Single-gene diseases among complex neuropsychiatric disorders and genetic complexity in supposed single-gene neurodevelopmental diseases

Atsushi Takata
Department of Human Genetics, Yokohama City Univ. & RIKEN Center for Brain Science

04/17/19 PSTC Japan Safety Biomarker Conference@RIKEN IMS
Current status of biomarker studies for neuropsychiatric disorders

There have been extensive efforts to identify biomarkers... However, this work has failed to deliver markers that can distinguish reliably between diagnoses and has similarly failed to identify disease subgroups. **Currently, there are no biomarkers in routine clinical use.**

O'Donovan and Owen, Nature Medicine 2016

*I think we need to rely on sths most robust.*

Genetic variation that never change since one’s birth (with very few exceptions)

-> **One of the most robust biomarkers**
Genetic studies of neuropsychiatric disorders

Common variants vs. Rare variants

Genome-wide association study (GWAS)

- >100 genome-wide significant (P<5x10^{-8}) schizophrenia (SCZ) loci identified.
- Each variant increases the risk up to 1.2 times.
- May have a limited diagnostic value.

Ripke et al. Nature 2014

- Only found in up to 1% of SCZ.
- Increase the risk >20 times.
- Allele frequency in the general population≈0 (extremely rare).
- May have a certain diagnostic value.
- Usually arises as *de novo*.

McDonald-McGinn et al. 2015
De novo mutations (DNMs) and neuropsychiatric disorders

Individually rare, but collectively common

- 74 de novo SNVs (single nucleotide variants) per diploid genome on average.
- 1 DNM per diploid exome (all protein coding exons)

Veltman et al. Nature Reviews Genetics 2012

Next generation sequencing has enabled comprehensive analysis

Next generation sequencing has enabled comprehensive analysis

Epidemiological data indicate a role of DNMs in neuropsychiatric disorders

- Paternal age correlates risk of autism spectrum disorder (ASD), SCZ etc.
- Consistent prevalence despite the reduced reproduction fitness.

Kong et al., Nature 2014
The contribution of *de novo* coding mutations to autism spectrum disorder

Likely gene disruptive (*LGD*: nonsense, splice site and frameshift; also referred to as loss-of-function [LOF]) DNMs are especially enriched in ASD cases when compared with unaffected siblings.
Synaptic, transcriptional and chromatin genes disrupted in autism

2,270 trios, 1,601 cases and 5,937 controls

### Table 1 | ASD risk genes

<table>
<thead>
<tr>
<th>dnLoF count</th>
<th>FDR ≤ 0.01</th>
<th>0.01 &lt; FDR = 0.05</th>
<th>0.05 &lt; FDR = 0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥2</td>
<td>ADNP, ANK2, ARID1B, CHD8, CUL3, DYRK1A, GRIN2B, KATNAL2, POGZ, SCN2A, SUV420H1, SYNGAP1, TBRI</td>
<td>ASXL3, BCL11A, CACNA2D3, MLL3</td>
<td>ASH1L</td>
</tr>
<tr>
<td>1</td>
<td>CTTNBP2, GABRB3, PTEN, RELN</td>
<td>APH1A, CD42BPB, ETFB, NAA15, MYO9B, MYT1L, NR3C2, SETD5, TRIO</td>
<td>VIC1</td>
</tr>
<tr>
<td>0</td>
<td>MIB1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

22 autosomal genes with FDR<0.05

---

De Rubeis et al. Nature 2014
Integrative analyses of DNMs in ASD (Total N of trios = 4,244)
61 genes significantly enriched for damaging (LOF or deleterious missense) DNMs after multiple testing correction
Comparison of the results with or without our new dataset

An analysis of a few hundreds of new trios contributes to identification of ten new genes
Of these, **ANKRD11**
**MED13L**
**GABRB1**
**PPP2R5D**
**DDX3X**

-> known NDD genes
(but with limited evidence as ASD genes)

**The other genes,**
**ATP2B2**
**GGNBP2**
**MCM6**
**AGO1**
**ATP1A3**

-> co-expressed genes are significantly enriched for known ASD genes
• Diagnostic single-gene rare variants can be identified in >1% of ASD.

