Evaluation of serum 1,5 anhydroglucitol levels as a clinical test to differentiate subtypes of diabetes

Running title: Serum 1,5AG levels in subtypes of diabetes

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Objective: Assigning the correct molecular diagnosis in diabetes informs treatment and prognosis. Better clinical markers would facilitate discrimination and prioritisation for genetic testing between diabetes subtypes. Serum 1,5 Anhydroglucitol (1,5AG) levels were reported to differentiate Maturity-onset diabetes of the young due to HNF1A mutations (HNF1A-MODY) from type 2 diabetes, but this requires further validation. We evaluated serum 1,5AG in a range of diabetes subtypes as an adjunct for defining diabetes aetiology.

Research Design and Methods: 1,5AG was measured in UK subjects with: HNF1A-MODY (n=23), MODY due to glucokinase mutations (GCK-MODY, n=23), type 1 diabetes (n=29), latent autoimmune diabetes of adults (LADA, n=42) and type 2 diabetes (n=206). ROC curve analysis was performed to assess discriminative accuracy of 1,5AG for diabetes aetiology.

Results: Mean [SD range] 1,5AG levels (μg/ml) were: GCK-MODY 13.06 [5.74-29.74]; HNF1A-MODY 4.23 [2.12-8.44]; type 1 diabetes 3.09 [1.45-6.57]; LADA 3.46 [1.42-8.45] and type 2 diabetes 5.43 [2.12-13.23]. Levels in GCK-MODY were higher than other groups [p<10^-4 vs. each group]. HNF1A-MODY subjects showed no difference in unadjusted 1,5AG levels from type 2 diabetes, type 1 diabetes or LADA. Adjusting for HbA1c revealed a difference between HNF1A-MODY and type 2 diabetes [p=0.001]. The discriminative accuracy of unadjusted 1,5AG levels were 0.79 for GCK-MODY vs. type 2 diabetes, 0.86 for GCK-MODY vs. HNF1A-MODY, but only 0.60 for HNF1A-MODY vs. type 2 diabetes.

Conclusions: In our dataset serum 1,5AG performed well in discriminating GCK-MODY from other diabetes subtypes, particularly HNF1A-MODY. Measurement of 1,5AG levels could inform decisions regarding MODY diagnostic testing.
An estimated 2% of diabetes in Europe is caused by monogenic disorders of the beta-cell (Maturity-onset diabetes of the young; MODY) (1). The two most common types of MODY in clinical practice are caused by mutations in the genes encoding hepatocyte nuclear factor 1-alpha (HNF1A) and glucokinase (GCK) (1). Making the correct molecular diagnosis allows individualisation of treatment, for example the use of low dose sulphonylurea as a first line in HNF1A-MODY (2). It also conveys important information about prognosis and guides investigation of family members. Despite these clear advantages, individuals with MODY are frequently misdiagnosed as having either type 1 or type 2 diabetes, or do not have confirmatory molecular testing performed even when MODY is suspected.

Although HNF1A- and GCK-MODY have distinct phenotypes (1; 3), in clinical practice differentiating these from each other and from common forms of diabetes can be challenging. Molecular genetic testing, if positive, is definitive but is currently too expensive for indiscriminate use. Therefore there is a need for novel biochemical screening tools to identify and direct efficient genetic analysis in those for whom a probable monogenic diagnosis of diabetes exists. Ideally such a test would be highly specific for a MODY subtype as well as allowing differentiation from both type 1 and type 2 diabetes.

A recent report suggests that measurement of serum 1,5 anhydroglucitol (1,5AG) may represent such a test, at least to discriminate HNF1A-MODY from type 2 diabetes (4). 1,5AG is a metabolically inactive monosaccharide that reaches steady state between ingestion and urinary excretion with near complete renal reabsorption at a specific fructose-mannose active transporter (5; 6). Due to structural similarity, glucose competitively inhibits this reabsorption, such that in times of significant glycosuria, 1,5AG is excreted in the urine and serum levels consequently fall (7). Thus poor glycaemic control is associated with low serum 1,5AG levels (8). A low renal threshold for glucose also results in a lower than expected serum 1,5AG level (9). As HNF1A mutations are characterised by low renal glucose threshold (10) due to decreased expression of the high-affinity low capacity glucose co-transporter 2 (SGLT2) (11), it was hypothesised that 1,5AG levels could be a biomarker for HNF1A-MODY. The initial report of serum 1,5AG levels in Polish subjects (4) found that mean 1,5AG levels were 50% lower in patients with HNF1A-MODY compared to those with type 2 diabetes matched for glycaemic control.

