Clinical characteristics.Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP), which includes both hereditary diffuse leukoencephalopathy with spheroids (HDLS) and pigmentary orthochromatic leukodystrophy (POLD), is characterized by executive dysfunction, memory decline, personality changes, motor impairments, and seizures. A frontal lobe syndrome (e.g., loss of judgment, lack of social inhibitors, lack of insight, and motor persistence) usually appears early in the disease course. The mean age of onset is usually in the fourth decade. Affected individuals eventually become bedridden with spasticity and rigidity. The disease course ranges from two to 30 or more years (mean: 8 years). Diagnosis/testing.The diagnosis is suspected in individuals with characteristic clinical and brain MRI findings and is confirmed by identification of a heterozygous pathogenic variant in CSF1R, the only gene in which pathogenic variants are known to cause ALSP. Management.Treatment of manifestations: Supportive management includes: attention to general care and nutritional requirements; antiepileptic drugs for seizures; and antibiotic treatment for general and recurrent infections. Prevention of secondary complications: Information about and support systems for the social problems and suicidal tendencies often associated with disease progression. Surveillance: Periodic brain MRI and clinical evaluation to monitor disease progression Agents/circumstances to avoid: Use of first-generation neuroleptics due to increased seizure risk and risk of additional parkinsonian signs; medications used to treat multiple sclerosis as they are of no benefit and have major side effects. Genetic counseling.ALSP is inherited in an autosomal dominant manner. Individuals with ALSP usually have an affected parent; de novo mutation can occur. Each child of an individual with ALSP has a 50% chance of inheriting the pathogenic variant. Prenatal testing is possible if the pathogenic variant in a family is known. GeneReview Scope Image Table Hereditary diffuse leukoencephalopathy with spheroids (HDLS) Pigmentary orthochromatic leukodystrophy (POLD) Diagnosis Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP), which includes both hereditary diffuse leukoencephalopathy with spheroids (HDLS) and pigmentary orthochromatic leukodystrophy (POLD) (see Nomenclature) should be suspected in individuals with the following clinical and brain MRI findings; however, definite diagnosis relies on identification of a CSF1R pathogenic variant. Progressive neurologic decline Presenting signs may include: Personality changes, cognitive impairments, memory decline, and depression; Motor impairments including paresis, gait dysfunction, bradykinesia, rigidity and tremor; In rare individuals, seizure. Later signs usually include dementia, seizures, and pyramidal and extrapyramidal signs. Family history consistent with autosomal dominant inheritance Brain MRI [Van Gerpen et al 2008, Sundal et al 2012c, Bender et al 2014, Konno et al 2014] The white matter lesions are hyperintense on T2- and FLAIR-weighted images, and hypointense on T1-weighted images. Bifrontal or bifrontoparietal T2/FLAIR hyperintensities in the deep, subcortical, and periventricular areas are typical. The white matter lesions are often asymmetric, especially in the early stages of the disease. Also early on they are patchy and focal, but with time become confluent. T2 and FLAIR hyperintensities are present in other areas, including the corpus callosum and corticospinal tracts. Cerebral atrophy manifesting as enlarged ventricles is typical, as well as cerebral atrophy corresponding to the white matter lesions. The following are absent: Significant grey matter pathology Brain stem atrophy Contrast uptake in the parenchyma Cerebellar abnormalities are minimal. Testing Brain biopsy. Prior to the definition of the molecular basis of ALSP, the only method of definitive diagnosis was the demonstration of white matter lesions with axonal spheroids in brain biopsy or at autopsy [Axelsson et al 1984, Baba et al 2006, Sundal et al 2012b]; see Clinical Description, Brain pathology. However, molecular genetic testing practically eliminates the need for performing brain biopsy for diagnosis. Molecular Genetic Testing Gene. CSF1R is the only gene in which pathogenic variants are known to cause ALSP. Table 1. Table 1. Summary of Molecular Genetic Testing Used in ALSP Testing Strategy To confirm/establish the diagnosis in a proband. Sequence analysis of CSF1R is recommended to confirm the diagnosis in a proband with suggestive clinical and brain MRI findings. An alternative genetic testing strategy is the use of a multi-gene panel that includes CSF1R and other genes of interest (see Differential Diagnosis). Note: The genes included and the methods used in multi-gene panels vary by laboratory and over time. Clinical Characteristics Clinical Description Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) is characterized by a constellation of findings including executive dysfunction, memory decline, personality changes, motor impairments, and seizures. A frontal lobe syndrome (including loss of judgment, lack of social inhibitors, lack of insight, and motor persistence) usually appears early in the disease course. The presenting problems and rate of progression vary among individuals and even within the same family harboring the same pathogenic variant. The mean age of onset is usually in the fourth decade, but ranges from early adulthood to the eighth decade of life [Sundal et al 2012c]. The disease course may be from two to 30 years or more with a mean of eight years. Signs and symptoms that usually occur during the disease course include the following: Personality problems, memory decline, executive dysfunction Disturbances of higher cortical function such as motor aphasia, agraphia, acalculia, and apraxia Depression Gait disturbance Pyramidal signs such as spasticity, hyperreflexia, extensor plantar response, hemiparesis, or quadriparesis Sensory deficits including some impairment of vibration, position, tactile and pain perception. The higher integrative sensory functions such as graphesthesia, stereognosis, and double simultaneous stimulation are also impaired. Parkinsonian signs such as rigidity, bradykinesia, tremor (resting and/or kinetic), shuffling gait, and postural instability. Hypomimic face and hypophonic voice are common. Lack of beneficial response to levodopa defines the parkinsonian signs as atypical. Bulbar/pseudobulbar signs: dysphagia, dysarthria, slurred speech, and palatal myoclonus Cerebellar signs with ataxia, dysmetria, and intention tremor Visual field defects such as homonymus quadrant- or hemi-anopsia Other signs of a movement disorder: dystonia, myoclonic twitches, dyskinesia, and akathisia Seizures in some (at times only a single episode at the onset of the illness) Progressive course Affected individuals eventually become bedridden with spasticity and rigidity. They lose speech and voluntary movements, and appear to be generally unaware of their surroundings. In the last stage of the disease, individuals lose their ability to walk and progress to a vegetative state. Primitive reflexes, such as visual and tactile grasp and mouth-opening reflex, as well as the sucking reflex, are present. Death most commonly results from pneumonia or other infections. Other findings. Cerebrospinal fluid (CSF): Normal cell count, glucose concentration, and proteins No inflammatory cells Usually normal isoelectric focusing and no oligoclonal bands; however oligoclonal bands have been demonstrated in samples from affected individuals with the pathogenic variants p.Asn854Lys or p.Val 838Leu [Karle et al 2013, Levin et al 2014, Schuberth et al 2014, Sundal et al 2015]. No identified CSF biomarker. The following preliminary findings in four persons with ALSP need to be interpreted cautiously and require further research [Sundal et al 2012a, Sundal et al 2015]: Normal A╬▓42 protein concentrations Minimally increased levels of total Tau protein concentrations Borderline normal phospho-Tau protein concentrations Elevated neurofilament light chain (NF-L) proteins (note that NF-L proteins are markers of neuronal death and axonal damage) Slight increase in glial fibrillary acidic protein (GFAP), indicating gliosis or astroglial cell damage Brain pathology. The following features may be seen on brain biopsy or at autopsy: White matter changes which are typically vacuolated and demyelinated Axonal spheroids in the white matter lesions that are immunoreactive for neurofilament, amyloid precursor protein (APP), and ubiquitin Bizarre astrocytes and lipid-laden and myelin-laden macrophages Unaffected or very mildly affected basal ganglia, thalamus, hypothalamus, hippocampus, substantia nigra, raphe nucleus, reticular formation, and cerebellar grey matter Absence or only traces of amyloid angiopathy in parenchymal or leptomeningeal vessels Pigmented changes of either iron or lipofuscin found in macrophages and other glia cells Genotype-Phenotype Correlations No genotype-phenotype correlation exists: individuals from the same family harboring the same CSF1R pathogenic variant do not necessarily share the same phenotype. In the end stage all have devastating multiple neurologic impairments. Penetrance Penetrance appears to be incomplete [Karle et al 2013, Sundal et al 2015]; however, estimates have not been calculated given the limited number of families reported to date. Although ALSP is a dominantly inherited disease, de novo mutation occurs and variable expressivity in terms of the phenotype and the disease course can be found in members of the same family sharing the same pathogenic variant. Nomenclature Hereditary diffuse leukoencephalopathy with spheroids (HDLS) is within the same disease spectrum as familial pigmentary orthochromatic leukodystrophy (POLD) [Wider et al 2009]. Because of the phenotypic and radiologic similarities of the two disorders, Wider et al [2009] proposed the following terminology for the combined entity: ΓÇÿadult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP)ΓÇÖ. Families with POLD have recently been found to have CSF1R pathogenic variants [Nicholson et al 2013], providing evidence that HDLS and POLD are a single disease entity. Prevalence ALSP was previously thought to be a rare disease but recent expanded publications in this field have demonstrated that it is more common than previously recognized; however; actual prevalence figures have not been reported. Genetically Related (Allelic) Disorders No phenotypes other than those described in this GeneReview have been associated with mutation of CSF1R. Differential Diagnosis The clinical presentation of ALSP often overlaps with other neurologic disorders. ALSP should be considered in previously healthy individuals who develop cognitive decline, memory problems, and personality changes in midlife with a progressive course and white matter lesions evident on brain MRI. Because the signs and symptoms in the early stages of ALSP are nonspecific, ALSP can often be confused with the inherited and sporadic disorders listed below. In individuals with ALSP, laboratory and/or genetic testing for these other disorders are normal. Autosomal Dominant Disorders Individuals with the adult form of Alexander disease may not have the white matter lesions seen in individuals with the childhood-onset form and may only have atrophy infratentorially. Clinical findings are highly variable with bulbar/pseudobulbar signs, ataxia and spasticity, in addition to myriad other symptoms. However, palatal myoclonus should lead the diagnostician to consider molecular genetic testing of GFAP. Adult individuals with Alexander disease frequently have normal cognitive function and MRI imaging that demonstrates distinct brain stem and spinal cord atrophy [Farina et al 2008]. Additionally, there are often abnormalities in the basal ganglia, thalamus, and brain stem with contrast enhancement of the cerebrum or brain stem [van der Knaap et al 2005, Sawaishi 2009]. Most individuals with the adult form of Alexander disease have a GFAP de novo pathogenic variant. Adult-onset autosomal dominant leukodystrophy (ADLD) (OMIM) with autonomic symptoms is characterized by white matter changes in a frontoparietal distribution involving the corticospinal tracts from the supratentorial regions to the spinal cord. Additionally, it involves the superior and middle cerebellar peduncles [Sundblom et al 2009]. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is characterized by multiple cerebral infarcts and white matter lesions including the characteristic temporal pools [Tikka et al 2009]. Frontotemporal dementia typically demonstrates frontal and/or temporal atrophy with far fewer white matter lesions than are seen in ALSP [Seelaar et al 2011]. (See: MAPT-Related Disorders; Frontotemporal Dementia, Chromosome 3-Linked; GRN-Related Frontotemporal Dementia). A study comparing the pattern of cerebral atrophy suggests that the pattern of atrophy is more widespread in persons with pathogenic variants in GRN (encoding progranulin) than in persons with pathogenic variants in MAPT (encoding microtubule-associated protein tau). C9ORF72-related FTD is associated with symmetric atrophy predominantly involving dorsolateral, medial, and orbitofrontal lobes, with additional loss in anterior temporal lobes, parietal lobes, occipital lobes, and cerebellum. In contrast, striking anteromedial temporal atrophy is associated with MAPT pathogenic variants and temporoparietal atrophy was associated with GRN pathogenic variants. The sporadic frontotemporal dementia group is associated with frontal and anterior temporal atrophy [Whitwell et al 2012]. Early-onset Alzheimer disease (EOAD) typically begins with subtle memory failure which becomes more severe leading to disability. Common findings include confusion, poor judgment, language disturbance, agitation, hallucinations, withdrawal, and mutism. Seizures, parkinsonism, myoclonus, and urinary incontinence can occur. The significant overlap in clinical presentation of EOAD with ALSP includes similar age of onset. The predominant finding of EOAD on brain MRI is evolving cortical atrophy; white matter changes are present, but much less pronounced than those of ALSP. CSF biomarker examination reveals elevated total-Tau and phosphorylated Tau protein concentrations and reduced A╬▓42 concentrations. A heterozygous pathogenic variant in APP, PSEN1, or PSEN2 is causative [Andreasen et al 2003, Lopez et al 2011, Cohn-Hokke et al 2012]. Autosomal Recessive Disorders Polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (Nasu-Hakola disease) is characterized by sclerosing leukoencephalopathy with progressive cerebral atrophy, cerebellar atrophy, or both. White matter lesions are diffuse and usually centrally located, with sparing