Comprehensive Arrayed Primer Extension Array for the Detection of 59 Sequence Variants in 15 Conditions Prevalent Among the (Ashkenazi) Jewish Population

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In the Ashkenazi Jewish population, serious and lethal genetic conditions occur with relatively high frequency. A single test that encompasses the majority of population-specific mutations is not currently available. For comprehensive carrier screening and molecular diagnostic purposes, we developed a population-specific and inclusive microarray. The arrayed primer extension genotyping microarray carries 59 sequence variant detection sites, of which 53 are detectable bi-directionally. These sites represent the most common variants in Tay-Sachs disease, Bloom syndrome, Canavan disease, Niemann-Pick A, familial dysautonomia, torsion dystonia, mucopolidosis type IV, Fanconi anemia, Gaucher disease, factor XI deficiency, glycogen storage disease type 1a, maple syrup urine disease, nonsyndromic sensorineural hearing loss, familial Mediterranean fever, and glycogen storage disease type III. Several mutations in the selected disorders that are not prevalent per se in the Ashkenazi Jewish populations, as well pseudodeficiency alleles, are also included in the array. The initial technical evaluation of this microarray demonstrates that it is comprehensive, robust, sensitive, specific, and easily modifiable. This cost-effective array is based on a diversely applied platform technology and is suitable for both carrier screening and disease detection in Ashkenazi and Sephardic Jewish populations. (*J Mol Diagn* 2007, 9:228–236; DOI: 10.2353/jmoldx.2007.060100)

group, which inhabited northern and eastern Europe since the 9th century C.E., accounts for ~90% of the 5.7 million Jews living in the United States today.¹ This Jewish community has remained distinct as a result of cultural factors such as religion, customs, and language. Concomitantly, a set of genetic disorders relatively specific to the AJ people has emerged for unknown reasons but for which hypotheses and speculations have ranged from random drift to selective advantages.² One or a small set of founder mutations, which account for almost all of the mutations in this population, characterizes each of these conditions.

The American College of Obstetricians and Gynecologists currently recommends AJ population-based carrier screening for four inherited disorders, Tay-Sachs disease, cystic fibrosis, Canavan disease, and familial dysautonomia, based on carrier frequencies of 1:40 or less.³ However, several additional disorders are prevalent in this ethnic group, and most of these are severely disabling or fatal. The detection rate in the AJ population for these additional disorders would be excellent because of the limited number of founder mutations and a few additional variants (Table 1).^{4–31} Most commonly, current genetic testing is performed sequentially or only for a subset of the disorders.

We report the development of a population- and disease-specific arrayed primer extension (APEX) microarray that includes 59 sequence variants for cost-effective, rapid, and reliable detection of carrier or affected status in 15 different disorders that are prevalent in individuals of primarily AJ extraction. Cystic fibrosis testing is not included in this microarray because the American College of Medical Genetics currently recommends a panel of 23 mutations be offered to anyone who considers having children, and therefore *CFTR* mutation analysis is already typically offered routinely and separately during the prenatal evaluation. Moreover, the *CFTR* mutations prevalent in AJs are already represented in this panel.^{32,33} To provide a fully comprehensive spectrum of

The contemporary Jewish population is subdivided into three discrete groups based on their long-term location of residence: Middle Eastern (also known as Oriental) Jews, Sephardic Jews, and Ashkenazi Jews (AJs). The latter

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mutations, however, the 23 CFTR mutations may be included in future diagnostic versions of this microarray.

The process of detecting mutations by APEX technology combines a microarray-based assay with Sanger sequencing. It is a process that is performed on a glass slide that contains a two-dimensional array of 5'-immobilized oligonucleotides to which a polymerase chain reaction (PCR)-amplified DNA sample is annealed. The oligonucleotide primers flank the target nucleotides to be identified. DNA polymerase uses four unique fluorescently labeled terminator nucleotides in a primer extension reaction. After stringent wash conditions, this procedure results in the identification of the one fluorescent base in the target sequence with a high signal-to-noise ratio.

To our knowledge, the APEX array reported here is the most comprehensive testing panel for the AJ population currently available. In addition, mutations common in other Jewish populations were included, as well as those few mutations specific for these conditions in other well-defined populations. Thus, diagnostic and risk evaluations are more optimally possible than without the inclusion of such mutations, especially in patients or couples of mixed ancestry.

Materials and Methods

Mutation Selection

The full set of mutations and pseudodeficiency alleles is listed in Table 1. The 59 variants on the APEX microarray were selected from the literature (Table 1), as well as the Online Mendelian Inheritance in Man (OMIM; <http://www.ncbi.nlm.nih.gov/OMIM/>) and Genetests (<http://www.genetests.org/>) databases. The selected mutations represent the most frequently identified mutations in AJs. In addition to pathogenic mutations for this population, we also included two pseudoalleles in the *HEXA* gene and several mutations that are associated with the conditions in our panel but are not exclusive to AJs (Table 1) to extend the diagnostic capacity of the microarray.

