RESEARCH NOTE

No major genes in autoimmune thyroid diseases: complex segregation and epidemiological studies in a large Tunisian pedigree

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Introduction

The autoimmune thyroid diseases (AITDs) include, Graves' disease (GD), autoimmune hypothyroidism (AH): Hashimoto's thyroiditis (HT) and atrophic autoimmune thyroiditis (AAT). These are prevalent autoimmune diseases, affecting up to 2% of the general population. A widely accepted model for the pathogenesis of AITDs suggests that each subject has a inherited background predisposition to autoimmunity, with additional environmental and hormonal factors that trigger or contribute to the development of disease. The importance of genetic factors in determining susceptibility to AITDs has been demonstrated by the increased risk of thyroid disease in siblings of affected individuals (Brix *et al.* 1998), the higher concordance rate in monozygotic compared with dizygotic twins (Brix *et al.* 2000) and the high value of λs (16.9) (Villanueva *et al.* 2003). The search for susceptibility genes involving genome scans and genomewide association studies has identified many regions linked and/or associated with AITDs on different chromosomes (Jacobson and Tomer 2007; Todd *et al.* 2007; Welcome Trust Case Control Consortium 2007). However, there is still one main question about the features of genetic factors in AITDs: is there a major gene, or are AITDs the result of occurrence of multiple minor genes? In fact, segregation analysis focussing on determining both features of genetic component and transmission mode of inheritance of AITDs are missing. Therefore, in an attempt to address this question, we have focussed on a particular multigenerational family (named Akr) with an exceptionally high prevalence of AITDs (Maalej *et al.* 2001).

In the present study, we have performed an epidemiological and a complex segregation analysis of AITDs in the region where the studied family lives. Our results provided evidence for the polygenic character of these diseases when compared with the full model ($\chi^2_6 = 68.31$; $P = 9 \times 10^{-13}$) suggesting that genetic susceptibility to AITDs results from numerous loci, each contributing small effects.

Materials and methods

Subjects

One hundred and thirteen patients with AITDs who visited the regional dispensary in the studied district (located at the central eastern part of Tunisia) were subjected to a regular clinical follow-up for over eight years (1992–2000). The diagnosis of GD and AH was performed as previously described (Bougacha-Elleuch *et al.* 2004). Seventy patients belong to the Akr family (Maalej *et al.* 2001). Among 113

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patients, 47 could be considered as probands. We have conducted an inquiry in the village and recruited 180 control individuals (90 males and 90 females) randomly chosen and serving as reference in comparisons. The most complete genealogies possible have been established for patients and controls.

Measure of consanguinity

The coefficient of consanguinity of an individual F_i was first defined by Malecot (1948).The mean coefficient of consanguinity (α) was estimated as $\alpha = \Sigma\, p_i\, F_i$, where p_i is the relative frequency of the subjects with consanguinity coefficient F_i.

Estimation of prevalence

The frequency f of the disease was estimated as: $f = n/N$ (Barrai *et al.* 1965). N is the corresponding population size, $n = A/\pi$. A is the number of affected cases in this area and living at that time and π is the probability that an affected case is a proband.

Estimation of incidence

The incidence was evaluated by using the age at onset of the disease (0–19; 20–39; 40–59; over 60 years) among affected cases and the mean population size in these different classes in the studied district. The annual incidence was estimated over a period of four years (1997–2000), per 1000 inhabitants. This incidence was then corrected by the ascertainment probabilities (Ben Arab *et al.* 1990) which must be evaluated prior to the analysis using the distribution of probands among affected cases in sibships (Morton 1958). The frequency of disease in each age–sex class was then obtained by cumulating incidences over classes and the overall frequency by weighting by the population size in each age–sex class.

Segregation analysis

Evidence for AITDs inheritance, in our study, was assessed with the statistical package SAGE 5.0 (version 5.0 2004 Department of Epidemiology and Biostatistics, Care Western Reserve University, Cleveland, USA) using the REGTL program for segregation analysis of binary traits with a variable age at the onset under the class A regressive logistic model (Bonney 1986). The calculation of the likelihood for family data is described by Elston and Stewart (1971). Briefly, the likelihood is function of the population frequencies of the three genotypes (AA, AB and BB), the transmission probabilities of these genotypes (τ_{AA}, τ_{AB} and τ_{BB}) where τ_{AA} for example is the probability that a parent of genotype AA will transmit allele A to his child, and the cumulative probability that an individual with particular genotype will develop the disease by a given age. Hence, for Mendelian transmission (as that for dominant, recessive or co-dominant modes) these parameters are fixed and should be equal to 1; 0.5 and 0, respectively. For the full model, these parameters are free and not fixed, and are estimated by maximum likelihood under the condition $0 \leq \tau_{AA}, \tau_{AB}, \tau_{BB} \leq 1$. For the environmental model (where there is no parent–offspring transmission or type, so $\tau_{AA} = \tau_{AB} = \tau_{BB}$), the two groups model was used with the hypothesis of two age-of-onset distribution in the population for the disease. In fact, for this model, one should specify two or three groups. Here, we only used the two-groups model.

