Clinical Research Article

Monogenic Causes in the Type 1 Diabetes Genetics Consortium Cohort: Low Genetic Risk for Autoimmunity in Case Selection

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Abstract

Hypothesis: About 1% of patients clinically diagnosed as type 1 diabetes have non-autoimmune monogenic diabetes. The distinction has important therapeutic implications but, given the low prevalence and high cost of testing, selecting patients to test is important. We tested the hypothesis that low genetic risk for type 1 diabetes can substantially contribute to this selection.

Methods: As proof of principle, we examined by exome sequencing families with 2 or more children, recruited by the Type 1 Diabetes Genetics Consortium (T1DGC) and selected for negativity for 2 autoantibodies and absence of risk human leukocyte antigen haplotypes.

Results: We examined 46 families that met the criteria. Of the 17 with an affected parent, 7 (41.2%) had actionable monogenic variants. Of 29 families with no affected parent, 14 (48.3%) had such variants, including 5 with recessive pathogenic variants of WFS1 but no report of other features of Wolfram syndrome. Our approach diagnosed 55.8% of the estimated number of monogenic families in the entire T1DGC cohort, by sequencing only 1.1% of the autoantibody-negative ones.

Conclusions: Our findings justify proceeding to large-scale prospective screening studies using markers of autoimmunity, even in the absence of an affected parent.
also confirm that nonsyndromic WFS1 variants are common among cases of monogenic diabetes misdiagnosed as type 1 diabetes.

**Key Words:** monogenic diabetes, type 1 diabetes, WFS1, HLA, autoantibody

Type 1 diabetes mellitus (T1D) is due to the autoimmune destruction of the beta-cells and characterized by early onset and immediate requirement for insulin treatment. Among patients presenting with this clinical picture, a small but nonnegligible proportion actually have monogenic diabetes, many forms of which can be more effectively treated with alternatives to insulin (1). Balancing the small proportion of such cases (estimated at >1%) (2) against the potentially life-changing benefit of therapeutic reassignment requires refined selection algorithms for the costly genetic testing.

A recent study combining autoantibody (aAb) negativity with indices of beta-cell function (2) performed well in detecting the mild cases due to GCK variants (OMIM 125851) but missed more than half of those linked to HNF1A (OMIM 600496) and HNF4A (OMIM 125850), cases which were more likely to be misdiagnosed as T1D, the main differential diagnosis in children and young adults. Genetic T1D risk behaves as a polygenic trait (3), dominated by the human leukocyte antigen (HLA) class II DR-DQ locus (reviewed in (3)). Therefore, we tested whether selecting for HLA genotypes that do not confer autoimmune T1D risk can improve the efficiency of selecting aAb-negative cases to test for a monogenic cause.

In our recent screening of aAb-negative Chinese patients diagnosed as type 1 diabetes, we did find a high prevalence of monogenic cases, as expected from the low genetic T1D risk in East Asians, based on ethnicity alone (4). In a recent comparison of 1963 T1D cases with 804 cases of maturity onset diabetes of the young (MODY) previously diagnosed by clinical criteria, a low T1D genetic risk score was found to be predictive of MODY (5). We undertook to test this principle prospectively, for the ab initio diagnosis of unsuspected monogenic cases with a rigorously ascertained T1D phenotype, which is likely to exclude most patients with mild hyperglycemia due to GCK mutation. Our secondary aim was to see whether the high prevalence of recessive WFS1 variants causing a T1D clinical picture without any of the other manifestations of Wolfram syndrome (WS; OMIM 222300) that we found in China (4) would also be observed in individuals of European ancestry.

**Participants**

The T1D Genetics Consortium (T1DGC), recruited multiplex families (2 or more affected children) for the study of T1D genetics (6). The majority (97.4%) were described as white Caucasians. Inclusion criteria were onset <35 years, uninterrupted requirement for insulin within 6 months of diagnosis, and at least 1 similarly affected sibling. Syndromic cases were excluded. DNA and clinical information were obtained from the NIDDK repository. The T1DGC consent authorized research on T1D genetics. The study was approved by the Research Ethics Board of the Montreal Children's Hospital.