Oligonucleotide Microchips

Oligonucleotide primers were designed according to the wild-type gene sequences for both the forward and reverse directions. The oligonucleotides were each 25 bp in length and carried 5' 6-carbon amino linkers (MWG, Munich, Germany). Typically, these oligonucleotides were designed to identify specifically 1 bp in the sequence. For deletions and insertions with the same nucleotide 1 bp downstream, the oligo was designed so that it extended further into the deletion or insertion for optimal discrimination. The oligonucleotides spotted on the APEX array and PCR primers are proprietary information at this time.

The microarray slides used for spotting the oligonucleotides were produced as described previously.³⁴ For each mutation under interrogation, two forward and two reverse strand oligonucleotides were spotted, for a total of four data points per possible sequence variant. To

reduce the background fluorescence and to avoid rehybridization of unbound oligonucleotides to the APEX slide, the slides were washed with 95°C distilled water and 100 mmol/L NaOH before the APEX reactions.

Genomic and Synthetic Templates and Preparation

Where possible, native genomic DNA was collected from blood and cell culture samples using standard, commercially available DNA purification procedures. DNA (>100 ng/sample) from 23 patient or cell line samples with known mutations were evaluated on the chip (Table 2). The presence of mutations in commercially available samples (Coriell Cell repositories, <http://locus.umdj.edu/ccr/>) was verified in the Molecular Pathology laboratory at Stanford Hospital and Clinics. Blood samples were obtained from individuals who had been seen for genetic counseling and been identified as heterozygous for one or more of the mutations in the APEX array by a commercial laboratory. These carriers provided informed consent for research use of their DNA, obtained from venous blood samples. This set of samples was deidentified. Where native genomic DNA samples containing the screened mutations were unavailable, synthetic templates of ~50 bp in length were designed according to the variant sequence. Templates were created for both the sense and anti-sense directions and optimized for melting temperature.

For template preparation, the genes were amplified from genomic DNA in 37 amplicons. The PCR reaction mixture (15 μ l) was optimized with the following: 10 $\times$ TaqDNA polymerase buffer; 2.5 mmol/L MgCl₂ (Naxo, Tartu, Estonia); 0.25 mmol/L dNTP (MBI Fermentas, Vilnius, Lithuania) (20% fraction of dTTP was substituted with dUTP), 10 pmol of primer stock, genomic DNA (~25 ng), SMART-Taq Hot DNA polymerase (1.5 U) (Naxo), and sterile deionized water. After amplification (MJ Research DNA Thermal Cycler; MJ Research, Inc., Waltham, MA), the amplification products were concentrated and purified using Jetquick spin columns (Genomed GmbH, Lohne, Germany). In a one-step reaction, the functional inactivation of the traces of unincorporated dNTPs was achieved by the addition of shrimp alkaline phosphatase (Amersham Pharmacia Biotech, Inc., Milwaukee, WI) and fragmentation of the PCR product was accomplished by the addition of thermolabile uracil *N*-glycosylase (Epicenter Technologies, Madison, WI) followed by heat treatment.³⁵

APEX Reactions

The APEX mixture consisted of 32 μ l of fragmented product, 4 U of Thermo Sequenase DNA polymerase (Amersham Pharmacia Biotech, Inc.), 4 μ l of Thermo Sequenase reaction buffer (260 mmol/L Tris-HCl, pH 9.5, and 65 mmol/L MgCl₂) (Amersham Pharmacia Biotech, Inc.), and 1 μ mol/L final concentration of each of the fluorescently labeled ddNTPs: Cy5-ddUTP, Cy3-ddCTP, Texas Red-ddATP, and fluorescein-ddGTP (Perkin-Elmer Life

