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Genetics of Absolute Pitch

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I dedicate this thesis to

my mother

Mrs. Khadijeh Milaninia

and

my father

Dr. Abolgassem Baharlou

Preface

I would like to express my deepest gratitude to my thesis advisors Dr. Nelson Freimer and Dr. Jane Gitschier. During the course of my thesis I have had exceptional opportunities to tap into their intellect, wisdom and experiences. I am forever grateful for their nurturing of my scientific curiosities, for the guidance they gave me and the friendship that we shared. I also like to thank all members of Dr. Freimer's group, in particular Ms. Sue Service who has made significant contributions in planning experiments and various statistical analysis required for the completion of this work. Ms. Service has been a co-author on both papers that has resulted from my thesis.

The auditory pitch-naming task was designed in collaboration with Dr. Paul Johnston and the laboratory of Dr. Michael Merzenich at UCSF. The MEG experiments were Conducted at UCSF in collaboration with Dr. David Poeppel with assistance from Ms. Susan Honma.

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Abstract

Genetics of absolute pitch

Siamak Baharloo

This dissertation describes my investigation into the genetic bases of absolute pitch, conducted in the laboratory of Dr. Nelson Freimer.

It has been long established that the success of any genetic mapping study relies on the availability and accuracy of many parameters needed to determine the course of the mapping study. The ability to identify the pitch of tones in the absence of a reference pitch is known as Absolute Pitch (AP). This intriguing behavioral trait has been the subject of many investigations yet at the time we began this work little was established about the genetic basis of AP. Aside from anecdotal reports only one other study had investigated AP as an inherited trait but their inferences based on a small study sample.

We began our work by defining the AP phenotype in such a way that it would represent a distinct and reproducible cognitive function. The AP phenotype was defined based on the findings from a large survey of musicians as well as the data from an acoustical pitch-naming test of musicians. The acoustical test revealed different pitch naming abilities by individuals who claimed to possess AP. A subset of these individuals possessed the ability to identify the pitch of tones instantaneously and with superb accuracy. We named these individuals as having the AP-1 phenotype.

A reliable phenotype is one of the necessary components of a genetic study. An assessment of the habitability of the trait is also important to determine the most effective genetic mapping strategy. Through the AP survey and pitch testing we determined the ratio of the siblings of AP-1 probands who possessed AP-1 to the number of AP-1 individuals from the general population. Our findings are indicating a strong genetic component for AP-1.

Finally, an acoustical pitch naming test was used to screen for AP-1 families with a high loading for the trait among many potential candidate families: we were able to identify 6 such families. A gene mapping study was performed using polymorphic markers with a map density of approximately 5cM. Considering the small size of the families, hence their low power to detect linkage, lod score analysis did not reveal evidence for linkage to any locus. Yet at a number of loci positive lod scores were observed for a stretch of markers constituting a haplotype. These regions are handled as candidate localizations for the AP-1 gene or genes and will be investigated in subsequent genetic mapping studies.

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CHAPTER I

Introduction

Environment plays an important role in the development of many complex behaviors. Yet it is likely that genetic variations within our genome predispose us for the development of distinct behaviors in the presence of necessary environmental influences. One such behavior that is greatly influenced by the environmental experiences of an individual is absolute pitch (AP). AP is a specialized form of auditory perception in which the possessors of this trait can identify the pitch of tones in the absence of a reference tone. My aim in this study was to; a) define the AP phenotype in terms that will be likely to be related to specific genetic predisposition(s), b) develop quantitative measures to assess AP ability and identify a suitable study sample, c) assess familial aggregation of AP, d) devise and implement genetic mapping strategies for the identification of genes involved in AP predisposition. AP represents a small spectrum of the larger phenomenon of musicality, and it may be an ideal phenotype for investigating gene and environment interactions in the development of complex human behaviors. Furthermore, since AP is reported to be developed at a very young age, identification of its molecular basis can help us better understand early developmental events involved in formation and crystallization of auditory behavior.

Absolute pitch has been a topic of interest for a wide range of investigators from diverse fields such as musicology and psychology to genetics and neurobiology. Following is an introduction to some of the cognitive and biological aspects of AP.

Neural Correlates of AP

Electrophysiological measures such as event related brain potentials (ERPs) have been employed to identify the physiological correlates of AP. Several of these studies have suggested that working memory mechanisms for pitch are different in AP individuals compared to non-AP musicians, and have attributed the superior ability of AP possessors in pitch identification tasks to their ability to "access permanently resident representation of tones" (Klein et al. 1984). In other words, AP possessors do not utilize working memory mechanisms used by non-AP individuals during pitch identification tasks. One component of ERP measurements, the P300 wave, is a measurement that has been suggested to be a manifestation of the updating process of working memory. The context-updating theory of P300 (Donchin and Coles 1988) states that in "oddball experiments," where subjects are asked, for example, to discriminate two tones of different pitch presented in a Bernoulli series in which one of the tones occurs more frequently than the other, the less frequent tones will elicit a larger P300 wave. In other words, the P300 wave is generated during the process of updating the content of the working memory; rare stimuli require greater restructuring of memory and hence elicit a larger P300 wave. Klein et al (1984) reported that AP individuals performing auditory "oddball" tasks demonstrate very small P300 peaks. This difference in P300 peak

amplitude was specific to the auditory system, as "oddball" visual probes in AP individuals elicited P300 peaks comparable in amplitude to those in controls. The authors concluded that AP possessors do not need to fetch and compare representation of tones for pitch identification, and instead possess a permanent memory of different pitches. Subsequent studies regarding the P300 activity in AP possessors have produced mixed results, some agreeing with observations made by Klein et al. (Wayman et.al. 1992, Hantz et. al. 1992), and some contradictory to those observations (Johnstone, P. personal communication). These inconsistencies have been attributed to variations in the criteria used by different groups in the classification of AP individuals (Dunchin, E., personal communication). In fact, if some level of stratification is applied to the group of AP possessors participating in the study by Johnston according to their performance in pitch naming tasks, results more consistent with that of Dunchin's group is obtained.

Positron Emission Tomography (PET) studies measuring cerebral blood flow were conducted by Zatorre et al. (1998) to observe differences in brain activity between non-AP musicians and AP musicians during pitch discrimination tasks. Their results showed that in AP individuals, there was increased cerebral blood flow, indicating higher activity, in the posterior portion of the dorsolateral frontal cortex, which receives projections from the planum temporale (PT), an area of the associative auditory cortex believed to be related to language processes. They concluded that AP perception may arise from differences in neural processing within the superior temporal region.

Other types of studies have been useful in providing relevant information on the neuroanatomical basis of AP. First are the case studies, the most reliable of which is the study of a young AP possessor who underwent a left temporal lobectomy to relieve epileptic seizures (Zatorre 1989). The subject was tested for AP prior to and after the operation. Despite the removal of portions of the left anterior and medial regions of his brain, he retained his AP ability, indicating that AP ability does not depend on the integrity of these areas.

Magnetic Resonance Imaging (MRI) studies have also elucidated some neuroanatomical correlates of AP perception. Schlaug et al. (1995), applied in vivo magnetic resonance morphometry to measure anatomical asymmetry of the planum temporale (PT) in AP possessors. The authors demonstrated a stronger leftward PT asymmetry in AP subjects compared to non-musicians or non-AP musicians. This evidence suggests that the PT may play a role in AP perception, but it is not clear whether such neuroanatomical asymmetry causes the development of AP, or if the cortical changes observed are a result of auditory processing in AP individuals. Although the main architecture of the PT in humans is completed between the 29^{th} and 31^{st} week of gestation, the maturation of fiber tracts and intracortical neuropil that are determinants of gyral shape continues until about age seven. Music training at an early age may facilitate activity dependent changes in AP individuals that can lead to asymmetry in the PT directly or indirectly as the result of activation of other neurocircuits that interact with the PT.

One thing that is clear from the studies discussed above is that AP is a distinct cognitive process that most likely involves the associative areas of the auditory cortex. However, there are different theories for the etiology of absolute pitch. Based on her work with native speakers of tonal languages, Diana Deutsche has put forward the hypothesis that all humans are born with the ability to develop AP, yet after the first year of life this potential is lost (personal communication). However, her work on absolute pitch does not contradict our findings, as she uses a much broader definition in categorizing AP individuals. According to her definition, individuals speaking tonal languages but who cannot associate musical tones with a name or label are considered to have absolute pitch. Since individuals speaking tonal languages associate a distinct meaning to a certain pitch of a word, Deutsche et al. consider these people to be AP possessors. We have identified individuals speaking tonal languages who have had early musical training, but do not possess AP according to our definition. This suggests that the abilities to associate a pitch with a musical label (musical AP), and a non-musical label (linguistic AP) are two distinct cognitive traits.

It is possible however, that the developmental mechanisms involved in these two forms of absolute pitch could somehow be related. While Deutsche's hypothesis argues that a critical period of one year exists for the development of linguistic AP in all individuals, our observations show that for the development of musical AP, the critical period extends to the first six or seven years of life, and that it is not present in all humans. A logical extension of Deutsche's hypothesis is that if there exists a developmental critical period during the first year of life when anyone can attain linguistic AP, perhaps this period is

extended in some individuals who can develop musical AP as they are exposed to music lessons in early childhood. Psychophysical tests in young musical AP possessors who do not speak tonal languages can be used to assess the ability to develop association between pitch and linguistic meaning beyond age one. These experiments could shed light on the question of critical period for development of linguistic AP verses musical AP. If such young AP possessors were able to develop associations between linguistic representation and pitch, one could conclude that similar mechanisms are involved in these two forms of AP.

It is reasonable to hypothesize that musical AP individuals possess some sort of innate biological predisposition for the development of AP. While AP development is dependent upon certain environmental factors (i.e. music training), AP is not developed by all musicians who receive music training before age 6 (as it will be demonstrated in the following chapter). One can conclude from this that early music training is necessary, but not sufficient in the development of AP, and that certain biological factors may predispose an individual to develop AP. Sequence variation(s) in a single gene or a number of genes could lead to biological changes that render an individual susceptible to the development of AP. One can hypothesize that any genetic factors that influence AP development may include genes involved in early brain development. This is supported by the results of our epidemiological study on AP (discussed later), which clearly demonstrate that AP cannot be developed by adult musicians. Furthermore, our results show that the critical period for AP is roughly limited to the first 7 years of life, which coincides with the period of development during which the human brain is more

amenable to changes in its neurocircuitry. If the processes that are activated by the variant gene(s) involved in AP are, indeed, developmentally regulated, understanding the molecular basis of AP may identify some of the molecular substrates that are involved in regulating early developmental events in the human brain.

Genetic basis of AP

Profita and Bidder 1988, reported families with multiple AP individuals and proposed an autosomal dominant mode of inheritance with incomplete penetrance for AP. These authors calculated a range of segregation ratios of 0.24 to 0.37 for AP (assuming a single and complete ascertainment, respectively), and concluded that these ratios were "too large to be consistent with a multifactorial determination." The study by Profita and Bidder was the only one of its kind that had attempted to establish some of the basic parameters necessary to begin the genetic study of AP. Yet, the number of families with multiple AP possessors was too few (N=10) to yield reliable segregation ratios. The other notable study on the familial basis of AP is the study of 103 individuals by Bachem (1955), where the AP possessors reported 41 relatives who were similarly endowed.

In search of the molecular components of AP, the goal of my thesis is to lay the groundwork necessary for identification of the genes predisposing individuals to develop AP. At the onset of any genetic study many pieces of information need to be in place. For example a well characterized phenotype and means for the identification of the individuals with the desired phenotype need to be in hand. Furthermore, measures of the heritablity of the trait are helpful to estimate the type and the size of the sample that is

needed for mapping of that trait. Although the cumulative work of research has illuminated many intriguing features of AP, at the onset of this study very little was known about AP as a genetic trait. At the heart of matter lay the very definition of AP. The definition most widely accepted at the time included any individual with the ability to identify pitch or produce a tone on demand in the absence of a reference pitch. Here is a brief introduction to the various classification schemes that have been used to identify AP possessors.

In Western music, an octave is defined as the interval formed by two pitches whose fundamental frequencies (smallest frequency component of a tone) have the ratio of 2:1. An octave is divided into 12 logarithmically equal parts, each corresponding to a different pitch class. The distance between two adjoining pitch classes is known as a semitone. A semitone corresponds to 100 cents, and the 12 intervals of the scale correspond to integral multiples of 100 cents (Blzano, 1984). The fundamental frequencies of two tones that are a semitone apart have a 2 1/12: 1 ratio. Most western orchestras are tuned to middle A or A4 = 440 Hz.

To measure AP ability, most commonly, subjects are presented with musical tones and are asked to identify the pitch of those tones. Great procedural variability exists among tests conducted by different groups. For example, in some tests subjects were presented with piano tones, rich in musical cues such as timbre and overtones, while others used sine wave tones which do not possess any musical qualities (Takeuchi and Hulse 1993). Large variability existed in the number of tones presented (12 to 180), and in the range of

the register of tones presented (C1 to C7 vs. E3 to D#5) to subjects in a given test. In most cases octave errors were ignored, and in some cases 0.5 or 1 semitone errors were also ignored. Finally, each separate study involved only a small number of participants ranging in number from 3 in the smallest study to 22 in the largest there are also differences in testing procedures. For example, some investigators allowed test subjects a few practice trials before beginning the tests, or provided them with unlimited time to identify the tones. Hence, the description of an AP possessor depends on the parameters that are used to gauge the AP ability in various studies.

In order to investigate its probable genetic components, the AP phenotype needs to be described in terms that are most likely to represent a singular and quantifiable biological process. The first aim of this study was to define a specific form of the AP phenotype that was most likely to lead to the identification of its underlying genetic components.

The survey of musicians described in chapter II was intended as a tool to gather first hand information on the range of pitch identification capabilities that self-reported AP individuals possessed. In this survey (see appendix IV), respondents were questioned about the different features of their AP ability such as: the speed and accuracy of their pitch identification, the length and type of their musical training, the age that they first began formal music training, and finally whether they had other family members who also possessed AP. The AP survey was distributed among musicians with varying degrees of music experience, but in general, the entire group of surveyed individuals was

highly proficient, with at least 3 and often more than 8 years of music training in various instruments.

Evaluation of responses to the survey questions indicated that while individuals reported varying fidelity and speed in pitch identification tasks, there was a group of individuals who reported instantaneous and very accurate ability in identifying the pitch of tones. We reasoned that such individuals are more likely to constitute a phenotypic group that was distinct from the others who reported varying fidelity in pitch identification.

It is possible that individuals with lesser fidelity in pitch identification tasks possess a superior form of relative pitch (RP) and not AP. As opposed to AP possessors that can assign a verbal label to a tone in absence of a reference tone, RP possessors can assign a label to the interval between two tones. If the RP individuals know the name of the reference tone they can identify the interval between the reference tone and the test tone, and assign a name to the test tone. For example if the RP possessors are presented with two tones, A as the reference tone and an unknown tone 700 cents away, the interval between the two tones is labeled as a "fifth," and the second tone is labeled as an E. Of course this process involves multiple time consuming computational steps such as identification of the interval and labeling of the tone based on that interval. Some individuals with RP may have developed one or a few internal references that they can replay in their head, or sing. When presented with an unknown tone, such RP individuals can identify that tone by determining its interval with their own internal reference. It is possible that some of the individuals who claim to be AP possessors, but whose pitch

identification is not instantaneous, are indeed RP possessors who have developed such internal references. RP possessors are also more prone to making errors in naming tones, as they need to rely on active memory based processes. Setting of stringent criteria for identification of AP individuals is likely to significantly reduce the chance of incorporation of phenocopies, such as RP individuals, into the study.

While analyzing the data obtained from the survey, we considered only those individuals who claimed that their AP was instantaneous and very accurate to be AP possessors. The results of the survey are discussed later in chapter II, but in summary, we observed a strong correlation between the age of initial music training and the development of AP. Ear training (learning to identify musical intervals) did not correlate with AP development. Correlation between the age of initial music training and AP had been proposed by other studies [(Sergant 1969, Miyazaki 1988b, Bachem (1940, 1955)], and our results, which were obtained from a very large sample size, confirm the role of early music training in AP. Although ear training does not appear to be involved in the development of AP, our results do not preclude the role of other influence such as the intensity of the musical training in the etiology of AP.

The results of the survey also point to the possibility that AP may be familial, and hence, may have a heritable component. In order to quantify the familial aggregation of AP, we decided to measure the prevalence of AP in families compared to its prevalence in the general population. The observations from this study are discussed later.

The next step after phenotype definition is to develop a quantitative measure for the assessment of AP ability in individuals. Our goal was to use this auditory test to categorize AP possessors into sub-groups based on their performance in the auditory test. We reasoned that the individuals with the most extreme phenotype, in this case, those who performed 3 standard errors above the mean in two different tests, qualified as our target sample group. The main objective for setting such stringent criteria was to decrease the phenocopy rate (i.e. to exclude individuals with RP). We required the subjects to identify the pitch of tones in a rapid manner in order to exclude those individuals who may rely on RP strategies to identify the pitch.

The pitch testing results on a group of self-reported AP possessors are reported in chapter II. Yet there are a few additional observations that are worth mentioning here. First, there is a group of AP possessors that are labeled as AP-4. These individuals have a superb ability to identify the pitch of piano tones but perform poorly in identification of the pitch of pure tones. At first we concluded that these individuals relied on the timbre of tones (perceived acoustic qualities of a tone not related to pitch) to identify their pitch, hence familiarity with the musical instrument was essential for pitch recognition. To test the validity of this hypothesis, we presented these subjects with computer generated pure tones along with various numbers of overtones of the same pitch. To our surprise, AP-4 subjects performed to the same level on this task that they did with piano tones. We concluded that the presence of the additional cues in the overtones, rather than the timbre of the tones, facilitated the pitch identification in AP-4 individuals. This may point to different mechanisms of pitch perception

The other interesting observation made was that AP possessors, who on average were over 45 years old (we encountered 19 such AP possessors in our study), reported that their pitch perception had shifted 1/2 of a tone sharper as they grew older (roughly after the age of 45). This shift in perception was difficult to measure during the auditory tests, as subjects constantly attempted to adjust by 1/2 tone their perception of the pitches. Yet in a few cases, in which subjects deliberately attempted not to adjust their perception, these subjects scored consistently one 1/2 tone sharper (>90%). In two individuals who were over 65 years old, this shift was a whole step. This shift in pitch perception as a result of aging has been anecdotally reported by others (Takeuchi and Hulse 1993). It is believed that this shift is the result of the loss of the outer hair cells (OHC) that modulate the inner hair cells. The loss of OHC is believed to affect the mechanical properties of the basilar membrane, which is one of the primary sensory organs of the auditory system. The loss of the outer hair cells is a consequence of normal aging, although environmental factors such as loud noise or infections may destroy these cells. We concluded that the shift observed in the perception of tones in older individuals is not related to AP and is the result of changes in the peripheral organs of the auditory system.