**Autoantibody Status**

Subjects had been tested for aAb to glutamic acid decarboxylase 65-kilodalton isoform and islet antigen 2 at the time of recruitment, at widely varying intervals after diagnosis. Radiobinding assays, calibrated against the World Health Organization standards were used. Families were included if all affected members were negative.

**HLA Risk**

HLA had been genotyped by allele-specific oligonucleotide hybridization, confirmed by our exome results. Families were included if none of the affected individuals carried a risk HLA haplotype (defined as DRB1*0301-DQB1*0201 or DRB1*040x-DQB1*0302 where DRB1*040x includes *0401, *0402, *0404, and *0405) or if at least one affected member had the highly protective DQB1*0602.

**Exome Sequencing**

Exome sequencing in one proband from each family, involved capture with the 50 Mb Agilent SureSelect array and sequencing on the Illumina HiSeq at a depth of 50x. Data were processed in a standard pipeline, using GATK, BWA-MEM, and variants were annotated by Annovar. Only protein-altering variants (missense, frameshift, splicing, stopgain, stoploss) were considered. These were filtered for frequency in three public databases (1000 genomes, ExAc, and gnomAD). Maximal allele frequency cutoff was 0.0001 in any population for the dominant genes and 0.005 for the recessive WFS1. We searched for copy-number variants by loss of heterozygosity and normalized read counts, using FeatureCounts (http://subread.sourceforge.net/). All variants reported here were confirmed with Sanger sequencing of proband, untested sibling, and, if available, parents.
Genes in the University of Chicago list, one of the most comprehensive available, were analyzed. (https://dnatesting.uchicago.edu/tests/neonatal-diabetes-mellitus-mody-panel).

Data Analysis

The variants were evaluated by the American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology criteria (7) and rated as pathogenic, likely pathogenic, and variants of unknown significance (VUS). The latter were deemed actionable and included because they cannot be ignored in clinical practice, and justify a sulfonylurea trial or, at least, genetic counseling. They include the majority of disease-causing missense variants, if novel. Our very strict allele-frequency cutoffs minimize the probability of a given patient having a VUS that is spurious.

To evaluate the probability of finding the variants in a given gene under the null hypothesis, we summed up the allele frequencies of all variants in the European gnomAD set that met the same criteria (allele frequency and PP3 prediction). This aggregate probability of being a carrier of any such variant was applied to our exome cohort to derive the probability on the Poisson distribution.

Results

Autoantibodies

Of 2836 families, all affected individuals were negative for both antibodies in 414 families (14.6%) (Fig. 1). Of these, 52 families (1.8% of the total cohort) were also negative for HLA risk. In these families, sibling allele-sharing at the HLA locus on Chr6p21 had a negative logarithm of the odds ratio. We had usable DNA from 46 probands.

High frequency (41.2%) of actionable variants in cases with an affected parent

Twenty of these 52 families (38.5%) had a parent diagnosed with T1D or T2D, more than twice as many as the remaining T1DGC cohort (17.1%, P = 0.0003). Of the 17 such families for which DNA was available, 7 [41.2%, 95% confidence interval (CI 95%) = 20.1%-74.6%] had variants segregating with the phenotype: 5 in HNF1A (MODY3), a known 1 in RFX6 (OMIM 612659) (8,9), and 1 in INS (OMIM 613370) p.96Cyst > Arg replacing 1 of the cysteines involved in a disulfide bond and altered by a different missense variant in neonatal diabetes (10).

Monogenic diabetes is also frequent (48.3%), in the absence of history of an affected parent

Previous studies (2,11) indicate a penetrance of monogenic diabetes much lower than 100%, and our results corroborate this. We sequenced 29 of the 32 probands with no record of affected parent (3 excluded for DNA availability/quality control). No parent had been diagnosed with T1D, but information about other types of diabetes was incomplete in some cases. For this reason, what we would term “clinical penetrance” (based on awareness by the care provider) may underestimate the true biological penetrance. We found variants likely to be pathogenic for monogenic diabetes in 14 cases (48.3%, CI 95% = 30.1%-73.8%). Four of 29 had a variant in HNF1A. Of our total of 9 HNF1A variants (5 + 4), 5 were rated as pathogenic, 3 as likely pathogenic, and 1 was a VUS. (Table 1). All 9 segregated with diabetes in the affected sibling (P = 0.002).