Table 1. Complete List of Mutations Detectable with the AJ APEX Assay

Disease	Gene	Mutations	Carrier frequency	Mutation detection with carrier screening	References
Bloom syndrome	<i>BLM</i>	736delATCTGAinsTAGATTC (2281del6/ins7) (from start codon 2206del6/ins7)	~1:102	>97%	4, 5, 6
Canavan	<i>ASPA</i>	Y231X (693C>A) E285A (854A>C) A305E (914C>A) IVS2-2A>G	~1:40	>99%	Genetests 7
Factor XI deficiency	<i>F11</i>	IVS14 + 1G>A E117X (576 G>T) (from start codon: E135X; 403G>T) F283L (1074 A>C) (from start codon: F301L; 901T>C)	~1:23	>96%	8, 9 OMIM
Familial dysautonomia	<i>IKBKAP</i>	IVS20 + 6T>C R696P (2397G>C) (from start codon: 2087G>C)	~1:30	>99%	Genetests 10
Familial Mediterranean fever	<i>MEFV</i>	Exon 2: E148Q (442 G>C) *E167D (501 G>C) T267I (800 C>T) Exon 3: P369S (1105 C>T) R408Q (1223 G>A) Exon 5: F479L (1437 C>G) Exon 10: R653H (1958 G>A) M680I (2040 G>C) ΔI692 (del2076–2078) M694V (2080 A>G) M694I (2082 G>A) K695R (2084 A>G) V726A (2177 T>C) A744S (2230 G>T) R761H (2282 G>A)	~1:5	~90%	Genetests
Fanconi anemia	<i>FANCC</i>	IVS4 + 4A>T (711 + 4A>T) R548X ^{NE and AJ} (1642C>T) 322delG ^{NE and AJ} (from start codon 67delG) Q13X ^{SI} (37C>T)	~1:80	>99%	Genetests 12, 13
Gaucher disease	<i>GBA</i>	84insG (from start codon 94insG) N370S (1226 A>G) (from start codon N409S) V394L ^{NJ} (1307 G>T) (from start codon V433L; 1297G>T) *L444P (1448 T>C) (from start codon L483P) IVS2 + 1G>A R496H ^{NJ} (1604 G>A) (from start codon R535H) *1035insG ^{NJ}	~1:10	>90%	Genetests 14, 15, 16
Glycogen storage disease Ia (von Gierke)	<i>G6PC</i>	R83C (326C>T) (from start codon R82C; 247C>T)	~1:71	~94%	17, 18
Glycogen storage disease type IIIa (N. African Jews)	<i>AGL</i>	1484delT (4455delT)	~1:35 ^{NAJ}	?All N. African Jews in Israel?	19
Maple syrup urine disease	<i>BCKHDB</i>	R183P (548 G>C) G278S (832G>A) E372X (1114G>T)	~1:80	~99%	20, 21
Mucopolipidosis type IV	<i>MCOLN1</i>	IVS3-2A>G delE1-E7 (511del6944)	~1:100	>95%	Genetests 22, 23, 24
Niemann-Pick type A	<i>SMPD1</i>	L302P (905T>C) (from start codon L337P;1010T>C) fsP330 (delC) (from start codon P365delC) R496L (1487G>T) (from start codon R1667L;1592G>T) *ΔR608 (from start codon R643)	~1:80	~95%	25, 26
Nonsyndromic sensorineural hearing loss	<i>GJB2</i>	35delG *167delT	~1:25	>60%	Genetests
Tay-Sachs	<i>HEXA</i>	G269S (805G>A) 1278insTATC IVS12 + 1G>C IVS9 + 1G>A	~1:28	>93%	27, 28 Genetests 29, 30

(table continues)

Table 1. *Continued*

Disease	Gene	Mutations	Carrier frequency	Mutation detection with carrier screening	References
Torsion dystonia	<i>DYT1</i>	R249W (745C>T) ^P R247W (739C>T) ^P IVS7 + 1G>A ^{FRC} *Δ7.6kb ^{FRC} ΔE302 ΔF323-Y328	~1:900	>95%	15, 31

P, pseudodeficiency allele; FRC, French Canadian; NE, Northern European; AJ, Ashkenazi Jewish; SI, Southern Italian; NJ, not Jewish specific; NAJ, North African Jews. Customary as well as conventional (counting from the A in the ATG start codon) amino acid and nucleotide numbering are used in the table, where the two are discrepant.

*A mutation is detected reliably in one direction only.

Sciences, Wellesley, MA). The DNA was first denatured at 95°C for 10 minutes. The enzyme and the dyes were immediately added to the DNA mixture, and the whole mixture was applied to prewarmed slides. The reaction was allowed to proceed for 20 minutes at 58°C, followed by washing once with 0.3% Alconox (Alconox, Inc., White Plains, NY) and twice for 90 seconds at 95°C with distilled water (TKA, Kaarst, Germany). A droplet of anti-bleaching reagent (AntiFade SlowFade; Molecular Probes Europe BV, Leiden, The Netherlands) was applied to the slides before imaging.

Data Points

The APEX array for Jewish disorders has a redundancy of data points to assure optimal sensitivity and specificity of the assay. For each mutation, four points are available for data analysis because both the forward and reverse primers are spotted in duplicate. This markedly reduces nonspecific signals, which could lead to false-positive interpretation, and enables easy differentiation between homozygous and heterozygous samples. The array images were captured by means of detector Genorama QuattroImager 003 (Asper Biotech Ltd., Tartu, Estonia) at

20-μm resolution. This detection instrument combines a total internal reflection fluorescence-based excitation mechanism with a charge-coupled device camera.³⁵ Sequence variants were individually identified using Genorama 4.2 genotyping software. This software allows automated base calls, which were subsequently verified through technical interpretation of each spot by review of an image of the four signals, a bar graph representing signal intensities, and review of the preliminary call at every mutation spot.