of the arcuate fibers. Basal ganglia atrophy and calcifications are often present. Lytic foci are also often evident on bone radiographs [Paloneva et al 2001, Kaneko et al 2010]. Vanishing white matter (VWM) and metachromatic leukodystrophy (MLD) both have more widespread and diffuse white matter changes and atrophy than ALSP [Eichler et al 2009, Bugiani et al 2010]. Lysosomal storage diseases that can present in adult life with white matter lesions include the adult form of Krabbe disease. While it can present with parieto-occipital white matter [Loes et al 1999] and can be unilaterally diffuse [Lemmens et al 2011], upper corticospinal tract involvement can also be the first presenting change [Wang et al 2007]. Leukoencephalopathy with brain stem and spinal cord involvement (LBSL) diagnostic MRI findings are white matter lesions that are either non-homogeneous/spotty or homogeneous and confluent [Scheper et al 2007]. Signal abnormalities are evident in the medullary pyramids, dorsal columns, and lateral corticospinal tracts. Additionally, signal abnormalities may be present in the splenium of the corpus callosum, superior/inferior cerebellar peduncles, and cerebellum. X-Linked Disorders X-linked adrenoleukodystrophy (X-ALD) rarely develops into a cerebral form; when it does, it may demonstrate symmetric, increased T2 signal intensities usually in the parieto-occipital region with contrast enhancement at the periphery of the demyelination zone [Eichler et al 2007]. Fabry disease can present with white matter lesions together with grey matter pathology [Reisin et al 2011]; however, the clinical presentation is different from that of ALSP [Meschia et al 2011]. Mitochondrial Disorders White matter lesions (WML) may also be present in adult mitochondrial diseases [Saneto et al 2008]. In Leigh syndrome the WML may involve the deep white matter, posterior centrum semiovale, and corpus callosum. The progression of WML is from posterior to anterior [Lerman-Sagie et al 2005]. MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes) occasionally presents with diffuse WML involving the periventricular white matter, centrum semiovale, and corpus callosum [Apostolova et al 2005]. Alpers syndrome may show T2 hyperintensities in the occipital lobe, deep cerebellar nuclei, thalamus, and basal ganglia. MNGIE (mitochondrial neurogastrointestinal encephalopathy) has diffuse WML (typically sparing the corpus callosum) and supratentorial cortical atrophy [Barrag├ín-Campos et al 2005]. In contrast to ALSP, the cranial MRI in mitochondrial diseases may demonstrate symmetric T1 hypointense and T2 hyperintense signal abnormalities in deep grey matter. These abnormalities are not restricted to vascular territories and the lesions often fluctuate over the course of the disease. Additionally, varying degrees of cerebral and cerebellar atrophy may be present [Saneto et al 2008]. Other Primary progressive multiple sclerosis (PPMS) is initially dominated by progressive central paraparesis. With advanced disease the clinical picture is more multifocal with typical MS symptomatology including internuclear ophthalmoplegia (INO) and optic neuropathy. Cerebrospinal fluid-enriched oligoclonal IgG bands support the diagnosis. MRI lesions tend to be periventricular with characteristic MS ΓÇ£right-angle lesions.ΓÇ¥ Diagnostic PPMS criteria are based on one year of steady clinical progression and MRI and CSF findings [Polman et al 2011]. ALSP can mimic MS, but does not fulfill the diagnostic criteria for MS [Keegan et al 2008]. Sundal et al [2015] describe diagnostic tools that may help the clinician distinguish ALSP from PPMS. SusacΓÇÖs syndrome typically presents with the triad of retino-cochleo-cerebral vasculopathy. MRI demonstrates centrally located lesions of the corpus callosum of varying shapes and sizes (without atrophy) that usually evolve into pathognomic central callosal ΓÇ£holesΓÇ¥ [Saenz et al 2005]. Most affected individuals improve with immunosuppressive therapy [Mateen et al 2012]. Frontotemporal lobar degeneration (FTLD) ranges from behavioral and executive impairments to language disorders and motor dysfunction. The clinical findings of FTLD differ from those of ALSP. Although the combination of FTD with atypical parkinsonism has features such as multisystem atrophy (MSA) and progressive supranuclear palsy (PSP), and the addition of amyotrophic lateral sclerosis (ALS) can mimic clinical ALSP, the neuroimaging is different. MRI demonstrates mainly cerebral atrophy without the characteristic white matter lesions found in ALSP. Management Evaluations Following Initial Diagnosis To establish the extent of disease and needs of an individual diagnosed with adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP), the following evaluations are recommended: Complete neurologic assessment Psychological and psychiatric assessments Brain MRI to determine the extent and localization of white matter changes, presence of cortical atrophy, and involvement of the corpus callosum and corticospinal tracts Assessment of feeding/eating, digestive problems (constipation, incontinence), and nutrition based on patient history EEG or video EEG if a seizure disorder is suspected; evaluation of the need for antiepileptic drugs Lumbar puncture to measure neurofilament light protein (NFL) in the cerebrospinal fluid (CSF) to follow the disease progression. An increased level of NFL on repeat CSF examinations may suggest faster disease course and thus worse prognosis. Assessment of family and social structure to determine the availability of adequate support system Clinical genetics consultation Treatment of Manifestations No specific therapy is currently available for ALSP. Management is supportive and includes: attention to general care, nutritional requirements, antiepileptic drugs for seizures, and antibiotic treatment for general and recurrent infections such as pneumonia or urinary tract infections. Other: L-dopa or other dopaminergic therapies have not been beneficial in individuals with ALSP or in those with an atypical parkinsonian phenotype, but may be worth trying. Antidepressant medications may be prescribed for depression but reports to date have demonstrated no long-term benefit. Antipsychotics are in general not recommended due to extrapyramidal side effects, but may be used in aggressive individuals. Anti-seizure medications should be initiated in any individuals with seizures and are reported to be beneficial. Prevention of Secondary Complications Social problems (unemployment, divorce, financial troubles, and alcoholism) and suicidal tendencies are often associated with the progression of the disease. Some of the social consequences may be avoided if family members are informed early about the nature of the disorder. Surveillance Periodic clinical evaluation to monitor for the following is appropriate: Changes in mobility, communication, and behavior, which could indicate a need to alter care and support systems (wheelchair/ personal assistance) Onset of seizures and need for antiepileptic therapy Contractures, which could indicate a need to change medical management and physical therapy Behavioral changes, inappropriate emotions and actions, problems following directions, memory loss, incontinence, which indicate curtailing of independence Difficulties in swallowing or weight loss, which trigger consideration for gastrostomy Need for physical therapy to minimize contractures and maintain locomotion Longitudinal MRI studies can potentially help with prognosis, as during the disease course the more rapid the confluence of patchy or focal T2 hyperintensities and the progression of cortical atrophy, the poorer the prognosis appears to be [Van Gerpen et al 2008, Sundal et al 2012c]. Agents/Circumstances to Avoid The following should be avoided: Use of first-generation neuroleptics, which increase seizure risk and risk of additional parkinsonian signs Treatment agents for multiple sclerosis, as these medications are of no benefit and have major side effects Evaluation of Relatives at Risk See Genetic Counseling for issues related to testing of at-risk relatives for genetic counseling purposes. Therapies Under Investigation Search ClinicalTrials.gov for access to information on clinical studies for a wide range of diseases and conditions. Note: There may not be clinical trials for this disorder. Genetic Counseling Genetic counseling is the process of providing individuals and families with information on the nature, inheritance, and implications of genetic disorders to help them make informed medical and personal decisions. The following section deals with genetic risk assessment and the use of family history and genetic testing to clarify genetic status for family members. This section is not meant to address all personal, cultural, or ethical issues that individuals may face or to substitute for consultation with a genetics professional. ΓÇöED. Mode of Inheritance Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) is inherited in an autosomal dominant manner; however, de novo mutation does occur. Risk to Family Members Parents of a proband Individuals with ALSP usually have an affected parent, although de novo mutation can occur [Rademakers et al 2011, Kinoshita et al 2012]. A proband with ALSP may have the disorder as the result of a de novo pathogenic variant. In 14 families with ALSP, one set of monozygotic (MZ) twins with a de novo pathogenic variant was identified [Rademakers et al 2011]. If the pathogenic variant found in the proband cannot be detected in leukocyte DNA of either parent, two possible explanations are germline mosaicism in a parent or a de novo pathogenic variant in the proband. Although no instances of germline mosaicism have been reported, it remains a possibility. Recommendations for the evaluation of parents of a proband with an apparent de novo pathogenic variant include the following: Clinical evaluation and brain MRI Molecular genetic testing if the pathogenic variant has been identified in the proband The family history of some individuals diagnosed with ALSP may appear to be negative because of failure to recognize the disorder in family members, early death of the parent before the onset of symptoms, or late onset of the disease in the affected parent. Therefore, an apparently negative family history cannot be confirmed unless appropriate evaluations have been performed on the parents of the proband. Note: If the parent is the individual in whom the pathogenic variant first occurred, s/he may have somatic mosaicism for the variant and may be mildly/minimally affected. Sibs of a proband The risk to the sibs of the proband depends on the genetic status of the probandΓÇÖs parents. If a parent of the proband is affected, the risk to the sibs is 50%. When the parents are clinically unaffected, the risk to the sibs of a proband appears to be low. The sibs of a proband with clinically unaffected parents are still at increased risk for ALSP because of the possibility of reduced penetrance in one parent. If the pathogenic variant found in the proband cannot be detected in the leukocyte DNA of either parent, the risk to the sibs is low but greater than that of the general population because of the possibility of germline mosaicism. Offspring of a proband. Each child of an individual with ALSP has a 50% chance of inheriting the pathogenic variant. Other family members. The risk to other family members depends on the status of the proband's parents. If a parent is affected, his or her family members may be at risk. Related Genetic Counseling Issues Considerations in families with an apparent de novo pathogenic variant. When neither parent of a proband with ALSP has clinical evidence of the disorder, the CSF1R pathogenic variant is likely de novo. However, possible non-medical explanations including alternate paternity or maternity (e.g., with assisted reproduction) or undisclosed adoption could also be explored. Testing of at-risk asymptomatic adult relatives of individuals with ALSP is possible after molecular genetic testing has identified the specific pathogenic variant in the family. Such testing should be performed in the context of formal genetic counseling and is not useful in predicting age of onset, severity, type of symptoms, or rate of progression in asymptomatic individuals. Testing of asymptomatic at-risk individuals with nonspecific or equivocal symptoms is predictive testing, not diagnostic testing. Testing of asymptomatic individuals younger than age 18 years who are at risk for adult-onset disorders for which no treatment exists is not considered appropriate, primarily because it negates the autonomy of the child with no compelling benefit. Further, concern exists regarding the potential unhealthy adverse effects that such information may have on family dynamics, the risk of discrimination and stigmatization in the future, and the anxiety that such information may cause. Testing is appropriate to consider in symptomatic individuals in a family with an established diagnosis of ALSP regardless of age. See also the National Society of Genetic Counselors position statement on genetic testing of minors for adult-onset conditions and the American Academy of Pediatrics and American College of Medical Genetics and Genomics policy statement: ethical and policy issues in genetic testing and screening of children. Family planning The optimal time for determination of genetic risk and discussion of the availability of prenatal testing is before pregnancy. It is appropriate to offer genetic counseling (including discussion of potential risks to offspring and reproductive options) to young adults who are affected or at risk. DNA banking is the storage of DNA (typically extracted from white blood cells) for possible future use. Because it is likely that testing methodology and our understanding of genes, allelic variants, and diseases will improve in the future, consideration should be given to banking DNA of affected individuals. Prenatal Testing and Preimplantation Genetic Diagnosis Once the CSF1R pathogenic variant has been identified in an affected family member, prenatal testing for a pregnancy at increased risk and preimplantation genetic diagnosis for ALSP are possible. Resources GeneReviews staff has selected the following disease-specific and/or umbrella support organizations and/or registries for the benefit of individuals with this disorder and their families. GeneReviews is not responsible for the information provided by other organizations. For information on selection criteria, click here. Australian Leukodystrophy Support Group, Inc. Nerve Centre 54 Railway Road Blackburn Victoria 3130 Australia Phone: 1800-141-400 (toll free); +61 3 98452831 Fax: +61 3 95834379 Email: mail@alds.org.au www.alds.org.au European Leukodystrophy Association (ELA) 2, rue