CHAPTER II

Absolute Pitch: An approach for identifying genetic and non-genetic components

Abstract

Absolute pitch (AP) is the ability to recognize a pitch without an external reference. By surveying more than 600 musicians in music conservatories, training programs, and orchestras, we have attempted to dissect the influences of early musical training and genetics on development of this ability. Early musical training appears to be necessary but not sufficient for development of AP. Forty percent of musicians who began training at age 4 or less reported AP, whereas only three percent of those initiating training after age 9 did so. Self-reported AP possessors were four times more likely to report another AP possessor in their families than non-AP possessors. These data suggest that both early musical training and genetic predisposition are needed for the development of AP. We developed a simple computer-based acoustical test which has allowed us to subdivide AP possessors into distinct groups based on their performance. The results of this study form the foundation for future investigation of the genetic basis of AP.

Introduction

Understanding of any complex behavior requires dissection of its genetic and non-genetic elements. Methods are available for elucidating the genetic basis of a given trait (Lander and Schork 1994); however, the non-genetic factors that contribute to particular behaviors are usually less well-defined and hence more difficult to investigate. Therefore, human behavior is likely to be understood best by studying traits for which it is possible to evaluate quantitatively both genetic and non-genetic factors that contribute to their development. In this paper we present evidence suggesting that absolute pitch (AP), also known as perfect pitch, exemplifies such a trait. We also indicate the steps that we have taken to lay the groundwork for future studies aimed at identifying genes responsible for predisposition to AP.

AP refers to the ability to recognize the pitch of a musical tone without an external reference pitch. There is general, although not universal, agreement that to be considered an AP possessor, an individual must have the ability to recognize pitches accurately and instantaneously (Takeuchi and Hulse 1993). Interpretation of the existing literature on AP is complicated by the fact that there has been substantial variation in how AP has been operationally defined and tested. For example, some investigators have insisted that AP possessors be capable of producing specific tones without reference, either vocally or using a tone generator (Petran 1932; Revesz 1953), while others have focused only on the ability to recognize a pitch (Balzano 1984; Takeuchi and Hulse 1991). Similarly, some investigators have tested subjects for the ability to actually identify the pitch of tones with

different timbres such as sine-wave tones or piano, while others have limited testing to tones of a single timbre (Rakowski et al. 1987; Miyazaki 1989).

One of the goals of our study was to achieve a working definition of AP that would permit reliable identification of AP possessors for genetic studies. As described in this paper, we attained this goal through a survey of musicians and by conducting auditory tests to measure the accuracy of pitch recognition by self-reported AP possessors.

Although much is known about the anatomy and physiology of the human auditory pathway, the specific neural substrates involved in pitch perception remain unclear. Psychophysical and physiological experiments suggest that high-level cortical processes are involved in pitch perception (Klein et al. 1984; Zatorre et al. 1993). Recent Positron Emission Tomography (PET) studies of musicians with and without AP indicate that anatomical asymmetry of the planum temporale (an associative auditory area of the brain) may be associated with the processing of pitch perception (Schlaug et al. 1995). Although these and other studies offer some information about the neurobiology of auditory perception, there is no evidence regarding underlying developmental mechanisms that may play a role in these processes. Isolating genes responsible for AP could illuminate the developmental basis of pitch perception. Genetic studies of human behavior present researchers with problems ranging from the phenotypic definition of a behavior and the selection of optimal methods for genetic analysis to the ethical and social issues inherent in the genetic study of human behavior. AP is an intriguing ability

that is free of social stigmas, and can provide a model for the genetic studies of behavioral traits involving human perception.

We hypothesize that development of AP could depend on both genetic and non-genetic influences. The genesis of neural circuits for many animal behavioral traits and attributes follows a developmental blueprint which is largely determined genetically. For example circadian activity patterns in mice can be substantially altered by mutations in a single autosomal gene (Takahashi et al. 1994). A number of studies suggest a genetic basis for AP (Bachem 1940 and 1955; Revesz 1953). Most recently Profita and Bidder (1989) presented pedigrees with a high familial incidence of AP. They suggested that, in these families, AP was inherited as an autosomal dominant trait with incomplete penetrance.

However, much evidence also supports the importance of non-genetic factors, during a developmental "critical period", for the genesis of AP. There is abundant evidence that neural activity in response to sensory stimuli during a particular stage of development is required for the formation of certain neural circuits (Goodman and Shatz 1993). The window of opportunity during which neural activity can determine the outcome of a particular circuit is known as its developmental critical period. Sergeant (1969), Miyazaki (1988a), and Takeuchi (1989) reported that a high proportion of musicians who began their musical training at a very early age developed AP. These studies suggested that there is a developmental critical period (before age seven) during which musical training will lead to development of AP. It has been argued that such a critical period coincides with the period during which children's speech perception becomes specialized

for the sounds of their native language (Takeuchi and Hulse 1993). The evidence is persuasive for the role of environmental elements, in the form of early musical training, in the development of AP; however, this clearly does not exclude the possibility that there is also a genetic component to the etiology of AP.

One of the difficulties in evaluating the evidence for both genetic and non-genetic influences on AP is that most previous studies have been conducted using small samples (Sergeant 1969; Miyazaki 1988a and Miyazaki 1988b; Profita and Bidder 1988; Takeuchi 1989). The evidence presented in this paper is derived from a large sample of musicians. Here we describe a survey of music students enrolled in music schools and conservatories and of professional musicians. The aim of the survey was to assess the role of musical training in the development of AP, and to evaluate whether this trait aggregates in families. Data in this paper support the hypothesis that both genetic and non-genetic factors contribute to the development of AP.

Subjects and Methods

The initial AP survey

In order to obtain background information on AP, a survey was distributed to musicians and music students in several music institutions and music performing groups. The survey was approved by the Committee on Human Research at the University of California San Francisco and additional approval was obtained from the appropriate

authorities at each institution or music performing group. A copy of the survey is included.

A total of 900 surveys were either sent out by mail for distribution or were distributed on site by the interviewer. Surveys were handed out to musicians in classrooms or before rehearsals. We received completed surveys from 612 individuals from the following music institutions and music performing groups: the San Francisco Conservatory of Music (110 surveys were distributed in the class rooms, 105 students and 2 faculty responded), the University of California San Francisco Symphony (40 surveys were distributed before a practice session, 32 musicians responded), the San Francisco Symphony Youth Orchestra (50 surveys were sent for distribution before a practice session, 46 musicians responded), the University of California Berkeley Music School (85 surveys were distributed in the class rooms, 79 students responded), the Peabody Conservatory of Music (200 surveys were sent out to be distributed in the class rooms, 133 students and 3 faculty responded), the Curtis Institute of Music (surveys were handed out to every student who walked through the main entrance, all 75 students who received the survey responded), the La Scala Opera (40 surveys were sent out for distribution, 30 chorus singers responded), the Aspen Music School (150 surveys were sent out for distribution before practices, 65 students responded) and the Interlochen Center for the Arts (150 surveys were sent out for distribution before practices, 42 students responded).

The text at the beginning of the survey explained the purpose of the survey and emphasized that it was important for our study to have all individuals respond, regardless

of whether or not they possess AP. In this survey we had three aims: 1) To estimate the percentage of musicians in our sample group who claim to possess AP, 2) to ascertain whether the age of first formal musical training correlates with development of AP, and3) to find out whether AP aggregates in families.

In order to determine whether respondents possess AP, we inquired about their ability to identify the pitch of tones in the absence of an initial reference. To make the responses of those who claimed AP as objective as possible, we asked specific questions dealing with the speed and accuracy of pitch judgment, whether the subjects' AP depended on the tones generated by a particular instrument, and whether the subjects could vocally produce tones without first hearing a reference tone. To assess the role of musical training in the development of AP we asked respondents about the extent of their musical training and the age of their first formal music lesson. In order to determine whether AP is aggregated in families we asked the respondents about the presence of AP among their first degree relatives. At the end of the survey we asked the respondents to include their name, address and phone numbers if they were willing to participate in our auditory tests for perfect pitch.

Associations between AP status and other variables were assessed with X^2 contingency table analyses or Fisher exact tests.

Testing for AP and follow-up interviews

Portable auditory tests were developed for assessing AP ability in the self-reported AP possessors. Two types of tones were used as the stimuli for the tests; pure sine-wave tones, and real piano tones. Digitized tones were stored on a portable computer (Apple 190), and were delivered to the subjects via headphone. Twenty nine self-reported AP possessors and ten musicians who did not report AP were tested.

Stimuli

Sine-wave tones were digitally synthesized (16-bit, sampling rate 44.1 kHz) as text files on a Silicon Graphics Indy workstation using MatLab software (The Mathworks Inc, Natic, MA), and converted to standard AIFF audio files using conversion utilities. Tones of different frequencies were synthesized with different amplitudes to equalize perceived loudness (as judged by SB & PJ). The tones had durations of 1000 msec, with onset and offset ramps of 100 msec. Sine-wave tones had frequencies corresponding to the 40 musical notes from C2 to G#8 based on A4 = 440 Hz. Frequencies in the first octave were not used, as pure tones in this range were not reproduced clearly with our equipment.

Piano tones were taken from a CD produced by McGill University ("McGill University Master Samples"), containing professionally sampled tones from a 9' Steinway grand

piano tuned to A4=440 Hz. 40 piano tones from C1 to G#7 were digitally recorded from the CD to a MacIntosh PowerPC and were edited using SoundEdit 16 software (Macromedia, San Francisco, CA) to have uniform durations of 1000 msec, with offset ramps of 100 msec. Half of the tones presented to the subjects were equivalent to those represented by the white keys on the piano and half of the tones were equivalent to those represented by the black keys. Tones from the 8th octave were not used because of the insufficient duration of such high notes on the piano.

Pitch testing procedure

Two tests were administered to each subject. The first test consisted of 40 pure tones, the second of 40 piano tones. These tests were divided into 4 blocks with 10 trials in each block, with three second intervals separating each trial. Tones were played in pseudorandom order with the constraint that successive tones were separated by more than two octaves and a semitone. As subjects listened to the tones, they were asked to make an instantaneous judgment of the pitch of each tone and write it on a sheet of paper. Subjects were not allowed any practice runs, nor was any feedback regarding their performance given until the testing was completed.

Analysis of test results

Responses to the auditory tests were scored in the following manner: All correct judgments were given one point. Because previous studies as well as anecdotal reports suggested that AP is accurate to within a semitone (Baggaley 1974; Miyazaki 1988a), errors of one semitone were given 3/4 of a point in order to distinguish between those AP possessors who make semitone errors and those who do not. Judgments further than a semitone from the actual pitch were given no points. Tones at the extremes of each (4 pure tones in the 8th octave and 4 piano tones in the first octave) were excluded from scoring, as performance of self-reported AP possessors and non-AP possessors was indistinguishable in these registers.

Scores on pure and piano tones for the 26 subjects examined with the auditory test were evaluated by a cluster analysis to identify different "subtypes" of AP ability. The Euclidean distance between subject's scores in the 2-dimensional space of pure and piano tones was used as a measure of distance between individuals in these two dimensions. The groups were identified from the results of a hierarchical clustering algorithm, employing the average method (Maridia et al. 1979). In hierarchical clustering, the 26 subjects initially compose 26 clusters, and are sequentially joined into larger clusters until all 26 individuals are in one cluster. Subjects are joined to the cluster to which they are closest in Euclidean space.

Family and musical history interview

Subjects who participated in the testing for AP were also interviewed. Detailed questions were asked regarding their musical training, such as the exact age of their first musical lessons, whether their early music lessons included any particular methods of ear training, and the reasons why they began music lessons when they did. A detailed pedigree was drawn of the family of each subject. For the first degree relatives of each proband the following information was obtained by report of the proband: the extent of musical training, the age of the first formal music lessons, and whether they possess AP.

Results

The initial AP survey

Out of 900 surveys that were distributed or were sent out for distribution among musicians for the purpose of obtaining background information on AP, 612 (68%) were completed and returned. As described in the Subjects and Methods section, we provisionally designated as AP possessors only those individuals who claimed that their pitch identification ability was instantaneous and who also reported that they rarely err in judging the pitch of tones. In the population that we surveyed, the frequency of self-

reported AP was 15% (92 of 612 respondents). There were no significant differences in proportion of AP possessors between male and female respondents (data not shown).

Age of first musical training

To determine whether the age of first formal musical training correlates with the development of AP, we grouped all the musicians participating in the survey based on the age of their first formal musical training. As shown in figure 1, 29 of 72 individuals (40%) who began their musical training before age 4 reported AP. In contrast, 43 of 160 individuals (27%) who had their first musical training between ages 4-6, and 13 of 161 (8%) of those who had their first musical training between ages 6-9 reported AP. Only 4 of 104 individuals (4%) who began musical training between ages 9 and 12, and 3 of 112individuals (2.7%) who began musical training after age 12 reported AP.

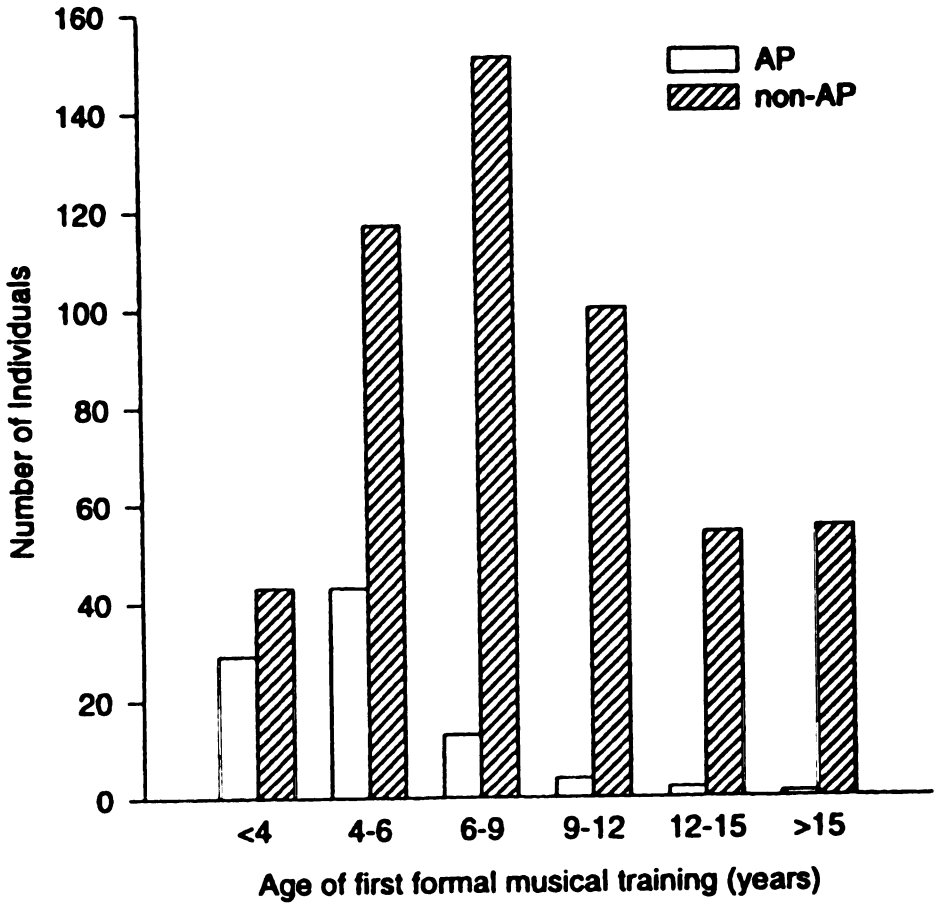
160
140
120
100
80
60
40
20
0
Number of Individuals
AP
non-AP
<4
4-6
6-9
9-12
12-15
>15
Age of first formal musical training (years)

Figure 1 Development of AP correlated with age at first formal musical training. The number of AP possessors and non-AP possessors is based on the self-report of the survey respondents.

Type of early musical training

We aimed to evaluate whether a particular type of early musical training (ear training) correlates with the ability to develop AP. Among the respondents to our survey, ear training is not correlated with development of AP. Of the self-reported AP possessors, only 28 of 62 individuals (45%) who began music lessons before age 6 and knew about the type of music lessons that they received reported that their early musical training included ear training. Only 41 of 119 (34%) non-possessors of AP who knew about the type of the music lessons they received before age 6 reported ear training as part of their early music lessons . There is not a significant association between the possession of AP and whether musical training included ear training (X^2=1.98, 1 d.f., p = 0.16).

AP attributes

Previous studies have suggested that AP possessors may have the ability to vocally produce any tone without a reference. In response to the question of whether subjects are able to vocally produce any particular pitch without first hearing a reference tone, 85 of 92 (92%) AP possessors replied positively.

Other studies have suggested that AP ability may depend on the timbre of a particular instrument with which individuals are most familiar. It has been hypothesized that timbre of familiar musical instruments may provide some AP possessors with cues necessary for

pitch identification (Ward and Burns 1982; Balzano 1984). In our survey, most of the AP possessors (73 of 92, 79%) indicated that they can identify the pitch of tones produced by any instrument. However, the results of our behavioral testing of a subset of these AP possessors indicates that timbre may be important for a subgroup of AP possessors (see below).