Results

Fifty-nine sequence variants, common to 15 genetic disorders that are most prevalent among Jewish populations, were selected for inclusion on the newly developed diagnostic APEX array. Mutations with a high allele frequency in the AJ population were especially selected because they are very well characterized. Our aim was to develop a comprehensive diagnostic panel enabling carrier and disease detection among Jewish individuals and among individuals affected with disorders most prevalent in Jewish populations (Table 1). That is, if a mutation was clearly associated with one of the conditions on the APEX array, but not specific to the AJ population, we aimed to include such a mutation to offer an optimally inclusive mutation panel. An example is the addition of the IVS7 + 1G>A splice site mutation and the Δ7.6kb deletion in the *HEXA* gene. Both of these mutations are relatively common in French Canadians. In addition, the two well-characterized pseudodeficiency alleles for the *HexA* gene were included to enable interpretation of apparent deficiencies found in the course of enzyme testing.

To evaluate all selected sequences present on the chip, sample DNA was amplified in 37 amplicons. All PCR mixes include a 20% substitution of dUTPs for dTTPs, which enables subsequent fragmentation with uracil *N*-glycosylase as reported previously.³⁵ Every sequence variant is identified by at least two unique 25-bp oligonucleotides, typically one for the forward and one for the reverse strand. When mutations occur in neighboring nucleotides, however, the method permits a smaller number of identifying oligonucleotides. A total of 118 oligonucleotides were annealed to the APEX microarray slide to identify 59 sequence variants.

Table 2. Genomic DNA Samples Used for Mutation Evaluation on the AJ APEX Array

Gene	Mutations validated with gDNA
<i>MCOLN1</i>	IVS3 – 2A>G
<i>MCOLN1</i>	Del E1-E7
<i>SMPD1</i>	L302P (from start codon L337P)
<i>SMPD1</i>	fsP330 (from start codon P365delC)
<i>SMPD1</i>	R496L (from start codon R1667L)
<i>G6PC</i>	R83C (from start codon R82C)
<i>BLM</i>	2281del6/ins7 (from start codon 2206del6/ins7)
<i>HEXA</i>	G269S
<i>HEXA</i>	1278insTATC
<i>HEXA</i>	IVS12 + 1G>C
<i>ASPA</i>	Y231X
<i>ASPA</i>	E285A
<i>FANCC</i>	IVS4 + 4A>T
<i>IKBKAP</i>	R696P
<i>IKBKAP</i>	IVS20 + 6T>C
<i>GBA</i>	84 insG (from start codon 94insG)
<i>GBA</i>	IVS2 + 1G>A
<i>GBA</i>	N370S (from start codon N409S)
<i>GBA</i>	R496H (from start codon R535H)

Figure 1. APEX analysis for sequence variant 2281del6ins7 (735delATCTGainsTAGATTC) in the *BLM* gene. Each numbered row represents the analysis of an individual patient sample. The row presents two sets of four-channel fluorescent images representing the bases adenine (A), cytosine (C), guanine (G), and thymine (T), respectively, for the sense strand (top) and anti-sense strand (bottom). The histograms to the right of the fluorescent images are of the fluorescent intensities of the four channels at the mutation analysis site (from left to right: A, C, G, and T). The letters to the right of the histogram represent the base(s) identified on each strand. Row 1 contains the results of heterozygous target DNA from an individual with genotype WT/2281del6ins7. In this case, the sense strand is extended by both the WT base A and base T, complementary for the mutation. The oligo that interrogates the mutation from the opposite direction demonstrates presence of a T (WT) and a G, the expected signal in a mutation carrier. This illustrates the separate primers used for deletions/insertions. Row 2 contains the results of normal DNA (WT/WT), in which the sense strand is extended by the WT base A and the anti-sense strand is extended by the WT base T. Row 3 is a negative control.

In 16 instances, the oligonucleotides originally designed for the microarray failed to perform the APEX reaction robustly. APEX primer failure is mainly because of self-annealing secondary structures that result in self-priming and extension or failure to hybridize altogether. On the assumption that these were the reasons for failure, we redesigned the initial 16 primers with either an incorporation of a single mismatch or the inclusion of a modified nucleotide at either the 5' end or internally. These changes can reduce primer self-complementarity without a negative effect on hybridization and extension. Our final set of APEX primers succeeded in eliminating secondary structure interference from all but six of the primers and detected 53 sequence variants (of 59 variants at 58 amino acid locations) in both directions. Four were detected from only the sense strand (when the anti-sense direction does not work reliably, mutation $\Delta 7.6$ kb in the *HEXA* gene, mutation E167A in *MEFV*, 167delT in *GJB2*, and L444P in the *GBA* gene), and two from only the anti-sense strand (the sense direction does not work reliably), mutation 1035insG in the *GBA* gene and $\Delta R608$ in the *SMPD* gene. The reason for detecting variants on both DNA strands is primarily confirmatory should there be a failure to obtain a good duplicate signal in one strand. Although having this second internally confirming strategy in place is desirable, experience with the microarrays confers confidence that a clear duplicate signal from one strand provides specific, reproducible, and reliable results.