Mi-les-Vignes B.P. 61024 Laxou Cedex 54521 France Phone: 03833093 34 Fax: 03833000 68 Email: ela@ela-asso.com www.ela-asso.com United Leukodystrophy Foundation (ULF) 224 North Second Street Suite 2 DeKalb IL 60115 Phone: 800-728-5483 (toll-free); 815-748-3211 Fax: 815-748-0844 Email: office@ulf.org www.ulf.org Myelin Disorders Bioregistry Project Email: myelindisorders@cnmc.org Molecular Genetics Information in the Molecular Genetics and OMIM tables may differ from that elsewhere in the GeneReview: tables may contain more recent information. ΓÇöED. Table A. Table A. Adult-Onset Leukoencephalopathy with Axonal Spheroids and Pigmented Glia: Genes and Databases Table B. Table B. OMIM Entries for Adult-Onset Leukoencephalopathy with Axonal Spheroids and Pigmented Glia (View All in OMIM) Gene structure. CSF1R comprises 22 exons. For a detailed summary of gene and protein information, see Table A, Gene. Benign variants. No benign variants have been reported. Pathogenic variants. To date, more than 40 different CSF1R pathogenic variants have been discovered. Encoded by exons 12 to 22, all are located in the intracellular tyrosine-kinase domain of the receptor. To identify the genetic basis of ALSP, an international consortium was established and one large kindred with clear autosomal dominant inheritance was selected for linkage analyses. Evidence for linkage was identified at loci on chromosome 5. Whole-exome sequencing identified CSF1R as the gene in which mutation is causative. Additional pathogenic variants were demonstrated in 13 probands with neuropathologically proven ALSP. CSF1R pathogenic variants cosegregated with the disease phenotype in all families. Ten missense variants, one single-codon deletion, and three splice site variants were identified in exons 12 to 22. No CSF1R pathogenic variant was found in any of the 660 controls [Rademakers et al 2011]. Table 2. Table 2. CSF1R Pathogenic Variants Discussed in This GeneReview Table 3 (pdf) lists the 44 CSF1R pathogenic variants reported to date. Normal gene product. The CSF1R protein is a cell-surface receptor primarily for the cytokine CSF-1, which regulates the survival, proliferation, differentiation, and function of mononuclear phagocytic cells, including microglia of the central nervous system. CSF1R comprises a highly glycosylated extracellular ligand-binding domain, a transmembrane domain, and an intracellular tyrosine kinase domain. Binding of CSF-1 to its receptor (CSF1R) results in the formation of receptor homodimers and subsequent autophosphorylation of several tyrosine residues in the cytoplasmic domain. CSF1R autophosphorylation precedes CSF1R-dependent phosphorylation of several proteins, including the phosphatase SHP-1 and the kinases Src, PLC-╬│, PI(3)K, Akt, and Erk [Rademakers et al 2011]. In the brain, CSF1R is predominately expressed in microglial cells. The link between pathogenic variants in CSF1R and the neuronal/ glial dysfunction remains to be elucidated. Abnormal gene product. The CSF1R pathogenic variants that cause ALSP affect the kinase activity and potentially the phosphorylation of downstream targets. Progressive behavioral and cognitive decline are features of frontotemporal dementia (FTD).1ΓÇô5 FTD syndromes (FTDS) are classified by whether they initially affect behavior, language expression, or semantic knowledge.6 Predominant unilateral or bilateral frontal or unilateral temporal presentations are described, and parietal lobe pathology is possible.7ΓÇô10 Histopathologic findings variably consist of Pick bodies, tau inclusions, ubiquitin inclusions, TAR DNA-binding protein 43 (TDP43) inclusions, or no apparent protein aggregations.11,12 Approximately 40% of patients with FTD have a positive family history.13 Mutations in the tau and progranulin genes on chromosome 17 produce autosomal dominant FTDS, and are associated with tauopathy or ubiquitin inclusions.14ΓÇô17 We now describe a multigenerational FTDS kindred with extensive subcortical gliosis and neither tau nor ubiquitin inclusions. Back to Top METHODS Back to Top Clinical descriptions. Six kindred members were evaluated at the Memory (five) and Movement (one) Disorder clinics of the University of Virginia (UVA). We report clinical and structural neuroimaging data, as well as results from relevant hematologic-serologic-urine, neuropsychological, and functional neuroimaging testing. Back to Top Pathology. Brain autopsies were available for six members of the kindred. We report essential gross and relevant microscopic findings. Back to Top RESULTS A progressive disorder of behavior and cognition is suspected across three generations (figure 1). One instance of intermarriage occurred between distantly related cousins, but intermarriage for most branches is not suspected. Individuals with strong clinical or autopsy evidence of subcortical gliosis are discussed in detail. Brief descriptions of putatively or potentially affected subjects are provided. Figure 1 opens a modal window Figure 1 Back to Top Subject IV-6. The UVA index case presented in 1995 at the age of 55 after 4 years of progressive behavioral change (including wandering), disorientation, poor memory, and decreased speaking. Examination revealed orientation only to person, markedly reduced spontaneous speech, and difficulty following multistep commands. There were nonfatiguing frontal release signs and appendicular paratonia. Brain CT showed severe, diffuse atrophy with frontal predominance and severe frontal lobe white matter attenuation. A differential diagnosis of Alzheimer disease (AD), Pick disease, and vascular dementia was considered. In subsequent years he developed mutism, bowel and bladder incontinence, hypertonia, myoclonic jerks, and gait abnormalities (small steps on a widened base). An FTD diagnosis was made before his death in 2001. At autopsy the brain weighed 1,120 g. There was atrophy of the bilateral frontal and temporal lobes with parietal and occipital sparing. Aside from the cerebellum where Purkinje cell loss was noted, neuron loss was minimal and very rare swollen neuronal cells were identified. No inflammation was seen. GFAP staining revealed hypertrophic astrogliosis within the superficial and deep white matter. There was loss of axonal processes with secondary myelin loss and prominent axonal spheroids (figure 2). Immunohistochemistry did not reveal tau, ubiquitin, or prion protein (PrP)-containing neuronal inclusions. Neurofilament staining occurred only in conjunction with axonal spheroids. Beta-amyloid staining revealed scant plaques and positive staining was present in a small proportion of arteries. Figure 2 opens a modal window Figure 2 Back to Top Subject III-3. A 1976 non-UVA autopsy report said the patient manifested progressive movement (postural changes and bradykinesia) and cognitive decline during his sixth decade. Parkinson disease (PD) and AD were diagnosed. Pneumoencephalogram showed ventriculomegaly. Lumbar puncture was unremarkable. He died at the age of 59. The brain weighed 1,150 g. Both frontal lobes were atrophic. No infarctions were seen. A marked increase in glia cells was noted. No Alzheimer changes or histologic evidence of PD were seen. Back to Top Subject III-7. We autopsied but did not clinically examine this patient. She was institutionalized for dementia during her eighth decade and died at age 77. The brain weighed 1,150 g. The frontal gyri were mildly atrophic. There was marked ventricular dilation and an old cerebellar stroke. There was microvacuolation (spongiotic change) of the cerebral cortex within the outer laminae, reflecting prominent neuronal loss, which was accompanied by gliosis. Glial fibrillary acidic protein (GFAP) staining revealed subcortical hypertrophic astrogliosis. Axonal spheroids were observed. Immunohistochemistry did not reveal tau, ubiquitin, TDP43, or PrP-containing neuronal inclusions. Neurofilament staining occurred only in conjunction with axonal spheroids. Neither Consortium to Establish a Registry for AlzheimerΓÇÖs Disease (CERAD) nor dementia with Lewy body criteria were met. Based on the clinical history of dementia, extensive gliosis, and paucity of AD histology, a final diagnosis of frontotemporal dementia with subcortical gliosis was made. Back to Top Subject IV-2. At the age of 58, this subject developed personality and behavioral changes. He neglected hygiene, lost interest in his hobbies, lost the ability to joke, began cursing excessively, developed stereotyped behaviors, and could not ΓÇ£finish what he started.ΓÇ¥ Otherwise, most activities of daily living were preserved. At age 61, outside neuropsychological testing revealed a dysexecutive state; MRI showed bifrontal atrophy and leukoariosis of the subcortical white matter capping the frontal horns (figure 3A). His vitamin B12 level was low. He was started on B12 and referred to the UVA MDC, where his Mini-Mental State Examination (MMSE) score was 25/30. He showed anosognosia, mildly impaired memory retention, and very impaired memory retrieval. Free hand and copy drawing were good. He named 11 animals over one minute and 1 F word over 1 minute. Set-shifting, motor sequencing, and ideomotor praxis were impaired. Aside from reduced odor discrimination and mild gegenhalten paratonia, his general neurologic examination was unremarkable. Figure 3 opens a modal window Figure 3 Behavioral and cognitive deficits progressed and at age 66 he died of pulmonary complications. The brain weighed 1,240 g. Severe frontal atrophy and diffuse white matter degenerative changes were evident (figure 4). There was extensive subpial gliosis with superficial laminar spongiotic change within the cortex (indicating neuronal loss), and extensive subcortical gliosis with moderate myelin loss. GFAP staining revealed hypertrophic astrogliosis (figure 4). White matter axonal dropout and dystrophic changes, including axonal spheroids, were seen. Immunohistochemistry did not reveal tau, ubiquitin, TDP43, or PrP-containing neuronal inclusions. Neurofilament staining occurred only in conjunction with axonal spheroids. Beta-amyloid immunochemistry revealed atypical cortical and white matter plaques with core but not mantle staining. There was amyloid deposition within multiple vessels. However, the hippocampus was unremarkable and other Alzheimer diagnostic features were lacking. Figure 4 opens a modal window Figure 4 Back to Top Subject IV-15. At age 41, this man was referred to UVA for progressive walking, speaking, and memory decline. He had been fired from his longstanding job about 10 months earlier, and recently gained 15 pounds. MMSE score was 25/30. Dysarthria, reduced repetition abilities, poor motor sequencing, and limb-kinetic apraxia were noted. Frontal release signs, extension plantar reflexes, and a parkinsonian gait were observed. Although an MRI obtained after a mild head injury 6 years earlier was reportedly normal, MRI at the age of 41 showed diffuse cortical atrophy and extensively increased white matter signal capping the frontal and occipital horns and rimming the rest of the lateral ventricles. Frontal leukoariosis was also observed on CT (figure 3B). Spinal fluid studies (including oligoclonal bands) were negative. Urine arylsulfatase A activity, serum arylsulfatase A activity, phytanic acid level, and plasma very long chain fatty acid levels were normal. Commercial spinocerebellar atrophy and triple repeat disorder genetic testing was negative. Progressive irritability, mutism, gait impairment, and bowel-bladder incontinence occurred. He died at age 43. The brain weighed 1,550 g and showed lateral ventricle enlargement with corpus callosum thinning. The hippocampus, midbrain, substantia nigra, pons, and cerebellum were normal. There was remarkable preservation of cortical neurons, which were nearly normal in density. Focal macrophage infiltrates were seen in all lobes and were present in white matter tracts and deep gray nuclei (figure 5). GFAP staining showed hypertrophic astrogliosis. Degenerating white matter showed myelin loss, numerous dystrophic axons, and axonal swellings and spheroids. Immunohistochemistry did not reveal tau, ubiquitin, TDP43, or PrP-containing neuronal inclusions. Neurofilament staining occurred only in conjunction with axonal spheroids. Electron microscopy revealed no features specific for lysosomal storage disease. Figure 5 opens a modal window Figure 5 Back to Top Subject IV-20. At the age of 46, this subject began manifesting a progressive inability to express himself, apathy, social withdrawal, and sleep disturbance. He had a home repair business and lost the ability to use his tools. He presented to UVA approximately 1 year after symptom onset. His general neurologic examination was remarkable only for frontal release signs. MMSE and Mattis Dementia Rating Scale (MDRS) scores were 29/30 and 121/144. Speech was effortful and contained paraphasic errors. There were deficits on tests of visuospatial reasoning, cognitive processing speed, mental flexibility, and conceptualization. MRI showed ventriculomegaly, diffuse mild atrophy, and periventricular white matter disease. Single photon emission tomography (SPECT) showed reduced left subfrontal perfusion. Commercial genetic testing for notch3 gene mutation was negative. A skin biopsy showed no evidence of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. Over the next 3 years, he gradually lost the ability to walk and swallow. At death, the brain weighed 1,100 g. Hydrocephalus ex vacuo and prominent frontal lobe atrophy were present. The vasculature showed minimal atherosclerosis. Gross examination revealed diffuse loss of anterior white matter with cortical sparing. Microscopic sections of the cortex showed generalized atrophy, mild neuronal loss, and gliosis. Scant ΓÇ£balloon-likeΓÇ¥ neurons were seen in the frontal cortex. In addition to extensive gliosis in subpial and gray cortical locations, GFAP staining showed white matter hypertrophic astrogliosis. The white matter was notable for significant loss of myelin and large eosinophilic balls indicative of axonal degeneration. These spheroids were accompanied by a prominent reactive astrocytosis. The thalamus showed focal neuronal loss and gliosis and the midbrain was notable for increased reactive type II astrocytes. Immunohistochemistry did not reveal tau, ubiquitin, alpha-synuclein, or PrP-containing neuronal inclusions. Neurofilament staining occurred only in conjunction with axonal spheroids. A few diffuse neuritic plaques were seen on beta-amyloid immunostaining. Back to Top Subject IV-19. This woman presented to UVA at the age of 53 with 1 year of language and gait problems. Lower extremity tone was increased. Her gait was markedly apraxic and somewhat magnetic. The noncognitive examination was otherwise unremarkable. MMSE and MDRS scores were 27/30 and 132/144. Terseness of expression