Anticipation in Schizophrenia and Bipolar Disorder Controlling for an Information Bias

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Anticipation was investigated in schizophrenia (SZ) and bipolar disorder (BP) while addressing several biases in 18 large families (154 subjects) from Eastern Québec densely affected by SZ, BP, or both over three generations. In particular, we controlled for an information bias using a measure of quality and quantity of clinical information (QOI) concerning the subjects' illness. Otherwise, spurious anticipation could have arisen because we found that QOI varied with the generations as well as with the severity of illness. Although anticipation was investigated separately for SZ and BP, both disorders were also included in one analysis that tested anticipation under the unitary hypothesis that the SZ and the BP spectrums represent a continuum of severity of the same disease. Age of onset (AOO) and five indices of severity were tested for anticipation. Two statistics were used: the difference in the mean AOO or severity between two successive generations, and the mean difference in parent–offspring pairs (POP). The study led to four main findings: 1) the choice of the statistics greatly influenced the results, POP yielding systematically greater biased estimates; 2) for SZ and BP, the evidence for anticipation with the five severity indices vanished after controlling for QOI; 3) as regards AOO a decrease of 8.6 years, $p = 0.0001$, and 5.3 years, $p = 0.009$ in AOO was found for SZ between Generations 1–2, and 2–3, respectively, despite controlling for QOI and addressing all biases; and 4) conversely for BP, anticipation with AOO may be due to censoring.

Findings suggest that future anticipation studies should also control for QOI. Am. J. Med. Genet. (Neuropsychiatr. Genet.) 96:61–68, 2000. © 2000 Wiley-Liss, Inc.

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INTRODUCTION

Family, twin, and adoption studies strongly support the role of heredity in the etiology of schizophrenia (SZ) and bipolar affective disorder (BP) [McGuffin et al., 1995; Tsuang and Faraone, 1990]. Even so, no success has yet been reached in identifying specific susceptibility genes predisposing to either syndrome. One major difficulty lies in the fact that SZ and BP are not transmitted following Mendelian expectation, but rather show a complex inheritance pattern that is not yet clearly understood.

Trinucleotide repeat expansions may provide an interesting clue to such complex inheritance. They represent kinds of mutations that increase in size over successive generations creating anticipation, whereby the severity of the disease increases with the number of repeats. This phenomenon of unstable DNA could offer an explanation for the many deviations from Mendelian inheritance observed in family studies of psychiatric disorders which were originally attributed to polygenic or oligogenic transmission [Petronis and Kennedy, 1995]. To date, repeat expansion has been shown in at least nine diseases including mental retardation and different neurodegenerative disorders [see Paulson and Fischbeck, 1996 for a review; Campuzano et al., 1996; Gusella and MacDonald, 1996].

For several trinucleotide repeat disorders (TRD), the relationship between repeat expansion and age of onset (AOO) or severity has been clearly demonstrated. For example, there is direct correlation between the size of the $(CAG)_n$ repeat expansion and the AOO of spinocerebellar ataxia type 1 [Orr et al., 1993]. Repeat size is associated with penetrance for fragile X syndrome [Warren and Ashley, 1995]. In myotonic dystrophy

[Jaspert et al., 1995], triplet size correlates significantly with muscular disability and, inversely, with AOO. Another example was provided by the X-linked spinal and bulbar muscular atrophy for which both the AOO and the age of stair-climbing difficulty correlated inversely with CAG repeat length [La Spada et al., 1992]. Although AOO was often related to the size of the repeat, a study of progressive myoclonus epilepsy provided at least one example where this was not the case [Lalioti et al., 1998].

Anticipation, as expressed by a decrease in AOO over successive generations, has been reported for SZ in several independent studies [Bassett and Honer, 1994; Bassett and Husted, 1997; Chotai et al., 1995; Gorwood et al., 1996; Heiden et al., 1999; Imamura et al., 1998; Johnson et al., 1997; Ohara et al., 1997; Stöeber et al., 1995; Thibaut et al., 1995]. The estimates of the average difference in AOO between generations varied among studies, sampling schemes, or both, but were generally between 4 and 16 years. Among the studies that have investigated anticipation in SZ using various severity indices, two did not find support for anticipation with severity [Johnson et al., 1997; Ohara et al., 1997], whereas others found evidence of an increase in severity of illness or in rates of hospitalization for psychotic illness [Bassett and Honer, 1994; Bassett and Husted, 1997; Heiden et al., 1999].