• Copy number variants and mutations in genes responsible for Mendelian disorders characterized with ASD and other symptoms (i.e. syndromic ASD) explain another >4%.

• On the other hand, these rare variants explains a small proportion of liability.

de la Torre-Ubieta et al., Nature Medicine 2016
DNMs in schizophrenia

1,043 ASD trios
1,021 SCZ trios
731 control trios

Takata et al., Neuron 2016

2,541 SCZ trios
2,216 control trios

Howrigan et al., bioRxiv 2018
Loss-of-Function Variants in Schizophrenia Risk and SETD1A as a Candidate Susceptibility Gene

Enrichment of LOF DNM in SETD1A (p = 2.4x10^{-6}), encoding H3K4 methyltransferase

Takata et al., Neuron 2014

<table>
<thead>
<tr>
<th>Gene name</th>
<th>$\mu_{\text{LoF}}$</th>
<th>$N_{\text{de novo}}$</th>
<th>$N_{\text{case}}$</th>
<th>$N_{\text{control}}$</th>
<th>$P_{\text{de novo}}$</th>
<th>$P_{\text{burden}}$</th>
<th>$P_{\text{meta}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SETD1A</td>
<td>6.6e-06</td>
<td>3</td>
<td>7</td>
<td>0</td>
<td>4.6e-07</td>
<td>0.0003</td>
<td>3.3e-09</td>
</tr>
<tr>
<td>TAF13</td>
<td>1.3e-06</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>3.7e-06</td>
<td>0.31</td>
<td>1.7e-05</td>
</tr>
<tr>
<td>HIST1H1E</td>
<td>2.4e-07</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0.00053</td>
<td>0.031</td>
<td>0.00019</td>
</tr>
<tr>
<td>BCAT1</td>
<td>1.9e-06</td>
<td>1</td>
<td>8</td>
<td>3</td>
<td>0.004</td>
<td>0.0058</td>
<td>0.00027</td>
</tr>
<tr>
<td>XIRP2</td>
<td>3.3e-06</td>
<td>0</td>
<td>41</td>
<td>35</td>
<td>1</td>
<td>3.5e-05</td>
<td>0.00039</td>
</tr>
<tr>
<td>KLHL17</td>
<td>3e-06</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>0.0065</td>
<td>0.0096</td>
<td>0.00067</td>
</tr>
<tr>
<td>HSP90AA1</td>
<td>3.1e-06</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>0.0066</td>
<td>0.013</td>
<td>0.00091</td>
</tr>
<tr>
<td>MKI67</td>
<td>1e-05</td>
<td>2</td>
<td>5</td>
<td>10</td>
<td>0.00024</td>
<td>0.53</td>
<td>0.0013</td>
</tr>
<tr>
<td>CAST</td>
<td>3.1e-06</td>
<td>0</td>
<td>15</td>
<td>6</td>
<td>1</td>
<td>0.00019</td>
<td>0.0018</td>
</tr>
<tr>
<td>ENDOV</td>
<td>2.2e-06</td>
<td>0</td>
<td>10</td>
<td>2</td>
<td>1</td>
<td>0.00031</td>
<td>0.0028</td>
</tr>
</tbody>
</table>

Table 2: Meta-analysis results for 1,077 trios, 4,264 cases and 9,343 controls. Only SETD1A reached exome-wide significance.