Familial aggregation of AP

To evaluate whether AP aggregates in families, subjects were asked to indicate if they were aware of any family members whom they believed to be AP possessors. Of the self reported AP possessors, 44 of 92 (48%) indicated that they had first degree relatives who also possessed AP. In contrast, only 72 of 520 (14%) AP non-possessors reported first degree relatives with AP ($X^2 = 38.6$, 1 d.f., $p < 10^{-5}$). Approximately equal proportions of self-reported AP possessors and non-AP possessors (28% and 32% respectively) reported that they did not know whether any of their first degree relatives possessed AP. These results suggest that AP is aggregated in families and may indicate that a genetic mechanism is involved in development of AP.

To gain information as to whether familial aggregation of AP may have a genetic basis, we performed an additional analysis in those families in which both the survey respondent (regardless of whether they have self-reported AP) as well as one or more siblings received musical training before age 6. Of 15 siblings of self-reported AP possessors who received early musical training, 9 were reported by the probands to

possess AP. Twenty-three siblings of respondents without self-reported AP received musical training before age 6, and only two were reported by the probands to possess AP This difference between the AP status of siblings of AP possessors and AP non-possessors was significant ($p = 0.0001$).

Testing for AP and follow-up interviews

To develop a quantitative measure of the AP ability of survey respondents we began testing these individuals using auditory tests designed in our laboratory. To date we have tested 16 individuals out of the 92 self-reported AP possessors identified through the survey as well as 10 self-reported AP possessors who came to our attention through word of mouth among musicians. The self-reported AP status was given to an individuals who climed that they rarely missed naming the pitch of a tone correctly. They also claimed that they could identify the pitch of tones in 3 seconds or less. These individuals are considered probands for future genetic studies. For three of the 26 individuals, we have also tested a sibling who was reported by the proband to possess AP. Interviews were conducted with these 26 individuals in order to determine the following information; their family pedigree structure, ethnic background, and history of musicianship among their family members. Pedigree drawings for these 26 families are shown figure 2. In ten families multiple siblings with AP were observed, and in 5 families a parent and at least one child were noted to possess AP.

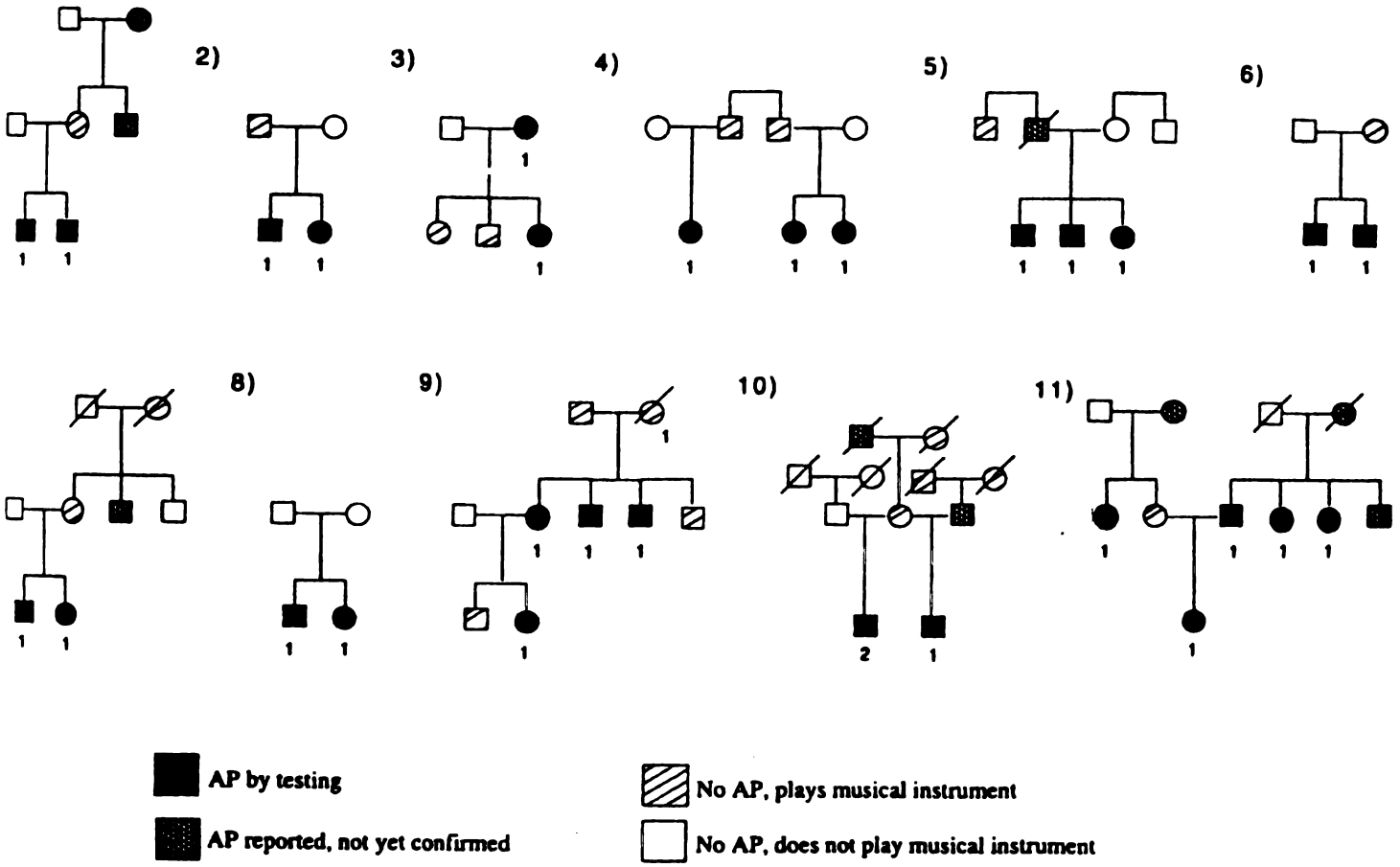
2)
3)
4)
5)
6)
8)
9)
10)
11)
AP by testing
AP reported, not yet confirmed
No AP, plays musical instrument
No AP, does not play musical instrument

Figure 2 Pedigree drawings for families of individuals with AP. For each pedigree, all available self-reported AP possessors were tested. Individuals were assigned to an AP category on the basis of their scores on our auditory tests, and the A_-group number is indicated bellow the symbol for each individual who was tested. Musical ability and AP status of family members who were not available for testing were reported by the proband, for each family.

Testing for AP was performed on the 26 self-reported AP possessors described above. Figure 3 demonstrates the distribution of the AP possessors based on their performance in acoustic tests, when either piano tones or pure tones are used as the stimuli. We observed clustering of absolute pitch subjects into distinct categories, using the procedure described in Subjects and Methods. Based on the observed clustering of the subjects' scores we divided them into three different groups. The maximum score that could be obtained in each test was 36. Individuals in group 1 (n=12) performed about equally well with piano tones (mean score of 35.6) and pure tones (mean score of 34.6). The members of group 2 (n=9) performed with less accuracy than the individuals in group 1, with mean scores of 32.8 and 28.8 for performance in tests with piano tones and pure tones respectively. The mean scores for the 4 individuals that we designated as being in group 3 was 23.4 with piano tones and 20.9 with pure tones. One individual could not be assigned to any of these groups, scoring 33 points in the test with piano tones but only 18 points with pure tones as the stimuli. In contrast, musicians who did not report AP displayed mean scores of 6.5 and 7.5 in the tests with piano tones and pure tones respectively.

The 12 individuals in group 1, constituting 46% of the total group of self-reported AP possessors whom we tested, were designated as "accurate" AP possessors. These individuals have an instantaneous and highly accurate AP ability which is independent of the timbre, harmonics, pitch class, and pitch register of the tones.

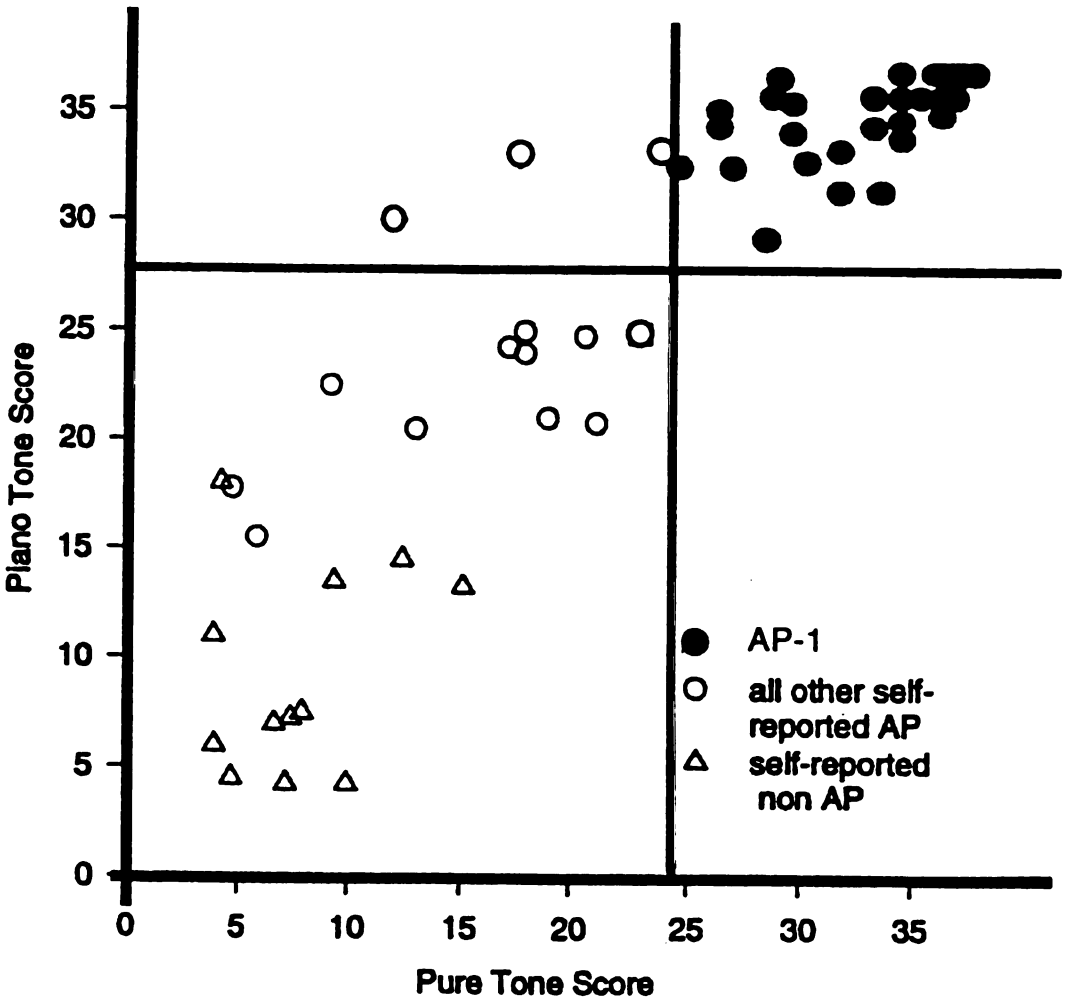
Piano Tone Score
Pure Tone Score
0
5
10
15
20
25
30
35
AP-1
all other self-reported AP
self-reported non AP

Figure 3 Scatter plot produced on the basis of pure-tone and piano-tone scores of a combined sample of self-reported AP possessors and self-reported non-AP individuals, examined with auditory test for AP. The mean scores and standard errors were obtained by calculating the average score of 12 randomly selected self-reported AP possessors and the 12 non-AP musicians. This procedure was repeated 100 times and the mean scores as well as 2 and 3 standard errors from the mean were calculated.The maximum score obtainable for piano tones and pure tones was 36. Subjects' AP status was assigned as described in the text. Vertical dashed line indicates the mean pure-tone score +2 SE; the vertical solid line indicates the mean pure-tone score +3 SE; and horizental solid line indicates the mean piano-tone score +3 SE.For the 26 probands mentioned above, the AP status of other family members was determined. Those first degree relatives who were reported by the probands to possess AP were described as having the same accuracy and speed in pitch identification as the probands themselves. In 3 families such an additional sibling was available for testing and was tested. In 2 out of the 3 cases where a proband and his or her sibling were tested, their scores placed them in the same group. In one case the proband belonged to group 2 and his sibling could be placed in group 1.

Discussion

This study was undertaken in order to obtain a more complete characterization of the AP phenotype than was available from previous studies; its ultimate goal was to set the stage for future studies aimed at identifying genes that predispose to AP. Our study of more than 600 musicians and music students from a wide range of institutions and groups has permitted us to draw broad conclusions about the development of AP with more confidence than was possible in previous studies conducted with samples of only a few individuals with AP or with less rigorous criteria. Our study clearly suggests that both inherited and environmental influences affect the development of AP.

As many of the main points made in this chapter are based on survey results, we must point out some caveats to our conclusions. We attempted to make the sampling process as unbiased as possible, by urging all survey recipients to respond. Furthermore in many instances surveys were distributed before classes or practice sessions and were collected immediately. Yet our sampling was not designed to be completely random and it is possible that response rates differed between individuals with self-reported AP and those without self-reported AP. Our results may not be generalizable to musicians as a whole, however, because of the diversity and the size of our sample we believe that it is sufficiently representative of the musicians for the purpose of this study. A second caveat regarding the interpretation of our survey derives from the fact that individuals were asked to judge their own AP ability and the AP ability of their family members. Although we have attempted to make their responses more objective by asking specific detailed questions regarding the nature of their pitch perception abilities, some

respondents may have been more critical than others in judging their own or their family members' AP ability. However, the results of our auditory testing suggest that self-reports of AP were in close accord with objectively measured AP ability; with the exception of two individuals all of the self-reported AP possessors whom we tested performed substantially better than any of the self-reported non-AP possessor musicians.

Previous studies have suggested that musical training at an early age is the single most important factor for the development of AP (Takeuchi and Hulse 1993). Authors of these studies have proposed that intensive musical training during the first 6 or 7 years of life leads to AP. The current study confirms that early musical training is indeed an important element for development of AP, as nearly all of the self-reported AP possessors in this study reported that their formal musical training began before age 6. The correlation between early musical training and AP could be explained by a developmental critical period for AP during which the brains of some individuals are particularly amenable to establishment of new circuits or fine-tuning of pre-existing circuits involved in pitch perception. The existence of such a developmentally critical period has been demonstrated previously for the singing behavior of song birds and for the development of language in humans (Doupe 1993; Neville 1991).

However, an additional possible explanation for the correlation between AP and early musical training is that some individuals inherit a predisposition for development of AP and that these individuals may be more likely than others to start musical training very early in their lives. According to this hypothesis, AP may be a part of the central

phenomenon of musicality, and an early interest in music could be the result of greater tonal acuity and increased awareness for sounds in predisposed children.

Musical training during a developmentally critical period does not entirely explain the development of AP, as the majority of the respondents in our study who reported that they had formal musical training before age 6 also claimed that they do not possess AP. Furthermore, we were unable to demonstrate qualitative differences in the type of early musical training received by AP possessors and non-possessors. Therefore, the results of our study are consistent with the hypothesis that early musical training is necessary but not sufficient for the development of AP.

Insufficiency of early musical training for the development of AP suggests that other factors must be present concomitant with early musical training in order for one to develop AP. We propose that one such factor, and perhaps a major one, is the inheritance of predisposing genes for AP. The likely role of genetic factors in AP is further evident from the familial aggregation of AP reported by the respondents in this study. The genetic components of AP may be elucidated using approaches suitable for genetic studies of complex traits.

A major obstacle in the genetic study of any complex trait is the identification of a reliable phenotype which is likely to be related to genetic variations in a small number of genes (McInnes and Freimer, 1995). A stringent definition of AP which identifies individuals with the fastest and most accurate pitch judgment may provide us with one

such phenotype. As described previously (Takeuchi and Hulse 1993), extensive musical training can enable some musicians to use learned strategies which help them in identifying the pitches of tones. However such conscious strategies for pitch identification involve multiple, time-consuming, memory-based processes. For example, individuals may have memorized an internal reference tone (often A440), and by determining the pitch interval between any given tone and their internal reference they are able to identify the pitch (Revesz 1953). Processes involved in active memory search and recall are slow in comparison to those processes which are innate. Pitch naming ability of musicians who rely on learned active processes for pitch identification diminishes as they are required to make their judgments of pitch at a faster rate. It is likely that individuals with the fastest and most accurate pitch identification ability employ the least number of learned strategies for pitch identification. We hypothesize that individuals whose pitch perception is least dependent on learned strategies may comprise a homogeneous group; we further suggest that this homogeneity may be on the basis of a similar genetic predisposition to AP. Therefore, it is essential in devising studies aimed at isolating genes responsible for such predisposition, to identify individuals whose pitch perception is immediate and accurate.

In order to define operational criteria for the identification of the most accurate AP possessors, we designed stringent auditory tests for AP and have already tested a substantial number of self-reported AP possessors. The unrelated individuals (probands), who reported that their AP was highly accurate and instantaneous could be placed into four groups based on the cluster analysis of their scores on our tests with pure tones and

piano tones. Individuals whom we designated as belonging to group 1 have an instantaneous and extremely accurate ability to identify the pitch of any tone based on the fundamental frequency (the lowest frequency component of any tone) of that tone. Individuals in group 2 are less accurate in identifying pure tones compared to piano tones. It is possible that this group of AP possessors need additional information contained in a musical tone, such as its timbre or its harmonics (frequency components of a tone which are multiples of its fundamental frequency), in order to accurately identify its pitch. Individuals in group 3 are less accurate than those in group 1 and 2 in tests with pure tones and piano tones. It is likely that individuals in group 3 are using conscious strategies for pitch judgment. The scores of all of the individuals tested were substantially higher than the scores of musicians without self-reported AP. Our results suggest that individuals in group 1, who exhibit the most extreme form of the AP phenotype, are likely to be the most suitable probands for studies aimed at identifying genes that predispose to AP.