For BP, anticipation was reported both in terms of a decrease in AOO over successive generations and an increase in episode frequency [Engström et al., 1995; McInnis et al., 1993; Mendlewicz et al., 1997; Nylander et al., 1994]. In one study [Grigoroiu-Serbanescu et al., 1997], a decrease of 6 to 10 years on average in AOO between generations could be observed only when the proband inherited the disorder from the paternal side.

Although several studies have suggested the presence of anticipation in SZ and BP, the question remains as to whether this evidence reflects biases rather than expansion repeats [Penrose, 1948; Gelernter, 1995]. The most relevant biases include: (a) the fertility bias, by which cases with an earlier onset are less likely to have children thus reducing the probability of finding parent–offspring pairs where the parent shows earlier onset; (b) the censoring of observations due to the fact that offspring in the lower generations may not have completed the period at risk [Heiman et al., 1996; Vieland and Huang, 1998]. Consequently, some late-onset cases in younger generations may be missed at the time of ascertainment, which can mimic anticipation; (c) an information bias whereby an increase in severity (or decrease in AOO) would result from increasingly improving clinical information. Regression to the mean had previously been suggested as a potential bias [Asherson et al., 1994], but became clearly recognized as a distinct phenomenon from anticipation [Hodge and Wickramaratne, 1995; Petronis et al., 1994].

The objective of the present study was to investigate anticipation while addressing the biases mentioned earlier, using 18 large families from Eastern Québec densely affected by one of the SZ or BP spectrum disorders over three generations. First, the availability of affected relatives other than parents and offspring (such as uncles and aunts) allowed correcting for the fertility bias [Grigoroiu-Serbanescu et al., 1997; Johnson et al., 1997]. Second, given that affected subjects covered three generations, a censoring bias could be detected if anticipation occurred only between the last two generations. The availability of three generations has already been suggested as an advantage for detecting statistical artifacts [Trubnikov and Golimbet, 1996] because, if anticipation truly acts in families, it should occur between each of the two pairs of successive generations. Third, this study had the unique advantage of controlling for an information bias using a rating of the quality of the clinical information (QOI) derived from medical records, semistructured interviews with the subjects, and information from relatives [Maziade et al., 1992; Roy et al., 1997]. Indeed, we found that QOI was related to both the generations and severity and, therefore, could spuriously create anticipation.

This study benefited from the availability of mixed families (in which both SZ and BP run), allowing for testing anticipation under the unitary hypothesis that different forms of SZ or BP represent different degrees of severity of the same disease [Crow, 1986]. Five indices of severity for SZ and BP were considered, as well as AOO. They included the severity of positive and negative symptoms, a global assessment score (GAS) of social functioning [Endicott et al., 1976], and the hospitalization rate. The investigation of various severity indices in anticipation studies is motivated by the following reasons: 1- Given that AOO was not the only indicator of anticipation in the TRD reviewed earlier, investigating various severity indices may help to better characterize the phenotype that expresses anticipation in psychiatric disorders; 2- At least one study has suggested that the severity of negative symptoms may be associated with repeat length in SZ [Cardno et al., 1999]; and 3- Severity indices may be less prone to biases such as censoring because, unlike AOO, the notion of time is not embedded into them. Therefore, finding evidence for an increase in severity in successive generations could provide further support for the repeat expansion hypothesis.

MATERIALS AND METHODS
Sample

The total sample consisted of 154 individuals affected by SZ, BP, or their spectrum disorders and from whom an informed consent was obtained. Diagnoses were made by a lifetime Consensus Best Estimate (CBE) method detailed elsewhere [Maziade et al., 1992; Roy et al., 1997]. Briefly, diagnostic information was gathered from a SCID interview with the subject [Spitzer et al., 1992], structural information from relatives, and medical records. The first diagnosis using the Diagnostic and Statistical Manual of Mental Disorders (III-R) [American Psychiatric Association, 1987] was made by the field team who was unblind to the psychopathology in relatives. Next, the raw information was reviewed independently by two blind research psychiatrists, who then met with two additional psychiatrists to decide on a final blind *DSM-III-R* CBE used in the present study. The SZ sample consisted of