We sought to evaluate this hypothesis in a larger independent sample set with a wider range of diabetes subtypes and to assess the performance of serum 1,5AG levels as an adjunctive test in identifying subtypes of diabetes.

SUBJECTS AND METHODS
Subjects were collected in Oxford, UK. The MODY samples comprise subjects with a confirmed mutation in either HNF1A (n=23 from 12 families) or GCK (n=23 from 10 families). Median family size was 2.5 members (range 1-6) and half the families comprised only one individual. Nineteen of the HNF1A-MODY subjects had diabetes, one had IGT and 3 were normoglycaemic. OGTT data from the time of sampling were available on all non-diabetic subjects.
The remaining subjects were from the Young Diabetes in Oxford (YDX) study, comprising subjects diagnosed with diabetes ≤ 45 years of age recruited from either primary (n=82) or secondary (n=198) care. Within the group are cases of classical type 1 diabetes, (n=29), Latent Autoimmune Diabetes of Adulthood (LADA, n=42) and type 2 diabetes (n=209). Type 1 diabetes was defined as permanent insulin treatment since diagnosis with additional evidence of severe β-cell dysfunction (C-peptide undetectable or HOMA %B < 10%), positive Glutamic acid decarboxylase antibodies (>14 WHO units/ml) or both. LADA was defined as diabetes with positive GAD antibodies but no requirement for insulin treatment within 3 months of diagnosis. Those not requiring permanent insulin treatment at diagnosis with negative antibodies were classified as type 2 diabetes. Subjects in the type 2 diabetes group did not meet current clinical criteria for MODY diagnostic testing (12) or had been tested and were negative for mutations in HNF1A/HNF4A (n=9) or GCK (n=4). Briefly, clinical criteria for HNF1A-MODY testing were young onset (<25 years) of familial non-insulin dependent diabetes and for GCK testing young onset of mild fasting hyperglycaemia (5.5-8mmol/l).

Two of the LADA subjects and 31 of the type 2 subjects were of non-European ethnicity (14 Asian, 11 Black, 1 Chinese, 7 mixed or other).

For all subjects clinical details, anthropometry and fasting blood samples were collected (Table 1). The study was approved by the Oxfordshire Local Research Ethics Committee and all subjects gave informed consent.

Intra-assay coefficient of variance (CV) was 0.46% and inter-assay CV ranged from 1.74-2.37%. GADA were measured by a radioimmunoassay using 3S-labelled full-length GAD65, and results were expressed in WHO units/ml derived from a standard curve calibrated from international reference material (National Institute for Biological Standards and Control code 97/550). Samples were considered positive if they had levels above 14 WHO units/ml (97.5th percentile of healthy school children) (14).

Values for age of diagnosis, duration of diabetes, BMI, creatinine, HbA1c, FPG and 1,5AG level were not normally distributed and were log₁₀ transformed. Geometric mean and standard deviation (SD) range were calculated. Analysis of variance (ANOVA) was calculated across the groups. For 1,5AG levels, pairwise comparisons (using T-test with Bonferroni correction for multiple testing) were also calculated between the different diabetes subtypes. We then examined the effect of correcting 1,5AG levels for HbA1c, and, in the type 2 diabetes and MODY subgroups, for the effects of treatment modality. As 1,5AG levels can be lowered in chronic renal failure (15), subjects with serum creatinine >150 μmol/l were excluded from the analysis (1 subject with LADA and 9 with type 2 diabetes).