The observation of familial aggregation of AP, the occurrence of multiple siblings with AP in several families that we have investigated, and the similarity of AP ability among siblings suggest the possibility of a genetic basis for AP. This suggestion is consistent with evidence from other studies (Profita and Bidder 1987; Gregersen 1996). While it is possible that a shared family environment may have contributed to familial aggregation of AP, strong evidence indicates that common environment has surprisingly little effect on the development of mental traits and abilities, in comparison to shared genotypes (Bouchard 1995). The evidence for the involvement of one or more major locus in the

development of AP could be strengthened by identification and pitch testing of additional pedigrees with multiple AP possessors.

Within the families that we have studied to date, the inheritance pattern of AP ability is consistent with an autosomal dominant mode of inheritance with incomplete penetrance. Under such a model, the penetrance of AP is influenced by early musical training. Therefore in many cases where an individual has not received early musical training it will not be possible to determine whether or not that individual has inherited a predisposing gene involved in development of AP.

In addition to the environmental factors that contribute to the complexity of behavioral traits, genetic studies of such traits may be further complicated by the existence of multiple genes involved in a particular behavior (genetic heterogeneity). One strategy that has proved successful for ameliorating the issue of genetic heterogeneity is to study particular traits in a genetically homogeneous population (Puffenberger et al. 1994, Lander 1996, Freimer et al. 1996). In a population that was established by a relatively small number of founders, and has remained genetically isolated, many of the individuals with a given phenotype are likely to have inherited a phenotype-conferring allele at the same locus from only a few common ancestors. Investigating such populations increases the likelihood that one may identify at least one single locus which plays a role in a trait that may have multiple genetic factors associated with it.

To minimize the impact of possible genetic heterogeneity on our search for AP loci, we will focus future genetic mapping studies of AP in genetically isolated populations. For example we have already began to recruit subjects from the Ashkenazi Jewish population. This population has been genetically isolated for the past few centuries; this fact has been previously exploited in genetic mapping studies of inherited neurological diseases (Risch et al. 1995). Today's Ashkenazi Jews are descended from a small population of Jews who lived in the 14th century in an area of Eastern Europe known as the Pale settlement. This population subsequently survived multiple historical bottlenecks (from pogroms) during the 15th and the 16th centuries and then experienced an era of exponential growth (Risch et al. 1995). Ashkenazi Jews living in the U.S constitute a relatively large and accessible community with a rich tradition of musicianship. We therefore anticipate that it may be possible to investigate a sufficient number of families from this population to enable us to identify genomic regions that are suggestive for AP loci.

Because of the likely etiological complexity of AP, any mapping strategy for identification of the gene or genes involved in this trait should include methods that are model-independent; for this reason non-parametric methods of linkage analysis may prove to be more successful than standard lod score approaches (Pericak-Vance 1996). Genetic mapping studies in isolated populations, such as the Ashkenazi Jewish population, may also benefit from the possibility of utilizing methods of analysis that are based on linkage disequilibrium between traits and polymorphic markers (Houwen et al. 1994).

In the past few years we have witnessed the development of new reference genetic linkage maps, and introduction of new paradigms and strategies to map complex human traits (Lander and Shork 1994). As a result, it is now possible for geneticists to take on the task of identifying the genetic components of complex human behavioral traits. One confounding factor involved in any genetic study of behavior is the issue of nature versus nurture. In many instances the environmental contributions to a particular behavior are mostly unknown, making the identification of a reliable phenotype for the purposes of genetic mapping difficult. For the development of AP, early musical training has already been determined to be a major environmental component. We have now operationally defined AP in a way that should allow us to pursue the identification of genes that, in conjunction with early musical training, can lead to the development of AP.

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CHAPTER III

Familial aggregation of absolute pitch

Absolute pitch (AP) is a behavioral trait that is defined as the ability to identify the pitch of tones in the absence of a reference pitch. We have proposed previously that AP is an ideal phenotype for investigating gene and environment interactions in the development of complex human behaviors. AP is distinct from most other such behaviors in that virtually all AP possessors have been exposed to a single, measurable environmental factor, namely, early musical training. Although previous studies have pointed to the possibility of a genetic predisposition for development of AP (Profita and Bidder 1988, Baharloo et.al.1998), there has been a paucity of systematic evidence for such a genetic contribution to AP. We have now attempted to quantify the familial aggregation of AP by estimating the lambda sib (λs) for a particularly distinct form of AP, termed AP-1. λs is defined as the risk to siblings of probands over the population prevalence, for a specific disease or trait and is a simple extension of the epidemiological concept of relative risk to genetics (Risch 1990a). AP-1 individuals are those who exhibit the most clear-cut AP ability on an auditory test developed in our laboratory. This test consists of identifying the pitch of 40 pure tones and 40 piano tones, presented to the subject at three-second intervals. Based on this test AP possessors were grouped into distinct phenotypic classes; AP-1 individuals are those who score at least 3 standard errors above the mean score of a randomized group of musicians (self-reported AP possessors and non-possessors) who received comparable musical training to one another (Baharloo et al. 1998). We therefore

consider AP-1 individuals as able to identify pitches accurately and instantaneously and without relying on reference tones.

The sibling recurrence risk (λs) has been estimated for a wide range of behavioral traits, for which prevalence data are available for siblings of probands as well as for the general population; for example, for schizophrenia the recurrence risk is about 9% in sibs vs. 1% in control populations, that is, λs is nine (Risch 1990). The degree of risk (we use this standard epidemiologic term for simplicity, although AP is obviously not a disorder) of the siblings of AP probands can be estimated by careful interviewing of AP probands (to determine which sibs are possible AP possessors) followed by direct auditory testing of the sibs. However, the population prevalence of AP is a potentially misleading measure, as early musical training is necessary, in almost all cases, for the development of this trait (Profita and Bidder 1988, Sergeant 1969, Miyazaki 1988a); we have no way of estimating the prevalence of AP in individuals who are not musically trained. Indeed each of the 74 AP-1 probands in the current study had musical training by age 6. Therefore for the purpose of determining λs it is important to estimate the prevalence of AP in control populations consisting of individuals who have experienced early music training.

AP-1 probands were previously recruited for participation in possible genetic mapping studies of AP, without our having knowledge of their family history for either musical training or AP. All of the AP-1 individuals recruited into the genetic studies at the time that we initiated the current investigation (N= 74) were asked whether they had siblings who had started musical training by age 6 (Table 1).

	Sibs of AP-1 Probands (n = 113)				Controls (n = 625)			
	Music Training at <6 Years of Age		Music Training at >6 Years of Age		Music Training at <6 Years of Age		Music Training at >6 Years of Age	
	AP	Not AP	AP	Not AP	AP	Not AP	AP	Not AP
band or self-report	25 (47.2%)	28 (52.8%)	1 (1.7%)	59 (98.3%)	19 (13.7%)	120 (86.3%)	39 (8.0%)	447 (92.0%)
for AP	13	0	0	0	19	120	39	447
	12 (92.3%)	...	...	...	4 (21.1%)	0	0	0

Table 1 Absolute Pitch (AP) in siblings of AP-1 probands and in a control sample.

Note. The rates for the sibs are based on reports made by the probands about their sibs. The rates for the controls are based on self-reports. All available sibs (n=13) and all controls were evaluated using a test for AP ability (Baharloo et al. 1998). From the results of these tests, we computed the rates of AP-1 in the sibs of probands (lower and upper bounds were 12/53 [22.6%] and 25/53 [43.5%], respectively) and controls (4/139 [2.9%]), considering only those individuals with music training beginning at <6 years of age.

These probands reported that they had 113 siblings, of whom 53 had started musical training by age 6. The probands reported that 25 of these 53 individuals possessed AP ability identical or very similar to their own AP abilities. Of 60 siblings who did not receive musical training before age 6 only one was reported by a proband to have AP. To obtain an estimate on accuracy of the AP reports about siblings by the probands, we tested 13 sibs to whom we had ready access and who were reported by the probands to possess AP; 12 of these 13 individuals (92%) were confirmed as AP-1. These 13 sibs were tested using the same auditory test and procedures as were used to test the probands.

To obtain an estimate of the population prevalence of AP-1 (the denominator of the λ_s equation) we surveyed and pitch tested all 625 students attending a summer music camp at the Florida State University (FSU). The music camp at FSU was randomly selected from a list of the first 10 summer camps identified in an internet search using "summer music camp" as the key word. Students attending the FSU camp consisted of two groups; 221 grade 7 and 8 students, with at least one year of music training, and 404 grade 9- 12 students with at least 2 years of musical training. Surveys were distributed to and completed by all students attending the camp during their regular class hours. The aim of the survey was to identify those students who had begun musical training by age 6. Immediately after the students completed the surveys they took the auditory test that we have used to assess AP status. The only difference in the testing procedure from that employed previously was that the students in each class (approximately 20 students per class) listened to the tones played over speakers rather than listening to them on headphones. To assess whether this difference in testing procedure from our prior

protocol could influence the results, we re-tested, using speakers, seven individuals who had previously scored in the AP-1 range when tested via headphones; all seven of these subjects continued to score as AP-1 in this re-test. Thus it does not appear that use of speakers as opposed to headphones leads to a more difficult test which could artificially deflate the rates of AP-1 in this control group versus our family study group.

Of the 625 camp students tested, 139 reported musical training by age 6 (Table 1). Nineteen of 139 students with early musical training reported AP; after auditory testing four (21.1%) of these students were determined to be AP-1.. Thirty-nine students with a history of musical training beginning after age 6 reported AP yet none of them tested as AP-1.

Because we were unable to test all the sibs of the AP-1 probands, we can only provide a range of estimates for the recurrence risk of AP-1 in this group. At the lower end, we identified 12 siblings who tested as AP-1, giving a lower bound estimate of 12/53 = 22.6% for this group. However, an additional 12 siblings were reported by history from the probands to have AP. If we assume the same rate of AP-1 among this group as among the 13 sibs we tested (i.e. 92.3%), we obtain an estimate of (12+11.8)/53 = 43.5%, which we use as the upper end of the range.

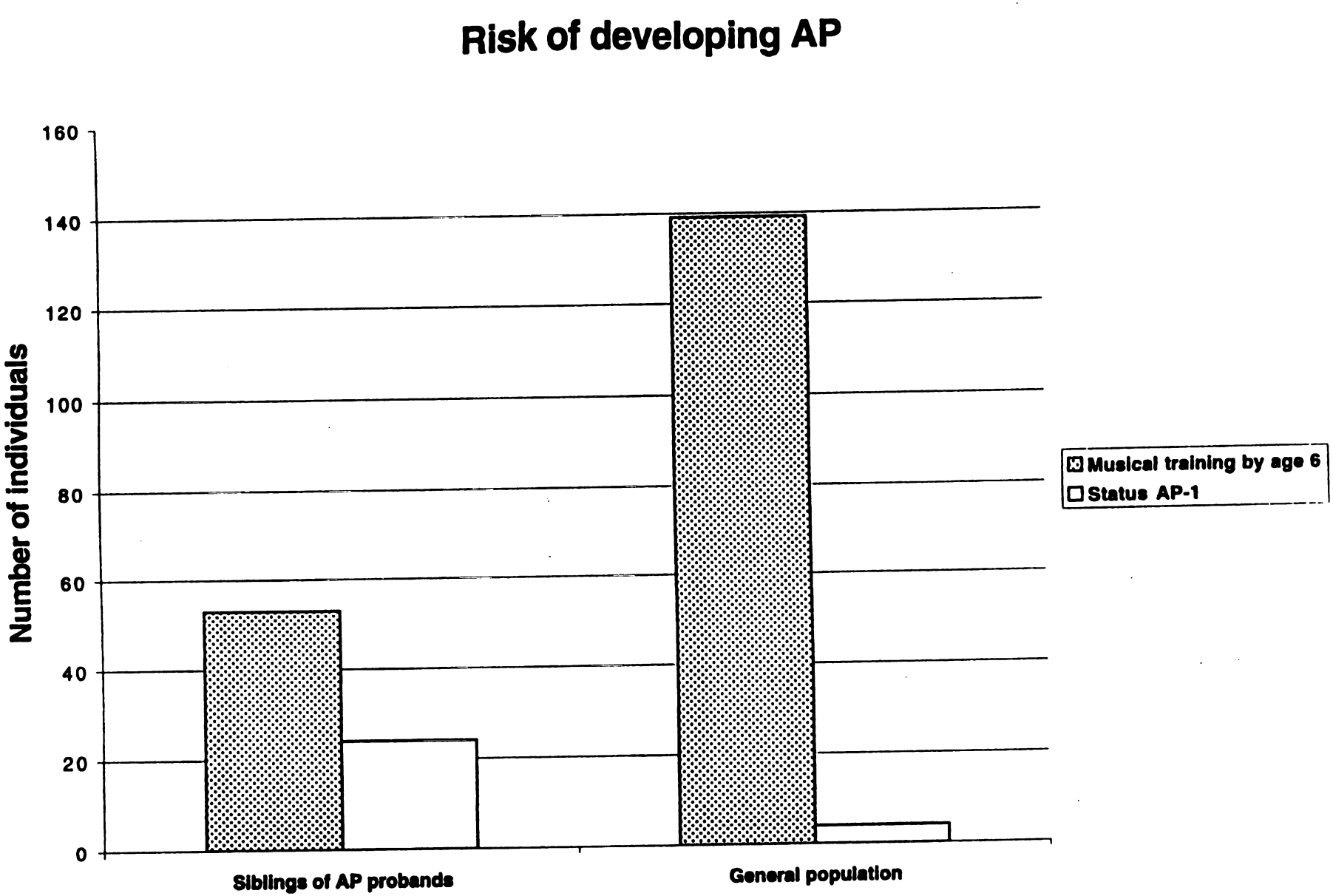
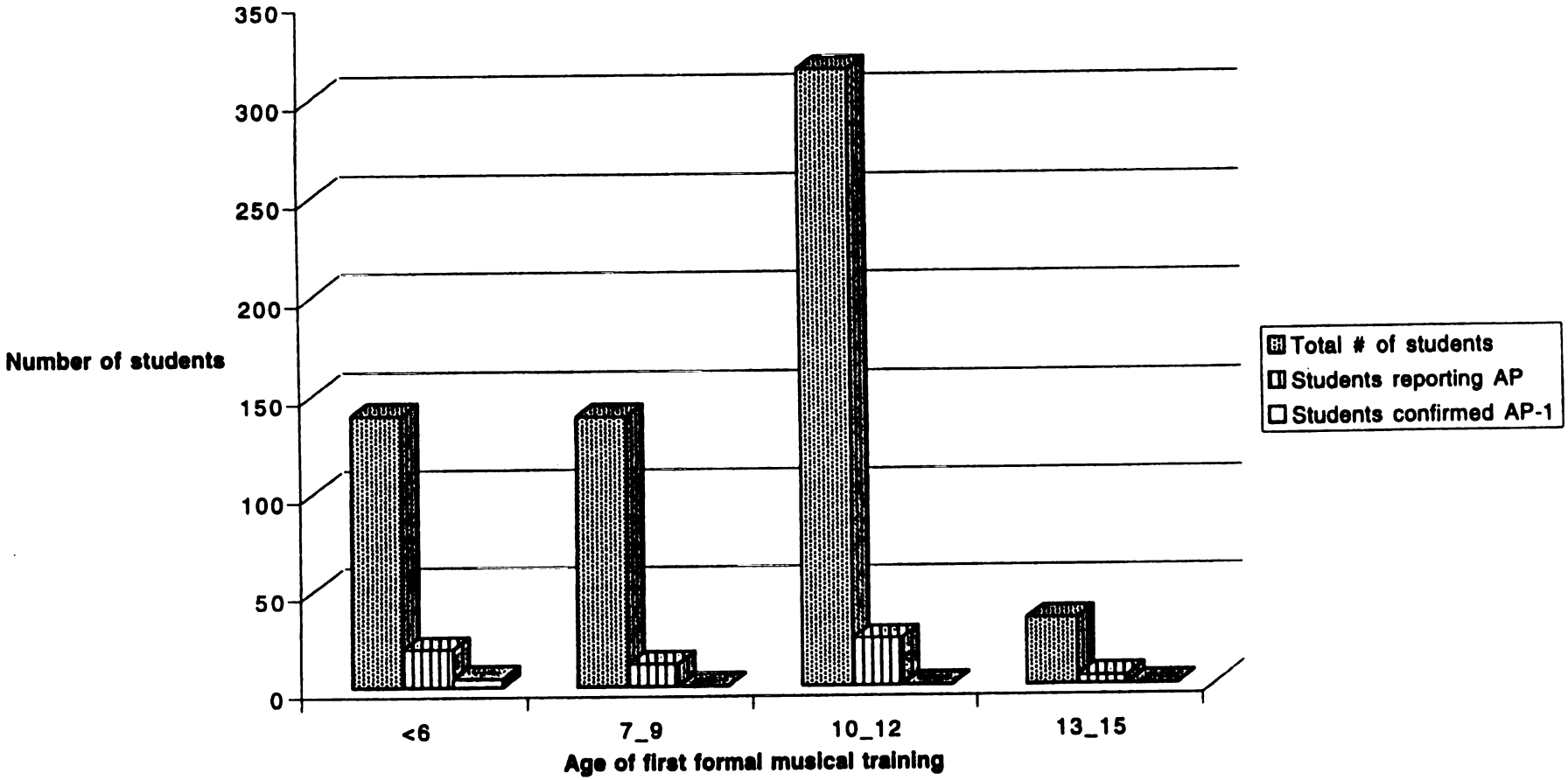

Risk of developing AP
Number of individuals
160
140
120
100
80
60
40
20
0
Siblings of AP probands
General population
Musical training by age 6
Status AP-1

Figure 4 Occurrence of AP-1 in siblings of AP-1 probands and in musicians from the general population.