Singh et al., Nature Neuroscience 2016

The only gene that surpassed the exome-wide significance (0.05/20,000 = 2.5x10^{-6})
Not only for discovery of diagnostic variants - analysis of properties of genes hit by damaging DNMs in ASD
Screening of compounds globally down-regulating DNM target genes

With CMAP (Connectivity Map) data
By DNENRICH (that considers gene sizes etc.) (Fromer et al., Nature 2014)

DNA topoisomerase inhibitors implicated in ASD (King et al. Nature 2013)

Most established maternal risk factor of ASD (Christensen et al. JAMA 2013)
Screening of compounds globally up-regulating DNM target genes (in cell lines)

All of these are cardiac glycosides, used for treatment of cardiac failure

GO enriched among the genes upregulated by cardiac glycosides

-> Previously unknown effect on regulation of gene transcription

Takata et al. Cell Reports 2018
Interim summary 1

• Diagnostic rare genetic variation, one of the most reliable trait biomarkers, can be identified in ~5% of ASD
• The journey of discovery of diagnostic variants for SCZ is still at the beginning stage, while \textit{SETD1A} is the strongest candidate to date.
• Analysis of genes hit by damaging DNM can delineate biological pathways and gene networks involved in ASD, and may help drug discovery

\textit{Let’s move on to genetic complexity in supposed single-gene neurodevelopmental diseases}
A supposed single-gene neurodevelopmental disease: developmental and epileptic encephalopathy (DEE)

A heterogeneous group of conditions characterized by the co-occurrence of epilepsy and developmental impairment or regression.

Many causal/diagnostic genes have been identified, but the diagnostic rate = ~25-40%
Case-control exome analysis of rare variants in DEE, with 743 DEE cases and 2,366 controls, focusing on URVs (variants only once observed in our overall case-control cohort and never seen in databases (ExAC etc.).
Patterns of excess of URVs in DEE

CD (consensus damaging) missense: missenses predicted to be damaging by 7/7 in silico prediction tools (e.g. PolyPhen, SIFT etc.)

Supplementary Table 3. List of 58 known EE/DEE genes

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Chromosome</th>
<th>CodingStart(hg19)</th>
<th>CodingEnd</th>
<th>Ensembl_Canonical_Tx_ID</th>
<th>OMIM_ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>GNB1</td>
<td>1</td>
<td>1718769</td>
<td>1756892</td>
<td>ENST00000378609</td>
<td>139380</td>
</tr>
<tr>
<td>SLC2A1</td>
<td>1</td>
<td>43392711</td>
<td>43424322</td>
<td>ENST00000426263</td>
<td>138140</td>
</tr>
<tr>
<td>KCNA2</td>
<td>1</td>
<td>111145904</td>
<td>111147404</td>
<td>ENST00000316361</td>
<td>176262</td>
</tr>
<tr>
<td>HNRNPU</td>
<td>1</td>
<td>245017751</td>
<td>245027609</td>
<td>ENST00000283179</td>
<td>602869</td>
</tr>
<tr>
<td>ZEB2</td>
<td>2</td>
<td>145147017</td>
<td>145274917</td>
<td>ENST00000303660</td>
<td>605802</td>
</tr>
<tr>
<td>MBDS</td>
<td>2</td>
<td>149216327</td>
<td>149270510</td>
<td>ENST00000404807</td>
<td>611472</td>
</tr>
<tr>
<td>SCN2A</td>
<td>2</td>
<td>166152333</td>
<td>166246334</td>
<td>ENST00000283256</td>
<td>182390</td>
</tr>
<tr>
<td>SCN1A</td>
<td>2</td>
<td>166847754</td>
<td>166930131</td>
<td>ENST00000303395</td>
<td>182389</td>
</tr>
<tr>
<td>SLC6A1</td>
<td>3</td>
<td>11058897</td>
<td>11078652</td>
<td>ENST00000287766</td>
<td>137165</td>
</tr>
<tr>
<td>HCN1</td>
<td>5</td>
<td>45262022</td>
<td>45696195</td>
<td>ENST00000303230</td>
<td>602780</td>
</tr>
<tr>
<td>MF2C</td>
<td>5</td>
<td>88018420</td>
<td>88119605</td>
<td>ENST00000340208</td>
<td>600662</td>
</tr>
<tr>
<td>PURA</td>
<td>5</td>
<td>139493766</td>
<td>139494735</td>
<td>ENST00000331327</td>
<td>600473</td>
</tr>
<tr>
<td>GABRA2</td>
<td>5</td>
<td>161277816</td>
<td>161324428</td>
<td>ENST0000023897</td>
<td>137160</td>
</tr>
<tr>
<td>GABRG2</td>
<td>5</td>
<td>161495005</td>
<td>161580374</td>
<td>ENST00000414552</td>
<td>137164</td>
</tr>
<tr>
<td>SYNGAP1</td>
<td>6</td>
<td>33388041</td>
<td>33419683</td>
<td>ENST00000418600</td>
<td>603384</td>
</tr>
</tbody>
</table>