the 68 subjects (31 males) affected by SZ or a spectrum disorder: SZ (51), schizoaffective disorder (SAD; 10), schizophreniform disorder (5), schizotypal personality disorder (1), and delusional disorder (1). The BP sample consisted of 96 subjects (34 males) affected by BP or a spectrum disorder: BP I (43), BP II (10), SAD (10), recurrent major depression (11), and single major depression (22). The preceding classification is derived from the results of published family studies, which is the reason the 10 SAD subjects were included in both the SZ and the BP sample. The subjects came from 18 families: 8 families were mainly affected (i.e., more than 85% of subjects within the family) by disorders within the SZ spectrum, 5 by BP spectrum disorders, and 5 others were mixed. The average number of affected individuals per family was 8.5. Subjects covered three generations within families. Generations 1 (oldest), 2, and 3 (youngest) contained 15 (7 male), 41 (18 male), and 12 (6 male) subjects, respectively, affected with one of the SZ spectrum disorders, and 51 (18 male), 41 (13 male) and 4 (3 male) subjects, respectively, affected with one of the BP spectrum disorders. In the pooled sample, the average current age of the subjects in each generation (including unaffected members) was 65.1 ($n = 188$), 42.1 ($n = 195$), and 27.7 ($n = 33$), respectively. The families were originally identified for linkage studies and were ascertained according to the criteria that there should be at least four affected subjects in the first-, second-, or third-degree relatives of a proband. A more detailed description of our ascertainment procedure is provided elsewhere [Maziade et al., 1997].

Age of Onset and Severity Indices

Age of onset (AOO) was defined as the age of appearance of the first probable or definite episode meeting the *DSM-III-R* criteria for a targeted disorder. We assessed 82 items from the CASH [Andreasen et al., 1992] describing the lifetime severity of symptoms of psychosis, mania, and major depression. A factor analysis of these items in our sample revealed that the dichotomous factor structure of positive (PS) and negative symptoms (NS) was replicable both in familial SZ and familial BP [Maziade et al., 1995]. Hence, PS and NS were used as severity indices both in the SZ and the BP sample. The score on PS was an average of the symptom scores in the four dimensions loading on the positive factor: hallucinations, delusions, bizarre behavior, and thought disorder. The score on NS was an average of four dimensions loading on the negative factor: affective blunting, alogia, apathy, and anhedonia. Other severity indices were a lifetime rating of the global assessment scale [GAS; Endicott et al., 1976], whose method has already been described in detail [Maziade et al., 1995; Roy et al., 1997], and the hospitalization rate (HR) defined as the number of times hospitalized divided by the duration of illness in years. Finally, a severity score on diagnosis (SOD) was derived by attributing a weight from 0.5 to 6.0 to each diagnosis according to the following order: SZ (6.0), SAD (5.0), BP I (4.0), BP II (3.0), schizophreniform disorder (2.5), recurrent major depression or delusional disorder (2.0), major depression, single episode or cyclothymia (1.0), and schizotypal personality disorder (0.5). The variables AOO, PS, NS, and HR were studied separately in the SZ ($N_1 = 68$) and BP ($N_2 = 96$) sample, whereas SOD was studied in the combined sample of 154 subjects.

Quality of Information

For each subject, the quality and quantity of clinical information (QOI) from the multiple sources (medical records, semistructured interviews with the subjects, and structural family history from relatives) was rated according to a method described previously [Maziade et al., 1992; Roy et al., 1997]. The values for QOI ranged from 1 (*weak*) to 4 (*excellent*). Using a χ^2 for trend in proportions [Armitage and Berry, 1994], we found a significant linear relationship between the four levels of QOI and the generations, $P = 0.0007$, where upper generations tended to have a lower QOI.