With the approach described above, sensitive and specific identification of the wild-type (WT) and mutation alleles has been achieved for each variant interrogated by the APEX method on this microarray. In unaffected mutation carriers of autosomal recessive conditions, one or more heterozygous mutations are expected in the

entire array, as long as the heterozygous mutations are not present in compound heterozygous form in the same gene. Strom and colleagues¹⁴ reported that in a limited panel of eight AJ disorders, approximately one in seven individuals is a carrier of at least one heterozygous mutation. Affected patients are expected to carry two mutations, either two different mutations in the same gene or a homozygous mutation. Figures 1 and 2 illustrate representative results for a mutation in the *BLM* gene (Bloom syndrome; 2281del6ins7) and in the *IKBKAP* gene (familial dysautonomia; R696P), respectively.

The APEX microarray for Jewish disorders was validated with 23 patient samples, of which five were blinded to the operator (Table 2), with 19 different mutations. Mutations for which genomic DNA from patients or carriers could not be obtained were tested with synthetic oligonucleotides (Table 3), the content of which was based on the wild-type sequence but with incorporation of the mutation of interest. All sites for which genomic DNA was available were tested with synthetic oligonucleotides as well, and results were congruent.

With this initial validation series, no false negatives or false positives were observed. Thus, sensitivity (TP/TP + FN) and specificity (TN/TN + FP) were each 100%. The APEX reactions are entirely reproducible under standardized and requisite testing conditions. These conditions include 1) the requirement of good quality of the DNA sample, 2) optimized PCR amplification, 3) successful fragmentation of the uracil *N*-glycosylase-treated PCR product, 4) optimized, and 5) individually validated arrayed primers. For this APEX array, each individual sequence variant was tested 3 to 10 times using synthetic oligonucleotides and some patient samples. The results were highly reproducible from array to array. In addition, individual batches of arrays undergo quality control test-

Figure 2. APEX analysis for sequence variant R696P in the *IKBKAP* gene. The results are presented as described in Figure 1. Row 1 contains the results of normal DNA (WT/WT), in which the sense strand is extended by the WT base G and the anti-sense strand is extended by the WT base C. Row 2 contains the results of heterozygous target DNA from a carrier (WT/R696P). In this case, the sense strand is extended by the WT base G and base C complementary for the mutation. The anti-sense strand is extended by WT base C and base G, complementary for the mutation. Row 3 is a negative control.

ing before they are put into use, in accordance with ISO 9001 quality standards by Bureau Veritas Quality International. However, complete validation of the AJ APEX assay, requiring a full complement of patient samples with mutations at each of the array sites and in a manner blinded to the operator, will be undertaken to the extent possible.

Discussion

This report extends the application of APEX microarray technology to a panel of 15 conditions prevalent among Jewish populations, primarily AJs. The technology has been previously applied to the detection >200 CFTR mutations in cystic fibrosis,³² 198 mutations underlying sensorineural hearing loss (mostly nonsyndromic),³⁶ Usher syndrome,³⁷ mutations in β -globin and G6PD genes,³⁸ and >400 mutations in the gene associated with five distinct retinal phenotypes.³⁹ The APEX technology is an integrated system of multiplex primer extension on a DNA microarray chip with fluorescence imaging and data analysis that is accurate, reproducible, and technically robust. It is ideal for molecular diagnosis and the detection of carriers in a targeted population in which there are multiple genes and multiple mutations to be tested.

At present, the Jewish people are scattered around the world, with the largest populations living in Israel and the United States. Despite the geographic distribution, the Jewish people continue to share a cultural background that may include religion, language, traditions, and history. Approximately 5.7 million Jews live in the United States, and of these, the vast majority are of Ashkenazi (German/Eastern European) Jewish descent. Ostrer¹ has summarized the molecular basis for the vast majority of >40 Mendelian conditions with higher frequencies in Jewish populations than in others. This information represents an expanded—and expanding—infor-

mation base on which individuals and couples can determine their status as carriers or affected individuals and the status of their fetus during pregnancy. Although information of this nature has most often been connected to family counseling and reproductive decision-making, it should not be overlooked that the outcomes of testing are most often associated with counseling that communicates that neither the individual, couple, nor fetus is at risk for the health problems associated with this subset of genetic disorders.

We have generated a microarray that expands the current repertoire of testing, represents the majority of the conditions found primarily in the AJ population, and lays the groundwork for extending this panel to other diseases with mutations shared among various Jewish groups. Among the 15 conditions represented on this array: 1) five result in mental retardation, neurological deterioration, and childhood or preadult death (Tay-Sachs, Canavan, maple syrup urine disease, Niemann-Pick type A, mucopolipidosis type IV); 2) three result in marked developmental delays and growth retardation (familial dysautonomia, Fanconi anemia, Bloom syndrome); 3) six have multiple system involvement, including skeletal, skin, bone marrow, and/or internal organs with variable severity and progressions (familial dysautonomia, glycogen storage disease type III, Gaucher disease, glycogen storage disease type I, Fanconi anemia, Bloom syndrome), the last three of which also have marked predispositions to cancer; and 4) one results in severe injury-related bleeding (factor XI deficiency), one in extreme fevers and synovitis (familial Mediterranean fever), one in later onset neurological manifestations (torsion dystonia), and one in childhood hearing loss (connexin-related sensorineural hearing loss).