Takata et al. In revision
d(amaging)URV: LOF and CD missense URVs

p(athogenic)URV: convincingly pathogenic
dURVs in 58DEE genes, e.g. those confirmed to be de novo

May have a modifier/oligogenic effects?
Expression patterns of genes with potential modifier URVs (=non-58DEE gene dURVs in DEE cases with pURV)

An analysis testing if potential modifier dURVs are enriched among genes specifically expressed in each tissue, when compared with the same type of dURVs in controls.

Takata et al. In revision
Common genetic variants contribute to risk of rare severe neurodevelopmental disorders

SNP-based genetic correlations between NDD (6,987 cases and 9,270 controls) against other traits.

- Common-variant risk was not significantly different between individuals **with** and **without** a known protein-coding diagnostic variant, which suggests that common variant risk affects patients both with and without a monogenic diagnosis.

Niemi et al., Nature 2018
Lastly, I would like to show some simple way that would ameliorate phenotypes in single-gene neuropsychiatric disease.
Loss-of-Function Variants in Schizophrenia Risk and \textit{SETD1A} as a Candidate Susceptibility Gene

Enrichment of LOF DNM in \textit{SETD1A} ($p = 2.4 \times 10^{-6}$), encoding H3K4 methyltransferase

Takata et al., Neuron 2014

Analysis of heterozygous \textit{Setd1a} KO mice

Mukai et al., bioRxiv 2019
Phenotypic abnormalities in *Setd1a*+/− mice

- **Altered short-term synaptic plasticity** (greater depression of fEPSP responses)
- **Morphological changes of axons and spines**
- **Specific deficits in working memory** (delayed non-match to sample T-maze task)
Postnatal restoration of *Setd1a* expression

![Diagram](attachment:image.png)
Possibly too simple working hypothesis

Setd1A KO ➔ H3K4me ➔

H3K4 demethylase

Histone demethylase inhibitors

There was compelling overlap between LSD1 and Setd1a bound regions (1,137/1,178)

LSD1=lysine-specific demethylase≠lysergic acid diethylamide
Summary

• Single-gene diagnostic rare variants with a large effect size can be identified in genetically complex neuropsychiatric disorders such as ASD and SCZ.

• On the other hand, comprehensive analysis of rare and common variants highlights that even among supposed single-gene NDD there is considerable genetic complexity.

• While there is substantial genetic complexity, a simple intervention can reverse SCZ-related phenotypes in a mouse model of SCZ with Setd1a deficiency.

Acknowledgement

**ASD DNM paper**
Noriko Miyake
Yoshinori Tsurusaki
Ryoko Fukai
Satoko Miyatake
Eriko Koshimizu
Itaru Kushima
Takashi Okada
Mako Morikawa
Yota Uno
Kanako Ishizuka
Kazuhiko Nakamura
Masatsugu Tsujii
Takeo Yoshikawa
Tomoko Toyota
Nobuhiko Okamoto
Yoko Hiraki
Ryota Hashimoto
Yuka Yasuda
Shinji Saitoh
Kei Ohashi
Yasunari Sakai
Shouichi Ohga
Toshiro Hara
Mitsuhiro Kato
Kazuyuki Nakamura
Aiko Ito
Chizuru Seiwa
Emi Shirahata
Hitoshi Osaka
Ayumi Matsumoto
Saoko Takeshita
Jun Tohyama
Tomoko Saikusa
Toyojiro Matsuishi
Takumi Nakamura
Takashi Tsuboi
Tadafumi Kato
Toshifumi Suzuki
Hirotomo Saitsu
Mitsuko Nakashima
Takeshi Mizuguchi
Fumiaki Tanaka
Norio Mori
Norio Ozaki
Naomichi Matsumoto