ROC curve analysis was performed to assess the discriminative accuracy of 1,5AG with regard to diabetes aetiology. The performance of 1,5AG level as a diagnostic discriminator was compared to HbA1c and FPG. All statistical analysis was performed in SPSS v16 and p<0.05 was assumed to be significant.

RESULTS
Table 1 shows the characteristics of the subjects and the results of the biochemical investigations. Geometric
Serum 1,5AG levels in subtypes of diabetes

Mean [SD range] 1,5AG levels (μg/ml) were: GCK-MODY 13.06 [5.74-29.74]; HNF1A-MODY 4.23 [2.12-8.44]; type 1 diabetes 3.09 [1.45-6.57]; LADA 3.46 [1.42-8.45] and type 2 diabetes 5.43 [2.12-13.23].

Firstly examining unadjusted data, we found no difference in mean 1,5AG levels between subjects with HNF1A-MODY and type 2 diabetes (p>0.05). There was also no difference in 1,5AG levels between HNF1A-MODY and either of the autoimmune groups (p>0.05). As previously reported (16), we found 1,5AG levels were higher in those with type 2 diabetes compared to the autoimmune groups (type 2 diabetes vs. type 1 diabetes, p=0.011, type 2 diabetes vs. LADA, p=0.015). After controlling for HbA1c, adjusted 1,5AG level was lower in HNF1A-MODY than type 2 diabetes subjects (p=0.001). Adjusting for HbA1c did not alter the relationships between 1,5AG levels in the other diabetic subgroups.

Reanalysis adjusting for shared family membership in the MODY cases and excluding the non-European individuals had no effect on these findings (data not shown). Duration of diabetes also did not have a significant effect on 1,5AG levels, but in the type 2 diabetes subjects we did observe a progressively lower 1,5AG level (and higher HbA1c) with escalating treatment requirement. This was not seen in the MODY subgroups.

The three panels in figure 1 show scatter plots of unadjusted 1,5AG levels plotted against HbA1c. These illustrate the considerable overlap between HNF1A-MODY and common forms of diabetes at all values of HbA1c. This suggests that, in our dataset at least, 1,5AG levels will not be a very useful clinical indicator of HNF1A-MODY. We investigated this question further by constructing ROC curves. For HNF1A-MODY and type 2 diabetes, the area under the curve (AUC) was 0.60 (Figure 2b), confirming that in our set of subjects unadjusted 1,5AG levels are poorly discriminative between HNF1A-MODY and type 2 diabetes. We then repeated the ROC curve analysis using 1,5AG levels adjusted for HbA1c. This improved the AUC to 0.75.

Our most striking finding was that subjects with GCK-MODY had a higher 1,5AG level than any of the other groups, p≤0.0003 for all pairwise comparisons (both uncorrected and corrected for HbA1c). Figure 1 illustrates that the GCK-MODY cases show good separation from other kinds of diabetes. ROC curve analysis to examine the discriminative accuracy of 1,5AG level for GCK-MODY (from type 2 diabetes) gave an AUC of 0.79 in our subjects (Figure 2a). Similarly for GCK-MODY vs. HNF1A diabetic subjects, the AUC was 0.86 (Figure 2c). Both these estimates were improved by adjusting for HbA1c to values of 0.94 and 0.96 respectively.

We calculated threshold values of 1,5AG designed to reflect maximum sensitivity and specificity in our dataset; for GCK-MODY vs. type 2, a 1,5AG >11μg/ml gave a sensitivity and specificity of 75% for identifying the GCK-MODY cases, while 70% of the type 2 diabetes cases fall below this cut off. For GCK-MODY vs. HNF1A-MODY, 1,5AG >7.5 μg/ml gave sensitivity of 86% and specificity of 84% for identifying GCK-MODY, while 89% of HNF1A-MODY diabetic subjects had 1,5AG levels below this.

In clinical practice, HbA1c<8% and FPG of 5.5-8.5 are often used as biochemical discriminators to identify those individuals with apparent monogenic diabetes most likely to have GCK-MODY (12). In our dataset these performed less well than 1,5AG. Although they gave similar
sensitivity to the above for identifying GCK-MODY cases, specificities were much lower at 42%, 58% and 66% for HbA1c, FPG and the combined criteria respectively.

