozygous patients; P₂: normal glucose tolerance vs. abnormal glucose homeostasis patients.

Figure 1

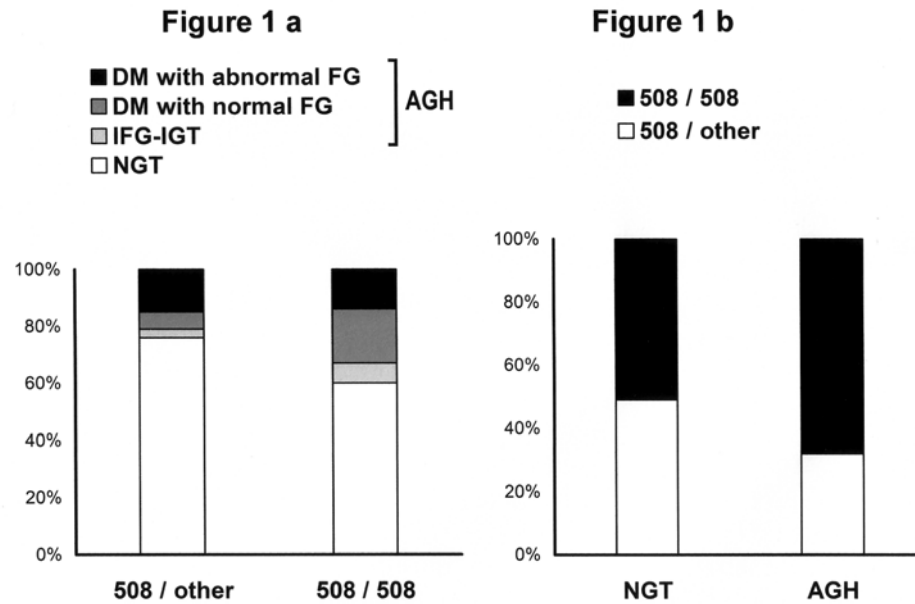


Figure 1a : prevalence of normal glucose tolerance (**NGT**), and of abnormal glucose homeostasis (**AGH**; i.e. impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) or diabetes (DIAB) with or without normal fasting glucose (FG)) in subjects with *Cystic Fibrosis Transmembrane Regulator* (CFTR) delta 508 mutation in the heterozygous (**508 / other**; n=33 subjects; *left of left panel*) or homozygous (**508 / 508**; n=43 subjects; *right of left panel*) state. **Figure 1b** : prevalence of *CFTR* delta 508 mutation in subjects with **NGT** (n=51; *left of right panel*) or **AGH** (n=25; *right of right panel*).

Figure 2

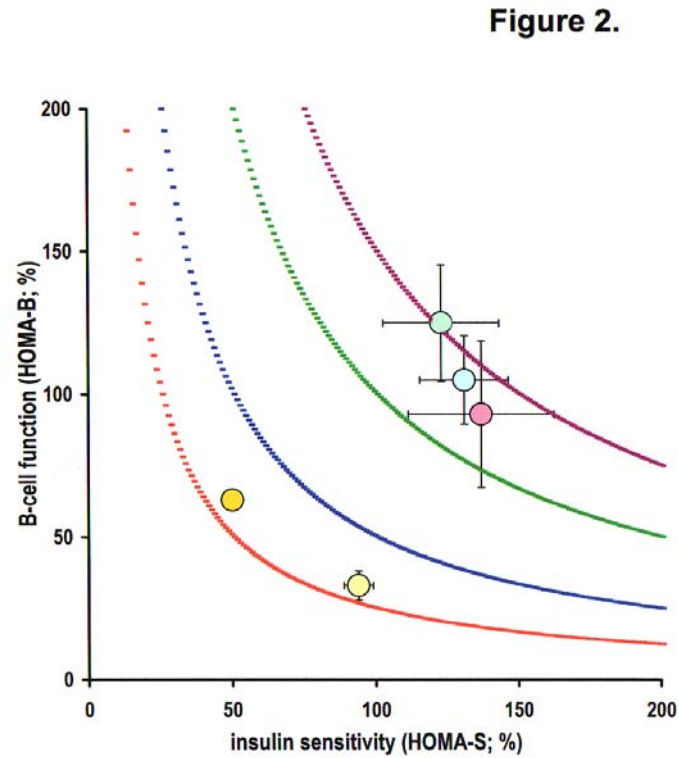


Figure 2. Hyperbolic product values (BxS) constructed from the means of HOMA insulin sensitivity and beta-cell function in subjects with type 2 diabetes mellitus (T2DM; n=450; orange circle), with chronic pancreatitis (CP; n=35; yellow circle), and in subjects with Cystic Fibrosis Transmembrane Regulator delta 508 mutation (CF; n=76; blue circle), either with normal glucose tolerance (CF-NGT; n=51; pale green circle) or abnormal glucose homeostasis (CF-AGH; n=25; pink circle). Errors bars represent the product's bidimensional standard errors of estimate adjusted for error propagation in product's terms (T2DM's error bars hidden by circle). The four dotted lines represent theoretical hyperbolic curves for constant values at 150% (mauve), 100% (green), 50% (blue) and 25% (red) of HOMA BxS for various combinations of S and B. Statistical differences in hyperbolic products ($p < 0.001$) were observed for T2DM vs. all CF subjects, T2DM vs. CF-NGT, T2DM vs. CF-AGH; CP vs. all CF, CP vs. CF-NGT and CP vs. CF-AGH.