We assessed the potential confounding effect of QOI by verifying if it was also related to AOO or severity. AOO slightly correlated with QOI for SZ, $r = -0.15$, $P = 0.12$, and more strongly for BP, $r = -0.20$, $P = 0.03$. PS tended to be correlated with QOI, both for SZ, $r = 0.20$, $P = 0.06$, and BP, $r = 0.20$, $P = 0.03$, whereas NS and GAS were not, $r < 0.10$, $P > 0.40$. HR correlated with QOI for BP only, $r = 0.22$, $P = 0.03$. In the pooled sample of SZ and BP, a significant correlation of 0.20, $P = 0.01$, was also found between SOD and QOI, where the most severe diagnoses tended to be assigned to individuals with a higher QOI. Hence, QOI was related to both the generations and severity, thus fulfilling the features of a confounding variable. Consequently, spurious anticipation could have occurred in our sample due to an information bias, i.e., the possibility that subjects in upper generations appeared less severely affected, or as having a later onset, than subjects in lower generations simply because of a poorer QOI about their illness.

Statistical Analysis

Three methods were previously used for estimating anticipation: 1- the difference (DIFF) in mean AOO or severity between two successive generations [Bassett and Honer, 1994]; 2- the mean of the differences in AOO or severity in parent–offspring pairs [POP; Chotai et al., 1995; McInnis et al., 1993]; and 3- the mean of the differences within all possible pairs (APP) that can be formed between two generations [McInnis et al., 1993]. In the present study, we performed DIFF because this method includes all available observations (e.g., uncles and aunts who did not have children), which allowed minimizing the impact of the fertility bias [Grigoroiu-Serbanescu et al., 1997; Johnson et al., 1997]. The APP approach also uses all available information. However, this method generates several unindependent observations. For example, if two generations contained 10 subjects each, then 100 pairs could be formed when in fact only 20 observations were available. Moreover, within a family, APP is mathematically equivalent to DIFF (see Appendix A). Hence, because APP is a similar but less conservative approach

(due to the exaggerated number of observations) than the DIFF method, APP was not used. The POP estimate is subjected to the fertility bias, as previously discussed. Therefore, we reported the POP estimate with AOO only to use it as an indicator of the presence of a fertility bias in our data. A second indicator of the fertility bias was obtained by comparing the average AOO of the 37 individuals who had children to the average AOO of the remaining 102 subjects of Generations 1 and 2 who did not have children using a Student t-test.

We tested the null hypothesis that there was no difference in mean AOO (or severity) between two successive generations (i.e., DIFF $= 0$) against the alternative hypothesis that the difference was significantly greater than 0 (or smaller than 0, with severity) using a Student test. A Wilcoxon rank–sum test was also performed and yielded similar results, thus they were not presented. One-sided P values were reported because the direction of the alternative hypothesis was clearly determined. A significance level of 0.05 was used. For each index of severity showing anticipation, the analysis was repeated while controlling for QOI by introducing QOI as a covariable in an ANCOVA. For AOO, survival curves were used to illustrate the whole distribution [Lawless, 1982]. Differences between survival curves were tested using the Wilcoxon test, and gender was tested as a covariable in order to detect a gender–generation relationship that could mimic anticipation [Davis, 1996]. No statistical testing was performed on POP estimates due to the limited number of parent–offspring pairs in our sample (7 and 20 in the SZ and BP samples, respectively).

In order to assess the degree of correction of the fertility bias provided by the DIFF method over the POP method, we simulated the AOO of 50 parents and 50 children in 50 independent samples where there was no anticipation. The two groups (parents and children) had an independent normal distribution of AOO with an equal mean of 25 and a standard deviation of 5. We introduced a fertility bias by excluding the children of

the parents who expressed the disease before the median age. Anticipation was then estimated with POP and DIFF.

RESULTS
Age of Onset (AOO)

AOO of SZ spectrum disorders significantly decreased between Generations 1 and 2, and between Generations 2 and 3 of 8.6 and 5.3 years, respectively (Table I). Even after controlling for QOI while withdrawing the most extreme observation (50 years) in Generation 1, the decrease in AOO remained significant, $P = 0.001$, between Generations 1 and 2, as well as between Generations 2 and 3, $P = 0.0063$. In parent–offspring pairs, the decrease in AOO was estimated to 20.0 years between Generations 1 and 2, and to 9.0 years between Generations 2 and 3. The survival curves in Figure 1 show that the subjects experienced their first symptoms at progressively younger ages across generations, Wilcoxon $\chi^2 = 19.53$, $df = 2$, $P = 0.0001$. Gender was not a significant covariate to generations, χ^2 increment $= 0.21$, $df = 1$, $P = 0.65$.