**DEE URV paper**
Mitsuko Nakashima
Hirotomo Saitsu
Takeshi Mizuguchi
Satomi Mitsuhashi
Yukitoshi Takahashi
Nobuhiko Okamoto
Hitoshi Osaka
Kazuyuki Nakamura
Jun Tohyama
Kazuhiko Haginoya
Saoko Takeshita
Ichiro Kuki
Tohru Okanishi
Tomohide Goto
Masayuki Sasaki
Noriko Miyake
Satoko Miyatake
Naomi Tsuchida
Kazuhiro Iwama
Gaku Minase
Futoshi Sekiguchi
Atsushi Fujita
Eri Imagawa
Eriko Koshimizu
Yuri Uchiyama
Kohei Hamanaka
Chihiro Ohba
Toshiyuki Itai
Hiromi Aoi
Ken Saída
Tomohiro Sakaguchi
Kouhei Den
Rina Takahashi
Hiroko Ikeda
Tokito Yamaguchi
Kazuki Tsukamoto
Shinsaku Yoshitomi
Taikan Oboshi
Katsumi Imai
Tomokazu Kimizu
Yu Kobayashi
Masaya Kubota
Hirofumi Kashii
Shimpei Baba
Mizue Iai
Ryutaro Kira
Munetsugu Hara
Masayasu Ohta
Yohane Miyata
Rie Miyata
Jun-ichi Takanashi
Jun Matsui
Kenji Yokochi
Masayuki Shimono
Masano Amamoto
Rumiko Takayama
Shinichiro Hirabayashi
Kaori Aiba
Hiroshi Matsumoto
Shin Nabatame
Takashi Shiihara
Mitsuhiro Kato
Naomichi Matsumoto

**Setd1a KO mouse paper**
Jun Mukai
Enrico Cannavo
Ziyi Sun
Gregg Crabtree
Anastasia Diamantopoulou
Pratibha Thakur
Chia-Yuan Chang
Yifei Cai
Stavros Lomvardas
Bin Xu
Joseph A. Gogos

**on behalf of the DEEPEN Consortium**

and many others including all study participants
Consortium membership
The Case-control Working Group of the DEEPEN Consortium*