Note. Only 13 of the 25 siblings of AP-1 probands that were reported to be AP-1 were tested. The accuracy of the report by the proband on the AP status of their siblings was 92.3% in the group that was tested. figure 5

AP in general population



All the individuals were tested for AP-1 using the procedure described in the text. These results strongly support familial aggregation of AP-1, even after controlling for early musical training. Using a rate of 4/139 = 2.9% as the population frequency of AP-1 among those with early musical training, the rate of AP-1 among the early-trained sibs of AP-1 probands is significantly increased, even using the lower end of our range (12/ 53 = 22.6% vs. 4/ 139 =2.9%, $\chi_2 = 17.2$, $p < .001$). Using the same sib recurrence rate figure (22.6%) gives a value of $\lambda s = 0.226/ 0.029 = 7.8$. If we use the more realistic upper bound estimate for the sib recurrence risk, we obtain a value of $\lambda s = 0.435/ 0.029 = 15.1$. Thus, a plausible range for λs is 7.8 to 15.1, although the higher end of this range is more likely to be appropriate.

There are several additional interesting observations from the data in Table 1. First, the data suggest that early musical training is itself familial. All the AP-1 probands had musical training before age 6. Among their 113 sibs, 53 (46.9%) had early musical training. By contrast, among the 625 controls, 139 (22.2%) had musical training by age 6. This approximately two fold excess in frequency is highly significant ($\chi_2 = 29.0$, $p < .001$). A second observation relates to the false positive rate of self-report and family history report of AP ability. We found a very low false positive rate among the tested siblings reported to possess AP by their AP-1 sibs (1 out of 13 = 7.7%), but a very high false positive rate among the controls self-reported to have AP (15/19 = 78.9% for those with musical training before age 6, 39/ 39 = 100% for those without musical training before age 6). These observations are opposite to what is typically found in family studies, namely greater accuracy of self-report compared to family history report. The

explanation in this case is not clear. It may be that individuals with true AP ability are excellent judges of similar ability in their sibs. Alternatively, as the great majority of the AP-1 probands were considerably older than the students at the music camps, it may be that younger people uniformly overestimate their AP ability. In any event, these results demonstrate the importance of direct AP testing, and indicate that investigators of AP should not rely exclusively on self-report or family history. If we had simply used the self report data for the controls, we would have obtained a population prevalence of 19/139 = 13.7% (for those with musical training before age 6), greatly attenuating the estimate of λ_s.

By contrast, it would appear that the false negative rate of AP-1 by self-report or family history is low. We have yet to encounter any subjects who tested as AP-1 and who did not previously report such ability, or had such ability reported for them by relatives.

In any case, the siblings recurrence rate for AP-1 appears to be significantly elevated compared to population prevalence, after controlling for early musical training. The sibling recurrence rate in combination with the population prevalence can also be used to estimate heritability of AP under various models of inheritance (Vogel and Motulsky, 1997). For example, the data are not compatible with a polygenic threshold model, even using the lower estimate of sib recurrence of 22.6%, since the heritability estimate comes out to be greater than 100%. Such observations often indicate major locus effects.

If the AP-1 phenotype is the result of interaction between multiple loci then λs per locus could be substantially smaller than our estimates for total λs. Estimation of risk to second and third degree relatives or identical twins can be used to estimate the probable number of loci involved and their contribution to the trait. For example λs for schizophrenia has been estimated as about nine, yet the recurrence risk data from different classes of relatives points to interaction of multiple loci each with recurrence risk < 2 (Risch 1990b). For logistical reasons we were not able to assess the risk of second-degree relatives of AP-1 probands.

A previous study that examined AP heritability, (Gregersen et al. 1999) estimated λ_S of 8.3 for AP by dividing the risk of the sibs of AP individuals participating in a survey of 2,707 music students, by the risk of the sibs of non-AP students participating in the same study. However their analysis did not involve direct testing of AP ability in all participants and did not differentiate between individuals who had early musical training and those who did not have such training. We previously showed that early musical training is a major environmental factor contributing to the development of AP-1 (Baharloo et al. 1998). Yet, estimation of λ_S may be confounded by the possibility that exposure to early musical training in itself is familial. Siblings in the same household are more likely to be exposed to early musical training (and hence having a higher likelihood of developing AP) if such training is provided for any one of them.

Indeed , we have provided evidence (Table 1) that early musical training is familial.

In considering our estimates of relative risk to the siblings of AP-1 probands and to members of the control population, one must keep in mind that concordance of the AP-1 phenotype in siblings may be partially due to experiencing similar environments aside from musical training which we have not considered or anticipated and thus our estimated λs of 7.8 to 15.1 may not entirely reflect genetic factors. Furthermore estimates used in the λs calculation were obtained in two different studies. While we only considered the prevalence of AP-1 in individuals with musical training before age 6, the value for the numerator was obtained from the study of sibs with a more rigorous music background (13 sibs tested had at least 5 years of musical training), and the denominator was obtained from a sample of students with perhaps less musical proficiency. This sampling scheme may have led to an inflated value for the numerator of the λs equation and thus an inflated estimate of λs. On the other hand it may also be true that children with AP ability are more musically oriented, and seek out musical training at an earlier age than children without such ability.

In summary we have obtained a λs value of about 8 to15 for AP-1, suggesting a strong role for genetic influences on the development of this trait. Furthermore the calculated sibling recurrence rates and the population rates suggest the possibility that the genetic predisposition to AP-1 may include a major gene effect. As early musical training is a requirement for the development of AP-1, we have only considered the fraction of the population with early musical training as the relevant population for λs estimation. This is the first study where the frequency of AP-1 in the control population was obtained through a randomized process and where the AP status of all individuals from the control

population was confirmed by an auditory test. The high estimates of λ_s, both from our study and from that of Gregersen et al. (1999), suggest that it may be possible to assemble a sufficient number of pedigrees or sib-pairs to map loci for AP-1.

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Profita J. & Bidder T.G. (1988) Perfect pitch. Am. J. Med. Genet. 29: 763-771.

Risch, N (1990a) Linkage strategies for genetically complex traits . I. Multilocus models. Am. J. Hum. Genet. 46: 222-228

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Chapter IV

Gene mapping study in 6 AP-1 families

Several investigators including Profitta and Bidder (1988) have suggested genetic bases for AP. Our evaluation of familial aggregation of AP-1 provides compelling evidence in support of genetic mechanisms involved in AP development. Hence it is reasonable to undertake mapping efforts for identification of genetic components of AP-1. Critical to the success of any mapping efforts is an appropriate choice of mapping strategies. Traditionally linkage analysis has been an effective strategy for mapping of Mendelian disorders, but in the case of complex traits multiple mapping strategies may be required. While recruiting subjects for the genetic mapping phase of our study, we encountered AP-1 individuals who reported at least 2 other family members with AP ability similar to their own. Since early music training is necessary for the development of AP, identification of large families with multiple AP-1 individuals is a difficult task. In fact, we very rarely encountered families where most members have had early music training. Observation of families with a high incidence of AP-1 can be indicative that within such families, a major locus for AP-1 may be in operation. Hence, for these families, genetic linkage studies may provide a reasonable strategy for identification of the major locus involved in AP-1.

Genetic linkage analysis

Genetic linkage analysis is an effective approach for the localization of the genes involved in monogenic traits. Genetic linkage is an indication that two loci are not inherited independently and they reside at close proximity to each other on the same chromosome. As parental chromosomes pair during meiosis, recombination occurs between homologous chromosomes. The probability of a recombinational event (crossover) occurring between two loci increases with distance. Genetic linkage indicates the probability that alleles at different loci are genetically coupled; the extent of genetic linkage is measured by the recombination fraction (θ). If two loci are far apart or reside on different chromosomes, they segregate independently, and the probability that recombinant and non-recombinant chromosomes are formed is equal, indicated by a recombination fraction of $\theta = 1/2$. Recombination fractions $< 1/2$ are indicative of the proximity of two loci on the same chromosome.

Genetic linkage between two or more loci can only be tested accurately if one can discriminate between a recombinant and non-recombinant transmitted chromosome within a family. The null-hypothesis (H_0) in linkage analysis states that if free recombination occurs between two loci, $\theta = 1/2$; the alternative hypothesis (**H1**) is that if two loci are linked, $\theta < 1/2$. In linkage analysis, the probability that a disease locus is

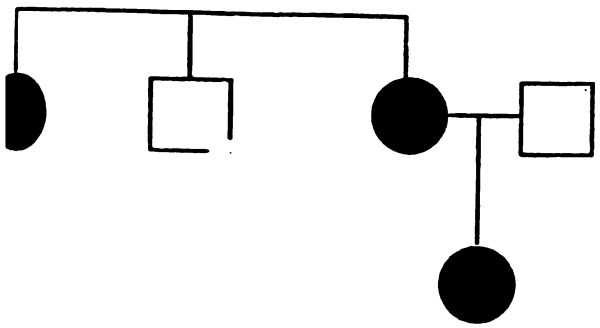
linked to a genetic marker is estimated based on recombination frequency between the markers and putative trait locus within a family. The likelihood ratio is a statistical measure of linkage between two loci and is based on the likelihood ratio between the likelihood of linkage (H_1) and free recombination (H_0). The Lod score is the logarithm of the likelihood ratio, and a minimum lod score of 3 (odds ratio of 1000:1) is generally accepted as evidence for linkage between two loci. Conversely, a lod score of –2 (odds ratio of 1:100) is evidence for the absence of linkage (Ott 1991). In order to provide evidence for linkage between a genetic marker and a disease, a sufficient number of informative meiosis are required in the pedigrees that are studied. To obtain additional power lod scores from different pedigrees with the same monogenic disease can be added together.

Recruitment of families

Careful family history was obtained from the probands reporting multiple AP family members. After obtaining permission (via probands), additional family members were contacted. Informed consent was obtained, participants were tested for AP, and DNA was collected from all those who either possessed AP-1 or added to the genetic informativeness of the families. DNA samples were extracted from the blood samples donated by the probands, but in many cases, because of inaccessibility of some family members, or lack of willingness on their part to donate blood samples, DNA was extracted from buccal cell samples. We began genotyping a total of 19 AP-1 individuals

as well as 11 of their family members. The status of these 11 individuals was considered as unknown for the purposes of linkage analysis. Since early musical training is necessary for the development of AP, and these 11 individuals did not have such training, absence of AP ability should not be taken as an indication of their phenotype. These individuals could in fact be non-penetrant carriers of AP. Incorrect categorization of potential non-penetrant carriers as unaffected will diminish the evidence for linkage in these families. The structure of these 6 AP-1 families is represented in figure 6. The AP-1 families come from diverse ethnic backgrounds; it is possible that different loci are involved in the predisposition to AP-1 in various families, a case of genetic heterogeneity. This diminishes the contribution of any given locus to the development of the phenotype at the population level. As the six AP-1 families in this study are small, the power to detect linkage in a given family is low. To increase power lod sores must be added across families. In case of genetic heterogeneity in genes involved in AP-1, addition of lod scores from different families will not improve the lod scores and could in-fact could decrease the evidence for the linkage at each locus.

(1)



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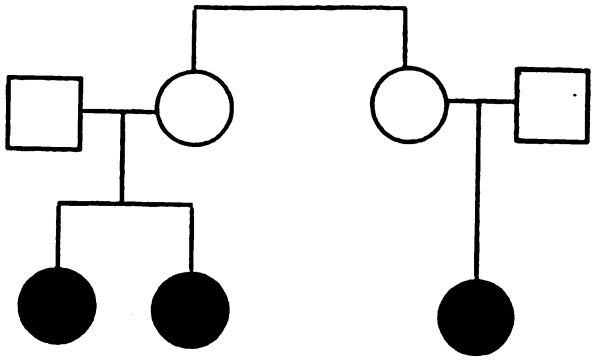

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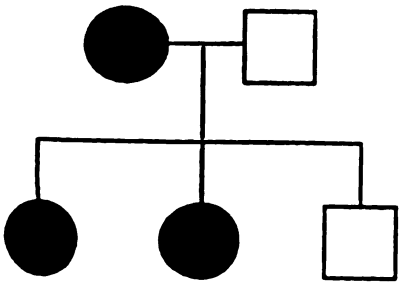

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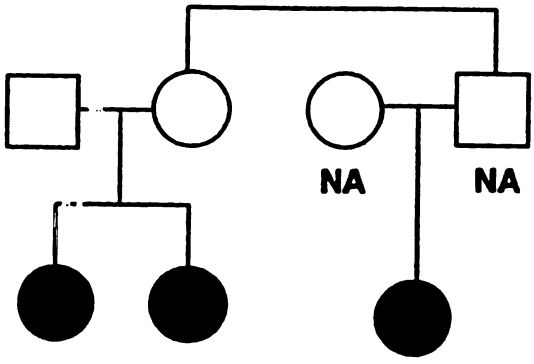

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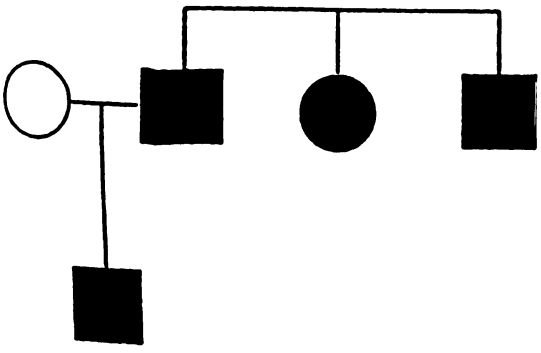

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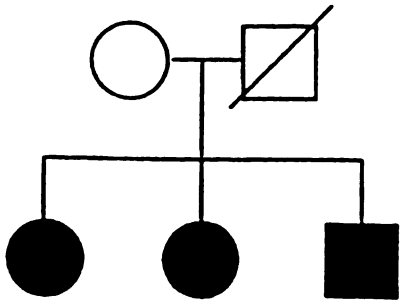


Figure 6 Pedigree drawings of six families that were recruited for the genetic mapping of AP-1. Only families 1,2,3 and 4 were used in the power simulations.

Power simulations

Before we began the genome screen for identification of the AP-1 locus, we performed a simulation analysis on families whose DNA samples were already collected. For the sake of simplicity simulations were done for linkage to a marker with four equally frequent alleles. An average marker map density of 5cM was selected as we expected to perform the genome scan for AP at a similar marker density. Four models were considered, of which two were dominant and two were recessive. For each model we classified non-AP-1 individuals in two ways. In the first case their phenotype was considered as unknown, and in the second case they were given the un-affected status if they had music training before age 6. Parameters used in these analysis are shown below. The codes for different models are as follows: D indicates dominant model, R, indicates recessive model. 1 indicates assumption of 1% prevalence in population and 2 indicates assumption of 0.1% prevalence in population. The population prevalences were selected arbitrarily to resemble the prevalence of other common traits in the population as well that of a less frequent trait. In families studied here we observed that a high number of individuals who received early music training possessed AP-1, hence we assumed the penetrance value of 0.9 in these families.

Model	Gene freq.	Penetrance	Penetrance	Penetrance	Prevalence
		DD	DN	NN	
D1	0.004	0.9	0.9	0.002	1%
D2	0.0005	0.9	0.9	0.0002	0.1%
R1	0.1	0.9	0.0025	0.0025	1%
R2	0.03	0.90	0.0002	0.0002	0.1%

These simulations indicate the low power of the families to detect linkage. For example under a dominant model, assuming prevalence of 1% and penetrance of 0.9, the power to detect a lod score of 1.0 is less than 40%. Under the 0.1% prevalence assumption, a lower trait gene frequency is expected in the population figure 7. Hence observation of a particular genotype in the affected individuals is less likely to be by chance alone. Similarly a recessive mode of inheritance increased the power of these families as linkage to a locus needs to be observed on both chromosomes and that is less likely to occur by chance. Simulations also illustrated that more power for linkage is provided by certain families, such as family Two, in which 3 cousins are AP-1 possessors.

Given the low power of the 4 families that were used in the power analysis, we continued our attempts to collect additional AP-1 families that were suitable for linkage analysis.

Dominant Models - All Families

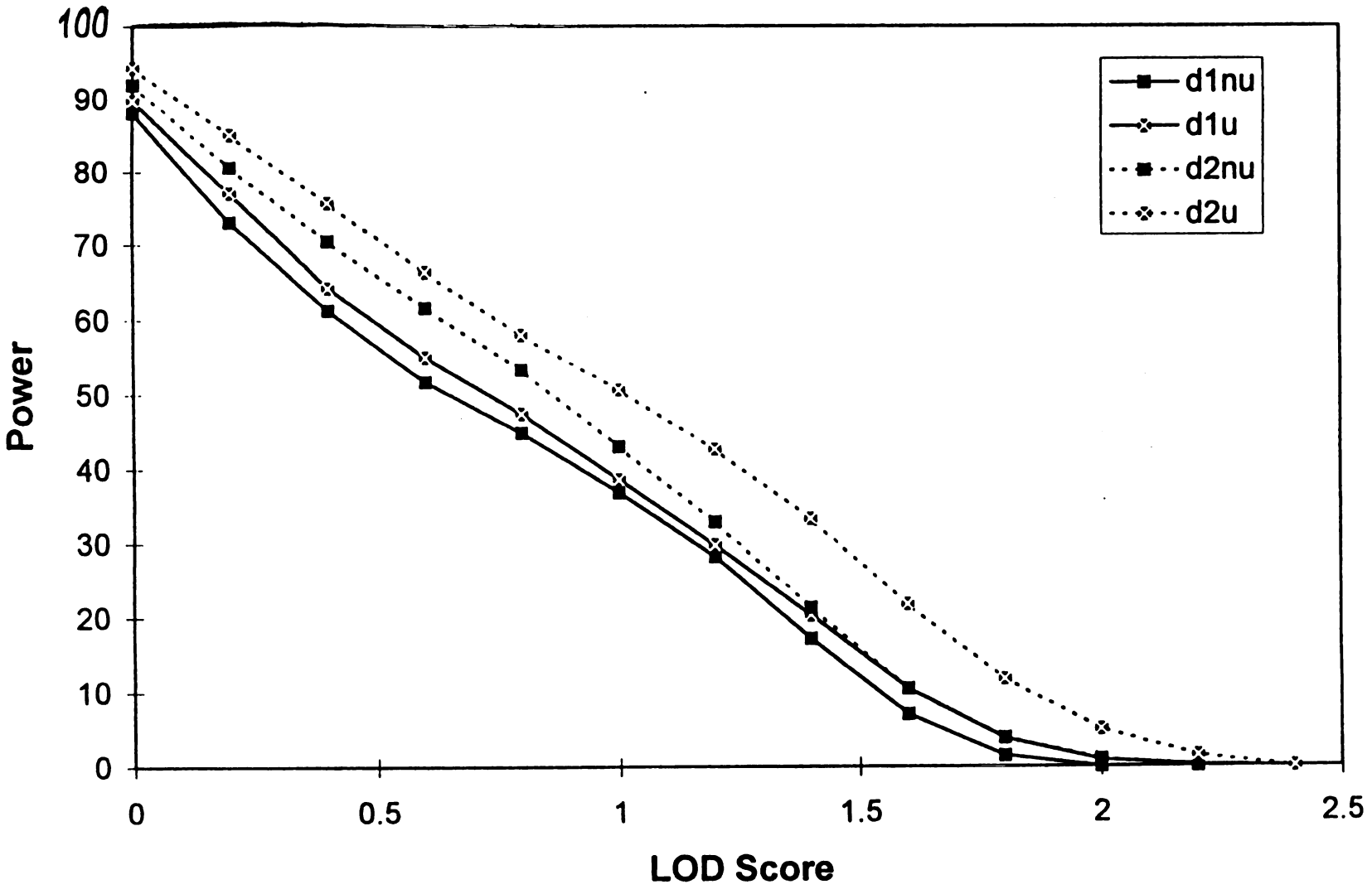


Figure 7 Simulations of lod scores vs. power in 4 AP-1 families. Simulations were performed under the following assumptions; dominant inheritance (d) and with disease frequency of 1% (1) and frequency of 0.1% (2), no information about the unaffected individuals (nu) and with information on the unaffected individuals (u).