We examined how serum 1,5AG could perform as a pre-selection for cases for GCK diagnostic sequencing. This was done by estimating the proportion of positive tests for GCK-MODY that would be identified by setting different prevalences of GCK-MODY in the baseline sample set using the threshold levels described above. If the type 2 diabetes and GCK-MODY groups from this study are combined, 25% of cases in this combined group with a 1,5AG value >11 μg/ml have a glucokinase mutation. This would be a similar rate of positive test pick-up to that achieved using standard clinical criteria by the UK diagnostic testing centre (17). However the prevalence of GCK-MODY in this combined group is 10%, which is somewhat higher than we would expect even in a group referred for genetic investigation. Recalculating based on a more realistic prevalence of GCK-MODY representing 5% of the diabetes cases would halve the positive test rate to 12.5%, which would probably still be an acceptable pick-up rate given the changes in management and prognosis that result from re-diagnosing type 1 or type 2 diabetes as a GCK mutation. This is also comparable to many other diagnostic genetic tests in the UK. Other models which add fasting glucose and HbA1c thresholds to the calculation do not improve these rates in our dataset.

DISCUSSION
Identifying monogenic forms of diabetes and assigning the subtypes correctly currently depends on recognising a clinical phenotype and arranging confirmatory molecular testing. Additional biochemical tests, which aid prioritisation of cases for genetic testing, would have great clinical utility.

1,5AG is an attractive candidate marker for HNF1A-MODY because it utilises the known characteristic of low renal threshold for glucose seen in HNF1A mutations. We confirm the previous finding (4) that 1,5AG levels are lower in HNF1A-MODY than type 2 diabetes but this difference is only apparent after adjustment for HbA1c. This is necessary because the lower HbA1c in our HNF1A-MODY group (7.2% vs. 7.8%, p=0.04) has the effect of diminishing the difference in unadjusted 1,5AG levels (the previous study was well matched at baseline for HbA1c). Adjusting for HbA1c increased the discriminative accuracy of 1,5AG to identify HNF1A-MODY from type 2 diabetes with the AUC of the ROC curve rising from 0.60 to 0.75. This is still of rather limited clinical utility (AUC of ≥0.8 representing a useful test) and would require further validation to design a suitable model that includes HbA1c. Ideally this validation would include more HNF1A-MODY cases with higher HbA1c as we have limited data on HbA1c>9%. A further limitation for a role of 1,5AG in a diagnostic strategy to detect HNF1A-MODY is that we found no difference in 1,5AG levels between HNF1A-MODY and either form of autoimmune diabetes. The most striking finding in our study was the higher 1,5AG levels in subjects with GCK-MODY compared to all other groups. This is likely to be explained by the known modest post-challenge glucose increment seen in those with GCK mutations (18). Post-prandial glucose levels rarely rise high enough to cause glycosuria, resulting in levels of 1,5AG that are mainly within the normal range. Our ROC curve analyses result in
promising estimates for the ability of 1,5AG to discriminate GCK-MODY from both type 2 diabetes and HNF1A-MODY. Though 1,5AG levels are likely to be influenced by recent dietary intake, we made no attempt to adjust for this. The ROC curve results we see are reassuring that this test will perform well in routine clinical practice where prior dietary information is not likely to be available. There are some limitations to extrapolating this finding to a general type 2 diabetes clinic population: the subjects in this study were selected for age of diagnosis ≤45 years, where the pre-test probability of possessing a GCK mutation is likely to be higher than in an unselected group of patients with type 2 diabetes (GCK mutations in fasting hyperglycaemia fall from a prevalence of 40% in children (19) to ~ 1% in adults diagnosed over 50 (20)). In the <45 yr age range the likely prevalence of GCK mutations is difficult to estimate but probably in the range 3-5%.

We found that duration of diabetes did not have a significant effect on 1,5AG level, however the type 2 patients on diet treatment had a higher 1,5AG than those on OHA or insulin. This suggests that post-prandial glucose excursion is not normalised by treatment of diabetes. Therefore we would predict that 1,5AG levels might be less useful in discriminating GCK-MODY from those with type 2 diabetes who are well-controlled on diet treatment.