Depending on the individual's geographic and ethnic history and the selection of the population carrier frequency on extracts from the literature, the likelihood of being a carrier for one of the autosomal recessive con-

Table 3. Synthetic Oligonucleotides Designed for Detection Evaluation on the AJ APEX Array

Disorder, gene, mutation(s)	Synthetic oligonucleotides
Bloom syndrome: <i>BLM</i> 736delATCTGAinsTAGATTC (2281del6/ins7)	5'-ttttatacttagATTCCAGCTACAT[ATCTGA] [ins TAGATTC]CAGGTGATAAGACTGACTCAG-AAGC-3'
Canavan: <i>ASPA</i> Y231X (693C>A) E285A (854 A>C) A305E (914C>A) IVS2 - 2A>G	5'-ATAAAATTATAGAGAAAGTTGATTA[C/A]CCCCGGGATGAAAATGGAGAAATG-3' 5'-ACCGTGTAACCCCGTGTGTGAATG[A/C]GGCCGCATATTACGAAAAGAAAGAA-3' 5'-AAGACAACATAAACTAACGCTCAATG[C/A]AAAAAGTATTCTGCTGCTGTTTACAT-3' 5'-aagaaagacgttttttgattttttttc[a/g]gACTTCTCTGGCTCCACTACCTGTC-3'
Factor XI deficiency: <i>F11</i> IVS14 + 1G>A E117X (576G>T) F283L (1074T>C)	5'-GGAAGGAGGGAAGGACGCTTGCAAG[g/a]taacagagtgttcttagccaatgga-3' 5'-CAGCTCAGTTGCCAAGAGTGTCAA[G/T]AATGCCAAGAAAGATGCACGGATGA-3' 5'-TTCTTCATTTTACCATGACACTGAT[T/C]TCTTGGGAGAAGAACTGGATATTGT-3'
Familial dysautonomia: <i>IKBPAP</i> IVS20 + 6T>C R696P (2397G>C)	5'-ATTTCGGAAGTGGTTGGACAAGtaag[t/c]gccattgtactgtttgcgactagtt-3' 5'-CTGCGGAAAGTGGAGAGGGGTTTAC[G/C]GATTTGTACTGTTGTGCCCCAGGA-3'
Familial Mediterranean fever: <i>MEFV</i> E148Q (442 G>C) E167D (501 G>C) T267I (800 C>T) P369S (1105 C>T) R408Q (1223G>A) F479L (1437 C>G) R653H (1958G>A) M680I (2040G>C) Δ1692 (del2076-2078) M694V (2080A>G) M694I (2082G>A) K695R (2084A>G) V726A (2177T>C) A744S (2230G>T) R761H (2282G>A)	5'-TGCCAGCCTGCGGTGCAGCCAGCCC[G/C]AGGCCGGAGGGGGCTGTGAGGAA-3' 5'-GCAACGCAGAGAGAAGGCCTCGGA[G/C]GGCCTGGACGCGCAGGGCAAGCCTC-3' 5'-ATTCTCCTGACTCTAGAGGAAAAGA[C/T]AGCTGCGAATCTGGACTCGGCAACA-3' 5'-AAGGAAGAGCCCGGGAAGCCTAAGC[C/T]CCCAGCCCTGCCACAGTGTAAGCG-3' 5'-CTGAGTCAGGAGCACCAAGGCCACC[G/A]GGTGCGCCCATTTGAGGAGGTCGCC-3' 5'-ACTTCTGAGCAGCAAGAGCATTT[C/G]TTTGTGGCCTCACTGGAGGACGTGG-3' 5'-TCTCCGAGTTTCCTCTCTGGCCGCC[G/A]TTACTGGAGGTGGAGGTTGGAGAC-3' 5'-CATCCATAAGCAGGAAAGGGAACAT[G/C]ACTCTGTGCCAGAGAATGGCTACT-3' 5'-CAGAGAATGGCTACTGGGTGGTGAT[AAT/-]GATGAAGGAAAATGAGTACCAGG-3' 5'-GAATGGCTACTGGGTGGTGATAATG[A/G]TGAAGGAAAATGAGTACCAGGCGTC-3' 5'-ATGGCTACTGGGTGGTGATAATGAT[G/A]AAGGAAAATGAGTACCAGGCGTCCA-3' 5'-GGCTACTGGGTGGTGATAATGATAGA[A/G]GGAAAATGAGTACCAGGCGTCCAGC-3' 5'-GTGGGCATCTTCGTGGACTACAGAG[T/C]TGAAGCATCTCCTTTTACAATGTG-3' 5'-AGCCAGATCCACATCTATACATTC[G/T]CCAGCTGCTCTTTCTCTGGGCCCT-3' 5'-CAACCTATCTTCAGCCCTGGGACAC[G/A]TGATGGAGGGAAGAACACAGCTCCT-3'