was noted. Neuropsychological testing showed executive dysfunction, constructional apraxia when attempting to copy complex but not simple figures (suggesting a deficit in visuomotor integration), and impaired fine motor dexterity. Her vitamin B12 level was borderline low, with an elevated homocysteine. MRI revealed moderate subcortical white matter disease without ventriculomegaly or extensive atrophy (figure 3C). SPECT showed decreased frontal cortical and subcortical perfusion. Vitamin B12 was repleted but gait and cognition continued to decline. She died at age 59. Autopsy was not performed. Back to Top Putatively or possibly affected subjects. Only one putatively or possibly affected subject was evaluated at UVA. This was IV-8, who presented at age 51 with memory complaints. Aside from mild difficulty with motor sequencing, the bedside cognitive and general neurologic examinations were unremarkable. An MRI did not indicate white matter pathology. Neuropsychological testing showed an MDRS score of 130/144, impaired executive function, poor figure copy, and good memory. Repeat neuropsychological testing 3 years later (her last UVA evaluation) showed an MDRS of 123/144 and deficits otherwise similar in quality and quantity to those originally seen. By family report, 2 years later the subject began displaying increasingly poor social judgment. Subject II-1 died in a state psychiatric hospital at the age of 71 with a diagnosis of Pick disease. Subject II-7 died in a state psychiatric hospital at the age of 65 with dementia but no specific diagnosis. Subject III-1 died with a diagnosis of AD, and apparently did not develop dementia until late in her eighth decade. Subjects III-12, III-13, III-17, and III-26 died with diagnoses of AD at the respective ages of 67, 68, 62, and 52. Subject III-27 had clinical dementia at the time of death at the age of 48. Subject III-28 died in a state psychiatric hospital at the age of 45. Subject IV-3 died in a nursing home in her eighth decade. Subject IV-5 has been diagnosed with a neurologic disorder of the CNS and has abnormal white matter on neuroimaging. Subject IV-7 died in her fifth decade with a diagnosis of Pick disease. Subject IV-9 died in her fifth decade. Her clinical diagnoses included multiple sclerosis and Pick disease. Subject IV-21 died at age 50 with a diagnosis of Pick disease. Back to Top DISCUSSION Paternal transmission indicates this is an autosomal dominant disorder. Penetrance is high but incomplete, and may result because senile symptom onset can occur. Absence of tau and ubiquitin pathology makes tau and progranulin gene mutation unlikely.17,18 Absence of ubiquitin and TDP43 staining also argues abnormal encoding or handling of TDP43 does not play a primary or etiologic role.19 With the exception of very limited congophilic angiopathy in two subjects, there was no evidence of beta-amyloid, alpha-synuclein, or prion protein pathology. Hypertrophic astrogliosis without neuronal loss or only minimal neuronal loss in at least some subjects suggests this is a primary disorder of glia, not neurons. The constellation of predominant white matter involvement, roughly equal degrees of demyelination and axonal loss, and axonal spheroids are consistent with membranous lipodystrophy,20,21 dermatoleukodystrophy with neuroaxonal spheroids,22 the adult-onset variant of vanishing white matter disease (VWM),23 and hereditary diffuse leukoencephalopathy with spheroids (HDLS).24 The age at onset and lack of non-CNS manifestations are incompatible with the first two entities. Relatively late symptom onset in many affected members of our kindred and an absence of VWM-characteristic oligodendroglial morphology argue against VWM. Our kindred does share several clinical, radiologic, and neuropathologic features with HDLS.25,26 Since leukoencephalopathy and axonal spheroids are general markers of subcortical damage, the nosologic relationship between this kindred and HDLS-designated kindreds is unclear. HDLS is itself a relatively recently described entity, and it will be interesting to see whether future studies define HDLS as an etiologically distinct disease or as part of a clinicopathologic syndrome/spectrum. Elucidating the genetic basis of our kindred and the published HDLS kindreds could definitively resolve this issue. There were no ultrastructural features of lysosomal storage or long-chain fatty acid disease. Progressive familial leukodystrophy of late onset and Binswanger disease can manifest white matter pathology with subcortical gliosis,27,28 but our kindred mostly lacks extensive demyelination and does not show arteriolar pathology. An FTD subtype, dementia lacking distinctive histologic features, can also show subcortical gliosis.29ΓÇô31 Subcortical gliosis as a pathologic entity was itself identified in 1949 and classified as a distinct disorder in 1967.32,33 Autosomal dominant subcortical gliosis kindreds are described.34 One was linked to chromosome 17q21-22 and shown to have an intronic tau gene mutation.35,36 Brains from affected kindred members had obvious tau pathology on immunohistochemical survey.36 In contrast, none of the brains from our kindred had any detectable evidence of tauopathy or abnormal tau immunohistochemistry. Executive dysfunction and frontal leukoariosis commonly occur in the absence of an amnestic state, and older individuals with this type of presentation may be diagnosed with the subcortical ischemic vascular dementia variant of vascular dementia.37ΓÇô39 Determining the basis of this kindredΓÇÖs autosomal dominant subcortical gliosis could potentially yield mechanistic insight into vascular-attributed cognitive impairment/dementia syndromes, as well as to what is commonly referred to as small vessel cerebrovascular disease. HDLS typically presents as an autosomal dominant disease associated with variable behavioral, cognitive and motor changes1-3. The onset of symptoms is usually in the fourth or fifth decade, progressing to dementia with death within six years. On magnetic resonance imaging (MRI), HDLS is characterized by patchy cerebral white matter abnormalities, often initially asymmetrical but becoming confluent and symmetrical with disease progression4-12. The changes predominantly involve the frontal and parietal white matter with evolving cortical atrophy affecting these lobes (Fig. 1a-b). Since neither the clinical symptoms nor the MRI changes are specific, a definite diagnosis of HDLS relies on pathological examination, showing widespread loss of myelin sheaths and axonal destruction, axonal spheroids, gliosis, and autofluorescent lipid-laden macrophages (Fig. 1c-i)1,4-8,10-12. Occasionally, brain biopsy has been used to confirm the diagnosis9. Figure 1 Figure 1 Neuroimaging (a-b) and neuropathological (c-i) findings in HDLS patient FL2-1 To identify the genetic basis of HDLS, we established an international consortium with ethical approval from the Mayo Clinic Institutional Review Board and collected clinical data, MRI studies, blood and brain tissue samples from families with at least one patient with autopsy- or biopsy-proven HDLS. In total, we collected 14 kindreds from the United States, Norway, Germany and Scotland (Fig. 2). Family VA was selected for genome-wide linkage studies, and non-parametric linkage analyses identified one locus with a lod-score>2.5 (chromosome 5; lod=2.67) and four loci with lod-scores>1.0 (Supplementary Fig. 1). Subsequent parametric linkage analysis identified significant linkage on chromosome 5q34 (lod=3.71, ╬╕=0 at rs13178296), while none of the other loci reached significance (Supplementary Fig. 1). Obligate recombinants narrowed the candidate region to 30.3cM between rs801399 and rs1445716 (Supplementary Fig. 2), corresponding to a ~25Mb genomic interval containing 233 candidate genes. Figure 2 Figure 2 Families with HDLS and CSF1R mutations To generate a list of potential disease-causing mutations, we performed whole-exome sequencing of two pathologically confirmed patients from family VA (VA-21 and VA-24, Fig. 2). We generated variant profiles for each patient and searched for shared heterozygous variants located within the chromosome 5q candidate region. We further predicted that mutations underlying HDLS are likely to be previously unidentified; therefore, we filtered all of the identified base alterations against dbSNP132. This led to the identification of two non-synonymous mutations: c.80C>T (p.S27L) in the gene encoding the 5-hydroxytryptamine receptor 4 (HTR4) and c.2624T>C (p.M875T) in the macrophage colony-stimulating factor 1 receptor gene (CSF1R). Both mutations segregated with disease in the extended family VA and were absent in 660 controls. We therefore searched for additional mutations in a cohort of 13 probands from autopsy- or biopsy-proven HDLS families (Fig. 2). Sanger sequencing of the 6 coding exons of HTR4 and 22 coding exons of CSF1R identified heterozygous CSF1R mutations in all 13 probands, whereas no other mutations in HTR4 were identified (Fig. 3; Supplementary Table 1). Segregation analyses confirmed transmission of the CSF1R mutations and co-segregation with the disease phenotype in all families where DNA from multiple affecteds was available (Fig. 2). We further confirmed the de novo occurrence of one CSF1R mutation in monozygotic twins from family NO, without a family history of HDLS (Supplementary Fig. 3). To confirm the rarity of these mutations, and to provide supporting evidence for pathogenicity, we also sequenced the CSF1R gene in 24 unrelated controls and genotyped the 13 novel mutations in at least 1436 Caucasian controls using Taqman genotyping assays. None of the mutations identified in HDLS patients and no other novel CSF1R mutations were found in controls. Figure 3 Figure 3 Genomic organization and protein domain structure of CSF1R with summary of CSF1R mutations The 14 CSF1R mutations identified in HDLS families are all located in the intracellular tyrosine-kinase domain of CSF1R encoded by exons 12-22. The mutations include 10 missense mutations and one single-codon deletion, all affecting residues highly conserved across species and within members of the CSF1/PDGF receptor family of tyrosine-protein kinases (Kit, FLT3 and PDGFR╬▒/╬▓)13 (Fig. 3). We further identified three splice-site mutations, leading to the in-frame deletion of exon 13 (NO) or exon 18 (CA2/FL2), deleting up to 40 consecutive amino acids within the tyrosine kinase domain (Supplementary Fig. 4). Detailed clinical information was available for 24 patients with proven CSF1R mutations from 14 HDLS families (Table 1). Mean age at onset was 47.2┬▒14.5 years (range 18-78 years), with mean disease duration of 6.0┬▒3.1 years (range 2-11 years) and a mean age at death of 57.2┬▒13.1 years (range 40-84 years). In some families (FL1/CA1/VA), age at onset or death differed by more than 25 years among family members, whereas a monozygotic twin pair (family NO) showed highly similar disease course with ages at onset and death within one year from each other, suggesting that currently unidentified genetic or environmental factors may be important determinants of the age-related disease penetrance. Presenting features and evolving clinical symptoms also varied significantly within and across families, and ante mortem clinical diagnoses in mutation carriers included frontotemporal dementia (FTD), CBS, Alzheimer disease (AD), multiple sclerosis (MS), atypical cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), and Parkinson disease (PD). Table 1 Table 1 Clinical characteristics of 24 patients from 14 families with genetically confirmed CSF1R mutations. Since most patients included in our study were not diagnosed with HDLS, we hypothesized that CSF1R mutation carriers may be present in clinical series of early-onset AD, FTD, CBS, MS and PD, or ischemic stroke patients with additional white matter changes. Sequencing analyses of CSF1R exons 12-22 encoding the protein tyrosine kinase domain in up to 93 Mayo Clinic patients affected with each of these neurological syndromes led to the identification of an additional CSF1R missense mutation c.2509G>T (p.D837Y) in a woman with clinical symptoms resembling CBS (Supplementary Tables 2-3). The identification of a CSF1R mutation in this limited patient series underscores that HDLS may be an under-diagnosed disease. CSF1R is a cell-surface receptor primarily for the cytokine CSF-1, which regulates the survival, proliferation, differentiation and function of mononuclear phagocytic cells, including microglia of the central nervous system14. CSF1R is composed of a highly glycosylated extracellular ligand-binding domain, a trans-membrane domain and an intracellular tyrosine-kinase domain15. Binding of CSF-1 to CSF1R results in the formation of receptor homodimers and subsequent auto-phosphorylation of several tyrosine residues in the cytoplasmic domain16. CSF1R autophosphorylation precedes CSF1R-dependent phosphorylation of several proteins, including the phosphatase SHP-1 and the kinases Src, PLC-g, PI(3)K, Akt and Erk16-18. In the brain, CSF1R is predominantly expressed in microglial cells, although low levels of CSF1R have been reported in cultured neurons19-21. An increase in CSF1R copy number and point mutations leading to constitutive activation of the CSF1R receptor have been associated with tumor development, including hematological malignancies and renal cell carcinomas22,23. To assess the functional importance of the CSF1R mutations identified in this study, we first studied the effect of the mutations on CSF1R in vitro. We transiently expressed DDK-tagged wild-type (CSF1RWT) and mutant (CSF1RE633K, CSF1RM766T and CSF1RM875T) CSF1R in cultured cells. Upon stimulation with CSF-1, autophosphorylation on multiple CSF1R tyrosine-residues was observed for CSF1RWT, while none of the mutants showed detectable levels of autophosphorylation (Fig. 4 and Supplementary Fig. 5). Since all mutations are in the CSF1R kinase domain, dimerization and/or cell surface expression are unlikely to be affected; however, we cannot exclude this at this time. These preliminary findings suggest that mutant CSF1R kinase activity is abrogated, likely affecting the phosphorylation of downstream targets. We speculate that mutant CSF1R might assemble into non-functional homodimers and wild-type/mutant heterodimers inducing a dominant-negative disease mechanism. Figure 4 Figure 4 CSF-1 induces autophosphorylation of wild-type but not mutant CSF1R To address whether CSF1R autophosphorylation is also disrupted in HDLS patient samples, we first subjected blood samples from a healthy control and HDLS patient CA1-1 to CSF1R immunoblotting, which revealed no apparent difference in CSF1R total or phosphorylation levels (Supplementary Fig. 6a). Further, CSF1R immunoblotting was performed in frontal cortex brain tissue of healthy