For BP, a significant decrease in AOO of 7.5 years was found between Generations 1 and 2, where most of the observations were gathered (Table I). Controlling for QOI while excluding the five most extreme observations from Generation 1 led to a still significant decrease in AOO, $P = 0.023$. The POP estimate was 8.7 years. Figure 2 shows the survival curves for AOO of BP, Wilcoxon $\chi^2 = 9.89$, $df = 2$, $P = 0.0071$. Gender was not a significant covariate to generations, χ^2 increment $= 3.05$, $df = 1$, $P = 0.0805$.

Severity Indices

An increase in positive or negative symptoms (PS and NS) between two generations would correspond to a negative value of anticipation in Table I. Hence, for the SZ spectrum disorders, subjects in Generation 3 were found to have significantly more severe PS than those in the preceding generation. No evidence for an-

TABLE I. Estimates of Anticipation and Corresponding Significance Level Obtained in the SZ and BP Samples Using the Difference in Mean AOO or Severity Between Successive Generations

	Generation	SZ Sample ($N = 68$)[a]				BP Sample ($N = 96$)			
		N	Mean	DIFF[b]	P value[c]	N	Mean	DIFF	P value
Age of Onset (AOO)	1	14	32.7	8.6	0.0001	48	34.1	7.5	0.0018
	2	39	24.1	5.3	0.0087	41	26.6	1.3	0.39
	3	11	18.8			4	25.3		
Positive	1	13	1.21	−0.11	0.31	50	0.55	−0.12	0.36
Symptoms (PS)	2	41	1.32	−0.57	0.0088	41	0.67	−0.10	0.23
	3	12	1.89			4	0.77		
Negative	1	13	2.07	0.54	1.00	50	0.77	0.22	1.00
Symptoms (NS)	2	41	1.53	−0.33	0.13	41	0.55	−0.51	0.043
	3	12	1.86			4	1.06		
GAS	1	11	53.6	−0.10	1.00	37	73.9	−3.3	1.00
	2	29	53.7	8.9	0.20	25	77.2	0.50	0.48
	3	4	44.8			3	76.7		
Hospitalization	1	12	0.28	−0.30	0.045	37	0.22	−0.30	0.015
Rate (HR)	2	30	0.58	−0.08	0.20	33	0.52	0.19	1.00
	3	10	0.66			3	0.33		

[a]Total number of available subjects. For a given severity index, missing values may occur due to missing information regarding an index.
[b]Difference in mean AOO or severity between Generation i and i + 1.
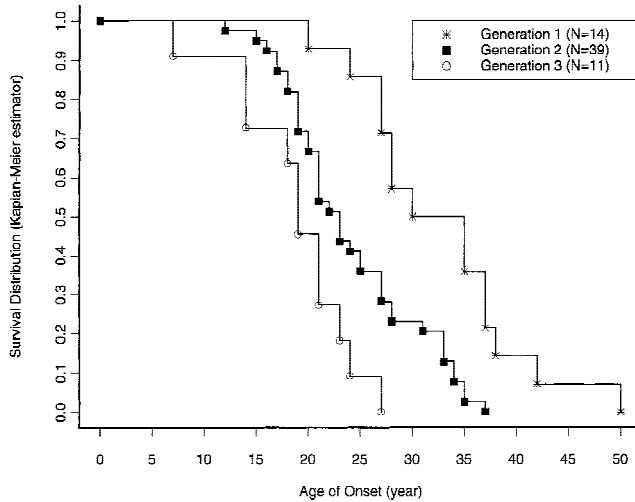[c]One-sided P value from the Student t-test.

Fig. 1.   Survival distribution of age of onset for SZ spectrum disorders in each of the three generations.

ticipation was found between Generations 1 and 2. Controlling for QOI did not affect the results significantly (details not shown). In the BP sample, PS did not increase significantly across generations. Severity in NS increased significantly between Generations 2 and 3 only in the BP sample (Table I). However, after controlling for QOI, the evidence was no longer significant, $P = 0.061$.

In the SZ sample, the increase in GAS of 8.94 between Generations 2 and 3 did not reach the significance level (Table I). Moreover, there was no evidence for anticipation between Generations 1 and 2. There was no evidence for an increase in GAS in the BP sample.