There was very little overlap between the 1,5AG levels in our GCK- and HNF1A-MODY groups. This discriminative performance benefits from the fact that 2 major characteristics of these subtypes of MODY (low renal threshold and low post-challenge increment) have opposite effects on 1,5AG levels and suggests 1,5AG analysis might have most potential as a discriminative test between these two MODY subtypes. Currently patients suspected of having MODY are frequently selected for GCK rather than HNF1A mutation testing on the basis of the characteristic pattern seen on OGTT: in GCK-MODY a mild fasting hyperglycaemia with a modest 2 hour increment (90th centile <4.6 mmol/l) (12), while in HNF1A-MODY diabetes FPG may be normal but a high 2 hour post-challenge level (mean > 5 mmol/l) is observed (18).

Although an OGTT is quoted as the gold standard investigation (12), it is time consuming, difficult to interpret in those on treatment and has large day to day variability (21). A 1,5AG measurement would in theory be a useful, more cost-effective and practical alternative to an OGTT as it reflects post-prandial glucose excursion from a single non-fasting blood sample. OGTT data were not available on our GCK-MODY cases so we were not able to directly assess the correlation with 2 hour glucose. However relationship of 1,5AG levels with OGTT has been previously examined; in subjects with IGT 1,5AG levels were strongly correlated (r=-0.8) with 2 hour glucose levels and this was greater than the correlation seen with FPG, or between HbA1c and either fasting or 2 hour values (22). Similarly 1,5AG levels showed good correlation with post-prandial CGMS readings (23) and 2-hour post-prandial capillary measurements (24). This supports the use of 1,5AG as a surrogate for post-challenge glucose and merits further validation in GCK-MODY.

Thus, in conclusion, we suggest future research should focus on the role of 1,5AG level as a tool to differentiate MODY subtypes in those already suspected of having a monogenic form of diabetes.
ACKNOWLEDGEMENTS
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Disclosure: There are no conflicts of interest to disclose.

Figure Legends

Figure 1 – Scatter plots of serum 1,5AG levels (μg/ml) vs. HbA1c (%) for the different subtypes of diabetes. For clarity the data points are plotted on 3 panels with a filled symbol to emphasize a different diabetic subtype in each panel. Lilac diamonds: subjects with autoimmune diabetes (type 1 + LADA combined); Blue circles: type 2 diabetes; Orange squares: HNF1A-MODY; Green squares: GCK-MODY. Panel A shows distribution of autoimmune diabetes; Panel B shows type 2 diabetes; Panel C shows both MODY subtypes. 3 subjects with HbA1c>12.5% are not shown for increased clarity of the figure but were included in the analysis.

Figure 2 – Receiver Operated Characteristic curves illustrating discriminative capacity of unadjusted 1,5 AG to distinguish diabetes subgroups. AUC=Area under the curve. Panel A - GCK-MODY from T2D, Panel B - HNF1A-MODY from T2D, Panel C - GCK-MODY from HNF1A-MODY
REFERENCES
Table 1. Characteristics of the subjects studied. Geometric mean [SD range] are reported, the p value refers to ANOVA across the diabetic subgroups.

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<th>Type 2 diabetes</th>
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<th>GCK- MODY</th>
<th>Non-Diabetic HNF1A mutation carriers</th>
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* Geometric mean [SD range], † Current age, ‡ Mean [±SD], adjusted for HbA1c
Manufacturer’s quoted reference range for 1,5AG: males 10.7-32.0μg/ml, females 6.8-29.3μg/ml
Figure 1

Panel A

Panel B

Panel C

Serum 1,5AG levels in subtypes of diabetes
Figure 2

Panel A - GCK-MODY from Type 2 Diabetes

Panel B - HNF1A-MODY from Type 2 Diabetes

Panel C - GCK-MODY from HNF1A-MODY

AUC = 0.79

AUC = 0.60

AUC = 0.86