Under the null hypothesis, the aggregate probability of any gnomAD variant satisfying our criteria (minor allele frequency < 0.0001 and ACMG PP3) to segregate with diabetes in 1 sibling pair was 0.0038. The probability that more than 1 such pair of HNF1A variants could occur by chance among our 34 undiagnosed pairs (46 sequenced minus 12 due to ACMG-pathogenic variants) was 0.0081, and the false discovery rate was calculated at 0.05 (calculation details in Table 2).
**Table 1. Monogenic variants found in 21 cases**

| Patient ID  | Gene        | GRCh37 position | CDS change | Protein | Hom/het | Age at diagnosis | Parent \(^a\) | Max MAF | Pathogenicity by ACMG criteria | PMID or accession no |
|-------------|-------------|-----------------|------------|---------|---------|------------------|-------------|--------|--------------------------------|---------------------|
|             |             |                 |            |         |         |                  |             |        | T1D | T2D |                                |                     |
| 24468904    | HNF1A       | 12:121416627    | c.C56T     | p.S19L  | Het     | 34               | No          | Yes   | 0   | VUS | PM2 PP3                         | —                   |
| 22082103    | HNF1A       | 12:121426713    | c.A404C    | p.D135A | Het     | 18               | No          | Unk   | 0   | Likely pathogenic PM1 PM2 PM5 PP3 | 18003757            |
| 20871403    | HNF1A       | 12:121426784    | c.G475T    | p.R159W | Het     | 19               | No          | Unk   | 3 × 10^-3 | Likely pathogenic PS1 PM2 PP3 | 9754819             |
| 42317204    | HNF1A       | 12:121431395    | c.G599A    | p.R200Q | Het     | 20               | Yes         | No    | 3 × 10^-3 | Pathogenic PS1 PM2 PM3 PP3 PP5 | 21224407            |
| 28836403    | HNF1A       | 12:121431435    | c.G659G    | p.I213M | Het     | 19               | No          | Yes   | 0   | Likely pathogenic PM1 PM2 PM5 PP3 | 23348805            |
| 224255704   | HNF1A       | 12:121432065    | c.G812A    | p.R271Q | Het     | 28               | Yes         | No    | 7 × 10^-3 | Pathogenic PS1 PM2 PM5 PP3 | 26853433            |
| 21373604    | HNF1A       | 12:121432077    | c.A824T    | p.E275V | Het     | 30               | Yes         | No    | 0   | Pathogenic PS1 PM2 PP3           | 27059371            |
| 21160303    | HNF1A       | 12:12143375     | c.1139delT | p.V380fs | Het | 16               | Yes         | No    | 0   | Pathogenic PVS1 PS1 PM2         | VCV000435426        |
| 26244503    | HNF1A       | 12:121435276    | c.1310-1G > A | splicing | Het | 22               | No          | Unk   | 0   | Pathogenic PS1 PM2 PM3 PP3      | —                   |
| 46247703    | WFS1        | 4:6296872       | c.G817T    | p.E273X | Het     | 15               | No          | No    | 2 × 10^-3 | Pathogenic PS1 PM2 PM3 PP3 | 10521293            |
| 41990004    | WFS1        | 4:6302483       | c.A961C    | p.T321P | Het     | 10               | No          | Yes   | 0   | Likely pathogenic PM2 PM3 PM5 PP3 | 24890733            |
| 48247803    | WFS1        | 4:6302884       | c.1362_1377del | p.Y454fs | Het | 10               | No          | No    | 0   | Pathogenic PVS1 PS1 PM2 PP3     | 12754709            |
| 47209403    | WFS1        | 4:6303394       | c.G1514A   | p.C505Y | Het     | 12               | No          | Unk   | 0.0137 | Pathogenic PS1 PM2 PM3 PP4 | 30014265            |
| 40670003    | WFS1        | 4:6303861       | c.G1839A   | p.W613X | Het     | 13               | No          | Yes   | 3 × 10^-3 | Pathogenic PVS1 PS1 PM1 PM2 PP3 PP4 | 15277431           |
| 20494604    | INS         | 11:12181129     | c.G2082C   | p.E694D | Het     | 0\(^b\)         | Yes         | No    | 0   | VUS PM2 PM3 PP3                  | —                   |
| 29863603    | INS         | 11:12182028     | c.174delA | p.A58fs | Het     | 6                | No          | No    | 0   | Likely pathogenic PM1 PM2 PM5 PP2 PP4 | —                   |
| 22358103    | GCK         | 7:44187340      | c.G277A    | p.G258S | Het     | 18               | No          | No    | 0   | Likely pathogenic PVS1 PM2 PP3   | —                   |
| 43752103    | KNCJ11      | 11:17408960     | c.G679A    | p.E227K | Het     | 9                | De novo     | 3 × 10^-3 | Pathogenic PS1 PM1 PM2 PP2 PP3 | 17021801            |
| 23676104    | RFX6        | 6:117198947     | c.224-12A > G | splicing | Het | 7                | No          | Yes   | 9 × 10^-3 | Pathogenic PS1 PM2 PM3 PP4 | 20148032            |
| 18891204    | SPINK1      | 5:147207385     | c.194 + 2T > C | splicing | Het | 23               | No          | Unk   | 0.0035 | Pathogenic PVS1 PS1 PM2 PP2    | 275378509           |
| 26036704    | KLF11       | 11:1018541      | c.G1026A   | p.M342I | Het     | 9                | No          | No    | 0.0001 | VUS PM2 PM3                    | —                   |