Fanconi anemia: <i>FANCC</i> IVS4 + 4A>T (711 + 4A>T) R548X (1642C>T) 322delG Q13X (37C>T)	5'-AAAACCTTAACCTCTGGATACAGgta[at]gagagtaaatcttgcctctgcacttc-3' 5'-AAGCCCTAGATCAGAAAACTGGCC[C/T]GAGAGCTCCTTAAAGAGCTGCGAAC-3' 5'-TTGGATGCAGAAGCTTTCTGTATG[G]GATCAGGCCTTCCACTTTGGAACC-3' 5'-TTCAGTAGATCTTTCTGTGATTAT[C/T]AGTTTTGGATGCAGAAGCTTTCTGT-3'
Gaucher disease type 1: <i>GBA</i> N370G (1226A>G) L444P (1448T>C) 84insG IVS2 + 1G>A R496H (1604G>A) V394L (1307G>T) 1035insG	5'-tcttttgctttgtccttaccttagA[A/G]CCTCCTGTACCATGTGGTCGGCTGG-3' 5'-CTGGTTGCCAGTCAGAGAAGACGACC[T/C]GGACGCACTGGCACTGATGCATCCC-3' 5'-TCATGGCTGGCAGCCTCACAGGATT[-/G]GCTTCTACTT CAGGCAGTGTCTGTGG-3' 5'-CTTCAGGCAGTGTCTGGGCATCAG[g/a]tgagtgaagtcaggcagtgaggagg-3' 5'-TCCATTACACCTACCTGTGGCGTC[G/A]CCAGTGATGGAGCAGATACCAAGG-3' 5'-GAACCCCGAAGGAGGACCAATTGG[G/T]TGCGTAACCTTTGTCGACAGTCCCAT-3' 5'-GCCTGGGCTTCAACCCCTGAACATCA[+G]GCGAGACTTCATTGCCCGTACCTA-3'
Glycogen storage disease type 1A: <i>G6PC</i> R82C (326C>T)	5'-tttccatagGATTCTCTTTGGACAG[C/T]GTCCATACTGGTGGGTTTGGATAC-3'
Glycogen storage disease type III: <i>AGL (GDE)</i> 1484ΔT (4455delT)	5'-TATAGTTTTGGTTAAAAATGTCTT[T]TCCCGACATTATGTTTCATCTTGAGA-3'
Maple syrup urine disease: <i>BCKHDB</i> R183P (548 G>C) G278S (832G>A) E372X (1114G>T)	5'-TTTAACTGTGGAAGCCTCACTATCC[G/C]GRCCCTTGGGGCTGTGTGGTCAT-3' 5'-GAGTGATGTTACTCTAGTTGCCTGG[G/A]GCACTCAGgtgagagcattgatcc-3' 5'-TGACACACCATTTCTCTACATTTTT[G/T]AACCATTTACATCCAGACAAATG-3'
Mucopolipidosis IV: <i>MCOLN1</i> IVS - 2A>G ΔEx1 → Ex7	5'-acaggccctcccccttctctgcccac[a/g]gTACCTGGCGTTGCCGTGACGTGTCA-3' 5'-cactgcagcctcgacctctgggct {caagcgatcctcc} > exon 1 → exon 7... < agATCAGCTTTGAC } AACAAAGCACACAGTGGGCGGATCC-3'
Niemann-Pick type A: <i>SMPD1</i> L302P (905T>C) fsP330 R496L (1487G>T) Δ608	5'-CGGGCCCTGACCACCGTCACAGCAC[T/C]TGTGAGGAAGTTCTGGGGCCAGTG-3' 5'-ACACCTGTCAATAGCTTCCCTCCCCCTTCATTGAGGGCAACACTC-3' 5'-cagccccacatccttgcagGTTACC[G/T]TGTGACCAAAATAGATGGAACACTACTCC-3' 5'-TGCCCGTGTGACAGCCCTGCTCTG[tee]CGCCACCTGATGCCAGATGGGAGCC-3'
Nonsyndromic sensorineural hearing loss: <i>GJB2</i> 35delG 167ΔT	5'-GGCACGCTGCAGACGATCCT[G]GGGGGTGTGAACAAACACTCCACCA-3' 5'-TGCAACACCC[T]GCAGCCAGGCTGCAAGAACGTGTGC-3'

(table continues)