After considerable efforts two more AP-1 families were collected. We did not expect that the addition of these two families would significantly increase the power of the study. Yet we proceeded with the genome scan of these 6 families as a first step in a broader effort for the genetic mapping of the AP-1. We reasoned that at minimum the results of this analysis would be additive to that of any future AP-1 linkage studies. Additionally, it was possible that this analysis in itself could yield candidate genomic regions that would be followed up in subsequent genetic studies.

Genome screen for AP-1

402 polymorphic microsatellite markers from the linkage map published by Genethon (Nature, 1996) were used to scan the entire genome of 19 AP-1 individuals and 11 relevant family members from 6 small pedigrees. Markers used belonged to the LMSV-2 panel of fluorescently labeled markers, developed by Applied Biosystems (ABI) for use with the ABI 377 sequencing instrument. DNA from the AP-1 family members as well as from a CEPH nuclear family (with known genotype for all markers tested) was dried down in 96 well PCR trays. PCR reactions were set and performed as described by Wissenbach et al. (1996), with the exception that Taq Polymerase gold (ABI) was used. PCR markers belonging to a panel were then pooled and loaded on an ABI 377 instrument. Using ABI Genescan and Genotyper software, the raw genotype data were analyzed. Markers were scored by two individuals and their scores were compared. Discrepancies between the two scorers were re-evaluated by the scorers and differences

were resolved. If the two scorers could not reach a consensus, a third scorer was brought in and made the final call. At times markers were redone for a single pedigree individual or the entire pedigree in order to obtain a genotype that was easier to discern.

Linkage analysis

Two point linkage analysis was performed with a program from the Linkage package (Lathrop et al. 1984). The frequency of the AP-1 gene with autosomal dominant inheritance was assumed to be 0.01% with 90% penetrance. The status of all the individuals who were not AP-1 was assigned as unknown. Since the 6 families genotyped were expected to yield low power to detect linkage, an arbitrary threshold of lod >0 5 was set to identify regions with mild positive lod scores. Markers at seventeen loci showed lod scores of 0.5 or better. In total, 349 markers were readable and were successfully scored for at least four of the six families. To optimize the information from markers in the areas of minimum lod scores, some markers with initially unreadable genotypes were re-typed.

The 25 loci with lod scores of 0.5 or better are indicated in the following table. Regions where more than one marker demonstrated lod scores higher than the minimum threshold are grouped together into 17 regions and separated from other markers by empty rows before and after them.

Marker	Lod score
D1S230	1.55
D1S484	0.81
D1S196	1.04
D1S238	0.64
D2S305	0.99
D2S347	1.31
D4S419	0.60
D4S391	1.03
D4S392	0.97
D5S419	0.62
D7S669	0.80
D9S158	0.86
D10S1653	1.11

D10S197	0 58
D10S1652	0.87
D10S1693	0.66
D11S1320	0.91
D15S1002	0.80
D16S3103	0.96
D16S3046	0.53
D16S3068	0.81
D18S464	0.77
D18s1161	0.60
D20S119	0.85
D22S539	1.05

Considering the low power of the families used in this analysis we decided to construct haplotypes across these 17 regions. This was done in order to obtain further evidence on whether these loci were involved in the predisposition to AP-1. We hypothesized that all or most of the affected individuals in a given family would share a haplotype at the AP-1 locus. Furthermore, barring locus heterogeneity, one would expect that while the actual haplotype may be different among different families, all or most families demonstrate the shared haplotype at the same region.

Haplotypes were constructed using two markers surrounding each marker showing lod > 0.5 by hand, assuming the minimum number of recombination between any two consecutive markers. In total, at least for two regions (D4S419-D4S392 and D16S3103-D16S3068) for the minimum of three families all individuals within a family shared the same haplotype.

These results indicate that the regions near D4S391 and D16S3103 are our best candidates to follow up in additional AP-1 families. One must be cautious and not over interpret this data. Judgment must be reserved until other mapping studies of AP-1 have substantiated these observations.

Chapter V

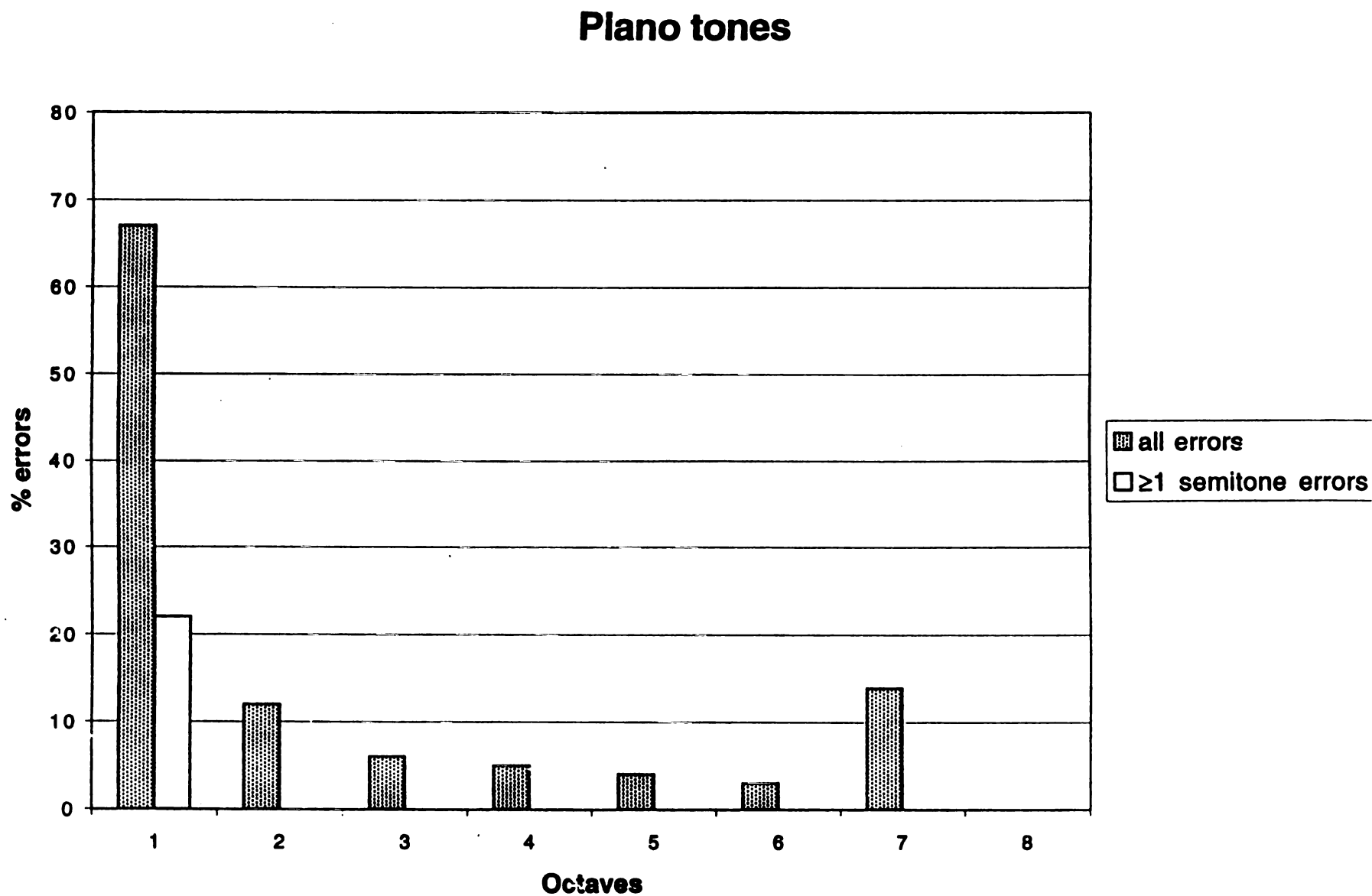
Additional aspects of AP phenotype

In chapter II we described how self reported AP possessors were classified into distinct phenotypic groups. This classification was critical in our efforts to recruit individuals with a similar phenotype that was likely to be related to a common genetic predisposition. Here we describe additional observations regarding the AP ability of individuals who were tested.

Effect of pitch register on AP-1

Many studies have suggested that since most musical pieces are written in central pitch registers, AP is most reliable within central registers (Takeuchi & Hulse 1993)). These reports claim that the repeated use of tones from middle registers make the task of pitch identification easier for AP individuals as they have become more familiar with these tones. To evaluate any differences in the reliability of pitch judgment by AP-1 possessors across pitch registers, the number of semitone errors as well as errors of over a semitone made in tests with pure tones and piano tones were graphed. In general AP-1 individuals performed very poorly in the 8^{th} octave in the pure tone test and also in the 1^{st} octave in the piano tone test. The performance of AP-1 individuals was indistinguishable from that of RP individuals in these ranges. For all other octaves in both tests, AP-1

individuals made very few errors larger than a semitone. While errors of less than a semitone occurred more frequently, such errors were fewer in the middle registers Figure 8 and Figure 9.


Piano tones
80
70
60
50
40
30
20
10
0
% errors
1
2
3
4
5
6
7
8
Octaves
all errors
≥1 semitone errors

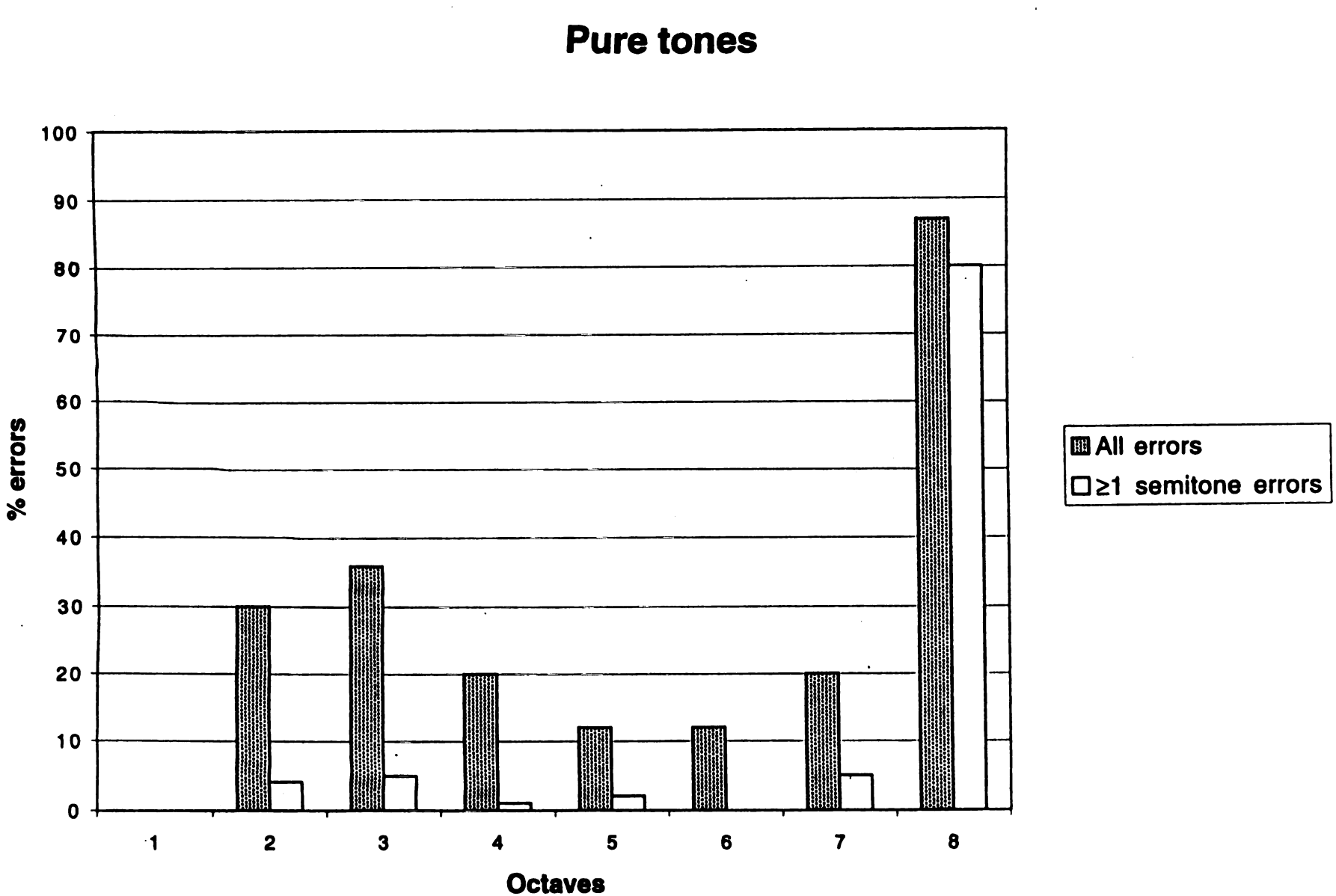
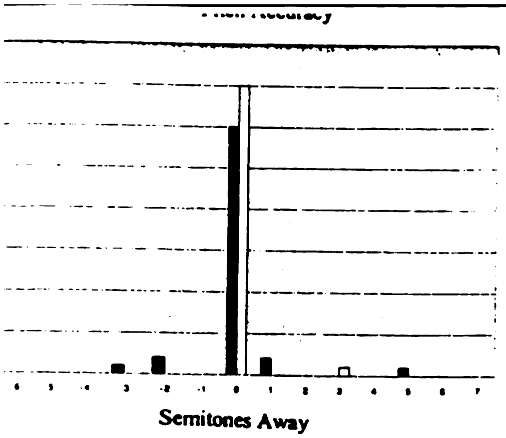

Pure tones
100
90
80
70
60
50
40
30
20
10
0
% errors
1
2
3
4
5
6
7
8
Octaves
All errors
≥1 semitone errors

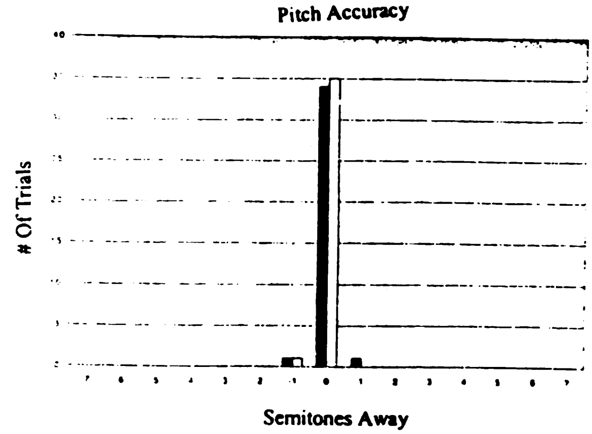
Figure 9 Reliability of pitch judgment by AP-1 possessors across pitch registers when testing with pure-tones.

Accuracy of pitch judgement

Earlier we described how AP status was assigned based on the score of each individual in auditory tests consisting of pure tones and piano tones. In order to investigate whether AP-1 individuals can be further sub-classified into distinct groups, we charted the accuracy of AP-1 as a function of their responses in the AP test.

AP-1 individuals are distinguished from non-AP individuals by the number of correct tones they identified in pitch naming tests. Furthermore, the variance around the correct tone is smaller for AP-1 possessors in comparison to non-AP individuals. Most of the errors committed by AP-1 individuals were within a semitone of the test tone, while non-AP controls made mistakes distributed over the entire range of tones. Closer examination of test results may allow further sub-grouping of AP-1 possessors as some of the AP-1 individuals made only semitone errors, while others made errors that were more than a semitone from the test tone. These differences may be useful in refining the AP-1 phenotype and in identifying a more homogeneous group of AP possessors suitable for gene mapping studies Figure 10-1 to 10-4 and Figure 11.