A similar and significant increase in hospitalization rate (HR) of 0.30 was observed both in the SZ and BP samples between Generations 1 and 2 (Table I). However, when QOI was taken into account after withdrawing two extreme observations from Generation 2,
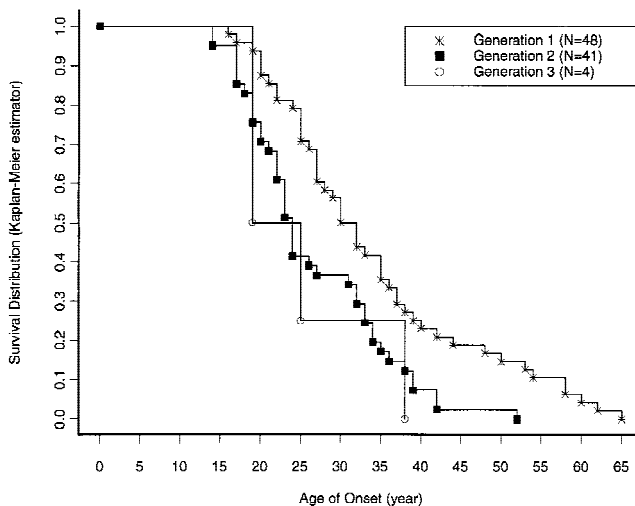
this evidence did not remain significant in either sample, $P > 0.07$. Moreover, between Generations 2 and 3, a decrease in HR rather than an increase was observed in the BP sample (Table I), arguing against anticipation.

Table II shows the results for index of severity of diagnosis (SOD) studied in the pooled sample of SZ and BP spectrum disorders. Negative differences in scores between two generations indicated an increase in severity. A significant increase of 0.86 in SOD was observed between Generations 2 and 3. However, when QOI was controlled for, the estimate did not remain significant, $P = 0.085$.

Fertility Bias

Among the 139 individuals affected by one of the SZ or BP spectrum disorders in Generations 1 and 2, the average AOO of the 37 individuals having children was significantly superior to the AOO of those having no children, 33.2 vs. 27.9 years, $t = 2.59$, $df = 131$, $P < 0.01$.

The average of the 50 DIFF estimates of anticipation obtained in the simulated samples of parents and children in which a fertility bias was introduced was only 0.28 years, with an average significance level of 0.45 (min = 0.08, max = 0.99). The POP method yielded an average estimate of 4.25 years, with an average significance level of 0.01 (min = 3.9×10^{-6}, max = 0.09).

DISCUSSION
Fertility Bias

From the methodological point of view, our study revealed that the choice of the statistics and the sample greatly influenced the estimate of anticipation. Indeed, the amplitude of anticipation with AOO obtained with POP was systematically larger than that obtained with DIFF. This strongly suggested that *reduced fertility* occurred in individuals who developed the disease particularly early. This bias could also be detected in our sample by our observation that, of the 139 individuals affected by one of the SZ or BP spectrum disorders in Generations 1 and 2, the average AOO of the 37 individuals having children was significantly superior to the AOO of those having no children (33.2 and 27.9 years, respectively). Using all affected individuals in families (with DIFF) seems to have corrected, at least partly, for such bias. Indeed, the simulation suggested

Fig. 2.   Survival distribution of age of onset for BP spectrum disorders in each of the three generations.

TABLE II. Estimates of Anticipation and Corresponding Significance Level Obtained in the Combined Sample of 154 Subjects Affected by One of the SZ or BP Spectrum Disorders for the Severity Index on Diagnoses (SOD)

| | | | | P value | |
| | | | | Without QOI[b] | With QOI[c] |
Generation	N	Mean	DIFF[a]		
1	64	3.77	−0.30	0.12	0.33
2	75	4.07	−0.86	0.035	0.085
3	15	4.93			

[a]Difference in mean SOD between generation i and i + 1.
[b]One-sided P value from the Student t-test.
[c]One-sided P values from an ANCOVA, using QOI as a covariable.

that the estimates of anticipation based on all affected individuals in a family, whether they had children, were not significantly biased by reduced fertility in upper generations. On the other hand, the estimates obtained with POP often detected significant anticipation, as suggested by the average estimate of anticipation of 4.25 years and the average significance level of 0.01, when in fact anticipation was not simulated in the data.