Table 3. *Continued*

Disorder, gene, mutation(s)	Synthetic oligonucleotides
Tay-Sachs disease: <i>Hex A</i> G296S (805G>A) 1278insTATC IVS12 + 1G>C IVS9 + 1G>A R249W (745C>T) R247W (739C>T) IVS7 + 1G>A Δ7.6kb	5'-TGGCCACACTTTGTCTCTGGGGACCA[G/A]gtaagatgatgtctctgggaccagag-3' 5'-CCCCCTGGTACCTG/AACCGTATATC[TATC]CTATGGCCCTGACTGGAAGGAA-3' 5'-ACACAAACCTGGTCCCCAGGCTCTG[g/c]taagggttttcggggggagggtgga-3' 5'-AGCTGGAGTCCCTTCTACATCCAGAC[g/a]tgaggaaggaaggaggggtcgggtggg-3' 5'-GGAGGTCATTGAATACAGCACGGCTC[c/t]GGGGTATCCGTGTGCTTGCAGAGTT-3' 5'-TGTGAAGGAGGTCATTGAATACGACA[c/t]GGTCCCGGGGTATCCGTGTGCTTGC-3' 5'-GGCCACACTTTGTCTCTGGGGACCAG[g/a]taagaatgatgtctctgggaccagagg-3' 5'-aattattattgactatagtccacct/attgtgctctcgaatagtatgtctt-3'
Torsion dystonia: <i>DYT1</i> ΔE302 ΔF323-Y328	5'-AGACATTGTAAGCAGAGTGGCT[cac]GAGATGACATTTTCCCCAAAGAGG-3' 5'-TCTCAGATAAAGGTGCAAAACGGT[CTTACCAAGTTACATTA]TTACTACGATGATTGACAGTCATGA-3'

Crossed-out sequences in the second column reflect nucleotides deleted by mutations listed in the first column.

ditions on the microarray (ie, excluding torsion dystonia) is approximately two in every five people. The diagnosis of any of these conditions is a foundation for appropriate medical care and interventions, whereas the determination of heterozygosity (carrier status) is the basis for genetic counseling. The primary value and use of this microarray is in its capacity to provide diagnoses of the diseases associated with the selected mutations. Application of the technology to screening for conditions that are associated with mild to moderate morbidity or disability may not be high priorities and poses considerations and implications beyond the scope of this report, ranging from the appropriateness of inclusion on a panel to the role of genetic counseling as the capacity to generate data expands. In a related manner, inclusion of the pseudodeficiency alleles for Hex A (Tay-Sachs disease) may be better applied to an individual who has a positive enzyme-based screening than for a prenatal test. However, this technology may be readily modified for various applications, including screening, at low cost and may, for example, be the entrée to targeted newborn screening. Ideally, patients and their physicians would be able to choose the information they wish to receive from the array through a process of selective interpretation. Whereas the data as a whole cannot be blocked selectively, review and interpretation ultimately occur spot by spot and should be guided by preselecting locations on the array, depending on whether the assay is performed for diagnostic, screening, or confirmatory purposes.

Tay-Sachs carrier screening is the prototype of successful population screening for inherited disease. It has been available in the United States since 1971 (by enzyme analysis). Carrier screening programs for this condition have enabled an international reduction of affected births by >90%.⁴⁰ Carrier screening guidelines by the American College of Obstetricians and Gynecologists advise to screen all AJ couples for cystic fibrosis, Canavan disease, Tay-Sachs disease, and familial dysautonomia.³ Of these, only cystic fibrosis carrier screening is already offered routinely to anyone who considers having children. Because several other disorders have only slightly lower allele frequencies (Table 1) and are clinically similarly devastating, we developed a single APEX assay to encompass the most frequent conditions and mutations. Thus, potential carriers seeking genetic coun-

seling and carrier screening can be tested with a single panel rather than sequentially or for a small subset of conditions. In addition, this assay is equally suitable for mutation detection in potentially affected patients. Finally, through inclusion of mutations that are specific to non-AJ groups and other select populations with a high carrier frequency for these disorders, the microarray is inclusive of mixed couples at risk and potentially affected non-AJ patients.

In summary, the APEX array system presents a new diagnostic approach for comprehensive screening of AJ and other Jewish mutations, through an integrated system consisting of a single DNA microarrayed chip, multiplex primer extension on the array, and automated data analysis.^{35,41} The large number and selection of mutations on the AJ microarray result in a higher mutation detection capability than assays currently available in most diagnostic laboratories. This APEX assay is highly sensitive, specific, reproducible, and technically robust in our initial technical validation. Because the array is based on a platform technology, it is suitable for the detection of a variety of genetic disorders. In addition, it can easily be modified to accommodate additional mutations. Although a large clinical study is necessary to establish further its diagnostic utility and effectiveness and a full validation with mutations in genomic DNA will be preferable, the AJ APEX microarray is comprehensive and will almost certainly allow a cost-effective approach for both carrier screening and disease detection in Ashkenazi and Sephardic Jewish populations.

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