controls as well as patients with HDLS. Brain samples from AD and ALS patients were included as neurodegenerative disease controls. Our data showed varied levels of total and phosphorylated CSF1R in these brain samples (Supplementary Fig. 6b); however, statistical analysis did not reveal a significant difference between any of the groups. Although these preliminary in vivo studies do not reveal a defect in autophosphorylation, these findings do not necessarily conflict with the data obtained in cultured cells. First, HDLS patients are heterozygous for the CSF1R mutations and therefore, in contrast to our in-vitro experiments, wild-type receptor is still present in these patients. In our cell culture experiments, CSF1R signaling was down regulated by serum deprivation to minimize basal signaling through this receptor before stimulation with the CSF-1 ligand. CSF-1 is a serum protein, so without this deprivation in vivo, immediate changes in CSF-1-induced CSF1R autophosphorylation may not be apparent as we cannot disregard wild-type receptors at the cell surface that have already been activated. Unfortunately, without access to an immortalized cell line derived from an HDLS patient, we are currently unable to accurately assess acute receptor activation in vivo. Finally, the post-mortem brain samples from HDLS patients included in these studies exhibit extensive degeneration, leaving the possibility that cells with greater disruption of CSF1R signaling are underrepresented in the tissue sample. Unraveling the genetic etiology of HDLS may significantly contribute to the understanding of other adult-onset leukoencephalopathies. De novo mutations in CSF1R could explain the disease in sporadic patients that have been described with clinical and pathological similarities to HDLS24-29. Future CSF1R mutation screening may also determine whether HDLS and pigmentary orthochromatic leukodystrophy (POLD) are part of a single clinicopathologic entity, as was recently suggested2. Moreover, the discovery of a mutation in a microglial trophic factor receptor may further elucidate the role of microglia in more common white matter disorders, particularly those associated with axonal dystrophy, such as BinswangerΓÇÖs disease24,30, multiple sclerosis31 and HIV encephalitis32. Interestingly, our findings also shed new light on Nasu-Hakola disease (NHD), a rare condition characterized by systemic bone cysts and dementia with striking similarities to HDLS33-35. NHD is caused by recessive loss-of-function mutations in the DAP12/TREM2 protein complex36,37, which was recently implicated in CSF1R signaling, establishing NHD as a primary microglial disorder38. We speculate that a partial loss of the CSF1R/DAP12 signaling cascade in microglia is responsible for the neurological phenotypes observed in HDLS and NHD, whereas a complete loss of this signaling cascade in bone marrow-derived macrophages is needed for the bone-cysts formation observed in NHD. In support of this hypothesis, a partial loss-of-function mutation in TREM2 in a family with early-onset dementia without bone-cysts was recently reported39. Also, no bone-cysts were reported in any of our HDLS patients and a bone scan in a patient CA1-1 did not show bone fractures, hypomineralization or any other bone structure abnormalities. In summary, we have shown that mutations affecting the tyrosine-kinase domain of CSF1R underlie the white matter disease of HDLS, establishing HDLS as an important novel member of the recently defined class of primary microglial disorders, called ΓÇÿmicrogliopathiesΓÇÖ40. Future molecular studies of CSF1R signaling might offer novel insights into microglial physiology and the involvement of this cell type in HDLS and neurodegeneration. Moreover, CSF1R mutation screening in neurodegenerative disease patient series will now allow an accurate diagnosis of HDLS and could facilitate detection of presymptomatic individuals, which is indispensable for therapy development and early treatment. The FMS gene encodes the functional cell surface receptor for colony-stimulating factor 1, the macrophageand monocyte-specific growth factor. Codons 969 and 301 have been identified as potentially involved in promoting the transforming activity of FMS. Mutations at codon 301 are believed to lead to neoplastic transformation by ligand independence and constitutive tyrosine kinase activity of the receptor. The tyrosine residue at codon 969 has been shown to be involved in a negative regulatory activity, which is disrupted by amino acid substitutions. This study reports on the frequency of point mutations at these codons, in vivo, in human myeloid maligances and in normal subjects. We studied 110 patients [67 with myelodysplasia (MDS) and 48 with acute myeloblastic leukemia (AML)], 5 patients being studied at the MDS and the later AML stage of the disease. There was a total incidence of 12.7% (14/110) with mutations in codon 969 and 1.8% (2/110) with mutations in codon 301. Two patients had mutations in the AML stage of the disease but not in the preceding MDS and one had a mutation in the MDS stage but not upon transformation of AML. This is consistent with the somatic origin of these mutations. FMS mutations were most prevalent (20%) in chronic myelomonocytic leukemia and AML type M4 (23%), both of which are characterized by monocytic differentiation. One of 51 normal subjects had a constitutional codon 969 mutation, which may represent a marker for predisposition to myeloid malian. The FMS gene encodes for the receptor of the macrophageand monocyte-specific growth factor, colony-stimulating factor 1 (CSF-1) (1-3). The protein product of the gene is a cell surface glycoprotein that is expressed on cells of the monocyte/macrophage lineages and possesses ligand-dependent tyrosine-specific kinase activity (3, 4). Binding of CSF-1 to its receptor is required for survival, proliferation, and differentiation of these cells in vitro. The FMS gene is the cellular homologue of the v-fms gene of the Susan McDonough feline sarcoma virus (5, 6). The v-fms gene product exhibits ligandindependent tyrosine kinase activity (7, 8) and will transform cells in vitro, whereas the normal human FMS gene will not (9, 10). DNA sequence analysis (11) has shown that the FMS and v-fms genes differ by a number of point mutations and by the replacement of the 50 amino acids at the carboxy C terminus of the human gene with 11 unrelated amino acids in the v-fms gene. This C-terminal deletion removes a tyrosine residue at codon 969 that negatively regulates the response of the gene product to CSF-1 stimulation (9, 12). In vitro studies have shown that substitution of the tyrosine residue at codon 969 to phenylalanine up-regulates the stimulation of the receptor to CSF-1 but is insufficient to confer transforming activity on the gene (9). Alterations in addition to Tyr-969 must therefore be necessary to fully activate the FMS gene in vitro. In the light of the sequence differences between the v-fis and FMS, studies of chimeric v-fms/FMS proteins have highlighted codon 301, in the extracellular domain, to be functionally important. The human, feline, and murine FMS genes all encode leucine at this position, whereas the viral gene encodes a serine residue (13). Substitution of Ser-301 for Leu-301 in the human FMS gene rendered the gene transforming in an in vitro assay (10). The mutation is believed to lead to a conformational change that mimics ligand binding, resulting in a constitutive tyrosine kinase activity. Mutant genes with Ser-301 and Phe-969 have increased transformation efficiency. The involvement of the FMS gene in myeloid malignancy has been implicated previously. It is known that the FMS gene is located on chromosome 5q33 (14), a region frequently altered in myelodysplasia (MDS) patients (15-17). Loss of one FMS allele has been demonstrated in some MDS patients with a 5q- refractory anaemia (14, 18). Expression ofthe FMS gene has been demonstrated in leukemia cells from acute myeloblastic leukemia (AML) patients but not in patients with acute lymphocytic leukemia. The highest levels were detected in AML type M5, which is characterized by a monocytic phenotype (19, 20). Coexpression of EMS and CSF-1 in the same leukemia cells has also been demonstrated in 5 of 15 AML cases studied (21), implicating autocrine stimulation of the receptor. Here we report on the frequency of mutations at potentially activating codons, 301 and 969. We have studied 67 patients with MDS, 48 with AML, and 51 hematologically normal individuals. MATERIALS AND METHODS Patient Material. Blood or bone marrow samples were obtained from 67 patients with MDS [13 with sideroblastic anemia (SA), 14 with refractory anemia (RA), 10 with RA with excess blasts (RAEB), and 30 with chronic myelomonocytic leukemia (CMML)] and 48 AML patients (9 type Ml, 14 type M2, 22 type M4, 2 type M5, 1 unclassified). Diagnosis of AML and MDS was made according to the FAB (FrenchAmerican-British) classifications (22, 23). Fifty-one normal blood samples were also studied, these being obtained from healthy blood donors, patients in the ophthalmology department, and healthy volunteers (17 aged 20-39, 13 aged 40-60, 21 aged 61-80). Fully informed consent was obtained from all individuals and the investigation was approved by the South Glamorgan Joint Ethics Committee. DNA Extraction. High molecular weight DNA was extracted from cells as described (24). Abbreviations: CSF-1, colony-stimulating factor 1; AML, acute myeloblastic leukemia; MDS, myelodysplasia; CMML, chronic myelomonocytic leukemia; SA, sideroblastic anemia; RA, refractory anemia; RAEB, RA with excess blasts; PCR, polymerase chain reaction. fTo whom reprint requests should be addressed. 1377 The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. ┬º1734 solely to indicate this fact. 1378 Medical Sciences: Ridge et al. 301 3' AGTTTCACTACCACCTCCGGAT 5' 5, ATGCCAGATGCTTG 3' 5' GATATCGCCCAGCCCTTGCT 3' ' Probes Amino Acid Sequence Probes Amino acid 5' GAGTGCCTACTTGAACTTGA 3' 5. ----------TCG.------- 3 5 ---- -----GTG------ 3' 5 . ATG - 3 51 ----------TTC------- 3' 5' ---------TTT------- 3 5, 969 wt 969 Tyr Phe Cys Asp Asn His Ser 31 \* 5' ---------.TAG------- 3' 5' AACAACTATCAGTTCTGCTG 3' 5' ------TTT----------- 3' 5' ------TGT----------- 3' 5' ------GAT----------- 3' 5' ------MAAT--------- 3' 5' ------CAT----------- 3' 5' ------TCT----------- 3' 5' ------TAG----------- 3' \*\* 5' ------ M----------- 3' FIG. 1. Sequences of primers and mutant-specific probes for codons 301 and 969. A single asterisk (\*) indicates the translational stop codon amber; a double asterisk (\*\*) indicates the translational stop codon ochre. Cloning and Sequencing. Four hundred thousand A phage from a human leukocyte library in EMBL3 (Cambridge Bioscience, Cambridge, U.K.) were screened (24) using a 2.3-kilobase (kb) Sst I fragment of Susan McDonough feline sarcoma virus containing 5' v-fms sequences (25). Two of 400,000 A phage clones were plaque purified. Restriction enzyme analysis showed these clones to be identical. A 2.5-kb BamHI fragment shown by hybridization to a codon 301 wild-type oligonucleotide probe (Fig. 1) to contain sequences around codon 301 was subcloned into a plasmid, pUC18. A sequencing primer (5'-TGAGGTTCTGCTCAGAGCTC-3') 3' to codon 301 was designed from the published cDNA sequence (11) and synthesized (Applied Biosystems). This was used to obtain intronic sequences 5' to Table 1. FMS mutations in MDS, AML, and normal subjects Amino acid Patient Disease Codon substitution 1 MDS (SA) 301 Phe/Ser 2 MDS (RA) 969 Cys 3 MDS (RAEB) 969 Cys 4 MDS (CMML) 969 Asn/Phe 5 MDS (CMML) 969 Ochre 6 MDS (CMML) 969 Cys 7 MDS (CMML) 969 Cys/Phe 8 MDS (CMML) 969 Cys 9A MDS (CMML) 969 Cys 9B AML (M5, post-MDS) 969 Cys 10 AML (M2) 969 Asp 11 AML (M4) 301 Phe 12 AML (M4) 969 His 13 AML (M4) 969 Asn 14 AML (M4) 969 Cys 15 AML (M4, post-MDS) 969 Cys 16 AML (M5, post-MDS) 969 His NS 969 Cys (constitutional) All mutations were confirmed by a second PCR and oligonucleotide hybridization analysis. NS, normal subject. codon 301. Two-hundred fifty base pairs (bp) of sequence was obtained by the Sanger dideoxy chain-termination method (26) using Sequenase (Cambridge Bioscience). All restriction enzyme digestions were carried out according to the manufacturer's recommendations (GIBCO/BRL). Polymerase Chain Reaction (PCR). Methods of amplification and hybridization were as described (27, 28) with modifications. Primers flanking codons 301 and %9 (Fig. 1) of the FMS gene were used. The primers were designed from cDNA sequences (11) and from sequences obtained by ourselves. Each sample was subject to two rounds of amplification each of 50 cycles with Taq polymerase (Thermus aquaticus DNA polymerase; Perkin-Elmer), using material from the first round as a template for the second. The samples were applied to the membrane without a vacuum. The filters were denatured in 0.5 M NaOH/1.5 M NaCl and neutralized in 1.5 M NaCl/0.5 M Tris, pH 7.5/0.001 M Na2EDTA prior to baking at 80┬░C. Filters were hybridized to mutant-specific oligonucleotide probes (Fig. 1) 5' end-labeled with [y-32P]ATP and polynucleotide kinase (Amersham). Autoradiography was carried out with intensifying screens at -70┬░C using XAR-5 Table 2. FMS mutations in disease subtypes Total no. No. of mutations Disease studied (amino acid position) SA 13 1 (301) RA 14 1(969) RAEB 10 1 (969) CMML 30 6 (969) AML M1 9 0 AML M2 14 1 (969) AML M4 22 5 (1 x 301, 4 x 969) AML M5 2 2 (969) AML UC 1 0 Total 115\* 17t None (NS) 51 1 UC, unclassified; NS, normal subjects. \*Five patients sampled twice in MDS and AML stages. tOne patient had an FMS mutation in MDS and AML stages. Proc. Natl. Acad. Sci. USA 87 (1990) 969 3' CAACTGCTGTCCCTCATGGT 5' -U- 301 wt 301 Leu Ser I? Sequence Val Met Phe Phe Trp \* '00or '\ Proc. Natl. Acad. Sci. USA 87 (1990) 1379 A B C D E F 1 \* \* - - - 2 a \* X 4 i 969Wt A B C D E F 969 His High stringency FIG. 2. Detection of a somatic FMS mutation. Hybridization of the AML sample of patient 16 (slot C2) to a His-969 probe at high stringency is shown. A previous MDS sample (slot B2) showed no evidence of the mutation. The same filter hybridized to the 969 wild-type probe is shown. This provides an estimate of quantitation of the DNA in each dot. (Kodak) film for 2 hr to 3 days. Potential mutants were rescreened on independent filters and reamplified to confirm the presence of mutations. Only those that stably hybridized to the mutant probes with significant signals were scored positive. Each mutant was therefore assayed for two independent PCR reactions. This was a stringent screen as, unlike RAS mutations where a biological transformation assay could be employed to confirm the presence of the mutations (28), there is no similar biological assay for FMS mutations. Statistical Analysis. Fischer's exact test (29) was