The cumulative probability is modelled using a logistic function that includes baseline parameters β (for details see Bonney 1986). The maximum likelihood under each model was compared by likelihood ratio test to that under the full model, in which all the parameters were estimated without restriction to assess whether the model of interest provided an adequate fit to the data.

Results

Characteristics of the epidemiological parameters of AITDs in the studied district

To determine the epidemiological parameters of the studied district, 113 patients affected with AITDs (belonging to Akr family and other families) were collected over the period between 1992 and 2000. Among them, 70 patients (39 GD and 31 AH) belong to Akr family, and the remaining 43 patients (20 GD and 23 AH) are from other families living in the same area. The mean ages were 44.04 years (20–81) and 49.07 years (14–81) for GD and AH, respectively.

Measure of consanguinity

The mean consanguinity was estimated at 0.03 in Akr family and 0.021 in the control group (table 1). The frequency of consanguineous marriages was estimated to be 60.5% in the Akr family and 38.3% in controls ($\chi^2 = 2.4$; $P < 0.05$) after removing the unknown class. The endogamy rate was 82.9% in Akr family and 95.2% in control group.

Estimation of AITD prevalence and incidence

The prevalence of AITDs in the studied region is about 43.6‰ (59.9‰ and 29.7‰ for women and men, respectively). The prevalence of GD and AH was 23.3‰ and 21.3‰, respectively. The estimated incidence of AITDs was 7.2 per 1000 inhabitants per year in the studied region. Table 1 gives the incidences cumulated and corrected by the ascertainment probability π that was estimated to 0.0746 (SE 0.00159). The cumulative incidence in women was twice that in men (56.15 versus 28.43, respectively).

Complex segregation analysis

Descriptive analysis of the studied families: Patients included in this study were subdivided according to the disease status of parents. Indeed, 83.5% of them have both parents unaffected.

Table 1. Characteristics of epidemiological parameters of AITDs in the studied district.

	Cumulative incidence*		Consanguinity coefficient	
Age class (years)	Males	Females	AKR family	Controls
0–19	0	3.95	$N = 4$ $C = 0.0425$	$N = 44$ $C = 0.0162$
20–39	8.22	23.54	$N = 23$ $C = 0.0305$	$N = 74$ $C = 0.0234$
40–59	17.55	50.61	$N = 13$ $C = 0.0293$	$N = 41$ $C = 0.0264$
≥60	28.43	56.15	$N = 3$ $C = 0.0044$	$N = 21$ $C = 0.0175$
Mean coefficient			$C = 0.030$	$C = 0.021$

*Cumulative incidence of AITDs corrected by the ascertainment probability per 1000 inhabitants according to age and sex.

The 47 probands are distributed among 47 pedigrees which exhibit a high degree of family aggregation. Among them, 15 pedigrees have two cases per family and eight are composed of more than two patients (3–10 patients). In the remaining families (24), each family has only one affected member and adds little to segregation analysis. Therefore, they were not included in this analysis.

Segregation analysis results: In the second step, we were interested in determining the segregation feature of AITDs in the studied region. For this reason, we performed a complex segregation analysis using the SAGE program. In this analysis, three hypotheses (Mendelian inheritance, environmental factors and no major type of transmission) were tested against the full model hypothesis. Table 2 shows the parameter estimates from the segregation analysis for the families ascertained in this study. The no major type hypothesis gave a significantly better fit to the data than both the monogenic and environmental hypotheses, implying the existence of more than one gene in the susceptibility to AITDs in the studied population ($\chi^2_6 = 68.31$; $P = 9 \times 10^{-13}$). By comparing the likelihoods for the group of genetic models, codominant Mendelian inheritance provided a significantly better fit to the data than both dominant or recessive inheritance ($\chi^2_3 = 60.89$; $P = 3.8 \times 10^{-13}$).

Table 2. Parameter estimates from segregation analysis of AITDs in the studied sample.

	Hypothesis					
	Mendelian inheritance			Environmental factors	No major type of	Full
Parameter	Dominant	Recessive	Co-dominant	Two groups	transmission	model
Allele frequency qA	0.763	0.252	0.258	0.971	–	0.25
Transmission prob*						
τ_{AA}	1	1	1	0.89	–	0.064
τ_{AB}	0.5	0.5	0.5	0.89	–	0.378
τ_{BB}	0	0	0	0.89	–	0.556
Baseline parameters						
β_{AA}	−1.44	287.98	0.98	−1.23	−1.00	9.07
β_{AB}	−1.44	−1.53	−0.59	−1.23	−1.00	−1.21
β_{BB}	120.30	−1.53	−2.18	6.44	−1.00	−1.46
−2(lnL) (#parameters)	202.25 (3)	202.41 (3)	211.53 (4)	194.80 (4)	218.95 (1)	150.64 (7)
χ^2 (df)[a]	51.61 (4)	51.77 (4)	60.89 (3)	44.16 (3)	68.31(6)	–
P value	$1.6\ 10^{-10}$	$1.5\ 10^{-10}$	$3.8\ 10^{-13}$	$1.4\ 10^{-9}$	$9\ 10^{-13}$	–