Semitones Away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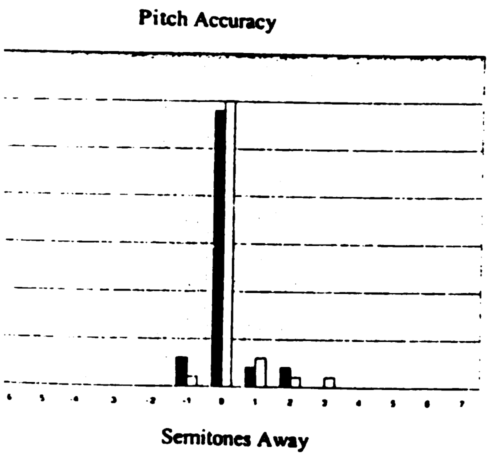
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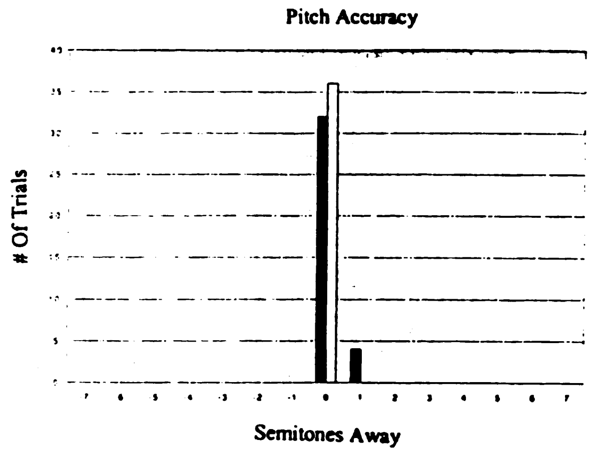
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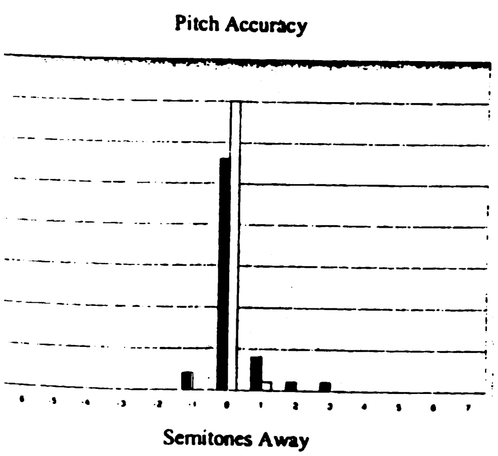
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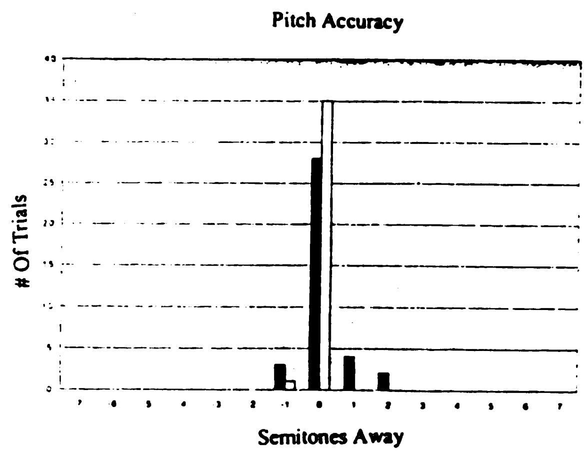
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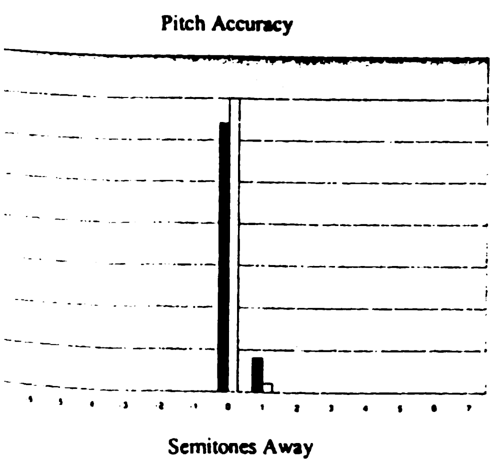
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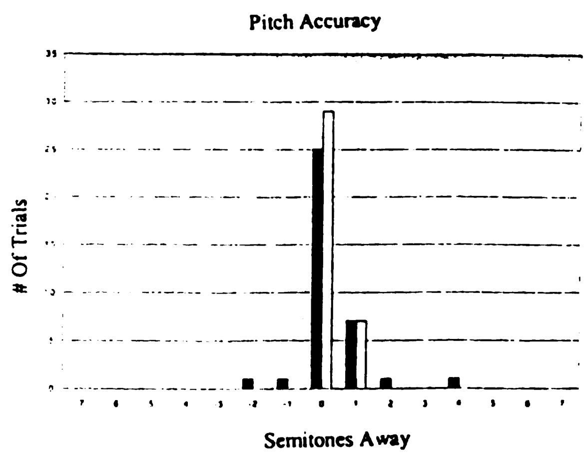
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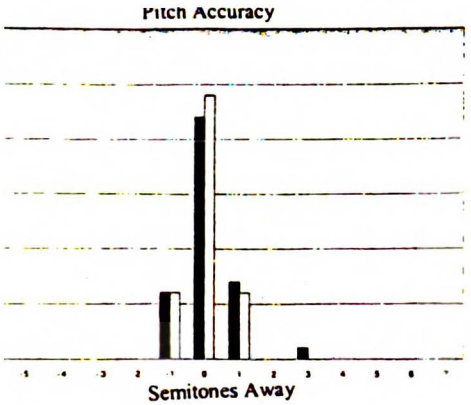
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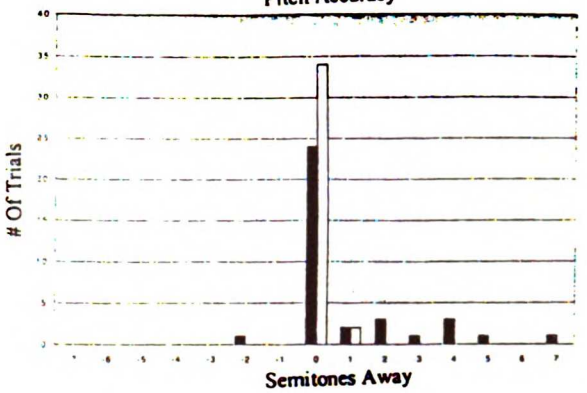
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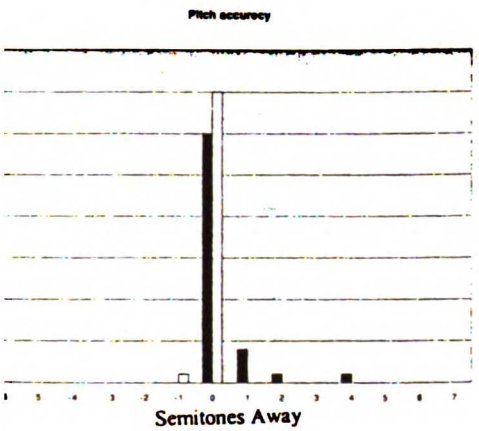
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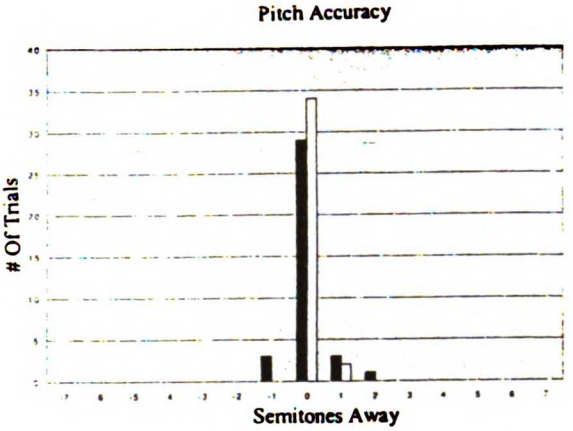
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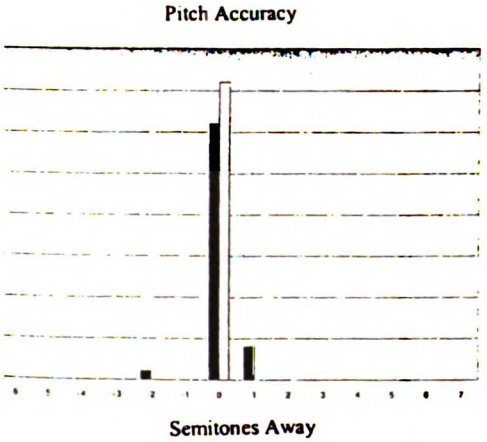
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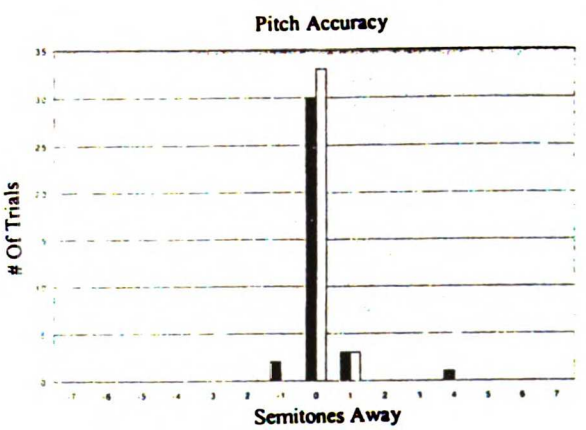
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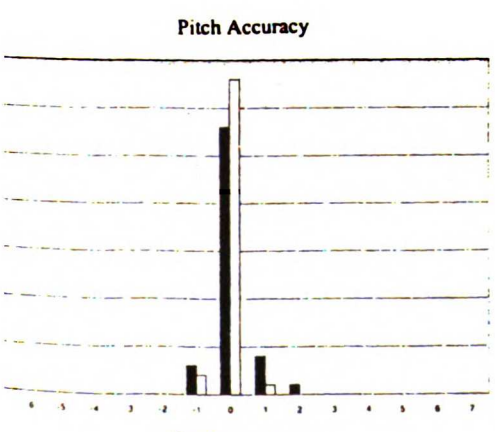
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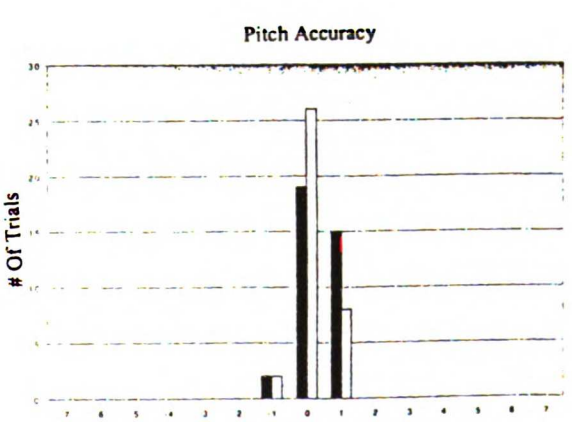
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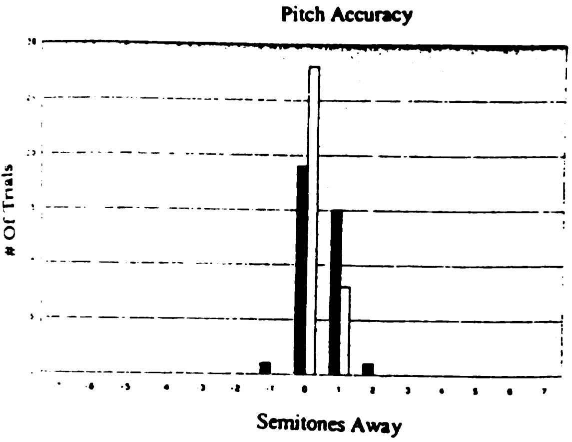
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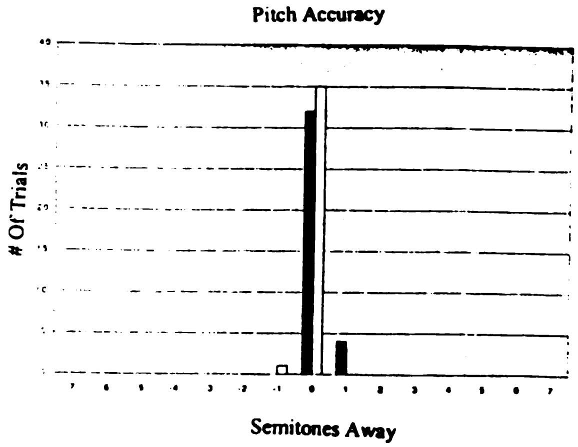
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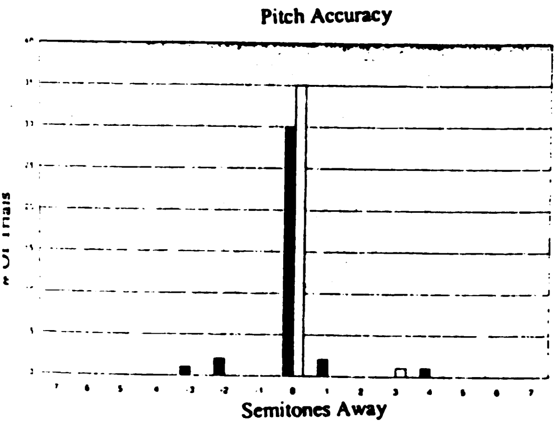
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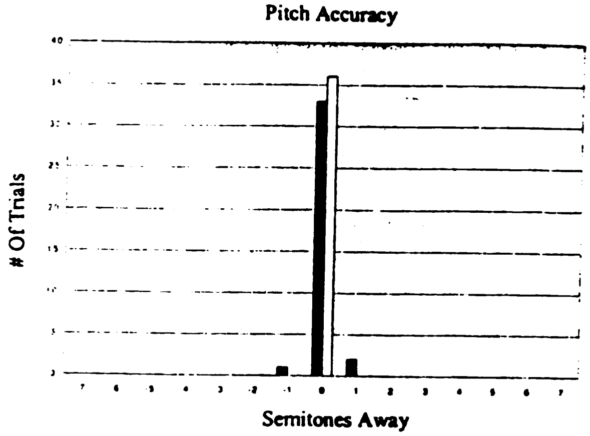
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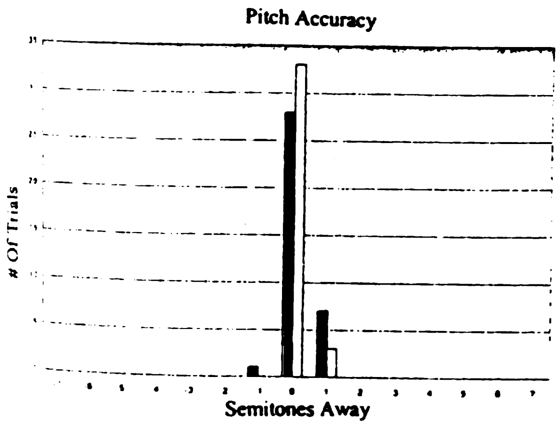
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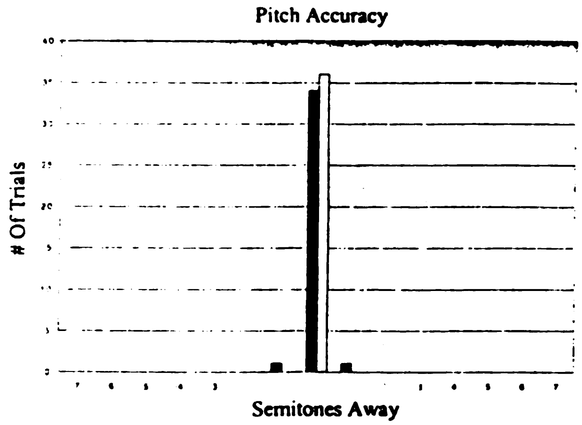
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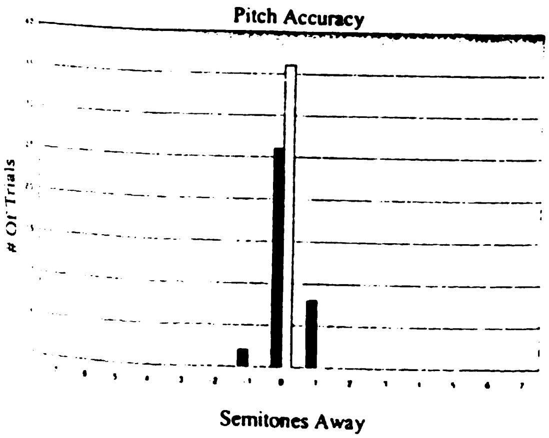
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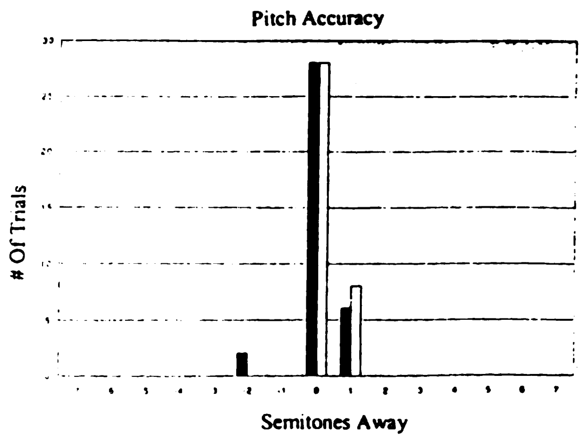
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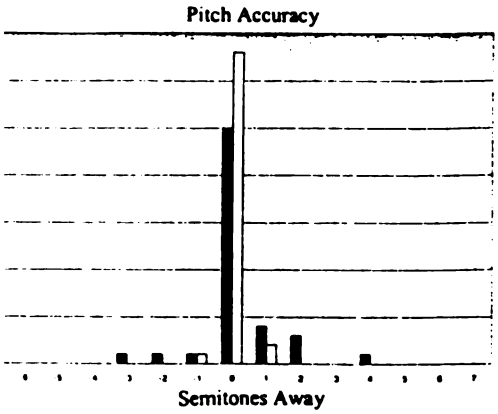
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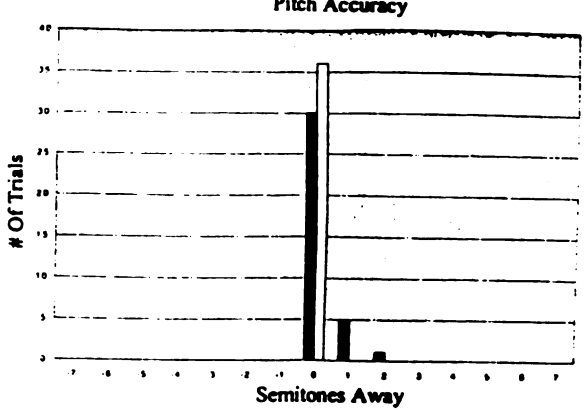
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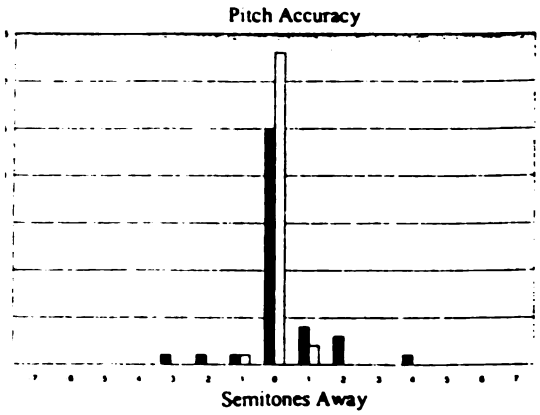
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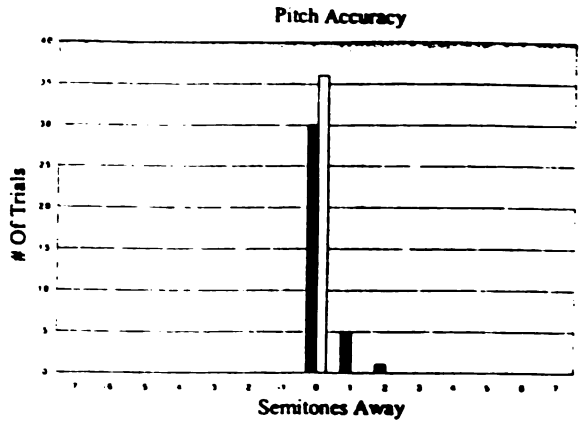
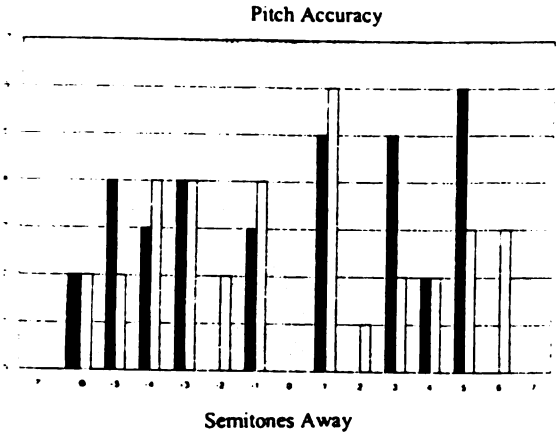
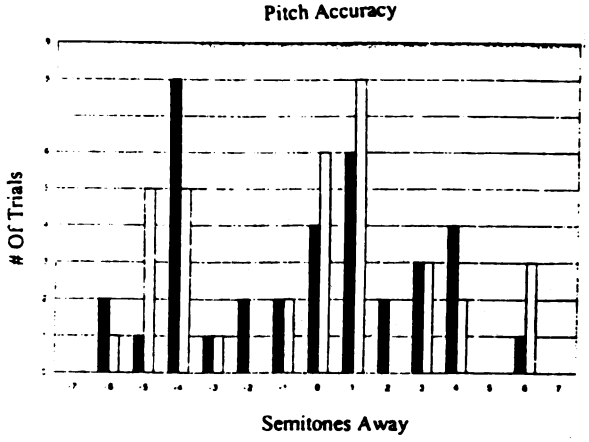