employed to determine if the differences found between the groups were significantly different from the normal incidence. RESULTS A total of 110 patients with MDS or AML was investigated for the presence of FMS mutations in their peripheral blood leukocytes (Tables 1 and 2). DNA from 16 of 110 (14.5%) patients was found to have mutations. Fourteen of 110 patients had mutations at codon 969. A cysteine substitution for the wild-type tyrosine was the most prevalent alteration. Two of 110 patients had mutations at codon 301. The FMS mutation in a single patient (9) was shown to be present in leukocytes from the MDS (9A) and AML M5 (9B) stages but not in buccal epithelium (data not shown). Patients 15 and 16 had mutations in the AML stage of disease but not in the preceding MDS (representative filter in Fig. 2). Patient 3 possessed a mutation at the MDS stage of the disease, which was apparently lost upon leukemic transformation. These results indicate that FMS mutations can occur at early and late stages of disease and hence may not represent an initiating event in the generation of the abnormal clone. These results are consistent with the somatic origin of these mutations. No patients were found to possess a mutation at codons 301 and 969. However, three patients (1, 4, and 7) showed evidence of two mutations at the same codon. CMML and AML M4 samples, both characterized by significant monocytic differentiation, had the highest rate of FMS A 1 \* B C D mutations [20% and 23%, respectively; Fisher's exact test, two-tailed analysis, P < 0.02 (29)]. In 1 of 51 (2%) normal subjects analyzed, a Cys-969 mutation was found to be present in peripheral blood. Analysis of buccal epithelial cells from this person showed the presence of the same mutation (Fig. 3). This finding suggests that the Cys-969 mutation is constitutional in this instance. DISCUSSION We have described the finding of point mutations at codons 301 (2/110) and 969 (14/110) of the FMS gene in vivo in myeloid disease and in 1 of 51 normal subjects. Our results indicate that a mutation at codon 969, rather than codon 301, is the more common lesion in these patients. We speculate that in the hemopoietic environment, in the presence of CSF-1, mutations at codon 969 that alter a negative regulatory site may up-regulate the response of the receptor to ligand binding and thus confer a growth advantage to the cell. In vitro studies imply that mutations at codon 301 may be of little advantage in an environment with normal or increased levels of CSF-1 (10). In patients with reduced CSF-1 levels, however, a codon 301 mutation may confer a growth advantage on a clone of cells. Studies are necessary to examine the relationship between serum CSF-1 levels and mutational status of the FMS gene in these patients. In the feline system, FMS mutations at codons 301 and 374 are required in addition to a C-terminal modification involving the loss of codon 969 for a fully transformed phenotype in Rat-2 cells (13), and it is possible that additional mutations may be found in myelodysplastic and leukemic patients. Cytogenetically, none of the present patients with FMS mutations has a gross deletion of chromosome 5q. We do not know, however, if a subpopulation of cells has microscopic deletions removing one allele, leaving only the mutant allele. We have demonstrated the presence of constitutional and somatic point mutations in the FMS gene using PCR and oligonucleotide hybridization. The somatic origin of FMS mutations in four patients and the presence of a constitutional A B C D 0 @ 969 wt 969 cys High stringency FIG. 3. Detection of a constitutional FMS mutation in a normal subject. Slots are DNA from a negative control (Al), original blood sample (Bi), repeat blood sample (Cl), and buccal mucosa (Dl); A2 is a Cys-969 mutant from a patient used as a positive control; B2, C2, and D2 are negative controls. Hybridizations of the filter to the 969 wild-type and the Cys-969 probes are shown. B1, Cl, Dl, and A2 hybridize strongly to the Cys-969 probe. Medical Sciences: Ridge et al. 1380 Medical Sciences: Ridge et al. mutation in one person have been shown. Lack of material prevents us from assessing the origin (somatic or constitutional) of the FMS mutations in the other patients. The appearance and disappearance of these mutations during the progression of disease in some patients suggest these lesions are not initiating events. Mutant RAS genes, also thought not to be an initiating lesion, are found in a high proportion of CMML patients (28, 30) possibly indicating that myelodysplastic or leukemic clones already showing potential monocytic differentiation may be especially susceptible to the transforming properties of these oncogenes. A constitutional mutation has been demonstrated in one hematologically normal individual. This mutant allele may have been inherited from one patient or may have arisen during embryonic development. In view of the high percentage of CMML (20%) and AML M4 (23%) patients with a mutation at codon 969 this may represent a lesion involved in predisposition to these particular malignancies. To our knowledge, a constitutional point mutation in a protooncogene at a regulatory domain has not been reported previously. Further studies are necessary to determine the functional significance of these mutations. Ongoing cancer genome characterization studies continue to elucidate the spectrum of genomic abnormalities that drive many cancers, and in the clinical arena assessment of the driver genetic alterations in patients is playing an increasingly important diagnostic and/or prognostic role for many cancer types. However, the landscape of genomic abnormalities is still unknown for less common cancers, and the influence of specific genotypes on clinical behavior is often still unclear. To address some of these deficiencies, we developed Profile, a prospective cohort study to obtain genomic information on all patients at a large tertiary care medical center for cancer-related care. We enrolled patients with any cancer diagnosis, and, for each patient (unselected for cancer site or type) we applied mass spectrometric genotyping (OncoMap) of 471 common recurrent mutations in 41 cancer-related genes. We report the results of the first 5000 patients, of which 26% exhibited potentially actionable somatic mutations. These observations indicate the utility of genotyping in advancing the field of precision oncology. Within the past decade the application of genome interrogation technologies to patient samples has greatly expanded our understanding of the spectrum of genomic alterations that underpin cancer initiation and progression and those events that contribute to the evolution of cancer and the emergence of resistance to targeted therapies. Studies such as the Cancer Genome Atlas (http://cancergenome.nih.gov) and the International Cancer Genome Consortium1 have comprehensively characterized >20 cancer types. Such studies have confirmed the incidence of many known oncogenes and tumor suppressor genes but have also identified hitherto unrecognized genes and pathways recurrently altered in cancers. In parallel with research endeavors, information gleaned from these studies has been translated to the molecular diagnostics arena to develop clinical tests that can detect somatic alterations in specific cancer types. Often, these clinical tests take the form of a gene- or alteration-targeted approach and can be used for diagnostic purposes (eg, BRAF testing to distinguish between subtypes of thyroid papillary carcinoma), prognostic indications (NPM1 and FLT3 testing in acute myeloid leukemia),2,3 predicting response to a targeted therapy (EGFR mutation analysis as an indicator for therapeutic response in metastatic non-small cell lung cancer), or detecting resistance to a targeted agent (ABL1 kinase domain mutational analysis for imatinib (Gleevec)-resistance in patients with chronic myelogenous leukemia). Moreover, clinical guidelines for some cancers encourage a sequential testing process, as exemplified by the testing guidelines for non-small cell lung cancer by the College of American Pathologists, International Association for the Study of Lung Cancer, and the Association for Molecular Pathology.4 Completing these tests is not necessarily a cost-effective exercise and uses substantial amounts of nucleic acid material, which may be limiting for many patients with cancer. In addition, expanding catalogs of cancer mutations challenge that these events are tissue specific or occur in isolation. For example, activating BRAF mutations have been described in >50% of papillary thyroid carcinomas5 and cutaneous melanomas but also at a lower frequency in lung cancer,6 colorectal adenocarcinoma,7 pediatric low-grade glioma,7 and multiple myeloma.8 These mutant BRAF proteins are potential targets for RAF inhibitors,9 and clinical trials have confirmed the utility of targeted therapies in some of these instances.10,11 An ever-expanding number of other targetable proteins, including phosphatidylinositol-4, 5-bisphosphate 3-kinase, catalytic subunit ╬▒ (PIK3CA), epidermal growth factor receptor (EGFR), and v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2 (ERBB2), are aberrant in multiple cancer types.7,12,13 Moreover, interrogating the mutational status of multiple genes has found clinical utility in some settings; for example, acquired resistance to EGFR tyrosine kinase inhibitors (TKIs) in non-small cell lung cancer can be due to the EGFR T790M mutation, MET gene amplification, or mutation of PIK3CA.14 A more rational approach to individualized cancer treatment, therefore, would be the application of multigene testing, using a small amount of DNA, to generate a more comprehensive assessment of mutations in several genes concurrently, in a more clinically relevant time frame. Although several challenges need to be overcome to implement such a paradigm,14ΓÇô18 such as generating high-quality genomic data from archival [eg, formalin-fixed, paraffin-embedded (FFPE)] tumor material, this information can inform a precision or individualized approach to clinical decision making, particularly in selecting an appropriate targeted therapy for a patient. Furthermore, the ability to combine multiple common targetable alterations in one assay greatly enhances the ability to identify patients who might be suitable candidates for clinical trials of investigational therapies and to implement such testing on all patients in the cancer center facilitates the implementation of basket trials that extend beyond specific anatomically defined cancer types. To this end, in the past few years we have developed a panel-based test that allows more broad screening of genomic alterations known to be informative in cancer. We and others have used mass spectrometric genotyping7 or allele-specific PCR technologies19 to establish personalized cancer medicine initiatives.7,19ΓÇô25 More recently, advances in next-generation sequencing technologies have enabled even more comprehensive genomic characterization in a massively parallel fashion and in a relatively short time frame, allowing the assessment of many types of genomic alterations (mutations, insertions and deletions, copy number alterations, structural rearrangements, and epigenetic changes) in hundreds or thousands of genes (targeted sequencing and whole-exome sequencing), whole-genome sequencing, transcriptome sequencing (RNASeq), and epigenetic interrogations (methyl-Seq and ChipSeq). Pilot studies that apply massively parallel sequencing technologies and/or integrative analyses in focused clinical settings for a few patients have also been reported.26,27 We implemented a prospective genomic characterization study called Profile that aimed to apply genomic technologies to advance the field of precision oncology by addressing some of the challenges described above. We obtained consent from >12,000 patients with cancer who came to Dana-Farber Cancer Institute or Brigham and Women's Hospital between August 2011 and June 2013, and OncoMap,7,28 a mass spectrometric genotyping assay that detects 471 unique mutations in 41 cancer genes, was performed in a laboratory certified by Clinical Laboratory Improvement Amendments. Test results were reviewed by laboratory staff and interpreted and reported by board-certified pathologists. Here, we report initial findings from profiles on >5000 patients with cancer. Go to: MATERIALS AND METHODS Patients and Tumor Tissue Collection Patients gave consent to the institutional review boardΓÇôapproved protocol 11 to 104 from the Dana-Farber Cancer Institute Office for the Protection of Research Subjects. Tumor specimens were obtained from Dana-Farber Cancer Institute and the Department of Pathology at Brigham and Women's Hospital. Patient charts were reviewed, and appropriate specimens for testing were selected with the following criteria: ΓëÑ30% viable tumor content (initially 50% tumor was required but this was decreased over time because performance metrics were comparable) and sufficient area (>3 mm in greatest linear diameter) for DNA extraction. Specimen types profiled included FFPE, fresh/frozen, and blood/bone marrow. DNA Extraction and Preparation For solid tumor specimens, tissue was sectioned and slides stained with hematoxylin and eosin were obtained. Tumor-rich areas of FFPE were manually dissected from unstained slides or whole FFPE blocks; fresh tissues were grossly minced and digested overnight with Proteinase K. DNA was extracted manually or by using an automated protocol (QiaSymphony) with Qiagen reagents (Qiagen, Valencia, CA). Blood or marrow samples with mononuclear hematological malignancies were enriched by Ficoll gradient before DNA extraction. Total DNA of 200 ng was required to proceed with OncoMap testing. DNA (100 ng) extracted from FFPE samples was whole genome amplified (GenomePlex Complete Whole Genome Amplification kit; Sigma-Aldrich, St. Louis, MO) for iPLEX analysis (see immediately below), whereas fresh frozen and blood DNA was processed with unamplified DNA. Confirmation of mutations was performed on unamplified genomic DNA by using homogenous mass-extend chemistry.29 OncoMap Assay Design and Genotyping Selection of cancer gene mutations for assay design and mass spectrometric genotyping was performed as previously described.7 Specimens were genotyped with OncoMap version 4, which assays 471 unique mutations in 41 cancer-related genes (Table 1). OncoMap version 4 identifies mutations in a high-throughput manner such that 48 to 96 patient samples are processed in parallel and consists of two chemistries (high complexity iPLEX5 and homogenous mass-extend) and a manual review step, as previously described.7 Genomic profiling was performed in an environment certified by Clinical Laboratory Improvement Amendments. Table 1 Table 1 Forty-One