[a] $\chi^2 =$ (−2ln L of the data under the hypothesis of interest)-(−2ln L of the data under the full model).
*For Mendelian transmission, $\tau_{AA} = 1$; $\tau_{AB} = 0.5$; $\tau_{BB} = 0$. For no transmission $\tau_{AA} = \tau_{AB} = \tau_{BB}$. For general model, parameters τ are free and estimated using maximum likelihood (see Demenais and Elston 1981).

Discussion

The Akr family with such high prevalence of AITDs is among the rare families in the world. Indeed, this large pedigree is issued from a restricted number of founders, and consequently, it could be considered as a genetically homogeneous sample. Therefore, it was an appropriate sample to detect major genes of AITDs if they exist. Segregation analysis performed here has proven that there are no major genes in AITDs development in the studied district. Indeed, the no major type hypothesis gave a significantly better fit to the data than either the monogenic or environmental hypotheses ($\chi_6^2 = 68.31$; $P = 9 \times 10^{-13}$), implying the existence of more than one gene in the susceptibility to AITDs in the studied population. This finding is consistent with genetic analysis already carried out on 'pseudo isolate'. In fact, to this date, many candidate genes involved in both immune process and thyroid physiology have been investigated and have shown different degrees of involvement in AITDs pathogenesis (Hadj Kacem *et al.* 2003; Maalej *et al.* 2004; Bougacha-Elleuch *et al.* 2004; Kammoun-Krichen *et al.* 2007, 2008). Therefore, none of these genes could be considered as the major one. On the other hand, a genome scan was performed on Akr family and has given linkage with a nonreplicated susceptibility region on chromosome 2 (Maalej *et al.* 2001). Until now, there is no segregation analysis performed on AITDs in order to determine transmission mode of inheritance of these diseases independently from disease parameters such as antibodies titres. Thus, two previous studies have reported evidence for genetic transmission of thyroid peroxidase auto antibodies in Old Order Amish families using the Pointer program (Pauls *et al.* 1993; Jaume *et al.* 1999).

In our study, the segregation analysis has shown that co-dominant Mendelian inheritance provided a significantly better fit to the data than either dominant or recessive inheritance ($\chi_3^2 = 60.89$; $P < 10^{-12}$). In other words, genetic components involved in AITDs have additive effects until they reach, together with environmental and endocrine factors, a threshold allowing occurrence of the disease. This finding is reinforced by transmission mode found for candidate genes investigated in Akr pedigree. Thus, possible implication of these genes was evaluated with the intrafamilial test: FBAT, which calculates the corresponding score under a particular transmission mode (dominant, recessive or additive) (Laird *et al.* 2000). The positive associations reported were either under the additive or the recessive modes (Bougacha-Elleuch *et al.* 2004; Maalej *et al.* 2004; Hadj Kacem *et al.* 2006; Kammoun *et al.* 2007, 2008). The more plausible mode was the additive one confirming that AITDs are a result of addition of several genes contributing each with a modest effect. Our hypothesis is also consistent with that of Vieland *et al.* (2008), who have recently proposed a multilocus model of the genetic architecture of AITDs, where many genes and loci, contributing only a small risk of disease, interact to cause clinical AITDs. In this model, the combination of particular alleles in different loci produces dramatic phenotypic effects at these loci.

In the studied district, the estimation of the consanguinity has given a value of 0.0209 which is nearly the same value found in the governorate of Sidi Bouzid (to which the studied district belongs). Indeed, in the Tunisian population, there is a gradient in consanguinity rates among governorates. Particularly, the governorate of Sidi Bouzid has the highest rate (0.0213) (Chalbi and Zakaria 1998). However, in Akr multiplex family (which is composed of 62%: 70/113 of the total number of affected individuals in the region), the inbreeding coefficient is higher (0.0299). This could explain the high prevalence of AITDs in this family. Also, due to high consanguineous rate, there is a reduced genetic and environmental heterogeneity which could increase the emergence of such diseases. The endogamy rate was similar to that reported in general population (92.02%) (Chalbi and Zakaria 1998).

To conclude, our findings suggest that the inheritance of AITDs in the studied district results from numerous loci, each contributing to small effects. The plausible hypothesis of transmission was the co-dominant Mendelian inheritance. The high incidence of AITDs in such a district (where the rural population was estimated at 84%) could be explained by the high rate of endogamy which is commonly reinforced by preferential consanguineous marriages. Such sample was particularly used for performing complex segregation analysis and to foresee transmission mode of genetic component of AITDs. Nevertheless, a larger sample and further segregation analysis on different populations should be investigated in order to give a general mode of transmission of AITDs.

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