Pitch Accuracy
Of Trials
Semitones Away

Figure 10 Distribution of tones identified by AP-1 individuals in AP test. The number zero on the X-axis indicates the location of the correct judgments. Numbers to the right indicate semitones higher and numbers on the left indicate semitones lower than the actual pitch. Dark bars represent the number of judgments made for the pure-tones and the white bars are the number of judgments made for piano-tones.


Pitch Accuracy
Semitones Away


Pitch Accuracy
Of Trials
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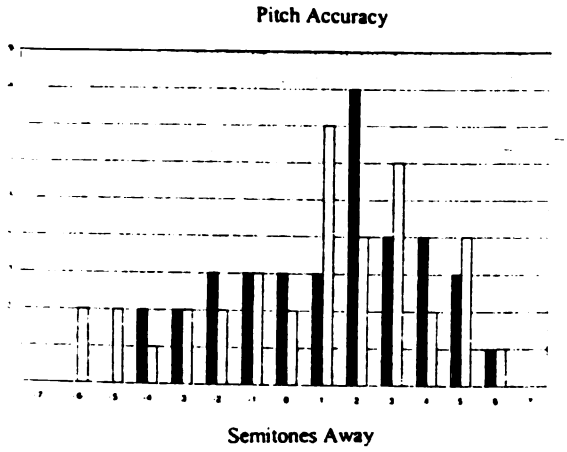
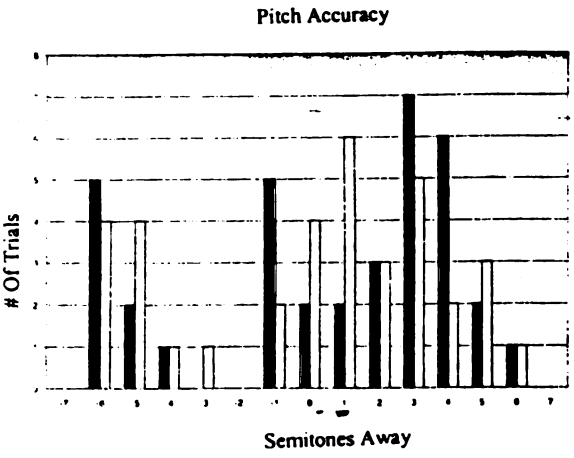

Pitch Accuracy
Semitones Away


Pitch Accuracy
Of Trials
Semitones Away

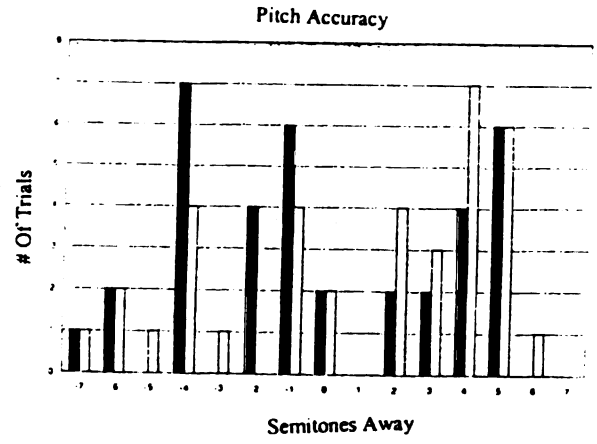

Pitch Accuracy
Of Trials
Semitones Away

Figure 11. Distribution of tones identified by non-AP musicians in AP test. The number zero on the X-axis indicates the location of the correct judgments. Numbers to the right indicate semitones higher and numbers on the left indicate semitones lower than the actual pitch. Dark bars represent the number of judgments made for the pure-tones and the white bars are the number of judgments made for piano-tones.

Magnetoencephalographic studies of AP-1

Magnetoencephalography (MEG) is an approach that is used to record very small changes in the magnetic fields generated by neurons as they are activated. MEG is non-invasive and is suitable for recording from awake and functioning subjects. Furthermore MEG recordings can localize the source of the neuronal activity with high precision to regions consisting of as little as a few thousand neurons.

In a recent MEG study by Pantev et.al. (1998), the authors recorded the auditory evoked neuromagnetic fields elicited by sinusoidal signals and piano tones from the left hemisphere of AP musicians, non-AP musicians, and nonmusical controls. Two effects were observed: First, piano tones generated larger responses than pure tone signals in musicians with and without AP. Second, this difference was not obtained in nonmusical controls. In particular, the dipole moment strength of the N1m/M 100 (occurring ~ 100 ms after stimulation onset), evoked field was increased in all musicians. On the basis of this finding, Pantev et al. argued for a use-dependent reorganization of the auditory cortex.

We undertook magnetoencephalographic studies of AP-1 subjects and controls in collaboration with a neuroradiology group at the University of California San Francisco (Howard Rowley and David Poeppel). We attempted to evaluate whether one can observe effects across hemispheres (as previously reported by Schlaug et. al.(1995) that are presumably independent of the amount of musical training. We recorded the auditory

evoked M100 field from hemispheres of two groups of musicians with the same levels of musical training, 5 AP-1 subjects and 5 RP subjects. 400 ms duration sinusoidal signals (100, 257, 440, 1000, 5000 Hz) were presented monaurally using Etymotic earphones. Each tone was presented at least 100 times to allow for signal averaging. For each stimulus frequency the threshold was determined, and then the loudness across stimuli was matched to allow for presentation at an approximately constant sensation level. The sensation level ranged from 45-65 dB. Using a 37-channel biomagnetometer (Magnes, Bti, San Diego CA), we recorded from the hemisphere contralateral to the ear of presentation. Epochs of 600 ms duration (400 Hz bandwidth) were acquired around each stimulus. The position of the recording device was adjusted to optimally capture the N1m/M100 evoked field elicited by a 1000 Hz pure tone. The M100 peak was determined by calculating the rms of evoked fields. Dipole strength Q, which is a model independent correlate of the strength of the evoked sensory fields, was also calculated.

No significant intra-hemispheric differences were observed in the latency of the M100 between AP-1 and RP musicians. Yet there were differences in the value of Q, which approached significance. To observe any differences in Q between the right and the left hemispheres, Q left was subtracted from Q right, (Q right – Q left = Q difference). We observed that the average Q difference in AP = 12.59, and the average Q difference of RP musicians = -5.94. In other words, the strength of dipoles created by auditory stimuli is possibly smaller in the left hemisphere of AP individuals than in their right hemisphere. Such differences were not observed in RP individuals.

This finding was surprising as we expected that the changes, if any, would indicate stronger evoked fields in the left hemisphere, consistent with the anatomical changes documented by Schlaug et al. Current dipole strength is believed to reflect the total number of synchronously firing neurons contributing to the stimulus-driven cortical response (Williamson, et. al. 1990). The reduced dipole strength could potentially indicate a reduction in the number of neurons contributing to the signal evoked in the left hemisphere of AP-1 individuals, or alternatively could indicate a decrease in the spatial or temporal coherence of evoked neuronal activity due to the participation of a larger number of neurons. Spengler et al. reports a similar decrease in the current dipole strength in the somatosensory cortex as a result of tactile training. Since both AP-1 and RP individuals have comparable music training, it is possible that the differences observed may be specific to the AP-1 phenotype.

Concluding remarks

The aim of my dissertation was to describe a path for the investigation of complex behavioral traits by establish the basis for the genetic analysis of absolute pitch as a model system. The result of this work makes a strong case for the involvement of a genetic predisposition in development of AP.

We defined a specific form of AP phenotype and reasoned that such phenotype is likely to be linked to specific genetic factor of factors. We demonstrated that early music training, most likely before age 6, was necessary for the development of AP phenotype. With the use of the acoustical test that we devised we were able to test a large number of musicians and obtained a value for the heritability of AP. Based on our estimates AP has a strong genetic component and the predispositition to AP may include a major gene effect.

We have collected DNA samples from hundreds of AP possessors, furthermore DNA samples from 6 families with three or more AP possessors were collected as well. Genetic linkage analysis in the 6 families revealed a number of candidate regions that will need to be followed with in additional AP family studies. Large number of the AP individuals that were collected are of Ashkenazi Jewish ancestry. High-density genotyping the AP candidate regions in these individuals may yield further evidence on the precise location of the AP gene.

Finally, we have provided preliminary evidence based on the MEG analysis of AP possessors that specific neuroanatomical differences may exist between AP and non-AP musicians. Such differences may provide clues on the functional pathways that underline this fascinating trait.

Appendix I

The AP survey

The following survey was administered to the musicians and music students at the various music institutions and programs.

Please take a few minutes and complete this questionnaire to help us in our Study of Perfect Pitch (ability to identify the pitch of a tone without a reference note). Your answers are extremely valuable for our study regardless of whether or not you possess perfect pitch.

The purpose of this survey is to find the frequency of perfect pitch among music students. After the completion of the survey you will be played a number of musical tones that you will be asked to identify by indicating their pitch name on a paper that we have provided. These questionnaires are anonymous however if you possess perfect pitch and would like to participate in the later stages of the study please indicate your name and contact information in the space provided.

The University of California San Francisco, Committee on Human Research, has approved this survey. Protocol # H5200-12059-01

1. Your age.

a. 10 or less

b. Between 11- 20

c. Between 21-30

d. Between 31-50

e. More than 50

2. Your gender

a. Female

b. Male

3. At what age did you began your music training in a **formal** setting, i.e. lessons from parents, from an instructor or in a music class?

a. 4 or less

b. Between 5-6

c Between 7-9

d. Between 10-12

e. Between 13-15

f. After age 15

4. How many years of music training have you had?

a. Less then 1 year

b. Between 2-5 years

c. Between 6-10 years

c. Between 11-20 years

d. More than 20 years

5. If you have had musical training before age 6, did it include ear training?

a. Yes

b. No

c. Don't Know

6. Do you have Perfect Pitch?

a. No, please proceed to question 12

b. Don't Know

c. Yes.

7. How do you rank your ability to identify the pitch of notes?

a. I am right less than half the time

b. I am right more than half the time

c. I am right almost all the time

8. How rapidly can you identify the pitch of notes?

a. Within minutes

b. Within 30 seconds

c. Within 15 seconds

d. Within 3 seconds

e. Instantaneously

9. At what age did you know that you have Perfect Pitch?

a. As long as I can remember I have had Perfect Pitch

c. By age 5

d. By age 12

e. After age 12

10. On which instrumen can you determine the pitch without a reference note?

a. Piano

b. Violin

c. Voice

d. Flute

e. Any instrument

11. Can you produce accurately any requested pitch vocally without a reference?

a. Yes

b. No

12. How many siblings do you have?

a. One

b. Two

c. Three

d. Four

e. More than four

13. Do any of your family members possess Perfect Pitch?

a. No

b. Don't Know

c. Yes, if the answer to 13 is yes,

14. What is their relation to you?

a. Mother

b. Father

c. Sister

d. Brother

e. Other

If you would like to be contacted about participating in the later stages of this study please complete the information below.

Name

Phone #

e-mail

Address

Appendix II

AP test

Two tests were administered to each subject. The first test consisted of 40 pure tones, the second of 40 piano tones. These tests were divided into 4 blocks with 10 trials in each block, with three-second intervals separating each trial. Tones were played in pseudorandom order with the constraint that successive tones were separated by more than two octaves and a semitone. As subjects listened to the tones, they were asked to make an instantaneous judgment of the pitch of each tone and write it on a sheet of paper. Subjects were not allowed any practice runs, nor was any feedback regarding their performance given until the testing was completed.

Stimuli

Sine-wave tones were digitally synthesized (16-bit, sampling rate 44.1 kHz) as text files on a Silicon Graphics Indy workstation using MatLab software (The Mathworks Inc, Natic, MA), and converted to standard AIFF audio files using conversion utilities. Tones of different frequencies were synthesized with different amplitudes to equalize perceived loudness (as judged by SB & PJ). The tones had duration of 1000 msec, with onset and offset ramps of 100 msec. Sine-wave tones had frequencies corresponding to the 40 musical notes from C2 to G#8 based on A4 = 440 Hz. Frequencies in the first octave were not used, as pure tones in this range were not reproduced clearly with our equipment.

Piano tones were taken from a CD produced by McGill University ("McGill University Master Samples"), containing professionally sampled tones from a 9' Steinway grand piano tuned to A4=440 Hz. 40 piano tones from C1 to G#7 were digitally recorded from the CD to a MacIntosh PowerPC and were edited using SoundEdit 16 software (Macromedia, San Francisco, CA) to have uniform duration of 1000 msec, with offset ramps of 100 msec. Half of the tones presented to the subjects were equivalent to those represented by the white keys on the piano and half of the tones were equivalent to those represented by the black keys. Tones from the 8th octave were not used because of the insufficient duration of such high notes on the piano.

The following is the exact order of the tones presented to subjects.

Pure tones:

1-10) F2,C5,C6,D4,G#2,F#4,B6,c#5,A6,F#2.

11-20) D#3,G8,D#7,A2,F#5,B2,D#4,G#3,B7,A#8.

21-30) D#2,G3,A5,C#3,E8,C#7,F#8,G5,C#4,E5.

31-40) E3,D5,A#5,F#7,G#5,F4,D6,A2,C4,F3.

Piano tones:

1-10) F2,C5,C6,D4,G#2,F#4,B6,c#5,A6,F#2.

11-20 G1,C#3,F#5,E4,B1,G#3,D#4,F7,C4,A7.

21-30).G#4,A1,F3,A#4,C#6,A#3,E7,A#5,F#6,G4.

31-40) a#2, g5, d#7, f1, d#3, g#5, a4, g7, c#2, d#5

Appendix III

AP web site

A web site for AP was designed that allows subjects to complete the AP survey and test via internet. Survey results and test scores are compiled in a database format that is amenable to searches for identification of subjects for the study.

The address to the AP web site is

www.**absolutepitchstudy.org**

Appendix IV

Synaesthesia and AP

Perception is typically described and understood in terms of sense modalities. The sense modality is the relationship between the physical object (sound), a receptor organ (ear) and a conscious phenomenology (conscious readiness to hear) (Posner and Rothbart, 1991). Synaesthesia is a "condition experienced by those individuals who report a life-long history of perceptual experience in two sense modalities under conditions where sensory simulation and overt orientation of receptor organs are adequate for perception in only one modality" (Grossenbacher, 1996). Synaesthesia links unimodal stimulation to bimodal experience. Colored hearing is a common form of synaesthesia where a synesthete experiences vivid sensation of color upon hearing sound. Auditory stimuli have been reported to induce synesthetic experience more frequently than stimuli of other modalities. There also have been reports that AP possessors are more likely to have synesthetic experiences. These anecdotal reports have been based mainly on self-report of some AP possessors. These observations suggest that further studies on auditory synaesthesia may shed some light on the complexities of absolute pitch.

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