Atsushi Takata, M.D., Ph.D.1, Mitsuko Nakashima, M.D., Ph.D.2, Hirotomot Suats, M.D., Ph.D.2, Takeshi Mizuguchi, M.D., Ph.D.1, Satomi Mitsuhashi, M.D., Ph.D.1, Yukitoshi Takahashi, M.D., Ph.D.3, Nobuhiko Okamoto, M.D., Ph.D.4, Hitoshi Osaka, M.D., Ph.D.5, Kazuya Nakamura, M.D., Ph.D.6, Jun Tohyama, M.D., Ph.D.7, Kazuhiro Haginoya, M.D., Ph.D.8, Saoko Takeshita, M.D., Ph.D.9, Ichiro Kuki, M.D., Ph.D.10, Tohru Okanishi, M.D., Ph.D.11, Tomohide Goto, M.D., Ph.D.12, Masayuki Sasaki, M.D., Ph.D.13, Yasunari Sakai, M.D., Ph.D.14, Noriko Miyake, M.D., Ph.D.1, Satoko Miyatake, M.D., Ph.D.1, Naomi Tsuchida, M.D., Ph.D.1, Kazuhiro Iwama, M.D., Manabu Minase, M.D., Ph.D.1, Futoshi Sekiguchi, M.D., Ph.D.1, Atsushi Fujita, M.D., Ph.D.1, Eri Imagawa, M.D., Ph.D.1, Eriko Koshimizu, M.D., Ph.D.1, Yuri Uchiyama, M.D., Ph.D.1, Kohei Hanamaka, M.D., Ph.D.1, Chihiro Ohba, M.D., Ph.D.1, Toshiyuki Itai, M.D., Ph.D.1, Yumi Aoi, M.D., Ph.D.1, Ken Saida, M.D., Ph.D.1, Tomohiro Sakaguchi, M.S1, Hiroko Ikeda, M.D., Ph.D.3, Kaito Yamaguchi, M.D., Ph.D.3, Kazuo Tsukamoto, M.D., Ph.D.3, Shibaku Yoshitomi, M.D., Ph.D.3, Taikan Oboshi, M.D., Ph.D.3, Kazumi Imai, M.D., Ph.D.3, Tomokazu Kimizui, M.D., Ph.D.15, Yuki Kobayashi, M.D., Ph.D.1, Masaya Kubota, M.D., Ph.D.16, Hirofumi Kashiwai, M.D., Ph.D.16, Shinpei Baba, M.D., Ph.D.11, Mizue Iai, M.D., Ph.D.12, Ryutaroh Kira, M.D., Ph.D.17, Munetsugu Hara, M.D., Ph.D.18, Masayasu Ohta, M.D., Ph.D.19, Yohane Miyata, M.D., Ph.D.20, Rie Miyata, M.D., Ph.D.21, Jun-ichi Takashani, M.D., Ph.D.22, Jun Matsu, M.D., Ph.D.23, Kenji Yokochi, M.D., Ph.D.24, Masayuki Shimono, M.D., Ph.D.25, Masayo Amamoto, M.D., Ph.D.26, Rumiko Takayama, M.D., Ph.D.27, Shinichi Hiramayashi, M.D., Ph.D.28, Kaori Aiba, M.D., Ph.D.29, Hiroshi Matsumoto, M.D., Ph.D.30, Shin Katabame, M.D., Ph.D.31, Takaaki Shihara, M.D., Ph.D.32, Hiroh Omao, M.D., Ph.D.33, Akito Watanabe, M.D., Ph.D.34, Asako Horino, M.D., Ph.D.35, Mao Fujioka, M.D., Ph.D.36, Takayoshi Koke, M.D., Ph.D.37, Hitoshi Ikeda, M.D., Ph.D.38, Tae Ikeda, M.D., Ph.D.39, Yasuhiro Suzuki, M.D., Ph.D.30, Keiko Yanagihara, M.D., Ph.D.31, Yukiko Mogami, M.D., Ph.D.32, Kazuhiro Muramatsu, M.D., Ph.D.33, Akihiko Miyayuchi, M.D., Ph.D.34, Karin Kojima, M.D., Ph.D.35, Akira Hojo, M.D., Ph.D.36, Shinichi Magara, M.D., Ph.D.37, Sato Suzuki-Muroomoto, M.D., Ph.D.38, Takehiko Inui, M.D., Ph.D.39, Yuki Mune, M.D., Ph.D.40, Ryotaro Endo, M.D., Ph.D.41, Ikki Kaba, M.D., Ph.D.42, Yoshihiro Watanabe, M.D., Ph.D.43, Hiisingi Kawakami, M.D., Ph.D.44, Go Takei, M.D., Ph.D.45, Atsushi Kumagai, M.D., Ph.D.46, Hiroshi Terashima, M.D., Ph.D.47, Toshio