Genes and 471 Mutations Interrogated in OncoMap Version 4 OncoMap Validation Validation studies were performed to determine precision, accuracy, sensitivity, specificity, and limit of detection by using blood, fresh frozen, and FFPE samples that had existing genomic characterization by using an orthogonal clinical test (eg, pyrosequencing, Sanger sequencing, PCR/electrophoresis, real-time PCR). Thirty samples with known mutations in KRAS, EGFR, BRAF, TP53, AKT, and PIK3CA were selected. Within this sample set, additional mutations identified in APC, P53, CTNNB1, and JAK3 were also assessed for a total of 28 genetic variants detectable by 53 individual assays. Normal (noncancerous) liver was included to verify detection of wild-type loci. To obtain sufficient quantities of DNA, several isolations were performed from each FFPE sample, pooled to ensure sample homogeneity, and then divided into aliquots to produce nine replicates. OncoMap was performed in triplicate across three experiments to determine intra- and inter-run precision. A total of 114 samples (41 wild-type and 73 mutant) were analyzed by reference methods (pyrosequencing, PCR/CE fragment analysis, Sanger sequencing, and allele-specific PCR) at the Center for Advanced Molecular Diagnostics, Brigham and Women's Hospital, and were subsequently analyzed in OncoMap to determine accuracy and concordance with gold standard methods for variants at the following loci: KRAS G12 and G13; NRAS G12, G13, and Q61; EGFR exon 19 (deletion); JAK2 V617F; EGFR L858R; and KIT exon 11 (deletion). To assess our ability to detect all 471 mutations in 439 assays, we designed synthetic oligonucleotides that harbored each of the genetic variants listed in Table 1 (IDT, Coralville, IA). These were pooled into groups of three nonoverlapping variants and spiked into normal liver DNA isolated from FFPE tissue such that the ratio of wild-type to variant was 1:1 and were analyzed with OncoMap. The limit of detection was determined by mixing genomic DNAs isolated from the following cell lines: THP-1 (NRAS G12D; ATCC, Rockville, MD), PC9-2 (EGFR E746-A750del), H1975 (EGFR L858R and T790M), and A-549-2 (KRAS G12S; gift from Dr. Pasi Janne, Dana-Farber Cancer Institute). Six replicates were prepared at defined ratios representing 0%, 5%, 7.5%, 10%, and 25% allele frequencies for five known variants and assessed in OncoMap. Ninety-five percent confidence intervals were calculated with R version 2.15.0 (R Project for Statistical Computing, Vienna, Austria; http://www.r-project.org) by using the binconf function in the Hmisc package. The calculation method used the Wilson score interval to generate the confidence interval. Go to: RESULTS Characteristics of Clinical Tumor Cohort Of 9950 patients with an available specimen accessioned in the pathology laboratory, 5372 (53.9%) were estimated by a pathologist to have sufficient and appropriate material to attempt DNA extraction. Of these, 5123 patients had sufficient DNA (200 ng or more) to proceed with OncoMap, and 99.9% (n = 5118) of attempted tests yielded an OncoMap result. Estimated tumor content exceeded 30% in all specimens as determined by pathological review (hematoxylin and eosin evaluation). The distribution of cancer types assayed in our cohort is depicted in Table 2, and consists of 24 main cancer types. This distribution of cases reflected the population of cancers for which the test was ordered and performed and was not necessarily reflective of the incidence of cancer types seen at our institutions; the distribution of cases was likely skewed by both differences in the availability of appropriate materials/specimens for testing and differences in ordering habits of the involved physicians. Specimens (n = 5118) yielded an OncoMap result. Of these, 451 specimens were obtained from frozen tissue, 74 from blood, 189 from bone marrow, and 4404 (86%) from FFPE blocks. Specimens (n = 2182; 42.6%) harbored one or more mutations. Specimens with mutations (74.4%) had one mutation identified by OncoMap, approximately one-third of reportable cases. Twenty percent of specimens with mutations (8.5% of reportable cases) had two mutations; the remainder had between three and five events. All specimens with four or more mutations (n = 22) were identified as either colorectal or endometrial adenocarcinoma. Table 2 Table 2 Distribution of Cancer Types Assayed in Our Cohort of Patient Samples, Consisting of 24 Major Cancer Types Performance of OncoMap To facilitate cancer gene mutation profiling in clinical tumor specimens, we used OncoMap version 4, a panel of genotyping assays that assessed the status of 471 mutations, across 41 cancer-related genes. The complete mutation profiling algorithm, including iPLEX chemistry, automated calling, manual review, validation by using homogenous mass-extend chemistry, and manual review by laboratory personnel and a pathologist, has been previously reported7; a schematic overview of the process, including the time taken for each step, is indicated in Figure 1. Figure 1 Figure 1 Technical and bioinformatics steps in the clinical diagnostics pipeline. The timeline from receipt of specimen to generation of a report is 3 to 4 weeks. hMe, homogenous mass-extend; QC, quality control. Validation studies have found 100% intra- and inter-assay precision (95% CI, 99.2%ΓÇô100%), because all 28 genetic variants (see Materials and Methods) were detected in nine of nine replicates in the expected 53 assays. Concordance studies that compared OncoMap with various validated methods for single gene testing reported 98.3% sensitivity (95% CI, 94.13%ΓÇô99.54%) and 100% specificity (95% CI, 98.1%ΓÇô100%) for KRAS, NRAS, BRAF, JAK2, KIT, and EGFR. Two samples gave a false negative OncoMap result for JAK2 V617F, both with allele frequency <1%. Further evaluation of all 471 genetic variants that used synthetic oligonucleotides found the expected mutation in 432 of 439 assays; 7 assays displayed poor performance and failed to detect the expected mutation. For each of the seven failing assays, at least one additional complementary assay (ie, the opposite strand) detected the variant in question, with no false positives, resulting in an overall sensitivity and specificity of 100% (95% CI, 99.19%ΓÇô100%). Limit of detection experiments performed on cell lines with known genetic variants mixed in defined ratios to produce allele frequencies that varied between 0% and 25% found successful detection (100%; 95% CI, 88.65%ΓÇô100%) to 7.5% mutant allele frequency for each of the five mutations monitored (EGFR T790M, EGFR L858R, EGFR E746-A750del, NRAS G12D, and KRAS G12S); EGFR T790M and EGFR L858R were also detected (100%; 95% CI, 75.75%ΓÇô100%) at 5% allele frequency. Expected Mutations in Well-Characterized Cancer Types In total, 2890 mutations were identified in our cohort. The spectrum of mutations by gene is shown in Figure 2. As expected, KRAS was the most commonly mutated gene in our cancer population, occurring in 538 samples (10% of all samples) or almost 20% of mutated samples. The next most commonly mutated gene was PIK3CA (497 instances; 17% of mutations), followed by TP53 (326 instances; 11%) and BRAF (202 instances corresponding to 7% of mutations). The TP53 mutation frequency was less than might be expected but may be explained by the ability of a genotyping technology to detect only specific, predetermined mutations incorporated into the assay design. This was a limitation when interrogating tumor suppressor genes such as TP53, which may have many loss-of-function mutations scattered throughout the gene (eg, this platform can detect only approximately 20% of the known TP53 mutations, by frequency, in the Catalogue of Somatic Mutations in Cancer30 database). The full landscape of mutations by cancer type is indicated in Figure 3. CDK4, CSF1R, FGFR1, and SRC were not detectably mutated in any of the cases. Figure 2 Figure 2 Incidence of mutations by gene in our cancer cohort. Of the 2890 mutations detected in 5118 patients, approximately 19% were KRAS mutations, followed by PIK3CA (17% of mutations), TP53 (11%), and BRAF (7%). Figure 3 Figure 3 Landscape of mutations by gene in our cancer cohort. The frequency of gene mutation (normalized by the number of samples in each category) is indicated on the y axis, genes mutated on the x axis, and cancer type on the z axis. GI, gastrointestinal. Interpretation of Mutations A tiering approach was designed to assess the import of genomic alterations in specific cancer types. Each mutation was assigned one of three tiers. In tier 1, the alteration has well-established published evidence to confirm clinical utility in this tumor type, in at least one of the following contexts: predicting response to treatment with a therapy approved by the Food and Drug Administration, assessing prognosis, establishing a definitive diagnosis, or conferring an inherited increased risk of cancer to this patient and family. In tier 2, the alteration may have clinical utility in at least one of the following contexts: selection of an investigational therapy in clinical trials for this cancer type; limited evidence of prognostic association; supportive of a specific diagnosis; proven association of response to treatment with a therapy approved by the Food and Drug Administration in a different type of cancer; or similar to a different mutation with a proven association with response to treatment with a therapy approved by the Food and Drug Administration in this type of cancer. In tier 3, the alteration is of uncertain clinical utility but may have a role as suggested by at least one of the following: demonstration of association with response to treatment in this cancer type in preclinical studies (eg, in vitro studies or animal models); alteration in a biochemical pathway that has other known, therapeutically targetable alterations; alteration in a highly conserved region of the protein predicted, in silico, to alter protein function; or selection of an investigational therapy for a different cancer type. Of all specimens tested, 26% have at least one tier 1 (10%) or tier 2 (16%) mutation, which may directly affect clinical decision making. As previously described, we identified known driver mutations in well-characterized cancers, KRAS mutations in colorectal cancer, endometrial cancer, and lung cancer; BRAF mutations in melanoma, papillary thyroid carcinoma, and colorectal adenocarcinoma; PIK3CA mutations in breast, lung, and endometrial cancers; EGFR and KRAS mutations in lung adenocarcinoma; and IDH1 mutations in gliomas. As expected, the distribution of mutations reflected patterns previously observed in human tumors, although the frequency of tumor suppressor mutations was lower (reflective of the reduced coverage of such mutations by OncoMap). Mutations Predicting Response/Resistance to Targeted Therapies Receptor Tyrosine Kinases EGFR, ERBB2, KIT, PDGFRA Our genotyping test robustly detected mutations that constitute established markers of response to targeted therapies. EGFR mutations predictive of response to erlotinib and gefitinib were identified at 8.6% frequency in non-small cell lung cancer, which is a little lower than expected. This is because of the inability of a genotyping approach to detect each of the possible, variable, EGFR exon 19 deletions. Of note, we identified two lung adenocarcinomas with co-occurring EGFR L858R and T790M mutations. L858R indicated sensitivity to a TKI therapy, and the presence of a T790M (usually) indicated that resistance to a TKI has emerged. In one case, the specimen tested was a post-TKI relapse specimen, with the L858R allele present at approximately 40% to 50% as determined by relative peak heights of Sequenom assays, and the T790M mutation present in approximately 2% of alleles. Interestingly, in the second case, the patient had a history of multifocal lung adenocarcinoma, and two specimens tested (one from 2008, one from 2012) were genomically distinct; the more recent tumor contained a baseline de novo T790M, which, although rare, has been previously reported in large series and predicts a poor response to EGFR TKIs.31 In this case, the allele fraction (as determined by peak height) of the L858R allele was also higher (10% to 15%) than the T790M allele (approximately 5%). Activating ERBB2 mutations were seen in 18 cases, 5 cases of lung adenocarcinomas, 4 cases of bladder cancer, 4 cases of female genital tract cancer, 3 cases of breast cancer, 1 case of colon cancer, and 1 case of kidney cancer. Interestingly, in three cases ERBB2 mutations co-occurred with canonical PIK3CA mutations, and in another two instances (one colon, one ovarian) an ERBB2 mutation co-occurred with a KRAS G12 or G13 mutation. Fifty-six samples harbored canonical KIT or PDGFRA mutations. Although 79% of these were gastrointestinal stromal tumors, and an additional 3.5% were noncutaneous melanomas, we also observed targetable mutations in mastocytosis (n = 3), germ cell tumors (n = 3), a glioblastoma, an acute leukemia, a thymus carcinoma, and an ovarian dysgerminoma. Mutation profiling also identified mutations that confer secondary resistance to targeted therapies (eg, resistance alleles arising during the course of targeted therapy). Six instances of PDGFRA mutation D842V or D842Y were identified in five gastrointestinal stromal tumors and one glioblastoma, and these alterations are predictive of resistance to imatinib in gastrointestinal stromal tumors32; recent in vitro data indicates potential response to newer inhibitors of platelet-derived growth factor receptor, ╬▒ polypeptide (PDGFRA) such as crenolanib.33 RAS/RAF/MEK/ERK Pathway BRAF V600E mutations linked to sensitivity to inhibitors such as vemurafenib were detected in 44% of papillary thyroid cancers and 34% of melanomas. In addition, activating BRAF mutations were also detected in rarer cancers or at lower frequencies such as Langerhans cell histiocytosis,34 hairy cell leukemia,35 metanephric adenoma36; pancreatic breast, ovarian, and prostate adenocarcinoma,37 indicating the utility of exploring a targeted inhibitor38 for these specific patients. Interestingly, we observed two cases of lung adenocarcinoma and one urinary bladder cancer with co-occurring BRAF and KRAS mutations; in each case the BRAF alterations were non-V600E mutations (G464E, G466E, L597V). One instance in a colorectal adenocarcinoma exhibited BRAF V600E and a KRAS G12D mutation. With mutations that confer heightened sensitivity to targeted therapies, OncoMap robustly detected mutations associated with resistance to several agents. Established examples include KRAS mutations in lung cancer (23%), colorectal cancer (42%), and endometrial cancer (20%) that confer resistance to erlotinib, gefitinib (lung cancer), or cetuximab (colorectal cancer).39ΓÇô41 HRAS mutations were identified in 2 of 10 