Censoring Bias

Anticipation as expressed by a decrease in AOO may be the consequence of a *censoring* bias, as formally demonstrated by Vieland and Huang [1998]. In other words, spurious anticipation can be induced by the fact that subjects in the younger generation may not have completed the period at risk. In our sample, the average current age of individuals in Generations 1, 2, and 3 (including unaffected relatives) was 65.1, 42.1, and 27.7 years, respectively. The average AOO of the subjects affected with one of the SZ spectrum disorders being 25.1, ($SD = 8.0$), one can conclude that most relatives in Generation 2 had already completed the period at risk for a SZ spectrum disorder. This suggests that our observation of a decrease of 8.6 years in AOO between Generations 1 and 2 did not result exclusively from a censoring bias. The evidence of anticipation of 8.6 years found in our sample is also consistent with previous findings reviewed in the Introduction. On the other hand, censoring cannot be ruled out in the BP sample because, in our sample, the BP spectrum disorders developed at an average age of 30.4 years, ($SD = 11.7$) and, consequently, some subjects of Generation 2 who could eventually develop the disease may have been missed at the time of ascertainment.

Information Bias

A decrease in AOO, or an increase in severity, in successive generations may also result from an information bias. Indeed, medical care and attitudes toward consultation have greatly evolved since 1920. Given that individuals in Generation 1 were born between 1920–1940, whereas those in Generations 2 and 3 were born between 1950–1975, an increase in severity across generations may simply reflect the increase in the quantity or quality of available clinical information, mimicking anticipation. In our family data, we found a clear evidence for this potential information bias. Indeed, QOI was related to both the generations and severity, thus fulfilling the features of a confounding variable. Our measurement of QOI allowed us to control for this information bias. Globally, controlling for QOI did not annihilate the evidence of anticipation obtained with AOO, whereas the increase in the severity indices was often confounded with QOI, as discussed later.

Cohort Effect

In a sample of families, the generations are necessarily confounded with birth cohorts. Therefore, the observation of intergenerational differences in AOO for SZ could reflect a cohort effect, i.e., a genuine accelera-

tion of the onset of SZ in successive birth cohorts that could arise, for example, from an increase in substance abuse or from a greater sensitization to appearance of psychiatric illnesses. However, we are unaware of any study that demonstrated a real cohort effect with age of onset of SZ. Heiden et al. [1999] found evidence for anticipation with AOO in their sample of 15 SZ families and addressed the cohort effect by using a control sample of two birth cohorts of patients with SZ. No significant difference in AOO was found between the two birth cohorts, which suggested that the evidence for anticipation found in their family data was unlikely due to a cohort effect. Studies aimed at verifying a cohort effect are also difficult to implement due to the methodological issues related to estimating AOO distributions in independently ascertained probands [Chen et al., 1992]. Nevertheless, although a cohort effect has yet to be clearly demonstrated, differences in birth cohorts remain a plausible explanation for a decrease in AOO in successive generations.

Recall Bias and Assortative Mating

Other potential biases in studying anticipation include recall bias, and assortative mating which could imitate anticipation if a double genetic contribution was transmitted to the children. In our study, recall bias was probably minimized by combining the information obtained from the subject's interview with the information from several relatives and from all available contemporary medical records [Maziade et al., 1992]. In addition, controlling for QOI allowed to indirectly control for a recall bias because QOI acts as a global measure of quality and quantity of information which takes into account, among several characteristics, the reliability and compliance of informants [Roy et al., 1997]. Assortative mating was also minimized in our sample given that there was no occurrence of families in which both parents were affected, and given that these families did not show evidence of bilineality, as requested for our linkage studies [Maziade et al., 1997].

Severity Indices

We did not find strong evidence for anticipation with the severity indices. Although anticipation with the hospitalization rate as defined by the percentage of subjects hospitalized for psychotic illness had been previously reported [Bassett and Honer, 1994] for SZ, we chose a different definition for HR as an index of severity to avoid any confounding effect with AOO. Indeed, AOO affects the duration of illness, which in turns affects the chances for a subject to be hospitalized across his/her lifetime. We attempted to avoid this confounding effect by dividing the number of hospitalizations by the duration of illness (in number of years). Using this definition, we observed an increase in HR between Generations 1 and 2 in the SZ and BP samples, but this could be attributed to the information bias and to the presence of extreme values. The severity of PS seemed to increase between Generations 2 and 3 in the SZ sample although no such evidence was found between Generations 1 and 2. This lack of con-