Hara, M.D., Ph.D.48, Seiichiro Yoshioka, M.D., Ph.D.49, Atsuko Yamamoto-Arisaka, M.D., Ph.D.50, Masahiro Ishii, M.D., Ph.D.51, Koji Tominaga, M.D., Ph.D.52, Yuji Inaba, M.D., Ph.D.53, Tomohiro Chiyonobu, M.D., Ph.D.54, Saori Tanabe, M.D., Ph.D.55, Noriyuki Akasa, M.D., Ph.D.56, Muneaki Matsuo, M.D., Ph.D.57, Yuji Kumagai, M.D., Ph.D.58, Shin-ichiro Hamano, M.D., Ph.D.59, Satoru Takahashi, M.D., Ph.D.60, Shinu Nogaya, M.D., Ph.D.61, Keitaro Yamada, M.D., Ph.D.62, Kyoko Takano, M.D., Ph.D.63, Mina Yokoyama, M.D., Ph.D.64, Kazuhiro Yamamoto, M.D., Ph.D.65, Atsuo Iida, M.D., Ph.D.66, Yuichi Takami, M.D., Ph.D.67, Yoji Sugawara, M.D., Ph.D.68, Hideki Hoshino, M.D., Ph.D.69, Gaku Yamanaka, M.D., Ph.D.70, Masahiro Ito, M.D., Ph.D.71, Takahiro Ichihara, M.D., Ph.D.72, Naoko Ishihara, M.D., Ph.D.73, Hisako Ishihara, M.D., Ph.D.74, Miki Tani, M.D., Ph.D.75, Kenji Saigo, M.D., Ph.D.76, Ayako Iida, M.D., Ph.D.77, Naoko Kanemoto, M.D., Ph.D.78, Katsuhiro Kobayashi, M.D., Ph.D.79, Tetsuhiro Fukuyama, M.D., Ph.D.80, Yusuke Aoki, M.D., Ph.D.81, Hisaya Kizu, M.D., Ph.D.82, Yuji Fujii, M.D., Ph.D.83, Hiroshi Ono, M.D., Ph.D.84, Nobuko Moriyama, M.D., Ph.D.85, Akira Kuma, M.D., Ph.D.86, Hiroshi Arai, M.D., Ph.D.87, Shinjiro Akaboshi, M.D., Ph.D.88, Masahiko Hiyane, M.D., Ph.D.89, Masami Toyama, M.D., Ph.D.90, Takeshi Matsushige, M.D., Ph.D.91, Hiroko Baben, M.D., Ph.D.92, Tatsuharu Sato, M.D., Ph.D.93, Sawesku, M.D., Ph.D.94, Keitaro Yamada, M.D., Ph.D.95, Kuriko Kagitani-Shimono, M.D., Ph.D.96, Atsushi Sato, M.D., Ph.D.97, Naoaki Sato, M.D., Ph.D.98, Takashi Matsushita, M.D., Ph.D.99, Ayako Iida, M.D., Ph.D.100, Ayako Hattori, M.D., Ph.D.101, Misaki Nakashina, M.D., Ph.D.102, Shouichi Ohga, M.D., Ph.D.103, Hiroshi Ohga, M.D., Ph.D.104, Kisho Tominaga, M.D., Ph.D.105, Takeshi matsushita, M.D., Ph.D.106, Takeshi Enokido, M.D., Ph.D.107, Kenji Ida, M.D., Ph.D.108, Shigeru Kanzawa, M.D., Ph.D.109, Naoko Aono, M.D., Ph.D.110, Kazue Kanzawa, M.D., Ph.D.111, Elina Taniguchi, M.D., Ph.D.112, Shigeo Nagai, M.D., Ph.D.113, Charles Marques Lourenc, M.D., Ph.D.114, Takahiro Yamamoto, M.D., Ph.D.115, Hiroko Kurashashi, M.D., Ph.D.116, Yoshihashi, M.D., Ph.D.117, Hiroshi Suzuki, M.D., Ph.D.118, Yoshinori Kobayashi, M.D., Ph.D.119, Mutsumi Sato, M.D., Ph.D.120, Takeshi Tsuji, M.D., Ph.D.121, Toshiro Hara, M.D., Ph.D.122, Fumihito Nozaki, M.D., Ph.D.123, Mariko Ikegami, M.D., Ph.D.124, Yoshio Makita, M.D., Ph.D.125, Kazuhiro Miya, M.D., Ph.D.126, Mari Matsuo, M.D., Ph.D.127, Takeshi Kamiyama, M.D., Ph.D.128, Mitsuhiro Kato, M.D., Ph.D.129, Naomichi Yoshimoto, M.D., Ph.D.130.
That’s it.