adrenal gland pheochromocytomas, as recently reported.42 Similarly, we identified MEK1 (MAP2K1) mutations in 11 specimens, four lung adenocarcinomas, one oral squamous cell carcinoma, three gastrointestinal tract adenocarcinomas, a breast cancer, a thymoma, and a hairy cell leukemia. MEK1 mutations have previously been identified in malignant melanomas43 whereby they often occur with BRAF or NRAS mutations; there is evidence that some MEK1 mutations may confer resistance to MEK [mitogen activated protein (MAP) extracellular signal-related kinase (ERK) kinase]/RAF inhibitors in melanoma. PI3K/AKT/Mammalian Target of Rapamycin Pathway Inhibitors of the PI3K/AKT/mammalian target of rapamycin pathway have found promise in preclinical and clinical trials in multiple cancer types.44 We identified gain-of-function AKT1 E17K mutations in several meningiomas (as recently identified by our group with the use of whole-genome and whole-exome sequencing45), an oral squamous cell carcinoma, a liposarcoma, and the more common events in breast, colorectal, ovarian,46 endometrial,7 and lung6 adenocarcinomas. This mutation may predict resistance to PI3K inhibition (and conceivably receptor tyrosine kinase inhibition) in some contexts.46 Three hundred sixty-nine additional samples (7% of all patients tested) across all cancer types (predominantly breast) harbored mutations in PIK3CA, PIK3R1, PTEN, or a combination thereof. These mutations might be expected to enrich for tumors responsive to the PI3K inhibitors currently in development. Metabolic and Other Signaling Pathways Several tumors harbored mutations that may have prognostic and therapeutic relevance. For example, IDH1 and IDH2 gain-of-function mutations have been identified in leukemias47 and glioblastomas48; in our cohort we identified IDH1 mutations in these cancers, less commonly in melanoma49 (n = 4), chondrosarcoma,50 cholangiocarcinoma,51 and prostate cancer52 but also in previously unreported cancers such as lung, colorectal, and endometrial adenocarcinomas and a urinary bladder carcinoma. We identified 28 samples with GNAS mutations across many cancer types, some known (lung, pancreatic, and colorectal adenocarcinoma) but also in breast, ovarian, and cervical cancers. All but one of the GNAS mutations were codon 201 in exon 8; there was one instance of codon 227 mutation in exon 9. Clinically, GNAS mutations in pituitary neoplasms have been associated with increased sensitivity to octreotide (somatostatin agonist) in some studies.53 Noncanonical Mutations in Potentially Actionable Genes Although genotyping assumes an a priori knowledge of specific regions in a gene that may be mutated, the mass spectrometric genotyping assay described here can be designed to incorporate additional sites in genes that may be known to be mutated at a lesser frequency. Although not as comprehensive as full-length sequencing of a gene, OncoMap nonetheless provides more information for some genes than current gold standard clinical tests such as pyrosequencing. For example, somatic mutations of BRAF occur at high frequency in numerous human cancers,54 and the BRAF V600E mutation (resulting in increased kinase activity) accounts for >90% of described mutations; pyrosequencing is often used to detect mutations in amino acids 599 to 601 only. In our cohort, of 202 BRAF mutations identified, 34 (17%) were non-V600 mutations, and 8 were indels at/near the V600 locus that were not the canonical c.1799T>A nucleotide change. Although we do not yet know the full implication of all these alterations, we know that V600E- or V600K-mutant tumors may indicate better response to targeted therapies than patients with wild-type tumors,55 and BRAF L597 mutations (seen in a colorectal adenocarcinoma and a bladder carcinoma in our cohort) may indicate sensitivity to MEK inhibitors in melanoma,56 indicating the utility of using a more comprehensive assay when performing molecular profiles of patients' tumors. In addition, BRAF N581S in a bone marrow myeloproliferative neoplasm was identified; this mutation has been seen rarely in several solid tumors,14,16,57 but to our knowledge this is the first BRAF mutation in myeloproliferative neoplasm. Missense Mutations in MET and JAK3 May Be Somatic or Germline MET T1010I (also known as T992I) mutations were observed in a reasonable frequency of our cohort (2.55% of cases). There is conflicting evidence in the literature about the transforming ability of this alteration58,59; it has also been identified as a heterozygous single nucleotide polymorphism in a normal (noncancer) population at a frequency of 2.49% (European American population; Exome Variant Server; National Heart, Lung, and Blood Institute Grand Opportunity Exome Sequencing Project, Seattle, WA; http://evs.gs.washington.edu/EVS, last accessed November 2013; P = 0.999, ╧ç2 test, no significant difference). Because our OncoMap tests were performed on tumor specimens and not matched germline samples, we cannot determine whether these represent somatic or germline events or a mix of both. [However, the allele frequencies (based on peak heights as the expected locations) for the T1010I allele (expected 50% if heterozygous single nucleotide polymorphism in diploid genome) ranged from 23% to 99% (mean, 46.5%; median, 46.4%), further supporting the likelihood this is a germline variant.] Similarly, evidence exists for the transforming ability of JAK3 alleles P132T and V722I,60 but both are also found in normal populations. The frequency of JAK3 V722I alterations is 2.67% in our cohort, compared with 2.56% in the Exome Sequencing Project database (P = 0.985), and JAK3 P132T occurs at a frequency of 23 of 5118 cases (0.45%), compared with an Exome Sequencing Project frequency of 0.05% (P = 0.0043). The difference in JAK3 P132T incidence in our cancer cohort and a normal cohort may be due to differences in ancestral populations (we used European American numbers as representative of our cohort) or might indicate that (in some cases) the single nucleotide polymorphisms might represent cancer susceptibility single nucleotide polymorphisms; thorough analysis of normal (noncancer) specimen would be necessary to support this. Cancers with Co-Occurring Actionable Mutations The presence of co-occurring mutations in known cancer genes may modify the clinical response to single-agent targeted therapy. In our cohort, 435 patient samples had two mutations, 101 had three mutations, and 22 had four or more mutations (Table 3). Of the 536 cases with two or three mutations, PIK3CA was the most frequently co-occurring mutated gene, with 204 specimens harboring mutant PIK3CA with another gene (most often TP53). Twelve specimens had two mutations within PIK3CA. Samples (n = 206) had a KRAS mutation and another mutation; 134 samples had a TP53 mutation with another mutation; and 61 specimens (mostly endometrial, breast, lung, and ovarian adenocarcinomas) harbored KRAS and PIK3CA mutations. As we previously noted, coincident mutations in these genes have been reported in cancers of the large intestine,61 but they have typically exhibited a mutually exclusive pattern of occurrence in endometrial cancer.55 Fifty-nine cases had KRAS and APC mutations. Table 3 Table 3 Samples with Co-Occurring Mutations in Our Cancer Cohort Go to: DISCUSSION One of the goals of precision cancer medicine is to combine genetic, genomic, and molecular characterization of a tumor with contextual information on anatomical site and other histological criteria to generate a more accurate diagnosis, prognosis, and/or choice of therapy for a patient. In contrast to many existing clinical tests that focus on one or a small number of gene alterations, newer technologies allow the simultaneous interrogation of many cancer genes. We previously reported the adaptation of genotyping-based mutation profiling for the characterization of both frozen and FFPE-derived tumor specimens in a research setting.7 The intent of the Profile study was to initiate an enterprise-level genomic characterization study wherein the logistical and scientific barriers to implementation of a precision cancer medicine approach could be identified and resolved. Although Profile testing generates clinical-grade results in a laboratory certified by Clinical Laboratory Improvement Amendments, most genotyping results from such a broad panel have no known clinical meaning for most patients with cancer. Therefore, we initially considered this to be a research test and developed a consenting process for patients. Of patients who consented, approximately 25% had a specimen at an outside hospital that was not available for testing. Of the 9950 that had consent and material available in our department for testing, >50% were estimated by a pathologist to have sufficient material to test, and, of these, approximately 95% yielded an OncoMap result. The success rate of generating a profile for a patient who gave consent can be enhanced by improving access to material at other institutions/pathology departments and by using a platform that requires less input DNA (eg, our experience with next-generation sequencing technologies is that they require less than half the amount of input DNA needed for OncoMap). A key performance characteristic is robust performance in samples derived from FFPE and/or archival tumor material, using a relatively small amount of DNA. Of patients with sufficient material to test, approximately 95% yielded an OncoMap result. Of all samples tested, <0.1% failed genotyping, indicating the utility of a robust platform to screen for cancer-driving mutations. Moreover, a variety of specimen types (solid, blood, bone marrow), fixation method (frozen, fresh, FFPE), specimen age (0 to 10 years), and quality performed well with this platform. OncoMap achieved 98.3% overall sensitivity (95% CI, 94.13%ΓÇô99.54%) and 100% specificity (95% CI, 98.1%ΓÇô100%), using clinically validated reference tests as a benchmark, in both fresh/frozen and FFPE-derived tumor DNA, indicating that false positive mutation calls are likely to be relatively rare. The sensitivity of OncoMap, here determined as 5% to 10%, is less than real-time PCR, comparable with pyrosequencing, and exceeds Sanger sequencing, all of which are common cancer molecular diagnostic technologies. As previously noted,7 however, achieving this level of specificity requires the implementation of an analytical algorithm in which genotyping data are subjected to automated and manual review of candidate mutations and validation of all candidates by using alternative genotyping chemistries. Thus, clinical implementation of this particular platform requires both genomic data generation and bioinformatic analysis in a molecular pathology or clinical diagnostic setting. The resultant 3- to 4-week turnaround time from specimen receipt to report generation (Figure 1), however, is less than ideal for some clinical cases. Advances in our understanding of biological driver events for some cancers, coupled with improvements in technologies used to detect somatic cancer alterations, have led to the establishment of personalized cancer medicine programs at several cancer centers in the United States.19ΓÇô25,27,41,52 Most of these programs use some form of genotyping to profile patient samples for alterations in a panel of potentially actionable or drugable gene mutations that may inform a therapeutic paradigm for patients. In this study, we report the clinical implementation of an updated panel of assays interrogating 471 unique sites in 41 known cancer genes. More than 5000 OncoMap profiles were generated over 2 years, from patients with cancer who gave consent, spanning all cancer types across both solid tumors and hematological malignancies. With the use of this panel, we robustly detected mutations in more than one-third of patients tested. Many of these mutations (26%) directly affect clinical use (tier 1 and 2 alterations) and/or predict resistance to existing agents such as TKIs (eg, EGFR and KRAS mutations) or investigational therapies currently in clinical trials. We also identified multiple gene mutations that may guide the use of emerging agents in specific cancer types, and we found the value of applying this OncoMap platform across a large cohort of patients with cancer. Some recent findings in rarer cancers (such as meningiomas) that were originally identified by large-scale whole-genome or whole-exome sequencing approaches were recapitulated by using genotyping, resulting in the description of mutations that may inform molecular classification and new therapeutic avenues. Finally, we determined that in approximately 10% of cases examined tumor specimens harbor expected and unexpected combinations of gene mutations, thus reinforcing that a broad profile of cancer-driving mutations is informative and may help to further elucidate differential patient responses to targeted therapies or why a long tail of clinical response is seen in patient populations selected for response to a particular therapy. Although this study indicates the clinical utility of a high-throughput, cost-effective approach to simultaneously detect mutations in multiple cancer genes, we acknowledge the technical limitations of genotyping, which restricts both the number of genes and fraction of base pairs interrogated, the type of alteration investigated (mostly single nucleotide substitutions and small insertions/deletions), the amount of input DNA required (high compared with some other molecular assays), and the labor-intensive and time-consuming nature of a two-chemistry process. In the past decade, major advances in massively parallel sequencing technologies will allow much more comprehensive assessment of the full spectrum of genomic alterations (eg, mutations, indels, copy number changes, structural rearrangements, and epigenetic changes), contributing to individual cancers. Initial reports that use such technologies capable of reading multifaceted genomic information in an efficient, timely, and cost-effective manner have found the utility of this approach for tumor mutation profiling and individualized cancer treatment.26,27 Our study represents the first large-scale, enterprise-level application of the OncoMap platform for mutation profiling of all types of cancer in a clinical laboratory. The proven effect of using mutation assessment in the selection of patients for targeted therapies (eg, in BRAF- and ALK-inhibitor phase 1 trials9,62) reiterates the need for molecular stratification of patients with cancer. The results of our study highlight several examples of informative oncogene mutations missed by standard single-gene clinical assays and describe a rational framework for enterprise-level tumor profiling to be used as a standard means to guide patient stratification and enrollment for targeted cancer therapies.