sistency across generations greatly impaired the plausibility of true anticipation with PS. There was no consistent evidence of an increase in severity of NS or GAS across generations in either the SZ or the BP sample. For NS, this lack of evidence was particularly surprising given the recent finding of an association between repeat length and NS [Cardno et al., 1999]. This could be due to a different way of measuring NS in the two studies. Our results were, however, consistent with those of Johnson et al. [1997] who also found no evidence for anticipation with NS. There was no conclusive evidence for anticipation with SOD as an index of severity: the increase in SOD observed between Generations 2 and 3 in the whole sample did not remain significant after controlling for QOI, and was not significant between Generations 1 and 2. This is not consistent with the evidence for anticipation found by Heiden et al. [1999], who used a similar but simpler approach that consisted of dividing the SZ spectrum disorders into only two categories, the most versus the less severe diagnoses. Again, the inconsistency between the two studies may depend on the method used to define the index of severity of diagnosis, on the use of QOI as a covariable in an analysis or both.

CONCLUSION

Our data allowed us to study anticipation for SZ and BP spectrum disorders across three generations while controlling for a potential information bias that, given the observed relationship between QOI and generations in our sample, could have also been present in previous studies. A fertility bias was obviously acting in our sample but could be at least partly circumvented by using all affected individuals in the families, as proposed by others [Grigoroiu-Serbanescu et al., 1997; Johnson et al., 1997; McInnis et al., 1993] and suggested by our simulation. Given the average current age of our subjects, a potential censoring bias seemed unlikely between Generations 1 and 2 and, thus, not responsible for the observed decrease of 8.6 years in AOO for the SZ spectrum disorders between these two generations. Conversely, the decrease in AOO for BP could be at least partly attributable to a censoring of observations in Generation 2. A cohort effect remains a plausible cause for a decrease in AOO in SZ, although studies that clearly demonstrate this cohort effect are still needed. As for the severity indices, an increase was not consistently found in our data across the two pairs of generations, or it could be attributed to an information bias. Although the finding of an increase in a severity index could have added support to the hypothesis of an underlying trinucleotide expansion, the lack of such evidence cannot preclude its existence.

Although we attempted to circumvent and minimize several biases as discussed earlier, none of them can be totally eliminated in such a study. Our measure of QOI seemed to have efficiently reduced biases in studying anticipation with several severity indices, but may not yet be the most appropriate measure of quality for the clinical information pertaining to AOO. Hence, although the present study does not provide a definite demonstration of anticipation in SZ or BP, our results nevertheless add support to the hypothesis of anticipation in SZ as expressed by a decrease in AOO. Consequently, we motivate further investigations of anticipation, particularly in samples not restricted to parent--offspring pairs and whose family members have already passed through the age at risk for the disorder. As studies relating repeat length and severity are emerging in psychiatric disorders [Cardno et al., 1999], the investigation of anticipation with various severity indices also seems timely to help to further characterize the phenotype that would result from a repeat expansion. Finally, given the potential information bias detected in our sample, we encourage all future anticipation studies to measure and control for the quantity and quality of clinical information in use.

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APPENDIX A

Within a family, the mean of the differences from all possible pairs (APP) between two generations is equivalent to the difference between the mean of the two generations (DIFF).

Let $X_1, \ldots, X_m$ be the values for a given severity index of m subjects in Generation 1, while $Y_1, \ldots, Y_n$ are the corresponding values for n subjects in Generation 2. Then, the mean $\bar{d}$ of the $m \times n$ differences from all possible pairs is given by:

$$
\begin{aligned}
\bar{d} &= \frac{1}{m \cdot n} \sum_{i=1}^{m} \sum_{j=1}^{n} (X_i - Y_j) \\
&= \frac{1}{m \cdot n} \left(\sum_{i=1}^{m} X_i - \sum_{j=1}^{n} Y_j \right) \\
&= \frac{1}{m \cdot n} (m\bar{X} - n\bar{Y}) \\
&= \bar{X} - \bar{Y},
\end{aligned}
$$

where $\bar{X}$ and $\bar{Y}$ are the mean of Generations 1 and 2, respectively.