Transcript accession numbers of each gene: HNF1A: NM_000545, WFS1: NM_00114585, INS: NM_001185098, GCK: NM_000162, KNCJ11: NM_000525, RFX6: NM_173560, SPINK1: NM_003122, KLF11: NM_001177716.

Abbreviations: Max MAF, maximal minor allele frequency in any population.

\(^a\)Diagnosis of diabetes in a transmitting parent or, if not determined, in either parent.

\(^b\)Although clearly neonatal, this case was declared as type 1 diabetes, never had genetic testing and was recruited for type 1 diabetes genetics.
As we found in China (4), the frequency of recessive WFS1 variants in patients not reported to have manifestations of WS rivaled that of MODY3. Five sibling pairs had WFS1 variants, 4 compound heterozygous and 1 homozygous. Five of the 10 subjects were recruited within a year of diagnosis, but the remaining had had diabetes for 5, 5, 6, 9, and 10 years without, we assume, having developed the diagnostic manifestations of WS, which would have disqualified them. A perhaps more important reason to believe that most are nonsyndromic cases, is their frequency, rivaling MODY3 and much higher than the very rare WS. By parental genotyping, the variants were in trans in all heterozygotes and all segregated with diabetes in the affected sibling (recessive logarithm of the odds = 2.4 at \( \theta = 0 \)). Only 1 of the 9 variants was rated VUS by ACMG criteria, a novel missense in trans to a pathogenic truncating allele (Table 1). We had no usable DNA from the sibling of the homozygous proband but, in the T1DGC SNP array linkage data (12), the 2 siblings are homozygous and identical-by-descent over a 31.8 cM telomeric region encompassing WFS1. This variant (p.R558C) is found only in Ashkenazi Jews, in whom homozygosity is known to cause a mild form of WS (13). Table 2 shows that the probability of recessive segregation of any 2 European gnomAD variants by chance alone

| Gene | (1) Carrier | (2) Diallelic | (3) Segregating | (4) In at least 1 of 34 undiagnosed | FDR (%) | (5) In more than 1/34 undiagnosed |
|------|-------------|---------------|-----------------|-----------------------------------|---------|----------------------------------|
| HNF1A| 0.0075      | N/A           | 0.00377         | 0.13                              | 3.2     | 0.0081                           |
| WFS1 | 0.035       | 0.0112        | 0.00031         | 0.011                             | 0.5     | 0.000055                         |

The European gnomAD carrier frequencies of all variants meeting our filtering criteria (missense or truncating, PP3 by ACMG criteria and allele frequencies ≤0.0001 or 0.005, respectively) were summed. This aggregate probability was used to estimate the probabilities, under the null, of (1) being a carrier (diallelic for any of these variants; (2) segregating with diabetes in 2 siblings; (3) segregating with diabetes in at least 1 of our 34 undiagnosed sibling pairs; and (4) segregating in more than 1 of these pairs on the Poisson distribution. Undiagnosed was defined as sibling pairs whose diabetes was not explained by variants found Pathogenic by American College of Medical Genetics and Genomics/Association for Molecular Pathology criteria (N = 34). The FDR was calculated as expected/observed cases with a segregating variant. The estimates are conservative, as most of the variants reported have frequencies drastically lower than the cutoffs.

Diagnostic considerations

As a proof of principle, our design sacrificed sensitivity to maximize specificity. Some aAb-negative pairs were not selected for sequencing because one sibling carried a risk HLA, not unusual in the general population, especially for the common DRB1\(^*\)0301-DQB1\(^*\)0201. The 45.7% estimate of monogenic cause should equally apply to them. Including them, the total number of monogenic families in the entire T1DGC cohort can be probabilistically estimated on the basis of 3 reasonable assumptions: (i) both siblings in a pair have diabetes for the same reason, (ii) monogenic cases are aAb negative, and (iii) HLA does not affect monogenic diabetes risk. Violations of 1 or 2 are too rare to materially alter the results and violation of 3 lacks biological plausibility.

Thus, out of 52 pairs negative for both aAb and risk HLA, we were able to sequence 46, and 21 (45.7%, CI 95% = 31.2%-64.7%) had an actionable monogenic variant.
estimated total of 37.6 pairs. Thus 55.8% (21/37.6) of the estimated total number of monogenic families in the entire T1DGC cohort were diagnosed by sequencing only 11.1% (46 of 414) of aAb negative probands.

Discussion

Our study was not designed to develop a diagnostic algorithm for screening T1D cases for monogenic diabetes. Selecting for an affected sibling enriches in monogenic causes, and therefore our percentages are an overestimate of those in the general T1D population. Our main aim was to use this convenience sample to showcase the power of genetic screening as an addition to auto-antibody testing. The T1DGC had no data on beta-cell function markers, and it is likely that they will further increase the power of future algorithms. However, it is worth pointing out that they may be less important in patients with a convincing T1D phenotype (note the very low prevalence of GCK cases, compared to clinically diagnosed MODY cohorts).

Our results clearly justify future prospective studies using additional aAb (anti-insulin and anti-ZnT8) and a more refined genetic risk assessment. Although HLA accounts for the bulk of the known T1D genetic risk, the INS and PTNPN22 loci also contribute, and more than 50 additional loci have small but measurable effects. Future case-selection algorithms can benefit from a number of genetic risk scores that are being developed (20,21), to explore deeper into those that were missed with HLA only. Moreover, given some reports of aAb-positive cases of monogenic diabetes (22), it may be worth also searching for monogenic diabetes in weakly positive patients who have a very low score for a single antibody.

Another important finding is the confirmation that the frequency of nonsyndromic WFS1 variants is a close second to that of HNF1A in subjects of European descent with a clinical diagnosis of T1D. Based on the results presented in this paper, the Canadian Institutes of Health Research have funded the Accurate Diagnosis in Diabetes for Appropriate Management study (ClinicalTrials.gov Identifier: NCT03988764), in which we aim to recruit 5000 pediatric patients diagnosed as T1D and sequence all aAb negative cases by exome sequencing. We expect that the results will generate a powerful algorithm for optimal selection of T1D patients for sequencing.

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Author Contributions: CP and ML developed the project and wrote the manuscript; LM managed the sequencing and data analysis; CL and IR added ACMG criteria and literature support; TAM, CL, LR, and ET managed the DNA samples and confirmed pathogenic variants with Sanger sequencing; and ACLB performed the analysis and statistics on the original T1DGC data set.

Additional Information

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Disclosure Summary: CP and ML are stock holders, and CP is Chief Scientific Officer of Zhejiang Maidagene Co., Ltd, a for-profit startup offering genetic testing services.

Data Availability: All the variants were submitted to Clinvar (https://www.ncbi.nlm.nih.gov/clinvar/). Exome results are available upon request for reasonable use.

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